Patterns of Plasma Human Immunodeficiency Virus Type 1 RNA Response to Antiretroviral Therapy

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Early identification of treatment failure among human immunodeficiency virus (HIV) type 1–infected patients receiving antiretroviral therapy could enable clinicians to modify inadequate regimens and to improve treatment response. Clinical definitions of treatment failure, however, may not be ideally suited for this purpose. This study empirically characterizes the patterns of HIV-1 RNA response to antiretroviral therapy in patients in 4 AIDS clinical trials. The approach assumed 2 patterns of HIV-1 response: “on track,” for eventual suppression to HIV-1 RNA levels below the limit of quantification, and “off track,” for deviation from this response. The results of this on- or off-track classification generally agreed with the protocol-defined outcomes of virologic success and failure, thus validating these commonly used definitions. Overall, only a minority of patients went off track because of suboptimal HIV-1 RNA response by the first follow-up visit. Most patients who went off track did so at later time points and had sharp unexpected rebounds without prior indication of a suboptimal response.

Despite the availability of potent antiretroviral drugs for the treatment of human immunodeficiency virus type 1 (HIV-1) infection, viral breakthrough, characterized by an increase or “rebound” in plasma viremia (HIV-1 RNA), occurs frequently and often is associated with resistance to ≥1 antiretroviral drug [1, 2]. For this reason, it is important to investigate the extent to which virus rebound can be predicted by the initial HIV-1 RNA response to treatment. If suboptimal initial responses could be identified with sufficient lead time before virus rebound, to permit the modification of inadequate regimens, then overall treatment responses could be improved.

A variety of definitions for virologic failure associated with viral breakthrough have been used in clinical trials to assess the efficacy of antiretroviral drugs for the treatment of HIV-1 infection. Although these definitions have been used for the development of guidelines for treating HIV-1–infected patients, their validity has not been established unequivocally. Thus, it is important to use objective methods to characterize the patterns of virologic response and to use these empirically identified patterns to verify the conventional definitions of virologic failure.

Here, we characterize patterns of HIV-1 RNA response to antiretroviral therapy in 1518 treatment-naive or treatment-experienced patients who participated in 4 clinical trials of antiretroviral therapy conducted by the AIDS Clinical Trials Group (ACTG). At each follow-up visit, we assumed that there were 2 possible states of HIV-1 RNA response: “on track,” for suppression of HIV-1 RNA levels to below the limits of detection, and “off track,” for deviation from this response. Conceptually, responses that are close to the pattern typical of patients whose HIV-1 RNA level will be suppressed are likely to be on track; as the responses stray further from the typical pattern, they are more likely to be off track. Instead of arbitrarily defining the on- and off-track status a priori, however, we used the observed HIV-1 RNA data to characterize the 2 states. To provide an analogy for the way we identified on- or off-track status, consider the example of the wild-type versus mutant genetic sequence of HIV-1. Instead of an arbitrary def-
inition of wild-type, large amounts of genotypic data were analyzed to define what constitutes the wild-type sequence and what constitutes a deviation from the wild-type sequence (i.e., mutant sequence). Here, we used statistical models to estimate the likelihood that a given patient’s response was on or off track at any visit. These results were compared with protocol-defined virologic failure, thus providing an independent confirmation of these definitions. By using this approach to characterize virologic outcome, we tested how well the early HIV-1 RNA response 2–4 weeks after the initiation of therapy can predict the later pattern of HIV-1 response.

Methods

Study population. Plasma HIV-1 RNA measurements (by standard or ultrasensitive reverse transcriptase–polymerase chain reaction [RT-PCR] method [3, 4]) were made for patients enrolled in 4 prospective clinical trials of antiretroviral therapy conducted by the ACTG. ACTG studies 343 [5], 368 [6], 359 [7], and 398 [8] (table 1) were selected because frequent plasma HIV-1 RNA measurements were available and because prior antiretroviral experience ranged from none (i.e., naive to treatment) to experienced with all 3 approved antiretroviral drug classes (nucleoside reverse-transcriptase inhibitors [NRTIs], non-NRTIs [NNRTIs], and protease inhibitors [PIs]).

