CONCISE COMMUNICATION

Patterns of Plasma Human Immunodeficiency Virus Type 1 RNA Response to Highly Active Antiretroviral Therapy in Infected Children

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This study examined the rate of decline in plasma human immunodeficiency virus type 1 (HIV-1) RNA levels to <400 and <50 copies/mL in children receiving highly active antiretroviral therapy (HAART) consisting of efavirenz, nelfinavir, and 1 or 2 nucleoside reverse-transcriptase inhibitors. Children receiving HAART achieved a plasma HIV-1 RNA level <400 copies/mL by a median of 4 weeks after initiation of therapy and a decline to <50 copies/mL by 20 weeks. Baseline plasma HIV-1 RNA levels affected the likelihood of achieving potent and sustained viral suppression, and children whose CD4 lymphocyte counts increased >70 cells/μL by 20 weeks on therapy were more likely to achieve durable virological and immunological benefit. These data provide time frames for virus suppression after the initiation of HAART that should be useful in evaluating the potential efficacy and durability of response of newly instituted combination antiretroviral therapy in HIV-1-infected children.

The availability of new antiretroviral drugs that effectively inhibit human immunodeficiency virus type 1 (HIV-1) in the United States has converted pediatric HIV-1 infection from an invariably fatal disease to a chronic illness. Development of reliable and reproducible markers of HIV-1-related disease progression has enabled the identification of treatment regimens that are best able to provide sustained benefit [1–5]. These markers can identify suboptimal regimens before the onset of clinical symptoms and allow for changes in antiretroviral therapy before the development of serious clinical diseases. Plasma HIV-1 RNA and CD4 lymphocyte counts and percentages are the most commonly used markers of HIV-1 status [6–8]. The current study was designed to define the rate of decline in plasma HIV-1 RNA levels in children with the introduction of new antiretroviral therapy and to assess the clinical relevance and usefulness of thresholds for standard and ultrasensitive assays.

Materials and Methods

Patient population. Pediatric AIDS Clinical Trials Group (PACTG) 382 was designed to determine the safety, pharmacokinetics, and efficacy of efavirenz plus nelfinavir in children. Study participants received efavirenz and nelfinavir plus 1 or 2 nucleoside reverse-transcriptase inhibitors. This study enrolled 57 HIV-1-infected children 3–16 years old from October 1997 to February 1998. Details of the study design, eligibility, data collection schedule, and patient demographics have been reported [9]. All participants were protease inhibitor and nonnucleoside reverse-transcriptase inhibitor naive. At study entry, the median age of subjects was 8.0 years. By standard assay, they had a median CD4 lymphocyte count of 699 cells/μL and a median log10 plasma HIV-1 RNA level of 4.0 (range, 2.6–5.7). The median length of follow-up was 49.1 weeks (25th–75th percentile, 48.1–52.6 weeks). The median length of study treatment was 48.7 weeks (25th–75th percentile, 47.7–52.4 weeks).

Quantitation of plasma HIV-1 RNA. Plasma, separated from EDTA-treated fresh whole blood within 6 h of collection, was stored at −70°C and tested for HIV-1 RNA by Amplicor assay (Roche Molecular Systems, Alameda, CA) [10] at baseline and at study weeks 2, 4, 8, 12, 20, 32, 40, and 48. Plasma samples with an HIV-1 RNA level <400 copies/mL (the stated limit of quanti-
of both assays and Ppt

There was a moderate correlation between the 2 assays (Kendall regression model. Virus suppression was defined as ≥2 consecutive intervals (CIs) were obtained using the Cox proportional hazards signficantly from zero (2 log10 difference among results obtained by the 2 assays differed

RNA assays.

Overall, 228 (41%) of 551 plasma samples run by standard assay with OD values below background, 225 (99%) were evaluated by the ultrasensitive assay. Of these 225 samples, 114 (51%) had OD values above background (defined on standard assay with OD values below background, 225 below the background of the assay used. Of the 228 samples that were retested using an ultrasensitive assay yielded optical density (OD) readings below the background of the assay. Of the 228 samples on standard assay with OD values below background, 225 (99%) were evaluated by the ultrasensitive assay. Of these 225 samples, 114 (51%) had OD values above background (defined as a plasma sample yielding a positive OD after subtraction of the OD value determined for a known negative control), of which 57 samples had ≥50 copies/mL on the ultrasensitive assay (table 1).

