

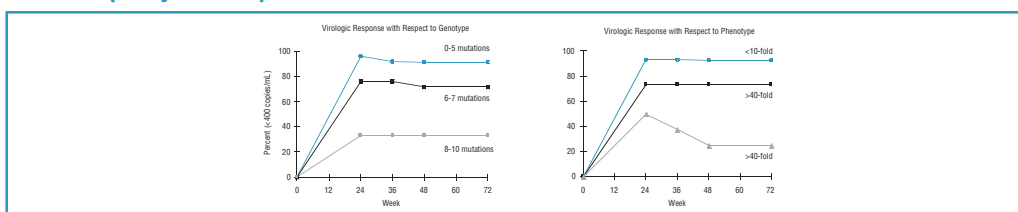
Quantitative Estimate of the Effect of Individual Baseline Mutations in HIV Protease on the Virologic Response to Lopinavir/Ritonavir Therapy in Heavily Antiretroviral-Experienced Patients

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INTRODUCTION

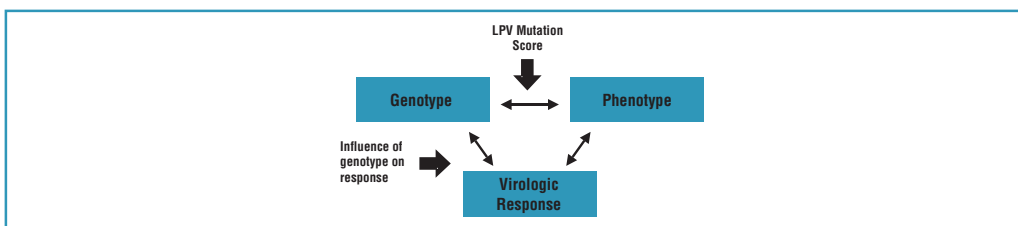
Virologic response to therapy with Kaletra (lopinavir/ritonavir), with reverse transcriptase (RT) inhibitors, has previously been shown to be associated with baseline phenotypic and genotypic susceptibility to lopinavir (LPV).¹ Although genotypic resistance to Kaletra has not been completely defined, 11 mutations in HIV protease have been found to be associated with significant changes in the *in vitro* susceptibility to LPV.² The total number of these 11 mutations constitutes the LPV mutation score. In Study M98-957, virologic response to Kaletra, efavirenz, and nucleoside RT inhibitors in multiple PI-experienced, non-nucleoside RT inhibitor-naive patients was highest in those patients with a baseline LPV mutation score of 5 or less. Intermediate response was observed in patients with a baseline mutation score of 6-7, and the lowest response was observed in those patients with a baseline mutation score of 8 or more³ (Figure 1).

Figure 1. Virologic Response to Kaletra, Efavirenz and NRTIs in Multiple PI-Experienced, NNRTI-Naive Patients Is Associated with Baseline Lopinavir Mutation Score and Lopinavir Phenotype (Study M98-957)



Although the LPV mutation score is a useful predictor of virologic response to Kaletra, the *de novo* analysis of response with respect to genotype (Figure 2) has not been adequately characterized. Furthermore, the LPV mutation score was based on an analysis of a limited number (112) of viral isolates from PI-experienced patients. Mutations that were underrepresented in that panel of isolates may not be included in the LPV mutation score, despite the possibility that some may contribute to significantly reduced susceptibility to LPV.^{4,5} In order to further characterize genotypic resistance to Kaletra, and to quantitatively assess the effect of individual mutations within the mutation score on virologic response, we analyzed data from the Kaletra ATU ("Authorisation Temporaire d'Utilisation", Provisional Authorization of Use) program conducted in France. The Kaletra ATU database is an observational database of 792 antiretroviral-experienced patients who initiated combination therapy including Kaletra, and who had genotype available prior to (median: 105 days) the change to Kaletra therapy.⁶ The availability of a large number of patients in this database allowed us to directly assess the influence of baseline genotype on virologic response (Figure 2). Furthermore, the influence of individual mutations included in (or excluded from) the LPV mutation score was studied.