Modeling of HIV-1 RNA responses as on or off track. Our goals were to identify patterns of HIV-1 RNA response to antiretroviral therapy and then to investigate whether the patterns could be predicted by changes in HIV-1 RNA level soon after initiation of therapy. Traditional methods for relating predictors (e.g., early changes in HIV-1 RNA level) to treatment failure include logistic regression (for dichotomous failure outcome) and Cox models (for time to failure) [9]. Although these are powerful methods, they were not adequate to address the goals of our analyses, because they rely on prespecified definitions of virologic failure, do not characterize HIV-1 RNA response patterns over time, and may not make full use of the data when some HIV-1 RNA values are missing.

Our approach assumed there are 2 possible HIV-1 RNA response states at any follow-up visit time: on track for HIV-1 RNA suppression or off track (patterns that deviate from the on-track state). By using flexible statistical models, as detailed in the Appendix, we used the patterns of HIV-1 RNA response within the raw data to define the 2 states. We first modeled the probability of going off track as a function of time since starting therapy and other predictors [10] and then modeled the distribution of HIV-1 RNA levels, given that a patient was either on or off track [11]. This approach allowed us to estimate the means and variances of the HIV-1 RNA responses that we would expect to observe for patients who were either on or off track at any visit time. From these estimates, we calculated the probability that a patient was on or off track at each visit time. Because such a probability can be anywhere between 0 and 1, the response of HIV-1 RNA can be characterized more flexibly than the categorical classification of treatment success or failure. Details regarding the model specifications, hypothesis testing, and estimation procedures [12] are provided in the Appendix.

The statistical model used has the following 5 features. First, a patient’s HIV-1 RNA response profile can change from being on track to off track at any time; second, the means and variances of the HIV-1 RNA responses change when patients’ HIV-1 RNA responses go off track; third, previous HIV-1 RNA levels are used to predict current values but in different ways for patients who are on or off track; fourth, all the observations can be included in the analysis, whether complete or not; and fifth, important covariates (e.g., baseline HIV-1 RNA level) can be included to predict the on- or off-track status at later time points.

Data presentation. We plotted the median, 25th, and 75th percentiles of HIV-1 RNA response profiles for all patients who went off track at each visit time. Because the determination of whether a patient was on or off track was expressed in terms of probability, the calculation of the median, 25th, and 75th percentiles was based on the contribution of each patient by the estimated probability. For example, if a patient had an estimated probability of 0.85 for going off track at week 8, a weight of 0.85 was assigned to the contribution of that patient to the calculation of the median response of patients who went off track at week 8.

Missing HIV-1 RNA level data and censoring below quantification limits. One advantage of our approach is that it can accommodate patients with missing visits. The model accommodates missing HIV-1 RNA levels easily because the patient’s status as being on or off track is expressed in terms of probability, and the estimation of that probability is based on all the available HIV-1 RNA values. The validity of our approach for accommodating missing

Table 1. Summary of AIDS Clinical Trials Group (ACTG) protocols 343, 368, 359, and 398.

<table>
<thead>
<tr>
<th>ACTG study</th>
<th>No. of patients</th>
<th>Prior treatment experience</th>
<th>Median baseline values</th>
<th>Protocol-defined antiretroviral treatment</th>
<th>Off track by week 2 or 4</th>
<th>Off track between weeks 2 or 4 and 24</th>
<th>Protocol-defined virologic failure at week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>343</td>
<td>455</td>
<td>Naive or Zdv</td>
<td>CD4 cell count, cells/μL</td>
<td>HIV-1 RNA level, copies/mL</td>
<td>Zdv + 3TC + Ifd</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>368</td>
<td>305</td>
<td>NRTI</td>
<td>437</td>
<td>11,310</td>
<td>Idv</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>359</td>
<td>277</td>
<td>NRTI + Idv</td>
<td>116</td>
<td>6014</td>
<td>Idv + Efiv ± ABC</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>398</td>
<td>481</td>
<td>NRTI, 1-3 PIs, NNRTI (44%)</td>
<td>202</td>
<td>9546</td>
<td>Sqv + Rtv or Nfv with Dlv, Adv, or Dlv + Adv</td>
<td>40</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>202</td>
<td>8620</td>
<td>Adv + ABC ± Efiv + Apv ± Idv, Nfv, or Sqv</td>
<td>25</td>
<td>46</td>
</tr>
</tbody>
</table>

NOTE. 3TC, lamivudine; ABC, abacavir; Adv, adenosine; Apv, amprenavir; Dlv, delavirdine; Efiv, efavirenz; Idv, indinavir; Nfv, nelfinavir; NRTI, nucleoside reverse-transcriptase inhibitors; NNRTI, non-NRTI; PI, protease inhibitor; Rtv, ritonavir; Sqv, saquinavir; Zdv, zidovudine; ±, with or without. 

