R57K Polymorphism in the Human Immunodeficiency Virus Type 1 Protease as Predictor of Early Virological Failure in a Cohort of Antiretroviral-Naïve Patients Treated Mostly with a Nelfinavir-Containing Regimen

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In 243 antiretroviral-naïve human immunodeficiency-infected patients starting a first-line-protease inhibitor (mainly nelfinavir)-containing therapy, the presence of the polymorphism R57K in the protease at the inception of therapy was independently associated with a higher rate of virological failure.

The relation between the response to protease inhibitor (PI)-including regimens and the natural variability of the human immunodeficiency type 1 (HIV-1) protease is not fully elucidated (1, 3, 16, 17). Our study aimed at identifying the determinants of early treatment failure in APROVIR, a virological substudy of the APROCO/COPILOTE French multicenter cohort including antiretroviral-naïve patients started on a PI-containing regimen.

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Patients were followed up every 2 months. Virological failure (VF) during the first year of therapy was defined as (i) viral rebound on two consecutive plasma specimens with HIV-1 RNA at >500 copies/ml after an initial response below 500 copies/ml or (ii) nonresponse with absence of viral drop below 500 copies/ml. The HIV-1 protease genotype was determined from plasma sampled at baseline for all the patients by the Agence Nationale de Recherches sur le SIDA consensus method (8). The definitions of the protease resistance mutations were those reported by the International AIDS Society-USA Panel (<http://www.iasusa.org>) on 1 October 2002. Major mutations corresponded to D30N, M46I/L, G48V, I50L/V, V82A/F/T/S, I84V, and L90M. The L10F/I/RV, K20M/R, L24I, V32I, L33F, M36I, M46I/L, I47V, F53L, I54V/L/M, A71V/T, G73S/A, V77I, and N88D/S changes were considered minor mutations. The L63P mutation and all other deviations from the protease subtype B consensus sequence, exclusive of the major and minor mutations, were considered polymorphisms. HIV-1

TABLE 1. Clinical and biological characteristics of the 243 patients in the APROVIR study at baseline therapy

Characteristic ^a	n	Value
Median age (yr) (IQR)	242	37 (32–43)
No. of men (%)	242	203 (84)
No. (%) in HIV transmission category:	240	
Men who have sex with men		103 (43)
Intravenous drug use		29 (12)
Sex between men and women		74 (31)
Others or unknown		34 (14)
No. (%) in CDC stage:	241	
A		111 (46)
B		51 (21)
C		79 (33)
Median CD4 ⁺ cell count/μl (IQR)	241	188 (58–385)
Median plasma HIV-1 RNA (log ₁₀ copies/ml) (IQR)	241	5.0 (4.5–5.5)
No. (%) with baseline PI of:	242	
RTV		12 (5)
IDV		46 (19)
NFV		160 (66)
Others (including PI combination)		24 (10)
No. (%) with baseline NRTIs of:	241	
ZDV, 3TC		89 (37)
d4T, ddI		63 (26)
d4T, 3TC		72 (30)
Others		17 (7)
No. (%) with HIV-1 subtype:	230	
A		12 (5.3)
B		208 (90.4)
C		3 (1.3)
E		4 (1.8)
Others		3 (1.2)

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^a Abbreviations: IQR, interquartile range; NRTIs, nucleoside reverse transcriptase inhibitors; ZDV, zidovudine; d4T, stavudine; ddI, didanosine; 3TC, lamivudine; CDC, Centers for Disease Control and Prevention.

FIG. 1. Frequency of baseline polymorphisms and resistance mutations in the protease gene in 236 patients started on a PI-containing regimen. Results are from the APROVIR substudy, 1997 to 2000. Resistance mutations are those reported by the International AIDS Society-USA Panel (<http://www.iasusa.org>). The L63P mutation was considered a polymorphism. Only codons with polymorphisms at a frequency >5% are shown.

subtype analysis consisted of a serotyping and heteroduplex mobility assay (2, 6) or an *env* phylogenetic analysis. The adherence to treatment was assessed at month 4 (M4) by self-administered questionnaires (5).