For plasma HIV-1 RNA determinations, the estimated mean log10 difference from zero resulted by the assays differed significantly from zero (−0.19; 95% CI, −0.30 to −0.09; P = .0003) when all observations were censored at 50 copies/mL. When HIV-1 RNA values were above the OD backgrounds of both assays and <400 copies/mL, the estimated corresponding difference was −0.17 (95% CI, −0.27 to −0.07; P = .001). There was a moderate correlation between the 2 assays (Kendall τ-b correlation, 0.55; P < .0001; n = 315 paired samples).

To assess the potential clinical utility of plasma HIV-1 RNA values determined by the standard assay as 50–400 copies/mL (range, 65–394 copies/mL), we compared the 66 plasma samples for which there were corresponding ultrasensitive assay values (table 1). Of the 66 plasma samples, 54 (82%) of the ultrasensitive assay values were ≥50 copies/mL, 10 (15%) were <50 copies/mL but had an OD above background, and 2 (3%) had ODs below background of the assay.

Rate of decline in log10 plasma HIV-1 RNA. Of the 57 patients, 53 had a decline in plasma HIV-1 RNA levels from baseline to week 2 of study, with an estimated mean of 1.43 log10 (95% CI, 1.26–1.60, censored at 50 copies/mL). Of these 53, 41 (77%) had a further mean decline in HIV-1 RNA levels from weeks 2 to 8 of 0.46 log10 (95% CI, 0.35–0.57). Of the 16 patients excluded from this analysis, 4 had an inestimable decline (i.e., their HIV-1 RNA levels remained <50 copies/mL), 5 had an increase in plasma HIV-1 RNA levels, and 7 had discontinued study medications by 2 weeks.

Time to first HIV-1 RNA level <400 or <50 copies/mL. Of the 56 patients receiving study medications who had baseline HIV-1 RNA levels >400 copies/mL, the proportions achieving <400 copies/mL at weeks 4, 12, 20, and 32 were 0.50, 0.72, 0.72, and 0.74, respectively; the proportions achieving <50 copies/mL were 0.09, 0.48, 0.56, and 0.65, respectively (figure 1A). Of the 40 patients receiving treatment who achieved virus suppression of <400 copies/mL, 37 (93%) achieved plasma HIV-1 RNA levels <50 copies/mL. The proportions of children who achieved <400 copies/mL with a confirmed plasma HIV-1 RNA level <50 copies/mL observed at weeks 2, 4, 6, 8, 12, 20, and 32 were 0.18, 0.43, 0.53, 0.65, 0.73, 0.80, 0.93, respectively (figure 1B). The median times to first plasma HIV-1 RNA level <400 and <50 copies/mL were 4 and 20 weeks, respectively. The median time to first plasma HIV-1 RNA level <50 copies/mL was 6 weeks from the time when virus suppression to <400 copies/mL was first observed for children receiving treatment (figure 1B).

Effect of rate of suppression on sustained suppression. Forty patients achieved plasma HIV-1 RNA levels <400 copies/mL while receiving treatment. The response rate of these patients was divided into a rapid-response group, whose RNA levels declined to <400 copies at 0–2 weeks (rapid responders), and a slow-response group, whose RNA levels were <400 copies/mL beyond week 2 (slow responders). Of these 40 patients, 12 (92%) of 13 rapid responders and 21 (78%) of 27 slow responders maintained virus suppression (<400 copies/mL) for ≥24 weeks (P = .39).

The 37 patients who achieved virus suppression (<50 copies/mL) while receiving treatment were also divided into rapid

**Table 1.** Comparison of human immunodeficiency virus type 1 plasma RNA assay values (in copies per milliliter), as determined by the Roche standard and ultrasensitive assays.

<table>
<thead>
<tr>
<th>Sample OD below background, copies/mL</th>
<th>Sample OD above background, copies/mL</th>
<th>Total samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50</td>
<td>57 (25)</td>
<td>68 (22)</td>
</tr>
<tr>
<td>≥50, &lt;400</td>
<td>55 (24)</td>
<td>109 (35)</td>
</tr>
<tr>
<td>≥400</td>
<td>2 (1)</td>
<td>25 (8)</td>
</tr>
<tr>
<td>Total samples</td>
<td>225</td>
<td>315</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%). OD, optical density.