* Data are percentage of patients.
values relies only on the assumption that whether or not a value is missing is independent of the unobserved value, given the observed data.

Because the HIV-1 RNA assays used have detection limits, many of the HIV-1 RNA levels are censored. To avoid computational complexity, we treated the level at which the HIV-1 RNA was censored as observed and then conducted sensitivity analyses to investigate the effect of the assumption. Two different HIV-1 RNA assays were used in the studies we analyzed: the ultrasensitive RT-PCR assay in ACTG 343 and ACTG 398 and the standard RT-PCR assay in ACTG 359 and ACTG 368. The limit of quantification for the ultrasensitive assay is 50 HIV-1 RNA copies/mL [4] and 400 HIV-1 RNA copies/mL for the standard assay [3]. The actual limits of the assay to detect HIV-1 RNA (i.e., the levels at which the assay are censored) can be below the limits of quantification, although the accuracy of the assay is reduced for values below these limits. To assess the effect of these limits of quantification on our results, we did several sensitivity analyses (described in the Appendix).

**Model validity.** We investigated the validity of the model in 2 different ways. First, we compared the observed median trajectory for patients who went off track at each time point with the median trajectory predicted by the model by using the baseline HIV-1 RNA levels for the population. Second, we evaluated whether an underlying assumption of the model, that the log_{10} of HIV-1 RNA levels are distributed normally, was reasonable, based on an evaluation of the residuals—the differences between observed and predicted values.

**Comparisons with protocol-defined virologic end points.** To assess the relationship between on- or off-track status and conventional protocol definitions of virologic failure, we compared the on- or off-track status, as determined by our model with the study-defined virologic failure end points in each of the 4 studies. For this analysis, patients whose probability of going off track was >50% at any time (using only the information up to that time) were classified as being globally off track; all other patients were classified as being globally on track. We chose 50% as a cutoff point because it assigns patients to the on- or off-track status to which they are more likely to belong. Similar results would be obtained by choosing any cutoff between 10% and 90% because, at all visit times, few patients have estimated probabilities of going off track of 10%–90%. This also indicates that the on- or off-track status accurately characterizes the patterns of HIV-1 RNA response of individual patients.

In ACTG 368, virologic failure was defined as the occurrence of any of the following events: (1) a confirmed increase of plasma HIV-1 RNA level of 1.0 log_{10} copies/mL above nadir; (2) any confirmed increase above baseline; (3) a single measurement after week 14 ≥20,000 HIV-1 RNA copies/mL among patients whose levels had never dropped to <500 HIV-1 RNA copies/mL; (4) a confirmed value ≥500 but <20,000 HIV-1 RNA copies/mL after week 14; or (5) a missing measurement at week 16, unless week 8 and week 24 measurements were <500 HIV-1 RNA copies/mL. We also used this definition for ACTG 359, which, like ACTG 368, used the standard RT-PCR HIV-1 RNA assay [3]. The definition of virologic failure used in ACTG 398 was the occurrence of any of the following events: a confirmed increase of plasma HIV-1 RNA level 1.0 log_{10} above nadir, any confirmed increase above baseline, detectable HIV-1 RNA (≥200 copies/mL) with <0.5 log_{10} copies/mL decrease in HIV-1 RNA level from baseline by week 8, or HIV-1 RNA level ≥200 copies/mL at week 24. This definition also was used for ACTG 343, which, like ACTG 359, used the ultrasensitive RT-PCR HIV-1 RNA assay [4]. The protocol-defined levels of HIV-1 RNA for failure criteria at week 24 were conservative and were above the limit of quantification for the approved ultrasensitive (50 HIV-1 RNA copies/mL) and standard (400 HIV-1 RNA copies/mL) assays [3, 4].

