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HIV-1 Genotypic Resistance Patterns Predict Response to Saquinavir–Ritonavir Therapy in Patients in Whom Previous Protease Inhibitor Therapy Had Failed

Andrew R. Zolopa, MD, **Robert W. Shafer, MD**, **Ann Warford, PhD**, **Jose G. Montoya, MD**, **Phillip Hsu, BA**, **David Katzenstein, MD**, **Thomas C. Merigan, MD**, and **Brad Efron, PhD**
Stanford University School of Medicine, Stanford, California

Abstract

Background—Tests for resistance to HIV drugs are available for clinical use; however, their predictive value has not been fully assessed.

Objectives—To determine HIV-1 genotypic predictors of a virologic response to saquinavir–ritonavir therapy in patients in whom at least one previous protease inhibitor–containing regimen had failed and to compare the predictive value of baseline genotype with that of standard clinical evaluation.

Design—Retrospective clinical cohort study.

Setting—University-based HIV clinic.

Patients—54 HIV-1–infected adults treated with saquinavir–ritonavir who had experienced virologic failure while receiving a protease inhibitor–containing regimen for at least 3 months.

Measurements—HIV-1 reverse transcriptase and protease gene sequences, CD4 cell counts, clinical characteristics, detailed antiretroviral treatment history, and plasma HIV-1 RNA levels at baseline and at three follow-up time points (median, 4, 12, and 26 weeks). Virologic failure was defined as a plasma HIV RNA level greater than 1000 copies/mL.

Results—In 22 patients (41%), a plasma HIV-1 RNA level less than 500 copies/mL was achieved by week 12; in 15 patients (28%), this response was maintained through week 26. Clinical characteristics predicting a poorer response included a diagnosis of AIDS, lower CD4 cell count, and higher plasma HIV RNA level ($P < 0.03$). Number of previous nucleoside reverse transcriptase inhibitors, previous protease inhibitor therapy, and duration of previous protease inhibitor therapy were predictors of poorer response ($P < 0.01$). Multivariate regression models revealed that protease mutations present at the initiation of saquinavir-ritonavir therapy were the strongest predictors of virologic response. A model of clinical features explained up to 45% of the variation in virologic outcomes by week 12, whereas the explained variance was 71% when genotypic predictors were included.

Conclusions—In patients in whom protease inhibitor–containing antiretroviral therapy fails, HIV-1 genotype is predictive of virologic response to subsequent therapy. This predictive capacity adds to that of standard clinical evaluation.

Requests for Reprints: Andrew R. Zolopa, MD, Division of Infectious Diseases and Geographical Medicine, Stanford University School of Medicine, Grant Building, Room S-156, Stanford, CA 94305; e-mail, azolopa@stanford.edu.
Current Author Addresses: Drs. Zolopa, Shafer, Warford, Montoya, Hsu, Katzenstein, and Merigan: Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Grant Building Room S-156, Stanford, CA 94305. Dr. Efron: Department of Statistics, Stanford University, Sequoia Hall, Room 132, Stanford CA 94305.

Combination antiretroviral therapy for HIV-1 infection has resulted in profound control of HIV replication in vivo, improved immune function, and significant decreases in AIDS-related morbidity and mortality (1–9). For many persons, however, this therapy does not provide sustained viral suppression or durable clinical benefit (10,11). Potential reasons for the loss of viral suppression include host immune defects, poor adherence to therapy, pharmacologic factors, and drug resistance (10–17). However, HIV-1 resistance to drug therapy is probably the central factor in the loss of viral suppression (18–22).

Mutations that result in reduced drug susceptibility have been demonstrated in vitro for all currently available antiretroviral agents, and some of these mutations have been associated with increasing plasma HIV-1 RNA levels and disease progression in clinical trials (19–30). Genotypic and phenotypic methods of measuring drug resistance are increasingly available to clinicians (31–37). However, the role of these tests in clinical practice has not been fully assessed. Many experts have been skeptical of resistance testing, although a recent consensus statement provides cautious support for testing in certain clinical circumstances (38–42).

Our objective was to determine the genotypic predictors of virologic response to saquinavir–ritonavir combination therapy in patients in whom therapy with at least one protease inhibitor–containing antiretroviral regimen had failed. We investigated whether HIV-1 reverse transcriptase and protease genotype predicts virologic response to saquinavir–ritonavir by week 12 and week 26 and compared those data with predictors from clinical and antiretroviral treatment history.

