Evolution of Lopinavir (LPV) Resistance in Protease Inhibitor-Experienced Patients Treated with LPV/r

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BACKGROUND

We have previously examined the virologic response of multiple PI- and NRTI-experienced, NNRTI-naive patients to treatment with lopinavir/ritonavir (LPV/r) plus efavirenz (EFV) and NRTIs with respect to baseline genotype and phenotype [Kempf et al., 2002]. Maximal activity was observed in patients with baseline viruses containing up to 5 mutations associated with LPV resistance and/or displaying up to 10-fold reduced susceptibility to LPV (lower clinical breakpoint). Although there was also a difference in clinical response rates between patients with baseline viral isolates displaying <40-fold and >40-fold reduced susceptibility to LPV, the ability to define an upper breakpoint for LPV/r activity in that study was limited by the relatively small number of patients with high-level baseline resistance and by the concomitant activity of EFV.

In separate Phase II and III studies, the development of resistance to lopinavir has not been observed among 508 antiretroviral-naive patients treated with a LPV/r-based regimen [Waimsky et al., 2002; Kempf et al., 2003, Stevens et al., 2003]. In contrast, the development of resistance to LPV/r has been observed in PI-experienced patients. In this investigation, we characterize the selection of incremental LPV resistance among PI-experienced patients with incomplete virologic response to LPV/r.

We also explored the selection of incremental LPV resistance in these patients as an alternate method for estimating an upper breakpoint for this boosted PI; lack of evolution among patients with high levels of baseline resistance may suggest a “no-effect” level if the drug(s) exert insufficient selective pressure to force the accumulation of additional resistance.

METHODS

Samples were analyzed from two Phase II studies and one Phase III study of LPV/r in combination with either nevirapine (NVP) or efavirenz (EFV) and NRTIs (Table 1).

Table 1. Clinical Studies in PI-Experienced Patients Used for Analysis of Incremental Resistance Development

<table>
<thead>
<tr>
<th>Study No.</th>
<th>Patient Population</th>
<th>No. of Patients Receiving LPV/r</th>
<th>Study Regimen</th>
<th>LPV/r Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>M97-765</td>
<td>Single PI-experienced, NNRTI-naive</td>
<td>70</td>
<td>LPV/r, NVP, NRTIs of choice</td>
<td>400/100 or 400/200 mg BID</td>
</tr>
<tr>
<td>M98-957</td>
<td>Multiple PI-experienced, NNRTI-naive</td>
<td>57</td>
<td>LPV/r, EFV, NRTIs of choice</td>
<td>400/100 or 533/135 mg BID</td>
</tr>
<tr>
<td>M98-888</td>
<td>Single PI-experienced, NNRTI-naive</td>
<td>148</td>
<td>LPV/r, NVP, NRTIs of choice</td>
<td>400/100 mg BID</td>
</tr>
</tbody>
</table>

For analysis of genotype and phenotype, samples were selected from among patients demonstrating virologic rebound or incomplete virologic response. Baseline samples were also analyzed for each patient. For patients with multiple rebound samples, the maximum fold change in LPV IC50 on therapy was considered in the analysis.


The effects of baseline genotype (number of PI mutations) and phenotype on the selection of additional resistance were assessed by logistic regression analysis. Number of PI mutations was based on the LPV mutation score, including the following mutations previously associated with reduced LPV susceptibility: L10F/I/R/V, K20M/R, L24I, M46I/L, I47A/V, I54A/V/L, N88D; (3) emergence of any other secondary mutation (L10F/I/R/V, K20M/R, M36I, A71V/T, G73S/A, V77I) accompanied by a ≥2-fold change in LPV IC50, between baseline (pre-LPV/r treatment) and rebound.

RESULTS

Selection of Incremental LPV Resistance

- Baseline and rebound genotypic results were available from 54 patients (41 single PI-experienced and 13 multiple PI-experienced).
- Phenotypic results were available from all 54 patients at rebound and from 45 patients at baseline. No patient was receiving any PI other than LPV/r.
- Selection of incremental lopinavir resistance was observed in 19 patients with viral rebound and resistance data available (19/54, 35%), including 14/41 (34%) single PI-experienced patients and 5/13 (38%) multiple PI-experienced patients.
- The most common mutations at baseline among the 19 patients demonstrating selection of incremental lopinavir resistance were at positions 10 (17 patients) 71 and 82 (17 patients each), and 50 (12 patients each). For analysis of genotype and phenotype, samples were selected from among patients demonstrating virologic rebound or incomplete virologic response. Baseline samples were also analyzed for each patient. For patients with multiple rebound samples, the maximum fold change in LPV IC50 on therapy was considered in the analysis.
- The most common mutations emerging at rebound among these patients included M46I/L (emerged in 10/13 [73%] patients without M46I/L at baseline), I54V (6/8 patients, 75%), L33F (6/18 patients, 33%), and V82A (2/7, 29%). The I50V mutation emerged in 2 patients with prolonged periods of detectable viral load (Figures 2a and 2b).
- For these 19 patients, the median (interquartile range) fold change in PI susceptibility to other protease inhibitors at the last available visit was ritonavir: 2.0 (0.9 to 4.2); indinavir: 2.0 (1.0 to 4.0); saquinavir: 2.0 (1.0 to 4.0). Notably, among patients not previously exposed to saquinavir, the median (IQR) fold change in PI susceptibility was 2.0 (1.0 to 4.0).
Genotypic Predictors of Additional LPV Resistance

- All patients demonstrating incremental resistance had at least one primary PI mutation (see Methods) at baseline: 19/39 patients with at least one primary PI mutation demonstrated incremental LPV resistance, compared with 0/15 patients without a primary PI mutation (p<0.001).