A multivariate Cox proportional-hazards model was used to analyze the predictive factors of VF during the first year of follow-up. The model included clinical, behavioral, and virological characteristics of the patients; the number of protease amino acid mutations (major plus minor mutations); and all the protease amino acid polymorphisms at M0 present in at least 5% of the patients. We used the principle of intent to continue analysis, i.e., ignoring changes or modifications of antiretroviral treatment.

Between January 1998 and June 1999, 243 patients were included in APROVIR (Table 1). The genotype of the protease at baseline was documented for 236 of the 243 patients (Fig. 1). Only two patients (0.8%) exhibited a major PI resistance mutation (L90M). Minor mutations were observed at codons 77 (24%), 36 (22%), 10 (12%), 71 (8%), 20 (2%), and 47 (0.4%). Polymorphisms were also observed at low frequencies at positions involved in PI resistance: L10M (0.4%), K20I/V (6%), L33I/V (4%), I47M (0.4%), V77M (0.4%), and V82I/L (2%). Polymorphisms at other residues in the protease were also frequently observed.

At M12, the patients exhibited a mean decrease of plasma HIV-1 RNA of $-2.6 \log_{10}$ copies/ml, 83% had a plasma HIV-1 RNA below 500 copies/ml, and the mean increase of CD4⁺

cells was 203/ μ l. Out of 220 patients with a follow-up at or after M4, 35 (16%) patients experienced a VF during the first year of follow-up: 8 were nonresponders, whereas 27 patients had a virological rebound. The frequencies of VF were 16, 15, 27, and 10% in the patients treated with nelfinavir (NFV), indinavir (IDV), zidovudine (ZDV), or another PI, respectively ($P = 0.6$). Adherence to PI therapy at M4 was reported by 132 of 220 (60%) patients. It was low for 20 (15%) patients, moderate for 35 (27%) patients, and high for 77 (58%) patients.

The number of PI resistance mutations at baseline, the number of protease polymorphisms at baseline, and the HIV-1 subtype were not associated with VF in the univariate analysis. In the multivariate analysis (Table 2), a higher plasma HIV-1 RNA at M0 and the failure of adherence to therapy at M4 were associated with a higher frequency of VF; an older age at inclusion was associated with a lower risk of VF. The presence of the polymorphic mutation R57K at baseline was also independently associated with a higher frequency of subsequent VF. This mutation was detected in 24 (10%) patients. VF occurred in 10 of 24 (42%) patients with the R57K mutation and in 25 of 196 (13%) patients with a wild-type protease codon 57. Among patients on NFV, 9 of 17 patients with the R57K mutation had a VF, compared with 14 of 140 patients without the R57K mutation. Although there was a trend for a stronger association between the R57K mutation and subsequent VF in those patients started on NFV (adjusted hazard ratio = 15.0; 95% confidence interval: 5.5 to 41.2; $P < 0.0001$),

TABLE 2. Determinants of VF in the first year of follow-up in 220 patients started on a PI-containing regimen, APROVIR substudy, 1997 to 2001 (multivariate Cox model)^a

Parameter	Initial multivariate		Final multivariate	
	HR (95% CI)	P	HR (95% CI)	P
Age (per 10 additional yr)	0.58 (0.34–0.98)	0.04	0.57 (0.36–0.91)	0.02
Female vs male	0.37 (0.10–1.44)	0.15		
HIV-1 RNA at M0 (per 1 log ₁₀ copies/ml higher)	1.28 (0.67–2.46)	0.46	1.81 (1.04–3.14)	0.04
PI at M0				
IDV vs other	0.23 (0.03–1.80)	0.16		
NFV vs other	0.19 (0.03–1.12)	0.07		
RTV vs other	0.50 (0.06–3.92)	0.50		
PI at M4				
IDV vs other	1.02 (0.10–10.59)	0.99		
NFV vs other	1.49 (0.19–11.92)	0.71		
RTV vs other	1.13 (0.09–14.23)	0.93		
No PI vs other	6.39 (0.74–55.54)	0.09		
Adherence to PI at M4				
Low vs high	5.50 (1.42–21.32)	0.01	5.43 (1.61–18.31)	0.006
Medium vs high	3.63 (1.04–12.65)	0.04	3.59 (1.07–11.96)	0.04
Unknown vs high	5.10 (1.68–15.48)	0.004	5.07 (1.79–14.33)	0.002
Codon V77: resistant vs WT vs polymorphism	1.26 (0.54–2.93)	0.59		
Codon R57: 57K vs 57R	6.00 (2.51–14.31)	<0.0001	5.46 (2.42–12.32)	<0.0001
Codon D60: polymorphism vs WT	1.83 (0.58–5.76)	0.30		
Codon H69: polymorphism vs WT	0.52 (0.14–1.84)	0.31		