Methods

Patients

Two of the investigators treated patients in a university-based clinic that provides primary care for 500 HIV-infected adults. We identified 54 patients who received saquinavir–ritonavir between October 1996 and February 1998 after therapy with at least one protease inhibitor–containing antiretroviral regimen had failed. Treatment failure was defined as a greater than 0.5 $\log_{10}$ copies/mL (more than three-fold) increase in plasma HIV RNA level from a nadir value, an HIV RNA level greater than 10 000 copies/mL, or detectable HIV RNA after the level had been below the threshold of detection (<500 copies/mL) during a therapeutic regimen for more than 12 weeks.

Study patients received 400 to 600 mg of saquinavir in a hard-gel formulation (Invirase, Roche Laboratories, Nutley, New Jersey) and 300 to 400 mg of ritonavir in capsule form (Norvir, Abbott Laboratories, Abbott Park, Illinois) twice daily. In addition to the two protease inhibitors, 47 patients (87%) received two nucleoside reverse transcriptase inhibitors, 4 received three nucleoside reverse transcriptase inhibitors, 2 received two nucleoside reverse transcriptase inhibitors and either nevirapine or delavirdine, and one patient received lamivudine.

Clinical and demographic variables were abstracted from the medical records. Adherence, as recorded in the patient's record, was categorized by the self-reported number of missed doses in the month before evaluation and was classified as none, one to two, three to seven, or eight or more. Plasma levels of HIV-1 RNA were monitored on average every 4 to 6 weeks, and samples were stored at −70 °C.

The Stanford University Panel on Medical Human Subjects approved this study (#M1272).

HIV Genotyping

Baseline HIV-1 genotype was evaluated in plasma specimens that were stored within 1 month before initiation of saquinavir–ritonavir therapy and were obtained while patients were still receiving an ineffective antiretroviral regimen. Plasma HIV-1 RNA was extracted, and nested polymerase chain reaction (PCR) amplification generated a 1.3-kb fragment encompassing protease and the first 750 nucleotides of reverse transcriptase (43,44). Direct bidirectional dideoxynucleotide terminator cycle sequencing of the PCR product was performed as described elsewhere (44). Sequencing reactions were analyzed by using an ABI 377 instrument (Perkin-Elmer, Foster City, California) and were manually proofread and edited. Sequences were compared to the HIV-1 clade B consensus sequence (Los Alamos database), and differences in amino acid sequence, including positions that contained a mixture of wild-type and mutant residues, were classified as mutations (45). Phylogenetic analysis of HIV-1 RNA sequence verified lack of cross-contamination (data not shown).

A priori, we decided to assess reverse transcriptase codons 41, 67, 69, 70, 74, 75, 151, 184, 210, 215, and 219 as predictors of virologic response. Mutations at these codons are known to be associated with resistance to one of the nucleoside reverse transcriptase inhibitors (25,45). In the protease gene, mutations at codons 30, 46, 48, 54, 82, 84, and 90 were evaluated as potential predictors. We chose these “major” mutations a priori because they are associated with in vitro resistance to a protease inhibitor or occur commonly in patients in whom therapy with currently licensed protease inhibitors is failing. We also evaluated all other protease codons as predictors of response.

Virologic Outcomes

Virologic response to saquinavir–ritonavir was measured at two time points between 3 and 18 weeks and again around week 24 (range, 22 to 36 weeks); the median time points of the three follow-up evaluations were 4, 12, and 26 weeks. Levels of HIV-1 RNA were measured by using the Amplicor HIV Monitor Assay (Roche Molecular Systems, Alameda, California). Specimens with HIV RNA below the level of detection (<500 copies/mL) on this assay were retested by using the ultrasensitive modification with a lower limit of detection of less than 50 copies/mL (43).

Virologic response to saquinavir–ritonavir therapy was categorized on the basis of the larger response from baseline to the first or second evaluation (median, 4 and 12 weeks). The ordinal categories were 1) complete response if the plasma HIV-1 RNA level was less than 500 copies/mL, 2) partial response if the reduction from baseline RNA level was 0.5 $\log_{10}$ copies/mL or more but was not less than 500 copies/mL, and 3) nonresponse if reduction from baseline values was less than 0.5 $\log_{10}$ copies/mL.

