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Exploration of Methodology for Estimating Upper Clinical Breakpoints for Lopinavir/ritonavir by Analysis of the Emergence of Resistance During Virologic Failure in Experienced Patients

M King, H Mo, A Molla, R Stevens, S Brun, D Kempf; Abbott Laboratories, Abbott Park, IL

BACKGROUND

Clinical breakpoints for protease inhibitors (PIs) are typically determined by analysis of virologic response with respect to baseline phenotype and/or genotype. However, definition of an upper breakpoint (the degree of resistance above which there is little evidence of antiviral activity) can be complicated by the need to recruit a sufficient number of study subjects with highly resistant virus in order to define a "no-effect" level of resistance. Moreover, interpretation of virologic response data can be complicated by activity of drugs other than the one of interest that are used in combination.

Complementary information on a "no-effect" level is available by examination of the fate of viruses in patients who either fail or incompletely respond to therapy. Under such circumstances, evolution of additional resistance in a particular target gene (e.g., protease or reverse transcriptase) suggests residual activity of the drug(s) of that class in the context of baseline resistance. In contrast, lack of evolution during either rebound or incomplete response might be anticipated under two sets of circumstances: (1) when baseline virus is drug-susceptible and the drug combination exerts a high pharmacological barrier to resistance emergence; and (2) when baseline resistance is sufficiently high that the drug(s) exert insufficient selective pressure to force the accumulation of additional resistance (i.e., "no-effect" level).

We have previously examined the virologic response of multiple PI- and NRTI-experienced, NNRTI-naïve 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 [Walmsley 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 explored the analysis of the selection of incremental LPV resistance in these patients during failure of LPV/r therapy as an alternate method for estimating an upper breakpoint for this boosted PI.

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

Study No.	Patient Population	No. of Patients Receiving LPV/r	Study Regimen	LPV/r Dose
M97-765	Single PI-experienced, NNRTI-naïve	70	LPV/r, NVP, NRTIs of choice	400/100 or 400/200 mg BID
M98-957	Multiple PI-experienced, NNRTI-naïve	57	LPV/r, EFV, NRTIs of choice	400/100 or 533/133 mg BID
M98-888	Single PI-experienced, NNRTI-naïve	148	LPV/r, NVP, NRTIs of choice	400/100 mg BID

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 IC_{50} on therapy was considered in the analysis.

Selection of incremental resistance was defined as having satisfied any of the following: (1) emergence of a new primary PI mutation (D30N, V32I, G48V, I50V, V82A/F/T/S, I84V, L90M); (2) emergence of a new secondary mutation that is not normally observed as a polymorphism (L24I, L33F, 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 IC_{50} between baseline (pre-LPV/r treatment) and rebound.

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, M46/I/L, F53L, I54L/T/V, L63P, A71I/L/V/T, V82A/F/T, I84V, L90M [Kempf, et al., 2001].

RESULTS

Selection of Incremental LPV Resistance

- Baseline and rebound genotypic results were available from 53 patients (40 single PI-experienced and 13 multiple PI-experienced). Phenotypic results were available from all 53 patients at rebound and from 44 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 (19/53, 36%), including 14/40 (35%) single PI-experienced patients and 5/13 (38%) multiple PI-experienced patients.

Genotypic Predictors of Additional LPV Resistance

- All patients demonstrating incremental resistance had at least one primary PI mutation (see Methods) at baseline.
- 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 1).
- Thus, no resistance emerged in the rebound isolates from 13 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 baseline PI mutations, respectively (Figure 2).

Figure 1. Logistic Regression Model of Predicted Probability (and 95% CI) of Incremental LPV Resistance Among Patients with Rebound

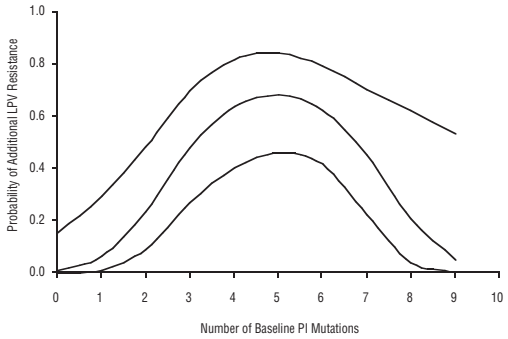
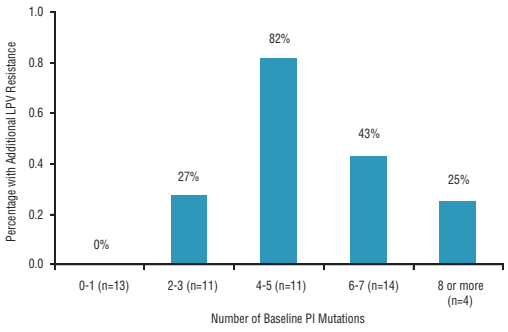