- A second-order logistic regression model indicated maximal selective pressure (highest probability of incremental LPV resistance) at 4-6 baseline PI mutations with little selective pressure below 2 or above 7 PI mutations (Figure 3).

- Thus, no resistance emerged in the rebound isolates from 14 patients with 0-1 baseline PI mutations, while in contrast, the selection of incremental resistance was evident in isolates from 3/11, 9/11, 6/14, 1/4 patients with 2-3, 4-5, 6-7 and 8-10 baseline PI mutations, respectively (Figure 4).

- Among patients with at least one primary mutation, a mutation at position 32 was statistically significantly associated with development of incremental LPV resistance: 4/4 patients with a V32I mutation developed incremental resistance, as did 15/35 patients without a V32I mutation (p=0.047). No other mutation was statistically significantly associated with incremental LPV resistance.
Phenotypic Predictors of Incremental LPV Resistance

- A second-order logistic regression model suggested a substantial drop in selective pressure beginning at 40- to 60-fold reduced baseline susceptibility to LPV (Figure 5). The probabilities (95% CI) of incremental selection of LPV resistance in patients with 40-, 60-, and 80-fold baseline LPV IC50 were 46% (25%, 72%), 31% (11%, 63%) and 20% (5%, 56%), respectively.
- Among patients with ≥4 baseline PI mutations, incremental resistance was selected in 13/19, 2/4, and 1/6 patients with <40-fold, 40- to 60-fold, and >60-fold baseline reduced susceptibility to LPV (Figure 6).

The magnitude of incremental phenotypic LPV resistance was highest among patients with at least 4 PI mutations but <60-fold baseline reduced susceptibility to LPV. Mean and median (IQR) changes in LPV susceptibility between baseline and rebound with respect to baseline genotype and phenotype are shown in Figures 7a and b. The majority of patients with 4 or more baseline PI mutations (27/29) demonstrated high-level NNRTI phenotypic resistance and Data Analysis Plan (DAP)-defined [DeGruttola et al., 2000] NNRTI resistance mutations at rebound.
The majority of patients treated with LPV/r in combination with NVP received 3 capsules (400/100 mg) of LPV/r BID. The mean C trough of LPV in 22 patients from Studies breakpoin for LPV/r (40- to 60-fold) using this method is not complicated by the concomitant therapy received by these patients. Notably, because the analysis of resistance emergence is class-specific and because of the high-level NNRTI resistance present at rebound, the estimation of an apparent upper breakpoint for LPV/r is derived primarily from patients with 4 or more baseline mutations, where the pharmacologic barrier to resistance is expected to be significantly eroded. In these patients, the selection of resistance by LPV/r is most likely in patients with baseline LPV susceptibility of ≤40- to 60-fold and in patients with 4-7 baseline PI mutations. Information on the upper clinical breakpoint for LPV/r is derived primarily from patients with 4 or more baseline mutations, where the pharmacologic barrier to resistance is expected to be significantly eroded. In these patients, the selection of resistance by LPV/r is most likely in patients with baseline LPV susceptibility of ≤40- to 60-fold and in patients with 4-7 baseline PI mutations.

Selection of LPV resistance did not occur during virologic rebound/incomplete virologic response on LPV/r based therapy in patients with 0-1 baseline PI mutations. This observation is illustrative of a high pharmacologic barrier to resistance and is consistent with results from extensive clinical studies in ARV-naive patients, where resistance to LPV/r has not been observed to emerge to date [Kempf et al., 2003, Stevens et al., 2003]. When 2 or more PI mutations are present at baseline (including a primary mutation), the pharmacologic barrier to resistance is compromised, and the emergence of additional resistance is possible. The likelihood of selection appears to be highest with 4 or more baseline mutations. Results were similar if the number of DAP-defined PI resistance mutations [DeGruttola, et al., 2000] was used instead of the LPV mutation score (data not shown).

In these patients, the selection of resistance by LPV/r is most likely in patients with baseline LPV susceptibility of ≤40- to 60-fold and in patients with 4-7 baseline PI mutations. Notably, because the analysis of resistance emergence is class-specific and because of the high-level NNRTI resistance present at rebound, the estimation of an apparent upper breakpoint for LPV/r is derived primarily from patients with 4 or more baseline mutations, where the pharmacologic barrier to resistance is expected to be significantly eroded. In these patients, the selection of resistance by LPV/r is most likely in patients with baseline LPV susceptibility of ≤40- to 60-fold and in patients with 4-7 baseline PI mutations.

In PI-experienced patients receiving LPV/r, the likelihood of emergence of additional resistance during virologic failure appears to be dependent upon both baseline genotype and phenotype. Incremental lopinavir resistance was not observed in patients without a primary mutation at baseline. Evidence of selective pressure during viral rebound may be a useful indicator for defining upper genotypic and phenotypic breakpoints for antiretroviral agents. The phenotypic upper breakpoint for LPV/r estimated in this analysis (40- to 60-fold) is consistent with the IQ pharmacological model for LPV/r activity.

**REFERENCES**


**Acknowledgments**

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