Statistical Analysis

Demographic, clinical, and genotypic variables were analyzed as potential predictors of virologic response by using bivariate linear regression and multivariable linear regression. In the multivariable models, we included a subset of the reverse transcriptase mutations (listed above) identified through stepwise regression as significant ($P < 0.05$) predictors. This subset of mutations was included in the model as a signed-sum variable. For the protease mutations, we included the signed sum of the seven major mutations listed above and the signed sum of three additional mutations at codons 10, 19, and 71, which were found to be statistically significant bivariate predictors.

The signed-sum variable is derived by a summation of the relevant mutations identified in the baseline sequence. A separate sum is derived from the seven major protease mutations, the three additional protease mutations, and the subset of statistically significant reverse

transcriptase mutations (codons 69 and 210). In the signed-sum variable, mutations that are positively associated with virologic outcome (such as protease mutation D30N) are assigned a positive sign (+1) and mutations negatively associated with outcome are assigned a negative sign (−1).

We used the Cook distance to assess skewing of the ordinal outcome variable in the final multivariable model (model 5, Table 3). The value of 0.11 indicated no significant skewing; this result supports the use of linear regression models (46). We also evaluated the multivariate models for bias that would result from overfitting of the data. We used a bootstrap technique to estimate bias ("optimism") in the explained variance values (R^2) for the models presented and found minimal upward bias; for example, model 3 in Table 3 has a bias of approximately one fifth of the SE (data for other models not shown) (47). A bootstrap technique was used to provide the 95% CI estimates for the R^2 values in Table 3. We selected 25 bootstrap samples of 54 with replacement from the original 54 patients to estimate the 95% CIs. The Wilcoxon rank test was used for comparisons between specific previous protease inhibitors in Table 1, and the *F* test was used for comparisons between models in Table 3. Two-sided *P* values are reported for all analyses. All analyses were conducted by using S-PLUS, version 4.0 (MathSoft, Seattle, Washington).

Results

Virologic Response to Saquinavir–Ritonavir Therapy

Of the 54 study patients, 22 (41%) achieved a "complete" response, with plasma HIV-1 RNA levels less than 500 copies/mL by the second follow-up evaluation (at a median of 12 weeks). Of these 22 patients, 10 (18.5% of the entire cohort) achieved a plasma HIV-1 RNA level less than 50 copies/mL. Fourteen patients (26%) had a partial response to saquinavir–ritonavir, and 18 (33%) were nonresponders (Table 1). The virologic response to saquinavir–ritonavir is shown by initial response category in Figure 1. The response waned somewhat in the partial and complete response groups by week 26: The HIV RNA level remained below 500 copies/mL in 15 patients (28%) and below 50 copies/mL in 10 patients (19%).

Predictors of Virologic Response from the Clinical and Antiretroviral Treatment History

Table 1 delineates baseline characteristics of the cohort as predictors of virologic response to saquinavir–ritonavir therapy by week 12. Plasma levels of the HIV-1 RNA and CD4 count within 4 weeks of the start of combination therapy and a history of an AIDS-defining opportunistic infection or malignant disease were predictors of response.

The cohort was heavily pretreated with nucleoside reverse transcriptase inhibitors. Only 24% of patients received a new nucleoside reverse transcriptase inhibitor along with saquinavir–ritonavir. Sixteen patients (29%) had received a non-nucleoside reverse transcriptase inhibitor (15 received nevirapine and 1 received delavirdine) before saquinavir–ritonavir but only 2 patients received a non-nucleoside inhibitor as part of saquinavir–ritonavir therapy. Before saquinavir–ritonavir therapy, the median number of protease inhibitors taken was two and the median duration of protease inhibitor therapy was 48 weeks. Previous protease inhibitor therapies are listed in Table 1.

The number and duration of nucleoside reverse transcriptase inhibitors and the number of protease inhibitors taken before saquinavir–ritonavir therapy were predictive of virologic response, and a trend was seen for duration of previous protease inhibitor therapy as a predictor. Patients who had had unsuccessful nelfinavir therapy as their sole previous protease inhibitor had a better response to saquinavir–ritonavir than did patients who had had unsuccessful indinavir therapy as their sole previous protease inhibitor ($P < 0.01$). No other statistically

significant differences were seen among the nine previous protease inhibitor treatment regimens listed in Table 1.

Adherence to Saquinavir–Ritonavir Therapy

Thirty-nine (81%) of the 48 patients with recorded self-reported adherence measures missed two or fewer doses of saquinavir–ritonavir in the month before the first and second evaluations. Although self-report of adherence was lower in the nonresponse group, the differences between response groups did not reach statistical significance. Furthermore, this measure of adherence did not add to the multivariate prediction models described below (data not shown).

