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The Pharmacologic Barrier to Resistance: Differential Patterns of Viral Evolution in Protease Inhibitor Naïve and Experienced Patients During Viral Load Rebound on Kaletra (lopinavir/r) Therapy

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INTRODUCTION

Lopinavir (LPV) is an HIV protease inhibitor (PI) that is co-formulated with ritonavir, which functions as an inhibitor of cytochrome P450 3A. Even at low ritonavir doses, there is a substantial increase in LPV exposure. At a dosage of 400 mg of LPV/100 mg ritonavir twice daily (3 co-formulated tablets BID), ritonavir concentrations are below those required for antiviral activity.[1] By contrast, the mean LPV C_{trough}/IC_{50} ratio (Inhibitory Quotient or IQ) for wild-type HIV is ≥75 when dosed 400/100 mg BID, potentially providing a barrier to emergence of viral resistance and activity against resistant virus. Lopinavir/ritonavir (LPV/r, marketed as Kaletra) has been studied in both antiretroviral naïve and experienced HIV infected patients. The objectives of this presentation are to:

1. Examine resistance patterns in viral isolates from antiretroviral-naïve and PI-experienced adult and pediatric patients who either failed to suppress plasma HIV RNA to <400 copies/mL or experienced a sustained rebound in plasma HIV RNA to >1000 copies/mL while on therapy with LPV/r.
2. Explore the impact of baseline viral genotype on the subsequent development of LPV resistance during viral load rebound on LPV/r therapy.
3. Present data on patterns of cross-resistance to other PIs following evolution of LPV resistance *in vivo*.
4. Discuss the concepts of the pharmacologic barrier to resistance and the zone of selective pressure as models that explain the observed resistance results in LPV/r clinical trials.

METHODS

Virologic Evaluation for Phase III Study M98-863

- Samples from all subjects with VL >400 copies/mL at least once at Week 24, 32, 40, 48 or 60 while on the assigned treatment regimen were submitted for analysis. Genotype (GeneSeq™) and phenotype (PhenoSense™) were performed by ViroLogic, Inc.
- Genotypic resistance to nelfinavir (NFV) was defined as the development of a D30N and/or an L90M mutation in protease. Genotypic resistance to LPV was defined as the development of any primary or active site mutation in protease (amino acids 8, 30, 32, 46, 47, 48, 50, 82, 84 and 90). Phenotypic analyses were performed on all samples obtained from LPV/r-treated subjects to confirm the lack of resistance to LPV. Resistance to 3TC was defined as the presence of an M184V and/or M184I mutation in reverse transcriptase.

Virologic Evaluation for Phase II Studies of LPV/r

- Baseline phenotypic susceptibility to commercially available antiretroviral agents, including LPV was measured by the PhenoSense™ (ViroLogic, Inc.) or Antivirogram (Virco, Inc.) methods and expressed as fold change in IC_{50} compared to standard, wild-type (wt) virus.
- Baseline genotype, including the entire protease and reverse transcriptase genes, was determined by population sequencing. The LPV mutation score for each isolate was defined as the number of mutations in protease out of the eleven mutations associated with reduced *in vitro* susceptibility to LPV as previously reported.[2] These eleven mutations are L10/F/I/R/V, K20M/R, L24I, M46I/L, F53L, I54L/T/V, L63P, A71I/L/T/V, V82A/F/T, I84V, and L90M.
- Following initial response, plasma samples from patients with plasma HIV RNA >1000 copies/mL without documented treatment interruption were submitted for phenotypic and genotypic analysis.