**Results**

The patients studied were from ACTG protocols 343, 368, 359, and 398. In ACTG 343, patients (n = 455) were either naive to all antiretroviral drugs (56%) or were experienced with zidovudine alone (44%). In ACTG 368, patients (n = 305) had prior exposure to NRTIs, but only 8% had exposure to NNRTIs and none to PIs. In ACTG 359, patients (n = 277) had exposure to NRTIs and the PI indinavir but were NNRTI naive. In ACTG 398, patients (n = 481) had exposure to NRTIs and ≥1 PI, and 44% had exposure to NNRTIs. In each of these studies, HIV-1 RNA level was measured at weeks 0, 4, 8, 16, and 24. In addition, HIV-1 RNA level was measured at weeks 12 and 20 in ACTG 343, at week 2 in ACTG 368 and ACTG 398, and at week 12 in ACTG 359. Table 1 summarizes each trial and the virologic outcomes of the study patients. The percentage of patients who missed any scheduled visit after baseline in these studies was 2%–20% (8% for all 4 study populations combined). For those who missed ≥1 scheduled visit, the percentage of all scheduled visits that were missed was 27%. By week 16, the proportion of patients with HIV-1 RNA levels below the limits of quantification were 72%, 74%, 27%, and 26% for ACTGs 343, 368, 359, and 398, respectively.

Figures 1–4 show the HIV-1 RNA response profiles among the 1518 patients studied. By using the methods described above, patient HIV-1 RNA response profiles were classified at any visit time as being on track for suppression of HIV-1 RNA to below the limit of quantification or as deviating from this trajectory (i.e., going off track). Each figure provides the median and 25%–75% range of plasma HIV-1 RNA levels for patients whose HIV-1 RNA response profile went off track after the initiation of therapy and for those whose profiles remained on track through week 24. The first graph in each figure (panel A) corresponds to patients whose HIV-1 RNA profiles went off track at the first visit after the initiation of therapy (i.e., weeks 2 or 4); the subsequent graphs correspond to patients whose profiles went off track at later time points. The last graph in each figure shows the HIV-1 RNA response profiles of patients who remained on track through week 24. The percentages of patients whose response profile went off track at each visit time are also provided.

Figure 5 displays the cumulative percentages of patients who had gone off track by each visit in all 4 studies. These results show that, of the HIV-1 RNA profiles that went off track in
ACTG 343 and ACTG 368, only a minority did so within the first 2–4 weeks after the start of therapy. By contrast, just over half the profiles that went off track in ACTG 359 and ACTG 398 did so by week 4. To illustrate, in ACTG 343, 3% of the profiles went off track within the first 4 weeks of initiation of therapy and 22.7% did so between weeks 4 and 24. In all studies, later off-track profiles were characterized by sharp rebounds in HIV-1 RNA level that were not predictable from the prior response profile. After week 4, the profiles of those who went off track did not differ from those who stayed on track until HIV-1 RNA level rebound occurred.

The proportion of HIV-1 RNA response profiles that went off track at any time point was higher in more heavily antiretroviral-experienced patients. Specifically, in ACTG studies 368, 359, and 398, the percentages of patients who went off track within the first follow-up visit were 6.7%, 40.3%, and 25.2%, respectively, and within the first 4 weeks, 7.9%, 40.3%, and 39.0%. This indicates that early off-track profiles are more common in drug-experienced patients, particularly those with prior PI exposure (ACTG 359 and ACTG 398). Patients who went off track early (weeks 2 or 4) were unlikely to have suppressed HIV-1 RNA at week 24; only 8%, 7%, and 5% of such patients in ACTG studies 368, 359 and 398, respectively, had HIV-1 RNA levels below the limit of quantification at week 24.

The HIV-1 RNA profiles that went off track between weeks 4 and 24 in more heavily antiretroviral-experienced patients again were characterized by sharp rebounds that could not be anticipated by the preceding HIV-1 RNA response. Thus, 2 patterns of going off track were apparent, regardless of treatment experience: a suboptimal initial decline in HIV-1 RNA level within the first 4 weeks of initiation of therapy and sharp unexpected rebounds in HIV-1 RNA level after an initial on-track response. Rather than showing a gradual loss of HIV-1 RNA suppression, patients tended either to remain on track or to rebound sharply without prior indication of a different HIV-1 RNA response.

In each study, there was a trend for patients whose profiles went off track at earlier time points to have higher HIV-1 RNA levels at the point of maximum virus suppression (i.e., the nadir level). This was particularly apparent in the first 8 weeks, when the nadir levels of HIV-1 RNA for most patients were above the limit of quantification and could be measured more reliably. In general, these higher nadir levels appeared to result from higher baseline HIV-1 RNA levels and an earlier time of rebound rather than to failure to achieve an initial HIV-1 RNA decline that was on track. In each study, the median nadir levels at week 4 for patients who went off track at week 8 were about twice the median levels at week 4 of patients whose profiles remained on track at week 8.

