Decreased Adherence to Antiretroviral Therapy Observed prior to Transient Human Immunodeficiency Virus Type 1 Viremia

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(See the editorial commentary by Gallant, on pages 1729–31.)

Background. To identify potential causes and clinical implications of transient increases in plasma viral load (hereafter, “blips”).

Methods. M99-056 and M02-418 were prospective, randomized trials evaluating the safety and efficacy of lopinavir/ritonavir (LPV/r) capsules administered twice per day or once per day to subjects infected with human immunodeficiency virus–1 (HIV-1). Plasma viral load was measured every 4 weeks (from baseline through week 24, excluding week 12 and week 20 in M02-418), every 8 weeks (from week 24 through week 48), and every 12 weeks (from week 48 through week 96). Blips were defined by 1 plasma viral load measurement of between 50–1000 copies/mL, immediately preceded and immediately followed by a measurement of <50 copies/mL. A medication event monitoring system was used to record the date and time subjects administered a dose of LPV/r.

Results. Of 228 subject enrolled, event monitor data were available for 223 (98%) subjects (92 of whom received twice-daily LPV/r therapy, and 131 of whom received once-daily therapy). Viral load blips (median plasma viral load, 82 copies/mL [range, 51–858 copies/mL]) were identified in 60 (27%) of the subjects (21 in the LPV/r twice-daily group and 39 in the LPV/r once-daily group). Neither the baseline plasma viral load nor the CD4 T cell count were associated with blips. During the week prior to a blip, the mean number of days that the subject administered the prescribed number of doses was lower than the number during a matched period for the same subject during which a blip did not occur (5.55 vs. 6.22 days; \(P = .007\)). Blips were not associated with virologic failure or the development of drug resistance.

Conclusions. Blips were associated with decreased adherence, but not with virologic failure or development of drug resistance in these studies of LPV/r.

Trial registration. Clinicaltrials.gov identifier: NCT00043966.

“Blips” are defined as intermittent episodes of detectable, low-level increases in plasma HIV-1 RNA (ie, viral load), which return spontaneously to an undetectable range without any change in treatment. Blips are observed frequently in HIV-1–infected individuals who receive antiretroviral therapy, both in clinical trials and in medical practice. Studies conducted to evaluate the potential causes and implications of blips have concluded that they are a random biological and statistical variation around a mean viral load below the lower limit of detection of the HIV-1 RNA assay [1] or that they are false elevations related to laboratory processing of samples [2]. Other studies argue against the hypothesis that viral load blips represent assay variation and suggest that other phenomena, such as the release of virus from latent reservoirs, may play a role [3]. The relationship observed between blips and plasma drug levels has been inconsistent from study to study. The association between blips and adherence to a drug regimen remains ambiguous, with some studies reporting no association and others suggesting that lack of adherence may play a role [1, 3, 4]. An absence of reliable and continuous...
measurements of adherence preceding and following a blip may limit the statistical power to identify such a relationship. The use of electronic monitors that record and store the date and time that subjects administer a dose may improve adherence assessment and better define the relationship between adherence and blips.

Studies have also evaluated the association between blips and various clinical outcomes. In general, such studies have shown no association between blips and virologic or clinical failure [1, 5–9]. Although earlier studies suggested an association between blips and resistance mutations [10, 11], more recent studies that used sensitive genotypic testing and complete assessment of baseline mutations have not identified such an association [1].

Lopinavir/ritonavir (LPV/r) is a coformulation of lopinavir and ritonavir and is widely recommended in treatment guidelines, in combination with 2 nucleoside/nucleotide reverse transcriptase inhibitors, for the treatment of antiretroviral-naive and treatment-experienced HIV-1–infected patients [12–15]. In the results from 2 similarly designed studies (M99-056 and M02-418) reported previously, subjects who received 800 mg of lopinavir and 200 mg of ritonavir once per day and subjects who received 400 mg of lopinavir and 100 mg of ritonavir twice per day had comparable virologic efficacy and immunologic improvement, and drug resistance developed at similar rates [16–18]. Differences in rates of treatment adherence for once-daily and twice-daily LPV/r regimens have been demonstrated in HIV-1–infected subjects, with subjects who received LPV/r once per day demonstrating higher rates of treatment adherence [19]. However, the differences observed in rates of treatment adherence do not appear to affect clinical outcomes through 96 weeks of treatment.