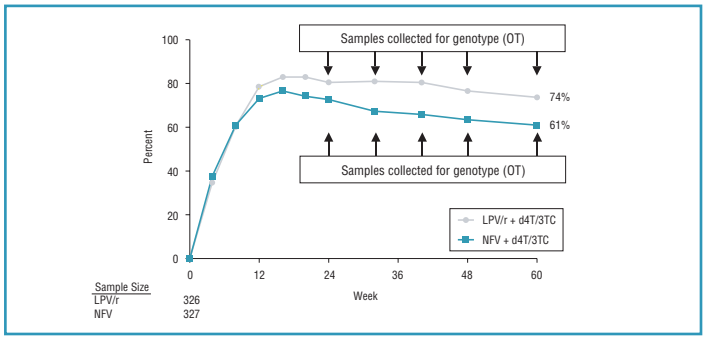
RESULTS

Emergence of Genotypic Resistance in Previously Antiretroviral (ARV)-Naïve Patients Treated with LPV/r or Nelfinavir: Phase III Trial M98-863

Phase III Comparative Trial of LPV/r vs. Nelfinavir (NFV) plus d4T/3TC in ARV-Naïve Patients (Study M98-863)

- Study M98-863 is a large, blinded, randomized, prospective study comparing the activity and safety of LPV/r plus d4T and 3TC to that of NFV plus d4T and 3TC in ARV-naïve patients. A total of 653 patients enrolled in the study and were assigned to receive d4T plus 3TC and either NFV or LPV/r. Through Week 60, significantly more LPV/r-treated subjects experienced viral suppression <400 copies/mL than NFV-treated subjects (74% vs. 61%, p < 0.001) (Figure 1).

Figure 1. Phase III ARV-Naïve Adults (M98-863): Proportion <400 Copies/mL (ITT NC=F)



Lower Incidence of Resistance in LPV/r-Treated Subjects

- Viral isolates from 40/65 LPV/r-treated subjects and 84/106 NFV-treated subjects could be amplified for resistance testing (Table 1).
- None of the 40 LPV/r-treated subjects demonstrated genotypic resistance to LPV.
- The absence of resistance to LPV was confirmed by phenotype in all LPV/r-treated subjects for whom phenotypic data were available (38/40).
- Thirty-one of 84 (37%) NFV-treated subjects demonstrated genotypic resistance to NFV.
- Baseline genotype was available for 30/31 NFV-treated subjects whose rebound sequence displayed resistance. The D30N or L90M mutation was not present at baseline in any of those 30 subjects.
- 3TC resistance was noted significantly more frequently in NFV-treated subjects than in LPV/r-treated subjects (81% vs. 38%, p<0.001).

Table 1. Phase III ARV-Naïve Adults: PI Resistance Through 60 Weeks

	LPV/r (n=326)	NFV (n=327)	P-value
Patients with HIV RNA >400 copies/mL and genotype available	40	84	
Resistance detected in protease	0/40 (0%)*	31/84†	<0.001
D30N		22/31	
L90M		8/31	
L90M and D30N		1/31	
3TC Resistance	15/40 (38%)	68/84 (81%)	<0.001

* For LPV, any primary or active site mutation (8, 30, 32, 46, 47, 48, 50, 82, 84, 90). Absence of genotypic resistance to LPV confirmed by phenotype.
† For NFV, D30N and/or L90M.

RESULTS CONT.

Appearance of Polymorphisms/Secondary Mutations in LPV/r-Treated Subjects

- Baseline genotype was available for 37/40 subjects with rebound genotype.
- Isolates were examined for the presence of any of the following polymorphisms/secondary mutations: 10, 20, 24, 33, 36, 53, 54, 63, 71, 77 and 88.[3]
- Each of the rebound sequences for 30/37 (81%) subjects displayed no new secondary mutations.
- The remaining 7/37 (19%) rebound sequences demonstrated one secondary mutation that was not present at baseline, including L10F (1), M36I (4), L63P (1) and A71T (1) (Table 2).
- At rebound, 2 subjects no longer had a secondary mutation that had been present at baseline.
- The presence of secondary mutations had no detectable effect on LPV susceptibility.