HIV-1 Genotype as Predictor of Virologic Response by Week 12

Mutations in the protease gene were strongly associated with virologic outcome. Each of the seven major mutations defined a priori, except for I84V, were predictors of virologic response ($P < 0.01$) (Figure 2). In addition to the seven "major" mutations, the "minor" mutations at codons 10, 19, and 71 were associated with virologic response ($P < 0.01$). All protease mutations evaluated except for L19Q/I and D30N were associated with a poor virologic response.

Linear regression models demonstrated a strong relation between the number of major mutations in the protease gene at baseline and the virologic response to saquinavir–ritonavir therapy by week 12 ($P < 0.001$). In Table 2, the relation between the seven major protease mutations present at the start of saquinavir–ritonavir therapy and the virologic response is shown. Patients without major protease mutations or with a D30N mutation had approximately a 100-fold (2 $\log_{10}$ copies/mL) reduction in plasma HIV-1 RNA levels at weeks 4 and 12 and an increase in CD4 count of 55 to 60×10^9 cells/L by week 12. Of the 6 patients with the L90M mutation, 2 had a complete response, 2 had a partial response, and 2 were nonresponders. Among the 10 patients with two major mutations, 2 had a complete response, 6 had a partial response, and 2 were nonresponders by week 12. In contrast, none of the 17 patients with three or more major protease mutations had a complete response, and only 4 (24%) had a partial response.

The 11 reverse transcriptase mutations associated with resistance to nucleoside reverse transcriptase inhibitors were evaluated as predictors of virologic response. Mutations M41L, D67N/G, L210W, T215Y/F, and K219Q/E, which are associated with zidovudine resistance, and mutation T69D/N, which is associated with didanosine and zalcitabine resistance, were bivariate predictors of poor virologic response by week 12 (Figure 2).

Multivariate Prediction Models

Table 3 compares multivariate prediction models using baseline clinical and antiretroviral treatment characteristics with models using baseline HIV-1 genotype. The clinical and treatment history model was derived through stepwise regression of the bivariate predictors in Table 1; baseline plasma HIV-1 RNA level, duration of previous reverse transcriptase inhibitor therapy, number of previous protease inhibitors taken, and previous treatment with nelfinavir were independent predictors of virologic response by week 12. The clinical prediction model (model 1) composed of these four variables explains 45% of the variation in virologic outcomes.

Prediction models based on all seven major protease mutations in multivariate stepwise regression analysis did not identify a stable subset of independent predictors. Given the difficulty in choosing a subset of these mutations for a multivariate model and the likelihood that these mutations are not independent events, we used a single variable: the signed sum of the seven major mutations. The signed sum explained 59% of variation in outcomes by week

12 (model 2, Table 3). It is important to note that mutation D30N is positively associated with virologic response, whereas all other mutations are negatively associated with response.

To further explore protease mutations as predictors of response, we took the three additional protease mutations found to be bivariate predictors and created a signed-sum variable. We then added this variable to the signed sum of the major mutations (model 2). The resulting protease genotype model (model 3, Table 3) that includes these two variables explains 66% of the variation in outcomes, an improvement over the model based only on the signed sum of the major protease mutations ($P < 0.01$).

Through stepwise regression, mutations at codons 69 and 210 were selected as the strongest predictors of the 11 reverse transcriptase mutations associated with drug resistance. In model 4 (Table 3), the signed sum of these two reverse transcriptase mutations was combined with the signed sum of the protease mutations (model 3), producing a genotypic model that explains 67% of the variance in outcomes. This additional variable did not significantly improve the predictive capacity of model 3 ($P > 0.2$).

Finally, in comprehensive model 1 (model 5), the genotypic predictors were combined with the clinical and antiretroviral treatment history variables of model 1 (Table 3). The protease genotypic variables from model 3 remained strong predictors of response; however, of the clinical and treatment history variables from model 1, only number of previous protease inhibitors made a borderline contribution to the overall predictive capacity of the model. The resulting model explains 69% of the variance in virologic response to saquinavir–ritonavir by week 12. Moreover, the genotypic predictors from model 3 remained significant predictors in comprehensive model 2, in which all of the clinical predictors of model 1 were forced into the model (model 6) ($P < 0.01$). The explained variance of 71% for comprehensive model 2 is slightly improved over that shown for model 5, but none of the clinical variables are statistically significant in this prediction model ($P = 0.16$ compared with model 3). It is therefore likely to be an overfit model.