The purpose of the current analysis was to identify potential causes and clinical implications of blips observed during these 2 clinical trials of LPV/r. We determined the predictors of blips and specifically evaluated the associations between blips and adherence. We also evaluated the impact of blips on virologic outcomes and the development of resistance.

**METHODS**

Studies M99-056 and M02-418 were prospective, randomized, open-label, multicenter, parallel arm clinical trials evaluating the safety, tolerability, antiviral efficacy, and pharmacokinetics of LPV/r soft-gel capsules, administered once daily (800 mg of lopinavir and 200 mg of ritonavir) or twice daily (400 mg of lopinavir and 100 mg of ritonavir), in combination with nucleoside reverse transcriptase inhibitors ( stavudine and lamivudine twice daily in M99-056, tenofovir disoproxil fumarate and emtricitabine once daily in M02-418) to antiretroviral-naive, HIV-1–infected subjects. The descriptions and results of these similarly designed studies have been published elsewhere [16–18]. Subjects were considered antiretroviral-naive if they had previously received antiretroviral therapy for less than 7 days. In addition, subjects were required to have plasma viral loads >50 copies/mL (M99-056) or >1000 copies/mL (M02-418) at screening. However, there was no CD4 T cell count restriction. Institutional review boards at the participating centers approved the studies, and all subjects provided written, informed consent prior to any study-specific procedures.

Blood collection for quantitation of HIV-1 RNA in plasma was conducted at baseline; at weeks 4, 8, 12, 16, 20, and 24 (excluding weeks 12 and 20 in M02-418); every 8 weeks thereafter, until week 48; and every 12 weeks thereafter, until week 96. For this analysis, a blip was defined as 1 plasma HIV-1 RNA measurement of 50–1000 copies/mL, immediately preceded and immediately followed by plasma HIV-1 RNA measurements of <50 copies/mL [4]. If a given subject had multiple episodes of transient viremia, only the first episode was included in the evaluation.

Rates of treatment adherence were assessed by use of electronic medication event monitors (Medication Event Monitoring System [MEMS]; Aardex) designed to record when patients administered a dose of their prescribed medications. These monitors recorded and stored the date and time that enrolled subjects opened a bottle of LPV/r throughout these studies. For the purpose of this study, a longitudinal assessment of subjects who received LPV/r either once or twice daily was undertaken. Each subject’s adherence pattern was summarized by a daily binary adherence sequence: on each consecutive day, adherence was defined as “correct” if the subject took at least the prescribed number of doses of LPV/r. This coding retains much of the temporal structure in the individual patterns of dose administration.

Correct adherence was summarized and depicted graphically for both daily and weekly intervals. For subjects who experienced a blip, correct dose administration during a period of time around the first blip was compared with correct dose administration for the same subject during the first period of time in which a blip did not occur. Sensitivity analyses were conducted by selecting periods without blips at random and by selecting the period without a blip closest to the period when the first blip occurred. Results were robust with respect to selection of the period without a blip (data not shown).

Virologic failure was defined 3 different ways for the purpose of evaluating its association with blips. These were as follows: (1) initial suppression of plasma levels of HIV-1 RNA to <50 copies/mL, followed by 2 consecutive plasma HIV-1 RNA measurements of ≥50 copies/mL or by a final measured plasma HIV-1 RNA level of ≥50 copies/mL; (2) initial suppression of plasma levels of HIV-1 RNA to <50 copies/mL, followed by a plasma HIV-1 RNA measurement >1000 copies/mL at the end of the study or the last study visit; and (3) initial suppression of plasma HIV-1 RNA to levels to <50 copies/mL, followed by 2 consecutive plasma HIV-1 RNA measurements of >200 copies/mL or a final measured plasma HIV-1 RNA level >200 copies/mL. Definition 1 was the protocol-defined criteria for failure in the clin-
ical trials, definition 2 was the more clinically relevant HIV-1 RNA threshold of 1000 copies/mL, and definition 3 was an approximate 0.5 log₁₀ increase above the lower limit of quantitation of the HIV-1 RNA assay used in the trials.

For study M99-056, resistance to lopinavir was defined as the presence of any primary or active site mutation in protease (at positions 8, 30, 32, 46, 47, 48, 50, 82, 84, and 90). Genotypic resistance to stavudine was defined as the emergence of 1 or more thymidine-associated mutations (M41L, D67N, K70R, L210W, T215F/Y, and K219Q/E/N) in reverse transcriptase. Lamivudine resistance was defined as the emergence of the M184V/I mutation in reverse transcriptase. For study M02-418, resistance to lopinavir was defined conservatively as the development of any mutation in protease at positions 8, 30, 32, 46, 47, 48, 50, 82, 84, and 90, with a corresponding decrease in phenotypic susceptibility to lopinavir of at least 2.5-fold, compared with wild-type HIV-1. Resistance to tenofovir disoproxil fumarate was defined as the emergence of any new mutation in reverse transcriptase at positions 41, 65, 67, 70, 210, 215, 219, or an insertion mutation in the vicinity of codon 69. Emtricitabine resistance was defined as the emergence of the M184V/I mutation in reverse transcriptase.