Table 2. New Polymorphisms/Secondary Mutations in LPV/r-Treated Subjects (M98-863)

Subject	Rebound Sequence
7	**L10L/F,** L33V, E35D, M36M/I/L, N37N/A/D/T, R41K, I64V, I72I/V, I93L
10	T12T/S/A, I13V, I15V, L19L/I, D60E, I62I/V, L63P/S, **A71T**
18	T12T/S, L19L/V, **M36M/I**
24	E35D/N, **M36M/I,** N37D, L63P, K70R, V77V/I
25	L19Q, **L63L/P,** V77I
28	**M36M/I,** R41K, D60E, I64V
36	**M36M/I,** R41R/K, Q61E, L63P, V77I

Model of the Pharmacologic Barrier to Resistance/Zone of Selective Pressure to Account for Differential Emergence of Resistance Between LPV/r and NFV

Model of the Pharmacologic Barrier to Resistance for Protease Inhibitors

- When mutations develop in the protease gene as a result of suboptimal viral suppression, a stepwise reduction in drug activity occurs, reflected by the fold change in susceptibility (as measured by the IC_{50}) relative to wt virus. As mutations accumulate, the IC_{50} for the mutated virus incrementally approaches drug trough levels.
- The Inhibitory Quotient (IQ), the ratio of the trough concentration (C_{trough}) to IC_{50}, provides a means to quantify the relationship between plasma drug concentrations achieved and viral drug susceptibility. In situations where a high IQ is achieved, more mutations in protease are required to generate sufficient reduction in drug susceptibility to adversely affect clinical response. Therefore, viral load rebound with the subsequent development of resistance mutations should be less likely to occur with a high IQ relative to circumstances where a low IQ is maintained (Figure 2).
- Inhibitory quotients were computed for randomly selected patients in the LPV/r and NFV arms of the M98-863 study using the protein corrected IC_{50} (50% human serum[4]) for wt HIV (Figure 3). The more frequent emergence of protease resistance in the NFV arm relative to the LPV/r arm is consistent with the lower IQ in the NFV arm.
- Potential explanations for the differential rebound/resistance patterns between treatment arms other than the pharmacologic barrier that results from a high IQ were examined.
  - Overall adherence as measured by pill counts was similar between LPV/r and NFV-treated patients with rebound genotype available and between the two treatment groups overall. Adherence was statistically significantly higher in virologic responders than nonresponders.[5]
  - Exposure to viral replication (baseline viral load, viral load at time of genotype, days with viral load >400 copies/mL and viral load area under the curve) was comparable between LPV/r and NFV-treated patients.[6]

Figure 2. Model of the Pharmacologic Barrier to Resistance for Protease Inhibitors

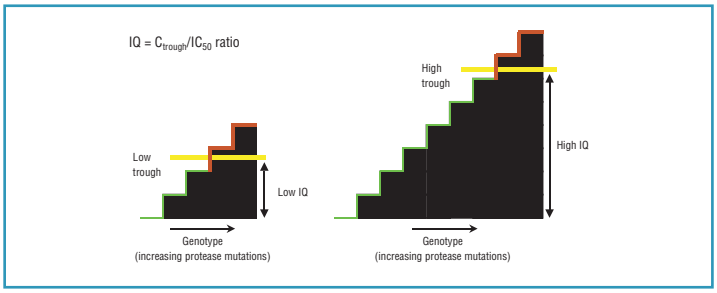
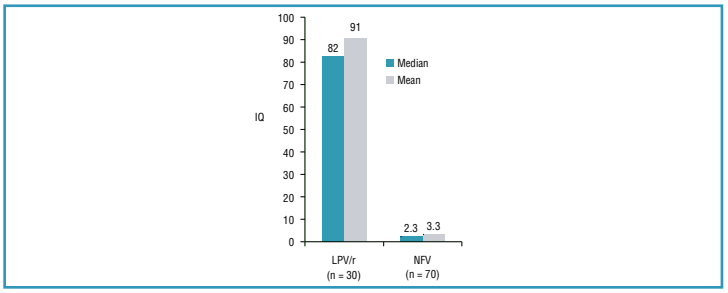


Figure 3. LPV/r and NFV Inhibitory Quotients (IQ) in ARV-Naïve Patients (M98-863)



Terminal Pharmacokinetics of LPV and the Zone of Selective Pressure

- While the pharmacologic barrier model explains the lower rate of virologic failure in LPV/r-treated patients with adequate adherence, the question remains as to why protease resistance does not develop during viral load rebound on LPV/r therapy in settings of suboptimal adherence. A semi-quantitative pharmacological model may account for the lack of resistance to LPV observed in this study despite the occurrence of viral replication (Figures 4A and 4B).

