Poster #082

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Identification of Individual Mutations in HIV Protease Associated with Virologic Response to Lopinavir/ritonavir Therapy

V. Calvez[1], I. Cohen Codar[2], AG. Marcelin[1], D. Descamps[1], C. Tamalet[1], J. Ritter[1], M. Segondy[1], H. Peigue-Lafeuille[1], F. Brun-Vezinet[1], E. Guillevic[2], J. Isaacson[3], R. Rode[3], B. Bernstein[3], E. Sun[3], D. Kempf[3], JP. Chauvin[2]

[1] ANRS HIV Resistance Group AC11, France; [2]Abbott Laboratories, Rungis, France; [3]Abbott Laboratories, Abbott Park, IL USA

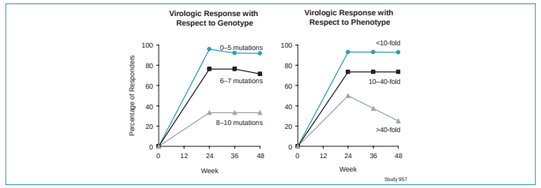
BACKGROUND

Lopinavir/ritonavir (LPV/r, Kaletra™) is a novel HIV protease inhibitor (PI) that has shown significant antiviral activity and tolerability in clinical trials to date.

The co-formulation of lopinavir with a low dose of ritonavir, acting as a pharmacokinetic enhancer, results in a mean LPV C_{trough}/EC_{50} ratio (inhibitory quotient or IQ) for wild-type virus >75 (at 400/100 mg BID dose), which contributes to the durability of the viral control and potentially provides a pharmacologic barrier to the emergence of viral resistance.

However, virologic control may be compromised by mutations induced by previous PI therapies. In study M98-957, the virologic response (viral load <400 copies/mL) to LPV/r therapy was associated with the baseline LPV mutation score (number of mutations in HIV protease known to be associated with reduced susceptibility to LPV) and with the baseline phenotype (Figure 1). In that study, however, the number of patients was too small to adequately explore the effect of individual mutations on the response rate.

Figure 1. LPV/r Genetic Barrier in Multiple PI-Experienced, NNRTI-Naïve Patients, Study M98-957



METHODS

From March 2000 to April 17, 2001, the French Drug Agency authorized the prescription of LPV/r to patients with no other treatment option available through an ATU (Autorisation Temporaire d'Utilisation-patient's name based program). Such programs are dedicated to drugs not yet registered but which could represent potentially life-saving medications. At the time of the ATU completion, as of April 17, 2001, the overall enrollment in the LPV/r cohort was 3,819 patients.

The patient population was defined using very strict entrance criteria in the beginning, i.e. CD4 <200/mm³ and viral load >4 log_{10} copies/mL; however, these immuno-virological criteria have been progressively removed to authorize the use of LPV/r in a larger PI-experienced population.

At baseline, demographic data, HIV-RNA and CD4, prior and current ARV medications, as well as genotype including the protease polymorphism, were collected. During the follow-up, HIV-RNA and CD4, as well as serious adverse events were collected.

Response was analyzed in all patients who had at least one viral load measurement a minimum of 10 days after the start of Kaletra therapy and a genotype that was not collected during a "wash-out" period. Fisher's exact test was used to measure the association between virologic response and mutations at each of the 83/99 amino-acids in HIV at which any change from the wild-type sequence was noted. A stepwise logistic regression analysis was also used to test the independent effect of specific mutations on virologic response.

RESULTS

A total of 700 patients were included in the analysis. Baseline characteristics of the population are provided in Table 1.

Table 1. Baseline Characteristics

Gender		**CD4 Count (cells/mm³)**	
Male	82.5%	<50	23.1%
		50 – 150	28.3%
Age (Years)		≥150	48.6%
Mean	41	Mean	177
Median	40	Median	144
		Standard Deviation	160
CDC Classification (N = 675)		Minimum	0
Asymptomatic (Stade A)	14.7%	Maximum	995
Symptomatic (Stade B)	31.3%		
AIDS – Indicator (Stade C)	54.1%	**HIV RNA (log_{10} copies/mL)**	
		<5 log_{10} copies/mL	56.6%
HIV Diagnosis Date (N = 610)		≥5 log_{10} copies/mL	43.4%
< 1990	41.6%	Mean	4.83
1990 – 1995	42.3%	Median	4.88
≥ 1995	16.1%	Standard Deviation	0.76
		Minimum	1.54
Prior ARV Use (median)		Maximum	6.98
PIs	3		
NRTIs	5		
NNRTIs	1		

Concomitant Antiretroviral Therapy

- Concomitant ARVs in the LPV/r-containing regimens for the study population are provided in Figure 2. Nearly all patients (638/700, 91%) received either two or three additional drugs.
- The majority of patients receiving 2 additional drugs (292/395, 74%) received two NRTIs along with LPV/r.
- The majority of patients receiving 3 additional drugs (179/233, 77%) were almost evenly divided between those receiving three NRTIs and those receiving two NRTIs and one NNRTI. A relatively low percentage of patients received another PI concomitant with LPV/r (115/700, 16%).