Figure 2. Relationships for Determining Genotypic Resistance to Lopinavir/



METHODS

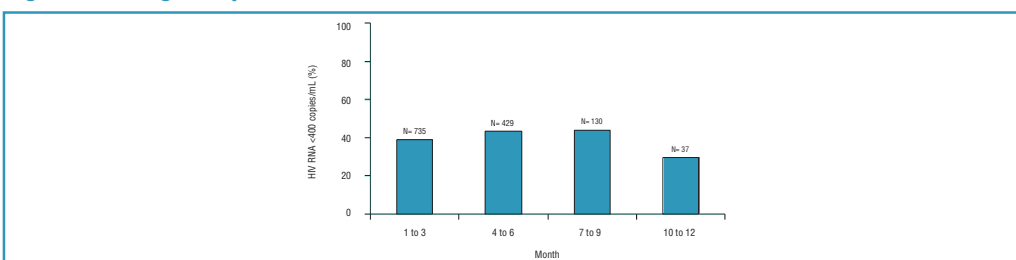
- Virologic response was defined as achieving a viral load <400 copies/mL within 12 months after initiation of Kaletra.
- Virologic response as a function of baseline LPV score was analyzed using logistic regression.
- The relative contribution of a given mutation included in the LPV mutation score was also analyzed via logistic regression. For each individual mutation, the LPV mutation score was computed excluding the specified mutation (e.g., 82). Logistic regression was then used to model response as a function of this mutation score (based on only 10 mutations) along with an indicator function for the mutation that was left out of the mutation score (0 if absent, 1 if present). The odds ratio for the indicator function of the excluded mutation is a measure of the relative contribution of this mutation to the LPV mutation score.
- For PI mutations outside of the LPV mutation score that were present in at least 1% of the patients (30, 32, 33, 36, 47, 48, 73, 77, 88), a similar logistic regression analysis was used to measure the additional contribution of each of these mutations in the presence of the LPV mutation score.

RESULTS

Virologic Response

- The percentage of patients responding (on-treatment) over time is shown in Figure 3.

Figure 3. Virologic Response over Time



Evaluation of Individual Mutations Within the LPV Mutation Score

- The distribution of isolates containing any of the 11 mutations from the LPV mutation score is provided in Table 1. Mutations at positions 24 and 53 occurred at relatively low frequency (i.e., <10% of isolates) in this patient population.

Table 1. Distribution of Isolates Containing Mutations Constituting the LPV Mutation Score

Mutation Score	Amino Acid Position											
	Total	10	20	24	46	53	54	63	71	82	84	90
1	81	9	2	0	4	0	1	49	4	2	0	10
2	76	17	3	0	16	0	6	40	20	15	6	29
3	73	36	3	1	16	0	9	49	38	10	10	47
4	124	77	10	1	54	0	38	96	71	43	24	82
5	145	115	25	6	86	4	68	108	96	76	40	101
6	125	123	33	9	70	5	66	112	114	76	43	99
7	106	103	43	13	76	10	96	100	102	87	29	83
8	23	23	16	2	17	8	23	22	22	22	10	19
9	3	3	3	2	3	2	3	1	3	3	3	1
Total	792	506	138	34	342	29	310	577	470	334	165	471

- The influence of individual mutations within the current LPV mutation score was evaluated using logistic regression in the following manner. The LPV mutation score was constructed using all mutations included in the LPV mutation score, with the exception of mutations at amino acid position 10. A logistic regression analysis was then conducted using this LPV mutation score (restricted to mutations at amino acid positions 20, 24, 46, 53, 54, 63, 71, 82, 84, and 90) and an indicator function (0, 1) for isolates either lacking or containing a mutation at amino acid position 10.
- Results from this analysis are presented in Figure 4. The histograms provide the observed response rates, based on the LPV mutation score, for isolates either lacking (blue) or containing (gray) mutations at amino acid position 10. In addition, the regression lines show the predicted response rates for isolates either lacking (blue) or containing (gray) mutations at amino acid position 10.
- A similar logistic regression analysis was performed for each individual mutation within the LPV mutation score. Results are provided in Figure 5.