Figure 4A. Estimating the Decay of LPV Through the Zone of Selective Pressure in ARV-Naïve Patients

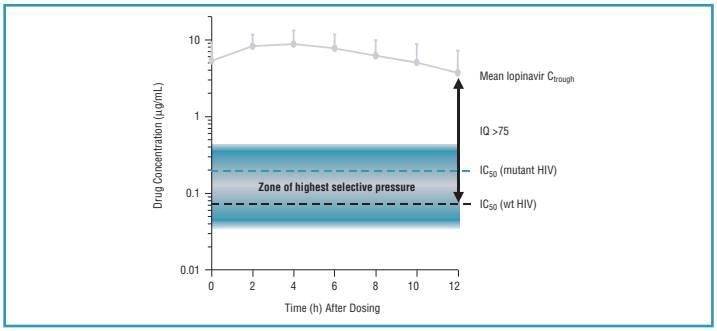
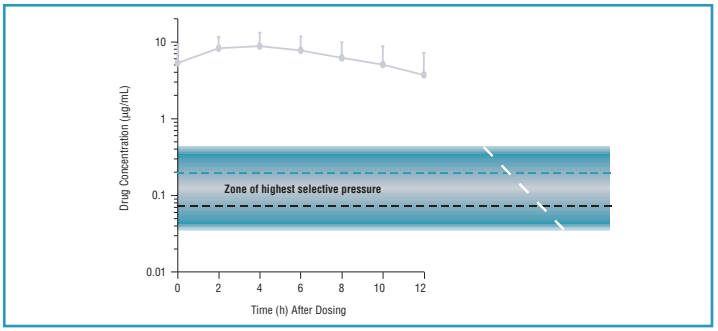


Figure 4B. Lopinavir Is Likely to Pass Rapidly Through the Zone of Selective Pressure as RTV Concentrations Decline



- During periods of adherence, plasma levels of LPV remain well in excess of the serum-adjusted IC_{50} against wt HIV (high IQ), and viral replication of both the wt virus and any pre-existing viral mutants are likely to be suppressed. Drug concentrations that lie between the IC_{50} values for the wt and mutant viruses are expected to provide the greatest selective replication advantage for the mutant (zone of highest selective pressure). During periods of non-adherence, plasma drug levels decline through the zone of highest selective pressure. As drug levels continue to decline below this concentration zone, overall replication increases, and, in the absence of drug, the wt virus has a fitness advantage over any mutants. Understanding the selection of resistance *in vivo* requires an estimation of the time during which significant selective pressure exists (i.e., how frequently and rapidly plasma drug concentrations decline through the zone of highest selective pressure).

- Single mutants display <3-fold reduced susceptibility to LPV.[7,8] Based on steady-state pharmacokinetic determinations, the median estimated time for plasma levels of LPV to decay to the upper boundary of the zone of highest selective pressure is 20.5 hours (Figure 4B). This time would approximate missing a scheduled LPV/r BID dose by 8 hours. By this time, plasma concentrations of ritonavir have declined to levels that no longer adequately inhibit the metabolism of LPV. Thus, as concentrations of LPV enter the zone of highest selective pressure, the clearance of LPV has increased substantially (short half-life). Thus, the estimated median time for LPV concentrations to decay through the zone of highest selective pressure is approximately 3.5 hours (range 2.5-5 hours). Since viral maturation through proteolytic processing by active (uninhibited) wt HIV protease is not instantaneous (estimated half-life up to 1.5 hours for production of mature, infectious particles)[9] and the kinetics of HIV protease containing primary mutations can be substantially impaired compared to wt protease,[10] the selective production of infectious virus containing primary resistance mutations may be minimal during this period.