Demographic characteristics and baseline clinical characteristics were evaluated using a Cochran-Mantel-Haenszel test for discrete outcomes and a 2-way analysis of variance model for continuous outcomes. Both statistical procedures were adjusted for clinical trial (M99-056 or M02-418). Further, the comparison of race was restricted to white and nonwhite subjects.

The average number of days with correct dose administration was compared between periods when a blip occurred and periods when blips did not occur by use of generalized estimating equation methodology. Confidence intervals (95% CIs) were also calculated within the framework of the generalized estimating equation methodology. The association between the occurrence of a blip and virologic failure was assessed using a survival methodology. The association between the occurrence of a blip and virologic failure was assessed using a survival methodology. Confidence intervals (95% CIs) were also calculated within the framework of the generalized estimating equation methodology. The association between the occurrence of a blip and virologic failure was assessed using a survival methodology. Confidence intervals (95% CIs) were also calculated within the framework of the generalized estimating equation methodology. The association between the occurrence of a blip and virologic failure was assessed using a survival methodology. Confidence intervals (95% CIs) were also calculated within the framework of the generalized estimating equation methodology. The association between the occurrence of a blip and virologic failure was assessed using a survival methodology. Confidence intervals (95% CIs) were also calculated within the framework of the generalized estimating equation methodology. The association between the occurrence of a blip and virologic failure was assessed using a survival methodology. Confidence intervals (95% CIs) were also calculated within the framework of the generalized estimating equation methodology. The association between the occurrence of a blip and virologic failure was assessed using a survival methodology. Confidence intervals (95% CIs) were also calculated within the framework of the generalized estimating equation methodology.

RESULTS

Frequency and timing of blips. Of 228 subjects enrolled in the 2 studies, 223 (98%) had medication event monitor data available (131 in the LPV/r once-daily group and 92 in the LPV/r twice-daily group). Baseline demographic characteristics for subjects with monitor data available are shown in table 1. With respect to demographic or baseline characteristics, there were no statistically significant differences between the subjects who received LPV/r once daily and those who received LPV/r twice-daily, after adjusting for study. However, study M99-056 appears to have enrolled fewer white subjects, compared with study M02-418 (31.4% vs. 54.8%; \( P = .014 \), Breslow-Day test).

The number of subjects in each study who experienced a blip is summarized in table 2. Transient viremia was identified in 60 (27%) of 223 subjects (39 in the LPV/r once-daily group and 21 in the LPV/r twice-daily group). A total of 51 (23%) subjects experienced a single blip, and 9 (4%) experienced 2 blips. A similar number of subjects experienced blips while receiving LPV/r once daily, compared with those who received LPV/r twice daily (30% vs. 23%), and the difference in the number of subjects who experienced a blip while receiving LPV/r once daily and the number who experienced a blip while receiving LPV/r twice daily was not statistically significant (\( P = .212 \), Cochran-Mantel-Haenszel test). The median blip magnitude was 82 copies/mL (range, 51–858 copies/mL). One subject had transient viremia with an HIV-1 RNA level >1000 copies/mL that did not meet the blip definition. Neither baseline viral load nor baseline CD4⁺ T cell count was associated with the occurrence of blips.

The median time to the first blip was 282 days (interquartile range, 169–448 days). Moreover, 23.3% of the first blip episodes occurred prior to week 24, 36.7% occurred between weeks 24 and 48, and 40.0% occurred after week 48 (see figure 1). The Kaplan-Meier estimated median time of follow-up was 673 days (95% CI, 670–676 days, or ~96 weeks).