The explained variances of the linear regression models shown in Table 3 were similar when a continuous virologic outcome of change in plasma HIV-1 RNA from baseline to either the first or second follow-up evaluation (whichever was larger) was used in place of the ordinal outcomes. For example, the R^2 value for the comprehensive model 5 was 0.66 when the continuous outcome variable maximal change ($\log_{10}$) in HIV-1 RNA level by week 12 was used.

Prediction of Virologic Response at Week 26

The regression models in Table 3 were also used to predict the virologic response at the third evaluation. Compared to the results in Table 3, the explained variance decreased for each of the models tested. Using the virologic response at this time point, the explained variance decreased to 27% for model 1; 45% for model 2; and 49% for models 3, 4, and 5. None of the clinical variables (model 1) made a statistically significant contribution to a prediction model with genotypic variables, and forcing the clinical variables into a comprehensive model did not improve the explained variance above that of model 3. The protease genotypic variables remained the strongest predictors of response at week 26 and remained independent of the clinical variables (data not shown).

Discussion

This study demonstrates that HIV-1 genotype is a strong predictor of virologic response to saquinavir–ritonavir in persons in whom previous protease inhibitor–containing antiretroviral therapy had failed to achieve maximal suppression of HIV replication. In the multivariate

analysis of virologic response, the baseline HIV-1 genotype adds significantly to the predictive capacity of baseline clinical features. A model of baseline clinical features in this study can, at best, explain 45% of the variation in virologic outcomes; in contrast, the explained variance of a model that includes genotypic predictors is almost 70%. This clinical cohort study, therefore, represents a proof of principle that HIV-1 genotypic resistance testing provides information that cannot otherwise be derived from standard clinical assessment of persons in whom antiretroviral therapy is failing virologically.

Our retrospective cohort study has several limitations. First, it does not prove that HIV-1 resistance testing will improve clinical outcomes in patients who are experiencing less-than-optimal virologic suppression with antiretroviral therapy, thus warranting a change in therapy according to current guidelines (48,49). However, two recent pilot studies comparing HIV-1 genotype–guided choice with standard practice choice of new antiretroviral agents for patients in whom therapy is failing suggested improved virologic outcomes with genotypic guidance (50,51). Second, the size of our study did not allow for detailed analysis of drug history in all its complexity, nor could we analyze all possible combinations of mutations that might affect outcome. Finally, our clinical cohort had uniformly good adherence to therapy; in groups with poorer adherence, the resistance predictors would probably not be as strongly associated with virologic response.

The sequencing technique used in this study cannot detect viral variants that make up less than 20% of the viral population present in the plasma (42). The relative lack of sensitivity to “minority” populations of viral quasi-species has raised concerns about the clinical utility of this technology, because these minority populations, if drug-resistant, may result in virologic rebound (40). An additional concern with the sequencing technique is that the results cannot indicate whether the drug-resistance mutations identified exist in a single quasi-species or are spread across multiple viral variants. Despite these limitations, HIV-1 genotype remained a strong predictor of virologic response to saquinavir– ritonavir therapy. Lack of standardized methods and interpretation of genotypic and phenotypic assays currently limit application of HIV-1 resistance testing, but progress is rapid (38,39,41,42).

A clinical diagnosis of AIDS, baseline CD4 counts, and HIV-1 viral load were bivariate predictors of virologic response, but only the baseline plasma HIV-1 RNA level remained significant in multivariate models. Previous antiretroviral therapy was also a predictor of virologic response. Both the number and the duration of previous therapy with nucleoside reverse transcriptase and protease inhibitors were bivariate predictors. Although the drug history variables were not as strong as HIV-1 genotype in predicting response to saquinavir–ritonavir therapy, the number of previous protease inhibitors contributed to the predictive capacity of a model with HIV-1 genotype predictors.

The baseline HIV-1 genotype proved to be a robust prognostic tool. In particular, the seven major protease mutations defined a priori were strong predictors of virologic response in this cohort. Although several reverse transcriptase mutations (at codons 69 and 210) had some predictive power, the protease mutations were much stronger predictors of response. This probably reflects the extensive previous exposure to nucleoside reverse transcriptase inhibitors in this cohort; as a result, the virologic response was primarily due to the dual protease inhibitor therapy. All eight patients with the D30N mutation (seven who had it as a single major mutation and one who also had the L90M mutation) were complete responders to saquinavir–ritonavir therapy. The “positive” association of this mutation with virologic outcome is more accurately viewed as the lack of negative association; negative associations were seen with the other major mutations. The virologic response in this group supports clinical trial results in patients switched to saquinavir–ritonavir therapy after nelfinavir-containing regimens failed (52).