Figure 2a. Description of LPV/r-Containing Regimens

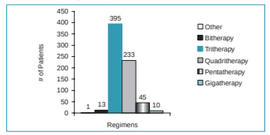


Figure 2b. Tritherapy: Antiretroviral Agents Combined with LPV/r

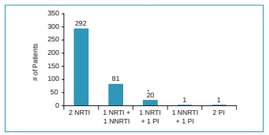
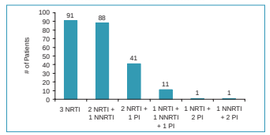


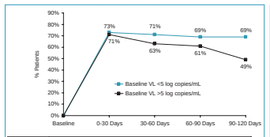
Figure 2c. Quadritherapy: Antiretroviral Agents Treatments Combined with LPV/r



Analysis of Virologic Response

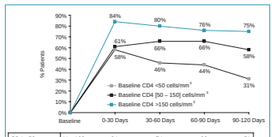
- Plasma HIV RNA data were available for analysis from a large group of patients through 4 months of LPV/r therapy for analysis. Virologic response was defined as a minimum viral load of <400 copies/mL and/or a decrease from baseline of at least 1.0 log_{10} copies/mL at any time point during treatment.
- Virologic response with respect to baseline HIV RNA and baseline CD4 are provided in Figures 3 and 4, respectively. Visit frequency was determined by standard of care; thus, for the assessment of response, 30-day intervals were arbitrarily adopted.

Figure 3. Virologic Response According to Baseline Viral Load



		Baseline	0-30 Days	30-60 Days	60-90 Days	90-120 Days
VL <5 log	N = 396	145	192	134	113	
VL ≥5 log	N = 304	110	139	135	94	

Figure 4. Virologic Response According to Baseline CD4 Count



		Baseline	0-30 Days	30-60 Days	60-90 Days	90-120 Days
CD4 <50	N = 160	64	74	66	51	
CD4 [50 – 150]	N = 196	61	96	76	57	
CD4 ≥150	N = 336	127	157	125	96	

Baseline Genotype

- The prevalence of baseline mutations in the study population was high (Table 2).
  - The median number of PI and NRTI mutations was 7 and 4, respectively.
  - 400 patients (57%) had 6 or more baseline PI mutations.
  - 476 patients (68%) had 4 or more baseline NRTI mutations.
  - 324 patients (46%) had at least 6 PI mutations and 4 NRTI mutations.

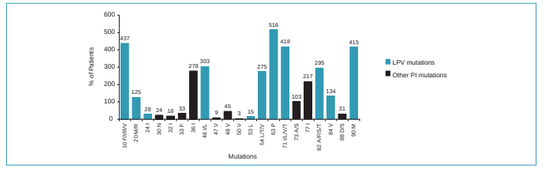
Table 2. Baseline NRTI and PI Mutations

No. of Mutations	NRTI Mutations*	PI Mutations*
0 – 3	224 (32%)	147 (21%)
4 – 5	280 (40%)	153 (22%)
6 – 7	169 (24%)	225 (32%)
8 – 10	26 (4%)	171 (24%)
>10	1	4 (1%)

* PI mutations include: L10F/I/R/V, K20M/R, L24I, D30N, V32I, L33F, M36I, M46I/L, I47V, G48V, I50V, F53L, I54L/T/V, L63P, A71I/L/V/T, G73A/S/T, V77I, V82A/F/S/T, I84V, N88D and L90M. NRTI mutations include: M41L, A62V, K65R, D67N, T69D, 69ins, K70R, L74V, V75I, F77L, Y115F, F116Y, Q151M, M184V/I, L210W, T215Y/F, K219Q.

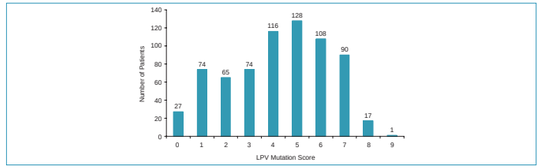
- The majority of patients (386, 55%) had at least one major NNRTI mutation (positions 103, 181 and/or 101), while 232 (33%) patients had no NNRTI mutations.
- Response was initially analyzed with respect to the LPV mutation score, which includes the number out of 11 protease mutations previously described to be associated with reduced susceptibility to lopinavir (amino acids 10, 20, 24, 46, 53, 54, 63, 71, 82, 84 and 90). The prevalence of mutations within the LPV mutation score is provided in Figure 5.

Figure 5. Baseline Prevalence of PI Mutations (700 Genotypes)



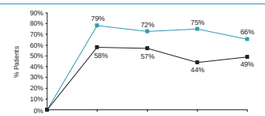
- The median LPV mutation score was 4. The majority of patients (63%) had a baseline LPV mutation score of 4-7 (Figure 6).

Figure 6. LPV Mutation Score



- Longitudinal virologic response with respect to categorical descriptors of the LPV mutation score (0-5 mutations and 6-9 mutations) is shown in Figure 7. The response in those patients with 0-5 mutations was significantly lower than compared to those with 6 or more mutations.