Figure 2. Proportion of Patients with Rebound Selecting Incremental LPV Resistance by Number of Baseline PI Mutations



- Changes in LPV phenotypic susceptibility between baseline and rebound for viral isolates from subjects with 0-1, 2-3, 4-5, 6-7, and ≥8 baseline PI mutations are shown in Figures 3a-e. Nine patients did not have baseline phenotype data, but rebound isolates from each demonstrated a LPV fold IC_{50} of 0.4 to 1.0 relative to wild type HIV. Therefore, these patients are represented in Figure 3a as having no change in phenotype between baseline and rebound.

Figure 3a. Change in LPV Resistance During LPV Treatment

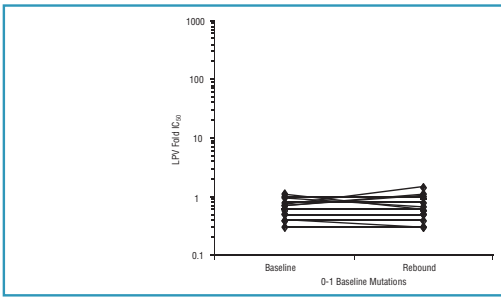


Figure 3b. Change in LPV Resistance During LPV Treatment

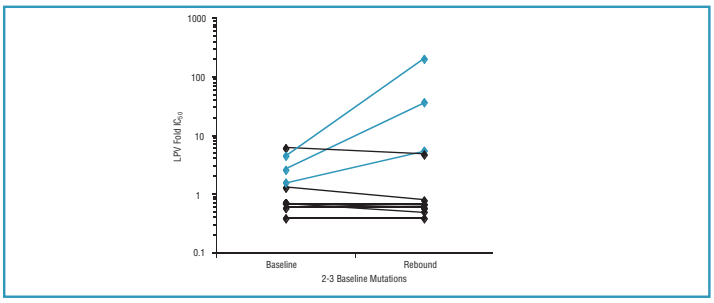


Figure 3c. Change in LPV Resistance During LPV Treatment

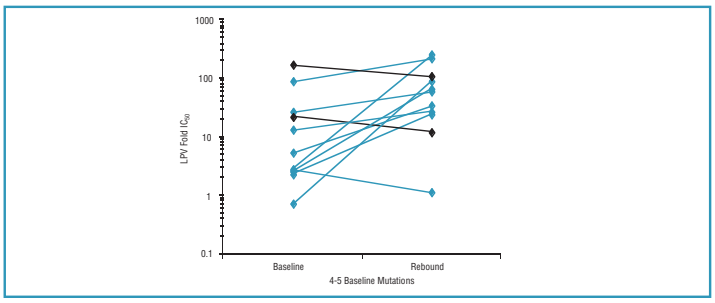


Figure 3d. Change in LPV Resistance During LPV Treatment

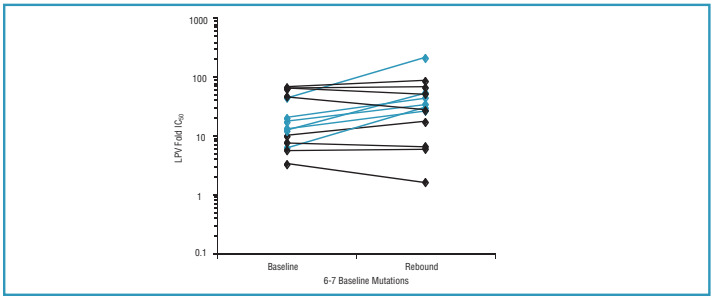
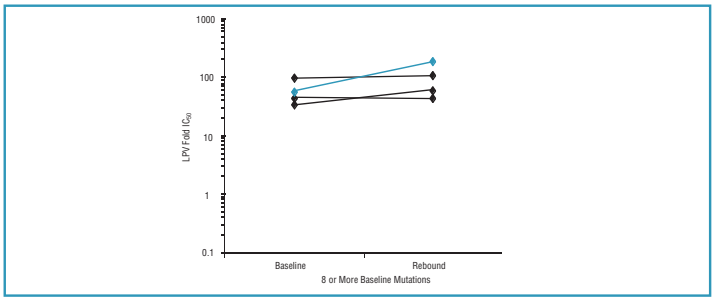


Figure 3e.Change in LPV Resistance During LPV Treatment



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 4). The probabilities (95% CI) of incremental selection of LPV resistance in patients with 40-, 60-, and 80-fold baseline LPV IC_{50} 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 5).