Our study has several notable features. First, the clinical cohort, with its complex and varied antiretroviral treatment histories, reflects the diversity of drug treatment in HIV-infected persons in clinical practice. Inclusion of patients with a wide variety of treatment histories allowed evaluation of treatment history as a predictor of virologic outcome. Second, HIV-1 genotype was not used as a criterion for selection of patients into the study. Knowing the genotype before enrollment could have introduced bias into subject selection. Third, the signed-sum method used in the multivariate analysis of the genotypic patterns helped to simplify complex data and avoid the potential problem of colinearity between mutation predictors. Finally, recent retrospective studies support our findings, although not all of these studies have shown that the predictive capacity of resistance testing is independent of standard clinical predictors (37,53–56).

Our results indicate that HIV-1 genotypic resistance testing provides prognostic information in patients who are experiencing less-than-optimal virologic response to antiretroviral therapy that contains protease inhibitors and that this information is not available through standard clinical evaluation. In randomized, controlled pilot trials (50,51), this prognostic capacity may translate into improved treatment outcomes of HIV-1 infected persons, but further study is required.

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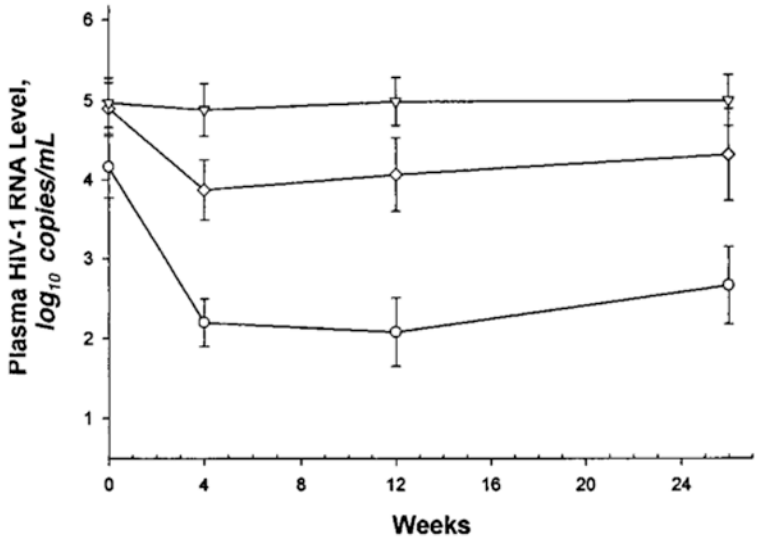

Figure 1. Virologic response to saquinavir plus ritonavir through week 26 based on response by week 12

See the Methods section for further explanation. Triangles represent nonresponders; diamonds represent partial responders; circles represent complete responders.