The response profiles for ACTGs 368 and 398 (figures 2 and 4) include results for week 2, whereas in ACTGs 343 and 359, the first follow-up visit was at week 4 (figures 1 and 3). These results show that going off track by week 4 reflects inadequate HIV-1 RNA declines by that time, whereas at all other times,
going off track was associated with rebound. Although figures 1 and 3 show that the patients whose profiles went off track at week 4 had, on average, a very small change from baseline HIV-1 RNA level, this observation may actually reflect an unobserved drop at week 2 and rebound at week 4, the pattern observed in ACTG 368 and ACTG 398 (figures 2 and 4, respectively).

**Model performance.** Comparison of observed and predicted HIV-1 RNA responses for patients whose profiles went off track at different times showed that the model fit well to the data; plots of residuals confirmed that the assumption of normally distributed log_{10} HIV-1 RNA levels was reasonable (data not shown). In addition, sensitivity analyses (see Appendix) showed that our results were not affected by different ways of handling measurements below the limit of HIV-1 RNA quantification. To demonstrate this, we repeated all the foregoing analyses by replacing HIV-1 RNA measurements below the limit of quantification with values obtained as described in the Appendix; this procedure did not qualitatively change any of the results.

**Comparison of off-track status with protocol-defined virologic failure.** Table 2 compares the global on- or off-track status of patients with the ACTG study definitions of virologic failure. As described in Methods, a patient was defined as being globally off track if the probability of going off track for that patient was >50% at any time when we used information up to that point. These results show a high degree of concordance between being globally off track and meeting the study definition of virologic failure. Only 34 (4.3%) of 783 patients classified as being globally off track did not meet the protocol-defined criteria for virologic failure. Of these 34, 16 (47%) had virus rebounds \(>0.5 \log_{10} \text{HIV-1 RNA copies/mL} \) and 12 (35%) had shallow initial HIV-1 RNA level declines \(<0.8 \log_{10} \text{HIV-1 RNA copies/mL} \). Of the remaining 6 patients, 4 had rebounds \(>0.3 \log_{10} \text{HIV-1 RNA copies/mL} \). The other 2 had high baseline plasma HIV-1 RNA levels and declines \(<1.5 \log_{10} \text{HIV-1 RNA copies/mL} \) by the third visit. Similarly, only 59 (8.0%) of 735 patients classified as being globally on track met the protocol definition of having virologic failure. Of these 59, 39 (66%) were classified as having virologic failure because of a missing plasma HIV-1 RNA measurement at week 24. The remaining 20 patients did not experience a sharp rebound in HIV-1 RNA level but had gradual increases in HIV-1 RNA level above the study-defined failure threshold. In ACTG protocols 343, 368, and 398, none of the on-track patients classified as having virologic failure had HIV-1 RNA levels \(>550 \text{ copies/mL} \) at week 24 and, in ACTG 359, none had HIV-1 RNA levels \(>1200 \text{ copies/mL} \).

**Predictors of going off track.** One important goal of this study was to determine whether baseline HIV-1 RNA level and the initial slope of HIV-1 RNA decline from baseline to the first follow-up visit (at week 2 or 4) predict the time to going off track among those who remain on track at least until the first follow-up visit. To accomplish this, we tested whether the baseline HIV-1 RNA level and the initial slope predicted the risk of going off track after the first postbaseline visit. By like-
Figure 3. Human immunodeficiency virus type 1 RNA response profiles for patients in AIDS Clinical Trials Group protocol 359 (n = 277) whose profiles went off track before the week 24 visit (A–E) and whose profiles stayed on track until week 24 (F). The percentage of patients having the illustrated profile is given in each panel. Solid black line, median response curve; dotted lines, 25th and 75th percentiles.

Table 2. Comparisons between global on- and off-track status and protocol-defined virologic failure for AIDS Clinical Trials Group (ACTG) studies 343, 368, 359 and 398.