Figure 4. Comparison of Estimated and Actual Response Rates for Patients with Baseline Isolates Containing or Lacking a Mutation at Position 10

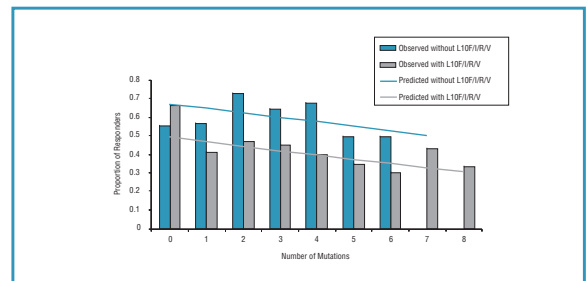
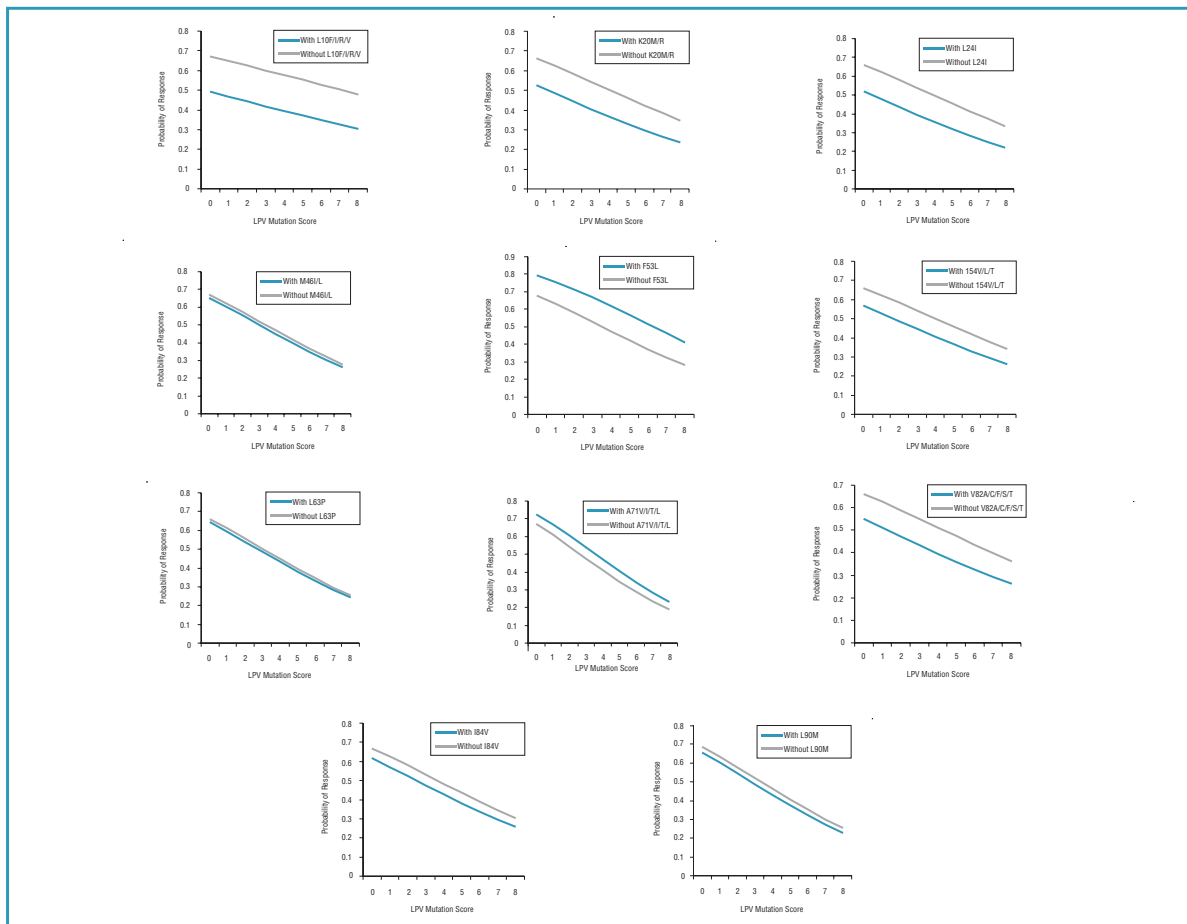


Figure 5. Logistic Regression Analysis of Single Mutations Within the LPV Mutation Score



- Mutations at positions 10, 20, 54, and 82 were found to statistically significantly influence virologic response in the context of the remainder of the LPV mutation score (Table 2).
- Although not statistically significant, mutations at positions 24 and 84 had an odds ratio of 0.8 or less in the logistic regression analysis. These mutations also appear to influence virologic response in the context of LPV mutation score.
- Mutations at positions 46, 63 and 90 have a smaller effect on the rate of virologic response (odds ratios between 0.8 and 1.25), while mutations at positions 53 and 71 appeared to be poor predictors of virologic failure in this population (odds ratios >1.25).