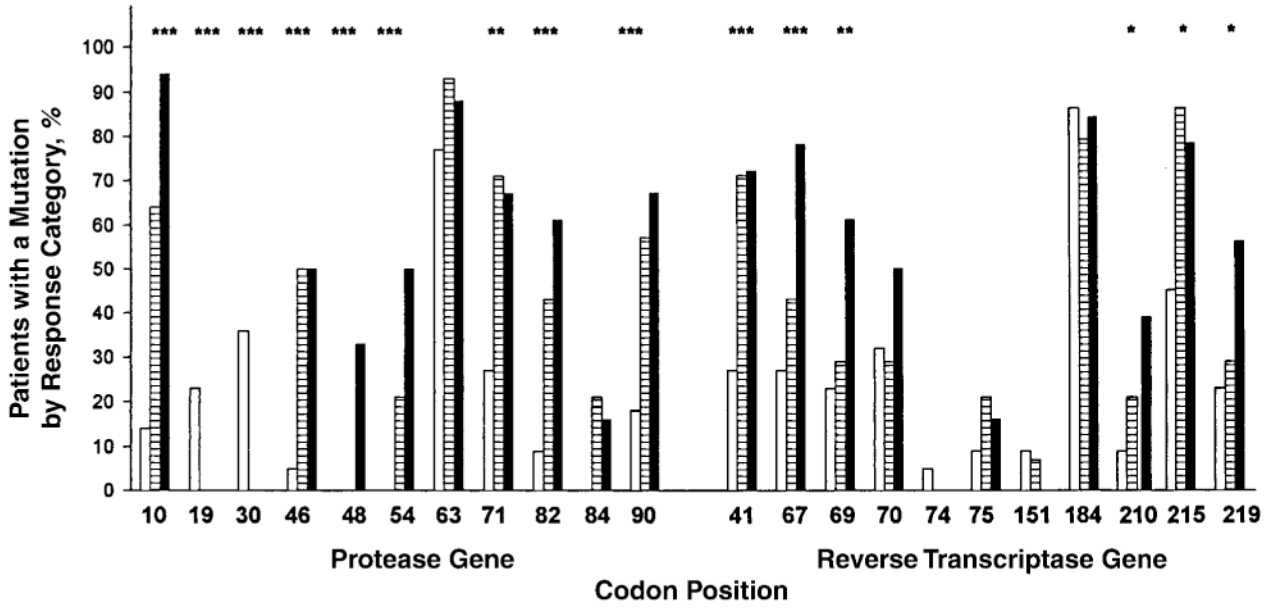

Figure 2. Frequencies of protease and reverse transcriptase mutations by virologic response at week 12

Mutation frequencies at baseline in complete responders (*white bars*), partial responders (*striped bars*), and nonresponders (*solid bars*) are shown. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 (as bivariate predictors).

Table 1

Baseline Demographics, Clinical Characteristics, and Antiretroviral Treatment History as Predictors of Virologic Response to Saquinavir–Ritonavir Therapy

Characteristic	Overall	Complete Response*	Partial Response†	Nonresponse‡	*P* Value§
Demographic characteristic					
Patients, *n (%)*	54	22 (41)	14 (26)	18 (33)	
Median age, *y*	41	39	42.5	38.5	>0.2
Men, *n (%)*	51 (94)	20 (90)	13 (93)	18 (100)	>0.2
White, non-Hispanic ethnicity, *n (%)*	39 (72)	14 (64)	12 (86)	13 (72)	>0.2
Baseline clinical characteristics					
AIDS diagnosis, *n (%)*//	20 (37)	5 (23)	5 (36)	10 (56)	0.03
Median CD4 count (range), $\times 10^9$/*L*	245 (5–1060)	295 (20–1060)	220 (5–460)	155 (9–470)	<0.01
Median $\log_{10}$ plasma HIV-1 RNA level (range), *copies/mL*	5.0 (3–5.9)	4.0 (3.0 –5.9)	5.0 (4.1–5.9)	5.0 (4.0 –5.9)	<0.01
Antiretroviral treatment history					
Median nucleoside reverse transcriptase inhibitors (range), *n*	4 (2–5)	3 (2–5)	4 (3–5)	4 (2–5)	0.01
Median duration of prior nucleoside reverse transcriptase inhibitor therapy (range), *wk*	188 (30–472)	118 (30–346)	226 (64–408)	334 (75–472)	<0.01
Patients with previous non-nucleoside reverse transcriptase inhibitor therapy, *n (%)*	16 (29)	8 (36)	5 (36)	3 (17)	0.19
Median previous protease inhibitors (range), *n*	2 (1–4)	1 (1–3)	2 (1–3)	2 (1– 4)	0.01
Median duration of protease inhibitor thearapy (range), *wk*	48 (12–216)	38 (12–104)	48 (32–216)	58 (24–156)	0.08
Patients who received new nucleoside reverse transcriptase inhibitors, *n (%)*	13 (24)	7 (32)	3 (16)	3 (17)	>0.2
Previous protease inhibitors, *n*					
Indinavir	14	5	4	5	—¶
Nelfinavir	5	5	0	0	—
Saquinavir then indinavir	14	4	4	6	—
Saquinavir then nelfinavir	9	6	2	1	—
Indinavir then nelfinavir	1	1			—
Ritonavir then indinavir	2	0	1	1	—
Saquinavir, nelfinavir, and indinavir	7	1	2	4	—
Saquinavir, ritonavir, and indinavir	1	0	1	0	—
All four protease inhibitors	1	0	0	1	—

*Defined as ≤500 copies/mL at 4 or 12 weeks.

†Defined as a >0.5 $\log_{10}$ or greater reduction in plasma HIV-1 RNA level but not <500 copies/mL at 4 or 12 weeks.

‡<0.5 $\log_{10}$ reduction at 4 or 12 weeks.

§Linear regression.

//Excludes CD4 cell count criterion.

¶No statistical test as a bivariate predictor.