<table>
<thead>
<tr>
<th>ACTG study</th>
<th>Off-track status</th>
<th>On-track status</th>
</tr>
</thead>
<tbody>
<tr>
<td>343 Virologic failure</td>
<td>96</td>
<td>25</td>
</tr>
<tr>
<td>343 No virologic failure</td>
<td>18</td>
<td>316</td>
</tr>
<tr>
<td>368 Virologic failure</td>
<td>100</td>
<td>21</td>
</tr>
<tr>
<td>368 No virologic failure</td>
<td>1</td>
<td>183</td>
</tr>
<tr>
<td>359 Virologic failure</td>
<td>214</td>
<td>8</td>
</tr>
<tr>
<td>359 No virologic failure</td>
<td>3</td>
<td>52</td>
</tr>
<tr>
<td>398 Virologic failure</td>
<td>339</td>
<td>5</td>
</tr>
<tr>
<td>398 No virologic failure</td>
<td>12</td>
<td>125</td>
</tr>
<tr>
<td>Total Virologic failure</td>
<td>749</td>
<td>59</td>
</tr>
<tr>
<td>Total No virologic failure</td>
<td>34</td>
<td>676</td>
</tr>
</tbody>
</table>
RNA level from baseline to the first follow-up visit does not predict the risk of going off track after this visit. Only patients who go off track at the first follow-up visit have distinctly different initial HIV-1 RNA level declines from those of other patients. This result is consistent with the observation that higher nadir values are associated with shorter times to virologic rebound. Our analyses indicate that higher baseline HIV-1 RNA levels and shorter time to rebound are more important than the initial rate of HIV-1 RNA decline in determining the relation between nadir and rebound.

We also compared the HIV-1 RNA response profiles between antiretroviral-naive and zidovudine-experienced patients in ACTG 343 (data not shown). The HIV-1 RNA profiles and the proportion of patients on or off track at each time point were similar for naive versus zidovudine-experienced patients. There was a slight trend toward a higher probability of going off track in zidovudine-experienced patients, but the difference between experienced and naive patients was small (27.8% vs. 23.9% off track by week 24). Analyses of NNRTI-naive versus NNRTI-experienced patients in ACTG 398 showed that, although both groups had similar patterns of HIV-1 RNA response and rebound, the NNRTI-experienced patients had a substantially higher likelihood of going off track at week 2 (40.5% vs. 13.2%).

Figure 6 compares the HIV-1 RNA response profiles between patients who continued to receive assigned study treatment and those who did not in ACTG 343. Patients who received assigned therapy were defined as those who never reported any missing or reduced dose for any drugs in the study regimen within the first 24 weeks (75% of ACTG 343 patients). These analyses clearly show that the response profiles (i.e., the shapes of HIV-1 RNA response) are similar for persons who continue to receive assigned therapy and for those who do not, but the probability of going off track is consistently higher for patients

Figure 4. Human immunodeficiency virus type 1 RNA response profiles for patients in AIDS Clinical Trials Group protocol 398 (n = 481) whose profiles went off track before the week 24 visit (A–E) and whose profiles stayed on track until week 24 (F). The percentage of patients having the illustrated profile is given in each panel. Solid black line, median response curve; dotted lines, 25th and 75th percentiles.

Figure 5. Cumulative percentage of patients whose profiles were off track by visit time in AIDS Clinical Trials Group (ACTG) protocols 343, 368, 359, and 398.
who did not continue to receive assigned therapy. Analyses of ACTG 368 and 398 data produced similar results (data not shown). We warn, however, that persons who did not continue to receive the assigned treatment might have done so because they experienced suboptimal responses.

**Discussion**

These retrospective analyses of ACTG studies demonstrate that the HIV-1 RNA response profiles are similar among patients in whom HIV-1 RNA is suppressed to below the limit of quantification and that departure from this successful on-track profile is most commonly characterized by abrupt rebound in HIV-1 RNA. An unexpected finding was that, until the time of rebound, the HIV-1 RNA response profiles do not differ between persons who experience rebound and those who do not. We expected to find evidence of either a plateau in the HIV-1 RNA response or a gradual loss of viral suppression that would precede the abrupt rebound and serve as an early warning signal. This could have allowed for the identification of impending treatment failure through monitoring of HIV-1 RNA level and treatment modification (e.g., intensification with an additional antiretroviral agent) to increase virus suppression and prevent rebound. In contrast, our analyses of HIV-1 RNA responses in >1500 patients indicate that impending treatment failure is not likely to be identified solely by monitoring the HIV-1 RNA response. This complicates the identification of appropriate patients for treatment modification or intensification to improve the success rate of antiretroviral therapy.