Table 2. Odds Ratios for Mutations Within the LPV Mutation Score

Mutation	Odds Ratio	95% CI	P-value
10	0.48	(0.34, 0.67)	<0.0001
20	0.56	(0.37, 0.83)	0.0045
24	0.48	(0.21, 1.02)	0.0680
46	0.92	(0.69, 1.24)	0.5880
53	1.80	(0.84, 3.88)	0.1310
54	0.68	(0.49, 0.94)	0.0176
63	1.07	(0.77, 1.49)	0.6890
71	1.29	(0.93, 1.80)	0.1270
82	0.63	(0.46, 0.85)	0.0028
84	0.80	(0.56, 1.14)	0.2110
90	1.14	(0.85, 1.55)	0.3840

Evaluation of Individual Mutations Outside the LPV Mutation Score

- The distribution of isolates containing mutations outside of the LPV mutation score, as a function of the mutation score, is provided in Table 3.
- Mutations occurred most frequently at positions 36, 73 and 77, while mutations at each of the remaining positions (30, 32, 33, 47, 48, 50 and 88) occurred in fewer than 10% of patients.

Table 3. Distribution of Isolates Containing PI Mutations Outside of the LPV Mutation Score

Mutation Score	Amino Acid Position										
	Total	30	32	33	36	47	48	50	73	77	88
0	36	2	1	0	10	0	0	0	0	5	2
1	81	7	0	2	27	0	1	0	0	27	7
2	76	9	0	2	24	0	5	1	1	26	7
3	73	5	1	0	27	0	5	0	14	28	9
4	124	1	1	6	44	0	12	1	23	43	3
5	145	3	12	14	53	8	14	1	30	58	4
6	125	0	3	11	55	2	10	0	32	35	2
7	106	0	4	6	55	3	6	0	17	24	1
8	23	0	1	2	15	0	1	2	0	4	0
9	3	0	0	0	3	0	0	0	0	0	0
Total	792	27	23	43	313	13	54	5	117	250	35

- In a manner similar to that above, the virologic influence of individual mutations outside of the current LPV mutation score was evaluated (Figure 6).
- Mutations at positions 33 and 36 were found to statistically significantly influence virologic response in the context of the LPV mutation score (Table 4).
- Although not statistically significant, mutations at positions 47 and 48 had an odds ratio of 0.8 or less in the logistic regression analysis.

Figure 6. Logistic Regression Analysis of PI Mutations Outside of the LPV Mutation Score

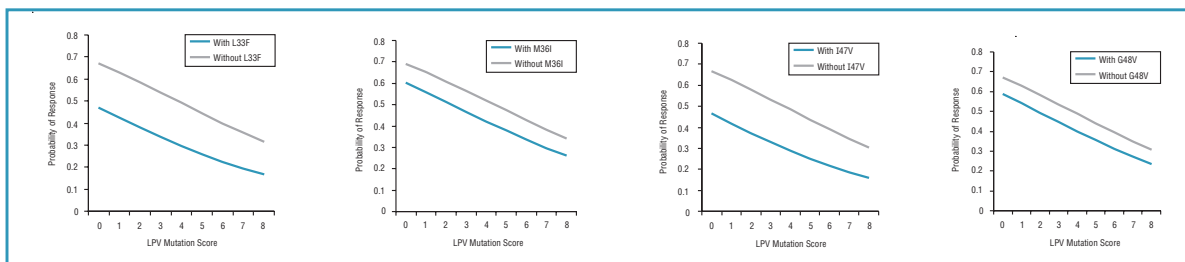


Table 4. PI Mutations Outside of the LPV Mutation Score That Apparently Contribute to Lowered Virologic Response

Mutation	Odds Ratio	95% CI	P-value
33	0.44	(0.21, 0.86)	0.0221
36	0.68	(0.50, 0.91)	0.0098
47	0.43	(0.096, 1.43)	0.2060
48	0.70	(0.39, 1.24)	0.2270

- Previous *in vitro* studies have suggested that the I50V mutation, in the context of other mutations, confers significantly reduced susceptibility to LPV.^{4,5} The prevalence of the I50V mutation in this study population was very low (<1%). However, none of the 5 patients whose genotype displayed the I50V mutation prior to initiation of LPV/ritonavir therapy experienced virologic response. Baseline genotypes of those 5 patients are provided in Table 5.