No Emergence of Protease Resistance Observed in Phase II Trials of LPV/r in ARV-Naïve Patients

- Results from phase II trials for LPV/r and d4T/3TC in ARV-naïve adults (n=100) treated for 144 weeks and ARV-naïve children (n=44) treated for 48 weeks were consistent with those from the phase III M98-863 trial. No protease inhibitor resistance was observed to emerge in these two study populations using the same definition of genotypic resistance (primary or active site mutations) employed in the M98-863 study (Table 3).

Table 3. LPV/r Phase II Program (ARV-Naïve)

Study	M97-720	M98-940
# Patients	100 adult	44 pediatric
Duration	144 weeks	48 weeks
<400 c/mL (On tx / ITT M=F)	99%/79%	86%/84%
Rebounds Analyzed	5	14
Evolution in Protease	0	0
Changes in Genotype from Baseline	Pr: E35D, M36M/I (n=1) N37E (n=1) V3I, S37D (n=1) RT: M184V/I (n=3)	Pr: L10I (n=1) L63L/P (n=1) RT: M184V/I (n=4)

Emergence of Resistance in Protease Inhibitor Experienced Patients Treated with LPV/r

Summary of Observations in PI-Experienced Patients with Viral Load Rebound on LPV/r-Based Therapy

- Phase II trials have been conducted in 70 single PI-experienced and 57 multiple PI-experienced patients (Table 4). Both populations were NNRTI naïve and received an NNRTI along with LPV/r in the phase II trials.
- Viral isolates from 27 patients with viral rebound on LPV/r were sent for genotype/phenotype. Patients with viral rebound were grouped on the basis of the baseline LPV mutation score (see Methods; Table 5).
- Significant evolution in protease was not observed in any of the 9 patients with 2 or fewer LPV associated mutations in their baseline viral isolates. In contrast, viral evolution was observed in isolates from 12/18 (67%) patients with 3 or more LPV associated mutations in baseline viral isolates (Table 5).
- Phenotypic susceptibility to LPV at baseline and the maximum value obtained post rebound are provided for individual patients lacking and demonstrating evidence of viral evolution at rebound in Figures 5A and 5B, respectively.
- Corresponding protease genotypes for patients lacking and demonstrating evidence of viral evolution at rebound are displayed in Tables 6A and 6B, respectively.

Table 4. LPV/r Phase II Program (PI-Experienced)

Study	M97-765	M98-957
Prior ARV	Single PI	Multiple PI
# Patients	70	57
Duration	96 weeks	48 weeks
Other ARV	NVP, NRTIs	EFV, NRTIs
<400 c/mL (On tx / ITT M=F)	81%/63%	80%/65%
Rebounds Analyzed	15	12

Table 5. Summary of Observations from PI-Experienced Patients with Viral Rebound on LPV/r Therapy

Number of LPV Associated Mutations at BL*	Number of Patients	Evolution in Protease	Evolution of NNRTI Resistance (>10-fold wt)
0-2	9	0	7 (78%)
3-9	18	12 (67%)	14/15 (93%)**

* Selected from 11 mutations associated with reduced *in vitro* susceptibility to LPV at protease amino acid positions 10, 20, 24, 46, 53, 54, 63, 71, 82, 84, and 90.
** Does not include 3 patients with BL viral isolates demonstrating >10-fold reduced susceptibility to NNRTIs.