**Results**

**Comparison of standard versus ultrasensitive plasma HIV-1 RNA assays.** Overall, 228 (41%) of 551 plasma samples run by standard assay and 113 (36%) of 315 plasma samples run by ultrasensitive assay yielded optical density (OD) readings below the background of the assay used. Of the 228 samples on standard assay with OD values below background, 225 (99%) were evaluated by the ultrasensitive assay. Of these 225 samples, 114 (51%) had OD values above background (defined as a plasma sample yielding a positive OD after subtraction of the OD value determined for a known negative control), of which 57 samples had ≥50 copies/mL on the ultrasensitive assay (table 1).

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The 37 patients who achieved virus suppression (<50 copies/mL) while receiving treatment were also divided into rapid
responders (those achieving RNA values <50 copies/mL by 8 weeks) and slow responders (those who achieved RNA values <50 copies/mL after 8 weeks). Of the 16 rapid responders, 12 (75%) maintained virus suppression (<50 copies/mL) for ≥24 weeks, versus 10 (48%) of 21 slow responders (P = .18).

Baseline plasma HIV-1 RNA levels were predictive of the nadir achieved and of sustained RNA suppression after the initiation of study antiretroviral drugs. Children with plasma HIV-1 RNA levels <10⁴ copies/mL were 2.3 times more likely to achieve <400 copies/mL (95% CI, 1.2–4.3; P = .01) or 2.2 times more likely to reach <50 copies/mL (95% CI, 1.1–4.2; P = .02), compared with those with a baseline plasma HIV-1 RNA level ≥10⁴ copies/mL. For each 10-fold increase in plasma HIV-1 RNA level, there was an increased risk for failure to suppress virus to <400 copies/mL (risk ratio [RR], 0.5; 95% CI, 0.3–0.8; P = .003) and to <50 copies/mL (RR, 0.5; 95% CI, 0.3–0.8; P = .008).

Additional factors that might affect the time to nadir and time to first viral rebound to a plasma HIV-1 RNA level >400 copies/mL were examined by Cox proportional hazards regression models. The models included response rate (rapid vs. slow), sex (boy vs. girl), ethnicity (black non-Hispanic vs. others), Centers for Disease Control and Prevention (CDC) clinical category (moderate or worse vs. mild or asymptomatic), switching nucleoside reverse transcriptase inhibitors within 2 weeks of entry, baseline age, and CD4 cell count and percentage. No variable was statistically significant. Baseline weight-for-age z score was predictive of virus suppression to <400 copies/mL (RR, 1.4; 95% CI, 1.1–1.9; P < .01) and <50 copies/mL (RR, 1.2; 95% CI, 1.001–1.2; P = .049).

Association of CD4 lymphocyte count and time to first viral rebound. Of the 39 patients with CD4 lymphocyte counts at week 20 and a confirmed plasma HIV-1 RNA level <400 copies/mL while receiving treatment, 7 (18%) experienced a virological rebound. Because the median CD4 lymphocyte count change from baseline at 20 weeks was 70 cells/μL, we examined the association of viral rebound with above or below the median change in CD4 cell count. There was a significant association between the rate of viral rebound and a CD4 cell count increase ≥70 cells/μL at week 20. Of the 24 study participants with a CD4 lymphocyte count increase of ≥70 cells/μL, only 1 (4%) rebounded to a plasma HIV-1 RNA load >400 copies/mL, compared with 6 (40%) of the 15 participants with a CD4 lymphocyte count increase of <70 cells/μL increase (P = .008, Fisher’s exact test). The RR for viral rebound at week 20 was 0.10 (95% CI, 0.01–0.80; P = .03) in favor of those with an increase.