^a Abbreviations: HR, hazard ratio; CI, confidence interval; WT, wild type.

the interaction between R57K and the type of initial PI was not significant. It is, however, noteworthy that none of the four patients on IDV with the R57K polymorphism had a subsequent VF. Finally, a robustness analysis showed that missing data on adherence could not have substantially influenced the results for codon 57.

In this study, we found previously reported covariates (9, 11, 12, 14, 18, 20) associated with VF: age, high plasma HIV-1 RNA at baseline, and failure to adhere to therapy. More surprisingly, the presence of the polymorphism R57K in the HIV-1 protease at baseline therapy was also strongly associated with VF in the first year of follow-up, conversely to the presence and number of major and minor PI resistance mutations at baseline. Very few major protease mutations were present at baseline in our cohort, a finding consistent with previous reports (1, 7), whereas minor PI resistance mutations were frequently present at baseline (10), although not associated with a subsequent VF (1, 3). Mutations other than R57K, such as A71V/T, particularly for patients on a first-line RTV therapy, and I93L and a high number of polymorphisms were shown to have some predictive value for failure of therapy (17). Studying patients from the ICONA cohort, mainly treated with IDV or saquinavir, Perno et al. (16) found that both a higher number of PI resistance mutations and the presence of the mutation L10I or M36I at baseline therapy were associated with higher risks of VF. We do not confirm these findings, in that we studied patients who were mostly started on NFV. Though the polymorphism R57K was highly associated with a

higher risk of subsequent VF in our cohort, its relevance needs to be further evaluated with other databases, in order to clarify if this finding is dependent on the nature of the PI. When we studied the evolution of the viral genotype at the time of virological failure in APROVIR (13), we found that the D30N mutation was the most frequently selected PI resistance mutation; interestingly, the R57K polymorphism was present at baseline and at the time of VF in six of nine patients with a selection of D30N (data not shown). The mutation L90M was detected in only one patient on NFV at month 10 and disappeared at month 12, with detection of a virus presenting both the D30N and the R57K mutations, suggesting the existence of a selective advantage for this combination of mutations. Moreover, two studies suggested that the NFV resistance pathway including the D30N mutation, which is generally used by HIV-1 clade B isolates (15), is underrepresented in HIV-1 subtype C- or CRF02_AG-infected individuals failing on NFV (4; P. Gomes, I. Diogo, F. Gonçalves, P. Carvalho, J. Cabanas, M. C. Lobo, and R. Camacho, Abstr. 9th Conf. Retrovir. Opportunistic Infect., abstr. 46, 2002). In APROVIR, all viruses with the R57K mutation belonged to clade B or to CRF01_AE. We did not detect the R57K polymorphism in the baseline samples from a cohort of recently infected patients from Abidjan (Ivory Coast) mainly infected with HIV-1 CRF02_AG (19) or in a cohort of ARV-naïve HIV-1 clade C-infected patients (unpublished data); taken together, these observations could also indicate a role for the R57K mutation in facilitating the D30N NFV resistance pathway. In vitro studies could also help

to evaluate the impact of the R57K polymorphism on viral fitness, in the presence or absence of the major NFV resistance mutations. If confirmed, our data suggest that genotyping before introduction of a first-line-PI-containing therapy could be of clinical use.

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