Figure 5A. Isolates without Evidence of Viral Evolution During Rebound on LPV/r

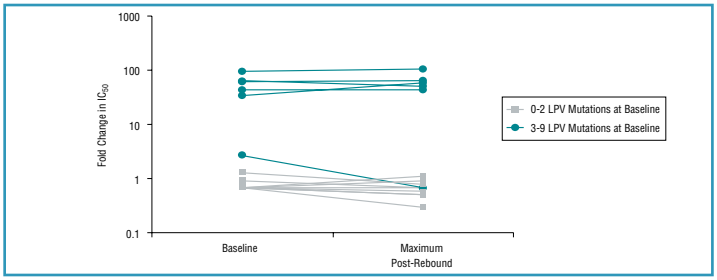


Figure 5B. Isolates with Evidence of Viral Evolution During Rebound on LPV/r

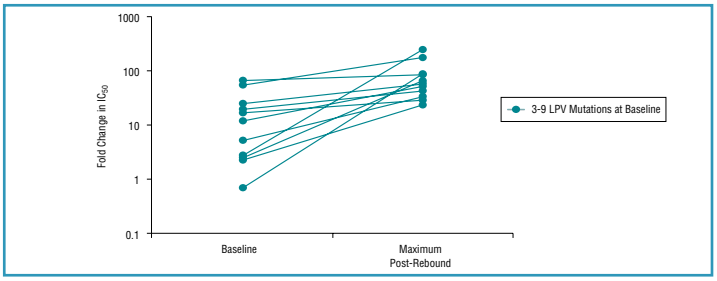


Table 6A. Summary of Observations from PI-Experienced Patients without Evidence of Viral Evolution in Protease During Rebound

Patient	BL LPV Mutation Score	Changes in Rebound Protease Sequence Relative to BL	Evolution of NNRTI Resistance (>10-fold vs. wt)
S1	0	R41R/K	Y
S2	1	No change	Y
S3	1	No change	Y
S4	1	No change	Y
S5	2	K20K/R, N37E, L63L/P	Y
S6	2	N88D/G	N
S7	2	No change	Y
S8	2	S37C	Y
M1	2	V77V/I	N
S9	4	I54I/V, L63A/T, C67C/D/G/Y, V82V/A	N
M2	6	H69H/Y	Y
M3	7	No change	Y
M4	8	No change	Y
M5	8	N37N/T	Y*
M6	9	No change	Y

* >10-fold NNRTI Resistance at BL.

Table 6B. Summary of Observations from PI-Experienced Patients with Evidence of Viral Evolution in Protease During Rebound

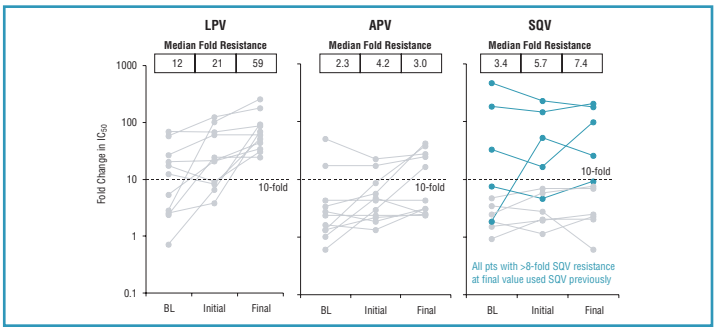
Patient	BL LPV Mutation Score	Changes in Rebound Protease Sequence Relative to BL	Evolution of NNRTI Resistance (>10-fold vs. wt)
S10	3	I47A, Q92K	Y**
S11	4	L24I, M36V, N37S, M46I, F53F/L, R57R/K, I64I/V	Y*
S12	4	L10F, L33F, N37D, M46I, I54V	Y
S13	4	L24I, I54V, L63P	Y
S14	4	L10I/V, K20K/T, M36M/I, M46M/I/L	Y
M7	4	L10V, M46I, I50V, K55R, I64I/L, A71V, I72R, V82A, L89I, Q92K	Y
S15	5	G16A, L33F, I54V, I62I/V, A71L, L76V, V82V/A, N88G	Y*
M8	6	M46M/L	Y
M9	6	L10F, K20I/M/V, E34K, M36I, I62V, H69H/Q, V82S	Y
M10	6	I64I/V, H69H/R, G73C	Y
M11	7	K14K/R, L33L/F, I72I/V	Y
M12	8	T4T/P, E34E/V, M46M/I, I47I/V, F53L, K55K/R	Y