Another important finding of our analyses is that a minority of patients have a suboptimal initial HIV-1 RNA response to therapy after 2–4 weeks; this response strongly depends on prior treatment experience. The percentages of patients who went off track during the first 4 weeks of treatment varied from very low (<5%) among treatment-naive patients (ACTG 343) to ~40% among highly experienced patients (ACTG 359). One potential explanation for this important observation is that, in naive patients, preexisting drug resistance is rare, whereas in experienced patients, drug-resistant and cross-resistant viral variants are present at the time of initiation of the new treatment regimen, resulting in a poor initial HIV-1 RNA response. Studies of baseline viral susceptibility and changes in susceptibility in groups with early versus late off-track profiles are planned and should address this hypothesis.

Data from the studies (ACTG 368 and 398) in which there were initial follow-up visits at week 2 indicate that failure resulting from inadequate initial drug activity occurs within 2 weeks and is manifested by an insufficient change in virus load from baseline. The failures at week 4 in these studies were characterized not by a shallow decline in HIV-1 RNA level from baseline to week 4 but by a decline from baseline to week 2 and a sharp rebound between weeks 2 and 4. Consequently,
we cannot exclude rebound within the first 4 weeks of therapy for ACTG 343 and ACTG 359 in which HIV-1 RNA level was first measured 4 weeks after the initiation of therapy. Studies with more frequent monitoring of HIV-1 RNA level will be required to differentiate these 2 possibilities.

Several investigators have reported that patients who experience treatment failure have higher plasma HIV-1 RNA levels at the point of maximal suppression (nadir) than those who have continued virus suppression [13–15]. This observation also was made in our analyses (figures 1–4). Three factors contribute to the nadir level of HIV-1 RNA: baseline HIV-1 RNA level, rate of decline of HIV-1 RNA level over time, and time of rebound. Our analyses suggest that, of these 3 factors, baseline HIV-1 RNA level and time of rebound are most important. In addition to identifying the factors that contribute to the relationship between nadir and time to rebound, our analyses indicated that the resulting figures can be used as benchmarks to evaluate whether a patient’s HIV-1 RNA level response has deviated from a successful pattern. This is preferable to basing evaluations on nadir values, since the nadir can be determined only retrospectively.

One important feature of our analysis was the avoidance of arbitrary definitions of outcomes and the use of the data to establish typical HIV-1 RNA response profiles and departures from them. Although logistically simple, external definitions of virologic failure are complicated by individual variability, measurement error, and missing data. Comparisons across studies can therefore be hard to interpret. In particular, missing HIV-1 RNA level measurements may make the determination of time of failure impossible, and variability of HIV-1 RNA level may make determination based on single measurements unreliable. Thus, it is of note that the definitions of virologic failure from ACTG studies agree well with the on- or off-track status from our analyses (table 2) and provide a retrospective confirmation that the virologic end points defined in clinical trials often perform well in identifying virologic failure, even without statistical modeling. Nonetheless, important differences between going off track and virologic failure exist. For example, in ACTG 368, patients whose HIV-1 RNA levels increased slightly above the low baseline levels were classified as having virologic failure. Inclusion of these patients as having virologic failure dilutes the predictive effect of baseline HIV-1 RNA level on time to failure because such an analysis artificially causes the low baseline HIV-1 RNA levels to be associated with virologic failure. This discrepancy suggests ways in which protocol definitions of virologic failure might be modified.

Although our analyses revealed important findings about HIV-1 RNA responses to therapy, several limitations of our study should be recognized. First, HIV-1 RNA level measurements were obtained at different time points in the studies we examined, and only ACTG 343 had measurements at week 20. This limited our ability to draw conclusions about patients who experience virologic failure between weeks 16 and 24. Similarly, the follow-up period examined was only 24 weeks, so we could not assess the relationship between HIV-1 RNA response profiles and treatment failure later than week 24. Third, prospective measures of medication adherence were not done in a standardized manner in the studies analyzed. We therefore used the most objective clinical measure available to us—reported discontinuation of study treatment—as a crude measure of adherence. By dividing patients into 2 groups, on the basis of whether or not they discontinued study treatment, we found that virus rebound was more common in those who reported treatment interruption in each study, but that the timing and patterns of rebound were similar in both groups. Improved measures of adherence may allow for the identification of stronger associations between medication nonadherence and the timing of rebound. Finally, HIV-1 drug resistance analyses and antiretroviral drug levels are needed to maximize understanding of the mechanisms involved in virologic failure. The lack of this information limits our ability to explain the differences in HIV-1 RNA response profiles, but it does not detract from the overall findings about the frequency and characteristics of different response profiles and the utility of HIV-1 RNA level monitoring in assessing the efficacy of antiretroviral therapy.