Table 2

Protease Mutation Patterns at Baseline and Response to Saquinavir–Ritonavir Combination Therapy*

Major Protease Mutations at Baseline†	Patients	Median Additional Baseline Mutations (Range)		Median Baseline CD4 Count	CD4 Count at 12 Weeks: Median Change from Baseline	Median Baseline Plasma HIV RNA Level	Follow-up Plasma HIV RNA Level during Saquinavir–Ritonavir Therapy			
		Protease	Reverse Transcriptase				Change from Baseline at 4 Weeks	Change from Baseline at 12 Weeks	<500 copies/mL	<50 copies/mL
	← n →			×10⁹ cells/L		← $\log_{10}$ copies/mL →			n (%)	
None	11	5 (1–7)	10 (0–17)	240	60	4.7	−1.8	−2.4	9 (82)	6 (55)
D30N	7	4 (3–9)	11 (9–14)	500	55	3.6	−2.0	−1.8	7 (100)	3 (43)
At codon 46, 82, or 84	3	11 (8–11)	10 (9–11)	200	50	4.6	−1.7	−1.8	2 (67)	1 (33)
L90M	6	6 (4–7)	10 (8–19)	345	5	4.6	−1.5	−1.2	2 (33)	0
Any two mutations	10	7 (0–10)	12 (4–16)	140	40	5.0	−0.9	−0.5	2 (20)	0
Three or more mutations	17	7 (2–10)	14 (7–24)	170	0	5.0	−0.2	0.0	0	0

*Response defined as <500 or <50 copies/mL by 12 weeks.

†Based on “major” protease mutations, defined as those at codons 30, 46, 48, 54, 82, 84, and 90 (see Methods section).

Table 3

Multivariable Linear Regression Models of Clinical, Antiretroviral Treatment History, and HIV-1 Genotypic Predictors of Virologic Response by Week 12 of Saquinavir–Ritonavir Therapy*

Model	Model 1: Clinical and Treatment History		Model 2: Major Protease Mutations		Model 3: Major and Minor Protease Mutations		Model 4: Protease and Reverse Transcriptase Mutations		Model 5: Comprehensive Model 1		Model 6: Comprehensive Model 2	
	Regression Coefficient	*P* Value	Regression Coefficient	*P* Value	Regression Coefficient	*P* Value	Regression Coefficient	*P* Value	Regression Coefficient	*P* Value	Regression Coefficient	*P* Value
Clinical and treatment history												
Plasma HIV RNA level at baseline	−0.312	<0.01	—	—	—	—	—	—	—	—	−0.10	>0.2
Duration of previous therapy with nucleoside reverse transcriptase inhibitors	−0.002	<0.05	—	—	—	—	—	—	—	—	−0.001	0.18
Number of previous protease inhibitors	−0.388	<0.01	—	—	—	—	—	—	−0.17	0.07	−0.11	>0.2
Previous nelfinavir treatment	0.491	<0.03	—	—	—	—	—	—	—	—	−.051	>0.2
Genotypic variables												
Signed sum of the major protease mutations†	—	—	0.413	<0.01	0.24	<0.01	0.20	<0.01	0.22	<0.01	0.21	<0.01
Signed sum of the minor protease mutations‡	—	—	—	—	0.45	<0.01	0.42	<0.01	0.43	<0.01	0.38	<0.01
Signed sum of the reverse transcriptase mutations§	—	—	—	—	—	—	−0.08	0.5	—	—	—	—
Explained variance of model, R^2 value (95% CI)	0.45 (0.31– 0.59)		0.59 (0.45– 0.73)		0.66 (0.52– 0.80)‖		0.67 (0.53– 0.81)¶		0.69 (0.57–0.81)**		0.71 (0.59–0.83)	

* Based on ordinal response categories and complete, partial, and nonresponse to therapy (see Methods section).

† Major protease codons were 30, 46, 48, 54, 82, 84, and 90. Mutation D30N was positively associated with a virologic outcome; mutations at all other codons were negatively associated with such an outcome.

‡ Minor protease codons were 10, 19, and 71. Mutation at codon 19 was positively associated with virologic outcome; mutations at condons 10 and 71 were negatively associated with such an outcome.

§ Reverse transcriptase codons were 69 and 210. Mutations at these codons were negatively associated with virologic outcome.

‖ $P < 0.002$ (model 3 compared with model 2; *F* test).

¶ $P > 0.2$ (model 4 compared with model 3; *F* test).

** $P = 0.07$ (model 5 compared with model 3; *F* test).