Figure 1. Kaplan-Meier plots. A, Time to first plasma human immunodeficiency virus type 1 (HIV-1) RNA level <400 copies/mL (solid line) and <50 copies/mL (dashed line). B, Time to first plasma HIV-1 RNA level <50 copies/mL for patients who achieved virological suppression to <400 copies/mL. Data in parentheses are 95% confidence intervals for proportion of patients with identified plasma HIV-1 RNA level declines at 4, 12, 20, and 32 weeks of study (A) and at 2, 4, 6, 8, 12, 20, and 32 weeks (B) after initiation of highly active antiretroviral therapy in nucleoside-experienced, but nonnucleoside- and protease inhibitor-naive, infected children.
Discussion

Our data indicate that the standard Roche HIV-1 RNA assay is useful not only for the quantification of plasma HIV-1 RNA levels >400 copies/mL but also for the qualitative detection of plasma HIV-1 RNA levels between 50 to 400 copies/mL, when the OD from a plasma sample is above background of the assay. However, the standard assay does not provide reliable quantitation at <400 HIV-1 RNA copies/mL, and 25% of samples considered to have <50 copies/mL by the standard assay had ≥50 copies/mL by the ultrasensitive assay. Thus, a plasma sample found to have detectable HIV-1 RNA by the standard assay provides useful information about the persistent presence of virus, whereas an assay with undetectable virus may have HIV-1 RNA present when tested by the ultrasensitive assay.

These data also provide useful information regarding the rate of decline of plasma HIV-1 RNA during effective combination antiretroviral therapy in children with no previous nonnucleoside reverse-transcriptase inhibitor or protease-inhibitor experience. As noted in previous studies, there is a biphasic decline in plasma HIV-1 RNA [11–15]. Within the first 2 weeks, there was a rapid decline in RNA levels of 1.43 log10, compared with a mean decline of 0.46 log10 during the subsequent 6 weeks. The median times to achieving a plasma HIV-1 RNA level <400 and <50 copies/mL were 4 and 20 weeks, respectively, for all children who remained receiving treatment. Therefore, half of the children with detectable virus loads by ultrasensitive assay will continue to have a decline in plasma HIV-1 RNA levels for 5 months after the initiation of a new course of antiretroviral therapy. Thus, detectable plasma HIV-1 RNA by the ultrasensitive assay at 50–400 copies/mL may still be associated with effective antiretroviral therapy that will eventually achieve optimal suppression. Of note, the median time from <400 copies/mL to <50 copies/mL was 6 weeks. The time to reach either point did not significantly affect the durability of RNA suppression in this 48-week study.

Baseline plasma HIV-1 RNA levels were predictive of the viral nadir and the durability of the response. For each log10 increase in baseline RNA, there was a 50% lower likelihood of a child’s achieving plasma HIV-1 RNA levels <400 and <50 copies/mL, respectively. Baseline weight-for-age z scores was also predictive of the likelihood of an excellent virological response. Other baseline factors (e.g., sex, ethnicity, CDC clinical category, age, and CD4 lymphocyte count) did not correlate with the virologic response.

We observed a striking association between achieving a sustained virological response and an increase in the CD4 lymphocyte count by ≥70 cells/μL. Although 60% of children with a CD4 lymphocyte count increase of ≥70 cells/μL had a durable suppression in virus load, 96% of those with an increase of ≥70 cells/μL achieved prolonged plasma HIV-1 RNA suppression. Thus, children who achieve viremological suppression and experience the largest increase in CD4 lymphocyte count are most likely to receive prolonged benefit from their current antiretroviral therapy.

The majority (96%) of the children in this study had prior treatment with nucleoside analogue reverse-transcriptase inhibitors and persistently detectable virus load. The median time to decline in plasma HIV-1 RNA levels to <400 copies/mL after initiation of effective combination therapy that included a nonnucleoside reverse-transcriptase inhibitor (efavirenz) and a protease inhibitor (nelfinavir) was 4 weeks. However, a continued decline in plasma HIV-1 RNA load was observed, with 20 weeks as the median time to <50 RNA copies/mL. Baseline plasma HIV-1 RNA levels affected the likelihood of achieving potent and sustained virus suppression, suggesting that, when antiretroviral treatment fails to suppress plasma HIV-1 RNA to <50 copies/mL, a modification in therapy might be considered before there are large increases in plasma HIV-1 RNA levels. In addition, monitoring of CD4 lymphocyte count provided important prognostic information as children with the greatest increases in CD4 lymphocytes were also most likely to achieve durable virological and immunological benefit from their antiretroviral therapy.

Acknowledgments

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References