### Table 1. Baseline demographic and clinical characteristics of study subjects who had medication event monitor data available.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>LPV/r once-daily group ( n = 131 )</th>
<th>LPV/r twice-daily group ( n = 92 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male ( n = 105 ) (80.2%)</td>
<td>Female ( n = 26 ) (19.8%)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>White ( n = 71 ) (54.2%)</td>
<td>Black ( n = 36 ) (27.5%)</td>
</tr>
<tr>
<td></td>
<td>Hispanic ( n = 15 ) (11.5%)</td>
<td>Asian ( n = 8 ) (6.1%)</td>
</tr>
<tr>
<td></td>
<td>Other ( n = 1 ) (0.8%)</td>
<td>Other ( n = 1 ) (1.1%)</td>
</tr>
<tr>
<td>Age, years</td>
<td>Mean ± SD 39.5 ± 11.9</td>
<td>Range 19–75</td>
</tr>
<tr>
<td>Time since HIV-1 infection</td>
<td>Mean ± SD 2.3 ± 4.1</td>
<td>Range 0.1–18.5</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA level,</td>
<td>Mean ± SD 4.85 ± 0.737</td>
<td>Range 3.44–6.44</td>
</tr>
<tr>
<td>CD4⁺ T cell count,</td>
<td>Mean ± SD 264.7 ± 206.75</td>
<td>Range 3–990</td>
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<tr>
<td>characteristic (cells/mm³)</td>
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</tbody>
</table>

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. LPV/r, lopinavir-ritonavir.

* Includes 1 subject who received once-daily therapy who selected “American Indian/Alaska Native” and 1 subject who received twice-daily therapy who selected “Other.”
Blips and adherence. The mean number of days that a subject administered the prescribed number of doses (correct dose adherence) during the week prior to a blip was significantly lower than the number during a matched period for the same subject during which a blip did not occur (5.55 vs. 6.22 days; \( P = .007 \)) (figure 2).

Correct dose adherence appeared to increase in the 3 weeks after detection of the blip and subsequently returned to baseline levels (figure 2). Subjects who had plasma HIV-1 RNA levels above the lower limit of quantitation were required by protocol to be contacted to have the viral load test result confirmed within 4 weeks; however, subjects presented for the confirmatory viral load testing after a median of 57 days (interquartile range, 36–79 days), which most likely corresponded to the next scheduled study visit.

Blips and treatment outcomes. The occurrence of blips was not associated with virologic failure under the 3 definitions of virologic failure previously described. The \( P \) value for the association under any of the definitions was \( \approx .33 \); the specific \( P \) values were as follows: definition 1, \( P = .33 \); definition 2, \( P = .50 \); and definition 3, \( P = .43 \) (table 3).

In a single subject in study M02-418, the infecting strain of HIV developed a protease M46M/I mutation, but phenotypic testing showed only a 0.5-fold change in lopinavir phenotypic susceptibility, compared with that of the wild type. No other primary or active site mutation was observed in protease in either study. No significant difference was noted in the development of drug resistance in reverse transcriptase at position 184 when subjects who did and did not experience a blip were compared (3 [5.0%] of 60 vs. 4 [2.5%] of 163; \( P = .39 \)). No other nucleoside reverse transcriptase mutations were observed in either study.

**DISCUSSION**

Baseline viral load and CD4 \( ^+ \) T cell count were not associated with the occurrence of blips. In these studies, blips were associated with decreased adherence to antiretroviral therapy. The subjects who experienced blips were less adherent to therapy during the week prior to the blip than they were during a matched week in which a blip did not occur. Differences in the results reported in this study and those of other studies may result from more precise monitoring of adherence (through the use of MEMS monitors); from evaluating adherence during a shorter, and possibly more clinically relevant, time period around the blip; or from increased power to detect a relationship. Studies that appeared to show an association between adherence and the occurrence of blips \([1, 3, 4]\) may have lacked adequate power to show statistical significance.

To be consistent with other published studies, we defined blips strictly as 1 plasma HIV-1 RNA measurement between 50–1000 copies/mL, immediately preceded and immediately followed by plasma HIV-1 RNA measurements of \( \approx 50 \) copies/mL. More refined assays may be able to detect HIV-1 RNA values below 50 copies/mL, but these assays are not clinically available and their clinical relevance is unclear. Also, a patient may have a viral load increase of \( >1000 \) copies/mL and subsequently have a viral load \( <50 \) copies/mL at the next visit. However, this occurred for only 1 subject in our studies, and exclusion of this single event is unlikely to bias our analysis. Finally, although the median time to confirmation of a viral load blip was 57 days, we feel that this interval of follow-up is reflective of actual clinical practice patterns and thus more clinically meaningful.