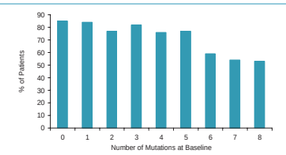
Figure 7. Longitudinal Response with Respect to Baseline LPV Mutation Score



		Baseline	0-30 Days	30-60 Days	60-90 Days	90-120 Days
≤5 mutations	N = 482	170	229	182	135	
>5 mutations	N = 218	85	102	87	72	

- Overall virologic response was fairly uniform in patients with LPV mutation score of 5 or less. Lower response was observed at mutation score of 6 or more (Figure 8). The overall response with LPV mutation score of ≤5 and ≥6 was 380/482 (79%) and 125/218 (57%), respectively, p <0.0001.

Figure 8. Overall Virologic Response with Respect to Baseline LPV Mutation Score



- Based on the above observations, the genotypic breakpoint for LPV/r using the LPV mutation score is best described as being between 5 and 6 mutations at baseline.
- Virologic response in the context of differences in baseline LPV mutation score, baseline HIV RNA and baseline CD4 is provided in Table 3. Overall, response was highest in those patients with >150 CD4 cells/mm³ and lowest in those with <50 CD4 cells/mm³. Baseline viral load appeared to be less important than baseline CD4 in determining virologic response.

Table 3. Virologic Response According to Baseline CD4, VL and LPV Mutation Score

Baseline CD4 / Baseline VL	<50 cells/mm³		50-150 cells/mm³		≥150 cells/mm³	
≥5 log_{10} copies/mL	>5 mut.	≤5 mut.	>5 mut.	≤5 mut.	>5 mut.	≤5 mut.
	14/43	47/69	19/38	46/57	20/26	58/69
	(33%)	(68%)	(50%)	(81%)	(77%)	(84%)
<5 log_{10} copies/mL	>5 mut.	≤5 mut.	>5 mut.	≤5 mut.	>5 mut.	≤5 mut.
	8/19	21/29	18/31	50/70	43/57	157/184
	(42%)	(72%)	(58%)	(71%)	(75%)	(85%)

- The effect of the LPV mutation score on response appeared to be the greatest at lower CD4 levels. In those patients with <50 CD4 cells/mm³, the response was twice as high in those with mutation score of 5 or fewer (68/98, 69%), compared to those with mutation score of 6 or higher (22/62, 35%). In contrast, the difference in response rates in patients with >150 CD4 cells/mm³ between the above two groups was <10% (85% vs. 76%, respectively).

Analysis of Response with Respect to Individual Mutations

- In univariate analyses, baseline mutations at positions 82, 54, 10 and 46 were statistically significantly associated with lower virologic response. Baseline mutations at positions 20, 63 and 33 were marginally associated with lower response (Table 4).

Table 4. Association of Virologic Response with Individual Mutations in HIV Protease

	Virologic Response		
Amino Acid Position	**With Mutation**	**Without Mutation**	**P-value**
82	193/308 (63%)	312/392 (80%)	<0.0001
54	178/287 (62%)	327/413 (79%)	<0.0001
10	291/438 (66%)	214/262 (82%)	<0.0001
46	205/306 (67%)	300/394 (76%)	0.0084
20	129/196 (66%)	376/504 (75%)	0.0241
63	399/567 (70%)	106/133 (80%)	0.0318
33	27/46 (59%)	478/654 (73%)	0.0414

- In a stepwise logistic regression analysis that considered all mutations in HIV protease, baseline mutations at positions 82, 54, 10 and 46 were shown to be independently associated with lower virologic response (Table 5).

Table 5. Multivariate Analysis of Virologic Response with Respect to Baseline Mutations

Mutation	Odds Ratio	95% C.I. for the Odds Ratio	P-value
54	1.60	(1.03, 2.47)	0.035
10	1.64	(1.10, 2.46)	0.015
82	1.47	(0.95, 2.27)	0.087
46	1.30	(0.92, 1.85)	0.141

CONCLUSIONS

- In 700 patients with heavy three-class ARV experience and a high prevalence of baseline resistance, the virologic response to therapy containing LPV/r is highly associated with baseline genotype.
- Virologic response was higher in patients with baseline LPV mutation score of 5 or less than in patients with baseline LPV mutation score of 6 or greater.
- These results confirm earlier studies that suggested a genotypic breakpoint between 5 and 6 mutations (out of 11 in the LPV mutation score) for LPV/r-containing regimens in PI-experienced patients.
- In univariate and multivariate analyses, 4 of the 11 mutations in the LPV mutation score (positions 82, 54, 10 and 46) were independently associated with lower virologic response. Two additional mutations from the LPV mutation score (positions 20 and 63) were marginally associated with lower response in univariate analyses. One mutation that is outside the LPV mutation score (position 33) was also marginally associated with lower response.
- LPV mutation score and baseline CD4 appeared to be more important predictors of virologic response than baseline HIV RNA.

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