Figure 4. Logistic Regression Model of Predicted Probability (and 95% CI) of Incremental LPV Resistance Among Patients with Rebound

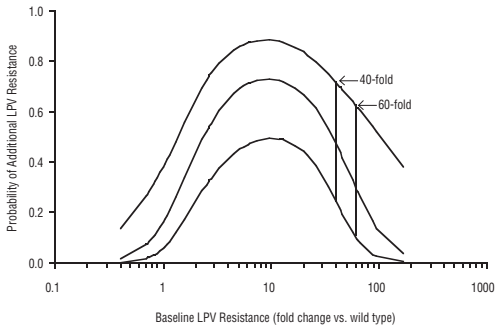
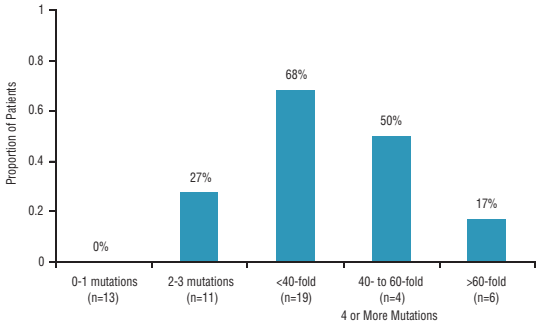


Figure 5. Proportion of Patients with Rebound Selecting Incremental LPV Resistance by Baseline Genotype and Phenotype



- 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 Figure 6.
- 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.

RESULTS *continued*

Figure 6a. Fold Change in LPV Resistance During LPV Treatment Among Patients with Rebound (Median and IQR)

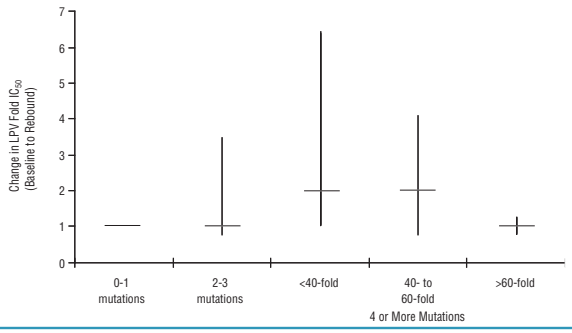
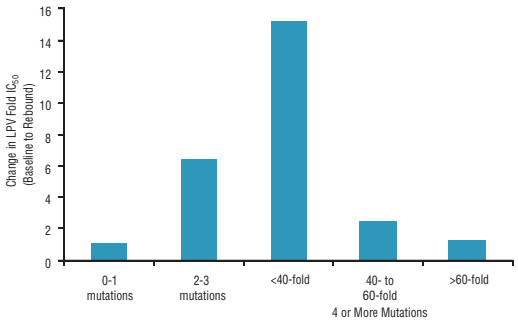


Figure 6b. Mean Fold Change in LPV Resistance During LPV Treatment Among Patients with Rebound



DISCUSSION

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-naïve 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).

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.

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 (40- to 60-fold) using this method is not complicated by the concomitant therapy received by these patients.

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 M97-765 and M98-888 was 3.87 µg/mL. Patients receiving EFV were given 3 or 4 capsules (400/100 mg or 533/133 mg) of LPV/r BID. The mean C_{trough} of LPV in patients from Study M98-957 was 2.16 µg/mL for the 400/100 mg BID dose (n=24) and 5.88 µg/µL for the 533/133 mg BID dose (n=26). Based on the serum-adjusted IC_{50} value for LPV (0.07 µg/mL) [Molla et al, 1998], the calculated average inhibitory quotient (IQ, C_{trough}/IC_{50} ratio) values for viruses of 60-fold reduced susceptibility, based on the above average C_{trough} values, range from 0.5 to 1.4. At IQ values ≤1 (i.e., C_{trough} lower than IC_{50}) substantial replication of the baseline virus would be expected and the selection of additional mutations might be disfavored, particularly if the more highly mutant viruses are less fit. Consequently, an apparent upper breakpoint of 60-fold is consistent with the IQ pharmacological model for LPV/r activity.

CONCLUSIONS

- 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.
- 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 PK/PD model for this regimen.

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