Table 5. Baseline Genotypes Containing the I50V Mutation

PI Mutations	Viral Load (# days on Kaletra)	Prior PI Therapy
10, 36, 50, 82	5.67 (-99), 3.48 (26), 5.49 (96)	NFV, RTV
10, 33, 50, 63, 71, 90	6.00 (-101), 4.80 (140), 4.80 (170), 4.80 (270)	IDV, NFV, SQV, RTV, APV
10, 33, 46, 48, 50, 54, 71, 82	4.69 (-28), 2.83 (56), 3.50 (83), 3.66 (130)	IDV, NFV, SQV, RTV, APV
10, 20, 33, 36, 46, 50, 54, 63, 71, 82, 90	5.29 (-35), 3.26 (14), 4.96 (91), 5.02 (147)	IDV, NFV, SQV, RTV, APV
10, 20, 33, 36, 46, 50, 53, 54, 63, 82, 90	4.89 (-89), 4.18 (23), 3.33 (91)	IDV, NFV, SQV, RTV, APV

Evaluation of the ATU Mutation Set

- Virologic response was analyzed by logistic regression using the set of mutations either statistically associated with lower response and/or displaying an odds ratio of 0.8 or less (positions 10, 20, 24, 33, 36, 47, 48, 54, 82 and 84). This set constitutes the ATU mutation set.
- The predicted response rates and odds ratios for the ATU mutation set and LPV mutation score were compared and are displayed in Figures 7 and 8 for the Kaletra ATU program and Study M98-957 (Week 72), respectively.
- In both studies, the odds ratio per mutation in the ATU mutation set was lower (more different than 1.0) than the odds ratio obtained using the LPV mutation score, suggesting that for this population, the ATU mutation set is a better predictor of virologic response than the LPV mutation score.
- In both the Kaletra ATU program and Study M98-957 (Week 72), a stepwise (forward and backward selection) logistic regression analysis of the LPV mutation score and the ATU mutation set selected the ATU mutation set as the better predictor of virologic response.

Figure 7. Estimated Response Rates for the Kaletra ATU Program as a Function of the LPV Mutation Score or the ATU Mutation Set

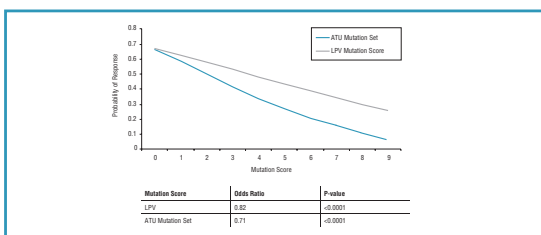
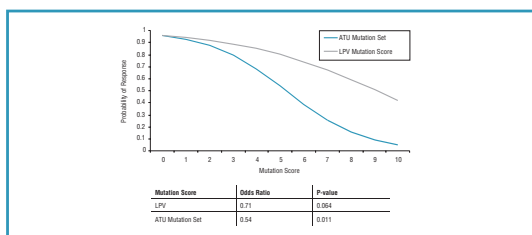


Figure 8. Estimated Response Rates for Study M98-957 (Week 72) as a Function of the LPV Mutation Score or the ATU Mutation Set



DISCUSSION

- The current LPV mutation score is based on a correlation of phenotype and genotype of a limited number (112) of isolates from PI-experienced patients. As clinical data become available on larger numbers of patients, the quantitative influence of individual mutations on virologic response and the effect of low-prevalence mutations can be assessed.
- In this study, a continuum of response with respect to the number of mutations was observed.
- The presence of mutations at positions 10, 20, 33, 36, 54 and 82 in the presence of multiple other mutations was statistically significantly associated with virologic failure. The presence of mutations at positions 24, 47, 48 and 84 also appeared to influence response, although the association was not statistically significant.
- The ATU mutation set based on the above 10 mutations was a better predictor of virologic response than the LPV mutation score in a separate study (Study M98-957).
- The prevalence of some mutations, particularly the I50V mutation, was too low for assessment of its effect on virologic response.

CONCLUSIONS

- PI mutation patterns that predict failure to enhanced PI regimens such as Kaletra are complex, and complicated algorithms and large study populations are necessary to quantify the effect of baseline genotype on virologic response.
- In this study, we identified a set of 10 mutations (positions 10, 20, 24, 33, 36, 47, 48, 54, 82 and 84) that predicted virologic response better than the current LPV mutation score.
- Data from this large observational cohort might be applicable to the development of weighted algorithms for predicting virologic response to Kaletra therapy.

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