Observing the pattern of dose adherence around the blip episode (figure 2) offers several interesting insights. First, subjects appear to improve their adherence in the few days immediately preceding the scheduled visit (week 0). This pattern, referred to

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**Table 2. Subjects who experienced transient plasma HIV-1 RNA viremia (blips), according to study.**

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients who experienced a blip, proportion (%)</th>
<th>Magnitude of blip, median (range), copies/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Once-daily LPV/r group</td>
<td>Twice-daily LPV/r group</td>
</tr>
<tr>
<td>M99-056</td>
<td>6/18 (33%)</td>
<td>7/17 (41%)</td>
</tr>
<tr>
<td>M02-418</td>
<td>33/113 (29%)</td>
<td>14/75 (19%)</td>
</tr>
<tr>
<td>Both</td>
<td>39/131 (30%)</td>
<td>21/92 (23%)</td>
</tr>
</tbody>
</table>

**NOTE.** LPV/r, lopinavir/ritonavir.
as “white coat compliance,” has been reported previously for a number of different diseases, including HIV infection [20], and our data provide additional support that this may occur in clinical trials. Further, subjects demonstrated a short-term improvement in adherence, more marked for those in whom a blip was detected, beginning several days after the clinic visit. This temporary increase in adherence may result from the subject being notified about the viral load test result, and the impact on adherence may be greater for those subjects with a detectable viral load.

With a LPV/r–based regimen, blips do not appear to be associated either with virologic failure or the development of HIV-1 drug resistance. These results are consistent with those of previous studies [1, 5–9]. Although some studies have suggested an association between blips and resistance mutations [10, 11], we did not detect an association in our study. LPV/r use in antiretroviral-naive patients has been demonstrated to result in relatively less development of resistance to all drug components of the antiretroviral regimen, compared with use of an unboosted protease inhibitor, such as nelfinavir, or a nonnucleoside reverse transcriptase inhibitor, such as efavirenz [21, 22]. The absence of resistance at the onset of virologic failure in antiretroviral-naive subjects has been reported with other ritonavir-boosted protease inhibitors [23, 24]. Further, because of intrapatient and interpatient variability, the relationship between adherence and plasma drug levels (as an indirect measure of adherence) may vary by subject and among antiretroviral agents. Therefore the lack of association between the occurrence

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**Figure 2.** Adherence of study subjects to correct dose administration during periods of transient HIV type 1 viremia (blips) and periods without blips. Gray lines, number of days with adherence to correct dose administration per subject, shown by week; black lines, mean values for all subjects; dashed lines, 95% confidence intervals.

**Table 3.** Association between HIV-1 RNA blips and the 3 definitions of virologic failure used in the study.

<table>
<thead>
<tr>
<th>Blip</th>
<th>Definition 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Definition 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Definition 3&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes, 2 consecutive</td>
<td>Yes, last measured</td>
</tr>
<tr>
<td></td>
<td>(N = 137)</td>
<td>110 (80)</td>
<td>131 (90)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21 (15)</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Yes (N = 60)</td>
<td></td>
<td>45 (75)</td>
<td>56 (93)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9 (15)</td>
<td>4 (5)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients.

<sup>a</sup> Initial suppression of plasma levels of HIV-1 RNA to <50 copies/mL, followed by 2 consecutive plasma HIV-1 RNA measurements of ≥50 copies/mL or by a final measured plasma HIV-1 RNA level of ≥50 copies/mL.

<sup>b</sup> Initial suppression of plasma levels of HIV-1 RNA to <50 copies/mL, followed by a plasma HIV-1 RNA measurement >1000 copies/mL at the end of the study or the last study visit.

<sup>c</sup> Initial suppression of plasma HIV-1 RNA to levels to <50 copies/mL, followed by 2 consecutive plasma HIV-1 RNA measurements of >200 copies/mL or a final measured plasma HIV-1 RNA level >200 copies/mL.
of blips and the development of resistance may not be the same for all antiretroviral agents.

In summary, our analysis suggests that plasma HIV-1 RNA blips are associated with decreased adherence to antiretroviral therapy, but they were not associated with virologic failure or with the development of drug resistance in subjects taking a LPV/r–based antiretroviral regimen. A blip should not be dismissed as a random phenomenon, and the occurrence of a blip may provide an additional opportunity to discuss the importance of adherence to the antiretroviral regimen with the patient.

Acknowledgments

We acknowledge the contributions made by the subjects who enrolled in these studies, as well as the investigators and study personnel who participated in these trials. In addition, we would like to acknowledge Renee Heuser, Kevin Niemi, and Karen Wikstrom for their role in collecting and analyzing the data.

References