Our most important finding was that, for patients who experience virologic failure at times other than the first follow-up visit, there is no indicator from their previous HIV-1 RNA profile that predicts this failure. Thus, it will be difficult clinically to identify patients who are destined to experience treatment failure solely on the basis of their HIV-1 RNA response profiles until the time of rebound. HIV-1 RNA sampling more frequent than every 4 weeks might provide a better indication of impending failure, although such frequent sampling is not likely to be practical. It is clear, however, that such failure is much more likely among persons who are not fully adherent to the treatment regimen or who are heavily treatment experienced. Thus, factors other than the initial HIV-1 RNA response profile, such as baseline HIV-1 RNA level, baseline viral susceptibility, ongoing medication adherence, and antiretroviral drug levels, may be better predictors of impending failure [16–20].

Nevertheless, frequent monitoring of the HIV-1 RNA response to therapy still is recommended for several reasons. First, suboptimal initial responses can be identified within 2–4 weeks of initiation of therapy and, although this profile is observed in a minority of all patients who experience virologic failure, its recognition will prevent the continuation of ineffective treatment. Second, frequent monitoring of HIV-1 RNA will reveal virus rebound earlier than will infrequent monitoring. Although the optimal time to switch therapy after virus rebound has not been determined, earlier identification of treatment failure may prevent the development of high-level HIV-1 resistance to drugs in the regimen, as well as cross-resistance to other antiretrovirals [21–23].
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Appendix

Statistical Analysis

Modeling of HIV-1 RNA response. We used $T_i$ to denote the time to going off track for patient $i$. Our approach treated $T_i$ as an unobserved variable and used the observed HIV-1 RNA data to estimate the distribution of $T_i$. For this purpose, we first characterized how $T_i$ depends on time since initiation of therapy and other predictors and then characterized the distribution of observed HIV-1 RNA levels given $T_i$.

The first model [10] is specified as $\log P(T_i = t | T_i > 0; X_{it}) = aX_{it}$, where $a$ is the vector of parameters that relates the probability to the predictor $X_{it}$ (the information up to time $t$). In the simplest analysis, $X_{it}$ only indicated visit times; for 2 other analyses, $X_{it}$ also included the baseline HIV-1 RNA level and the slope from baseline to the first postbaseline visit.

The second model [11] describes how the current HIV-1 RNA level depends on the prior HIV-1 RNA levels for patients at each of 3 possible stages: on track, in transition, and off track. This model suggests that the way in which the current value of HIV-1 RNA depends on the previous value differs according to whether patients are on or off track. The model specification is as follows: $Y_{it} = b_0 + b_1Y_{i,t-1} + \epsilon_{it}$, where $Y_{i,t}$ is the value of HIV-1 RNA for patient $i$ at time $t$ and where $b_{it}, b_1$, and the variance of $\epsilon_{it}$ are allowed to depend on whether the patient is on track ($t < T_i$), in transition ($t = T_i$), or off track ($t > T_i$). We also allowed the parameters to depend on visit time for patients who were on track or in transition and on the time since going off track for patients who are off track.

Estimation and testing. Because $T_i$ was not observed, we could not estimate the parameters in these models separately. Instead, we used an expectation-maximization algorithm to estimate the parameters simultaneously [12]. This also allowed us to calculate the probability that a patient was on or off track at any visit time. Tests of predictors of going off track that included baseline HIV-1 RNA level and initial slope were done by including the predictor in the equation for model 1 and then calculating the log of the ratio of the likelihoods for the models with and without the predictor.

When we applied our methods to data from all 4 studies, >91% of patients at any time had an estimated probability of being on track of either <10% or >90%. This allowed for unambiguous classification regarding on- or off-track status for most patients. Most patients could be well categorized as being on or off track, and few patients were in the middle "gray zone."

Sensitivity analyses. To assess the sensitivity of results to values of HIV-1 RNA below the limits of quantification, we conducted analyses in which we replaced all values below these limits (i.e., 50 HIV-1 RNA copies/mL in the ultrasensitive assay and 400 HIV-1 RNA copies/mL for the standard assay) with values that were sampled from different probability distributions. These distribution were chosen to be both peaked (normal) and flat (uniform) and to have variances that were similar to estimates for the HIV-1 RNA levels for the appropriate study, as well as variances that were 50% greater and 33% less. The primary analyses in this report used all HIV-1 RNA measurements, regardless of whether they were below the limit of quantification.

References