* >10-fold NNRTI Resistance at BL. **Phenotype not available (K103N in RT appeared at rebound)

Cross-Resistance of Viruses Selected by LPV *In Vivo*

- Phenotypic data were successfully generated for 11/12 PI-experienced patients with rebound viral isolates on LPV/r therapy demonstrating evolution in protease (Figure 6).
- In general, viral isolates demonstrating increased phenotypic resistance to LPV at rebound either remained resistant (if resistant at baseline) or developed cross-resistance to ritonavir, indinavir, or nelfinavir.
- By phenotypic testing performed at the first time of confirmed rebound, all viral isolates without substantial reduction in amprenavir sensitivity at baseline (<10-fold reduced susceptibility relative to wt) either remained sensitive or developed at most modestly reduced susceptibility to APV (<8.5-fold relative to wt).
- Similarly, in patients without prior saquinavir experience, all viral isolates tested at the initial time of confirmed rebound remained sensitive or developed only modestly reduced susceptibility to SQV (<7-fold relative to wt).
- Extended therapy with LPV/r following initial rebound lead to the emergence of more substantial reduction in APV susceptibility (>10-fold relative to wt) in 3 patients with ongoing viral replication. These isolates with evidence of further reduction in APV sensitivity following initial rebound contained multiple mutations in protease, including amino acid positions 82, 84, 90 and also 50 in one instance.[11]

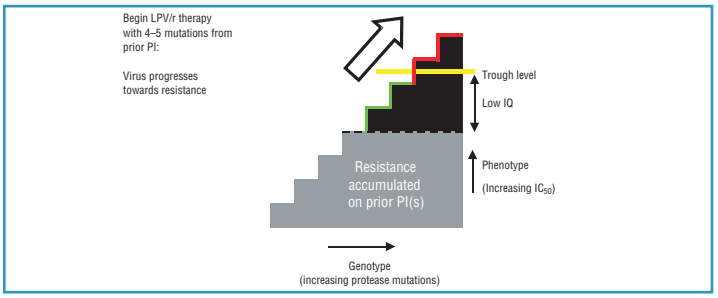
Figure 6. Phenotypic Susceptibility to APV and SQV in Viral Isolates with Evolution of Phenotypic Resistance to LPV



Model of the Pharmacologic Barrier to Resistance to Account for Emergence of LPV Resistance in Patients with Pre-existing Protease Mutations

- In patients who begin LPV/r-based therapy with virus containing 4 or more baseline protease mutations as a consequence of prior therapy, the pharmacologic barrier is compromised as the LPV IC_{50} values of these viruses are already above that of wild type virus and are starting to approach drug trough levels particularly in circumstances of suboptimal adherence (low IQ; Figure 7). Consequently, few incremental mutations are required to increase the IC_{50} to a level at which plasma drug concentrations are no longer able to adequately suppress viral replication. In this situation, selective pressure is high, and virologic rebound with evolution of resistance is much more likely to occur than in situations where the pharmacologic barrier is fully intact, as is the case with PI-naïve patients.

Figure 7. The Barrier to Resistance Is Compromised with Pre-existing Protease Mutations



CONCLUSIONS

- LPV/r appears to provide a high pharmacologic barrier to resistance in ARV-naïve patients, as a likely consequence of the elevated and sustained plasma concentrations achieved.
  - LPV resistance has not been observed in 470 ARV-naïve patients with ≥1 year of LPV/r-based therapy.
  - Significantly lower incidence of protease resistance mutations was observed for LPV/r-treated vs. NFV-treated patients through 60 weeks in a blinded comparative clinical trial.
- The barrier to resistance is compromised by protease mutations accumulated during prior PI therapy.
- Based on cross-resistance patterns, SQV or APV with RTV PK enhancement may be useful for salvage therapy when LPV resistance is present—clinical investigation is underway.

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