

Prevalence and Predictive Value of Intermittent Viremia With Combination HIV Therapy

Diane V. Havlir, MD

Roland Bassett, MS

Diane Levitan, MD

Peter Gilbert, PhD

Pablo Tebas, MD

Ann C. Collier, MD

Martin S. Hirsch, MD

Caroline Ignacio, BA

Jon Condra, PhD

Huldrych F. Günthard, MD

Douglas D. Richman, MD

Joseph K. Wong, MD

IN PATIENTS INFECTED WITH HUMAN immunodeficiency virus (HIV), antiretroviral therapy (ART) is directed at achieving and maintaining HIV RNA levels below the limit of detection of currently approved assays.^{1,2} A lower nadir of HIV RNA in response to therapy is an independent predictor of long-term virologic suppression.³ In 1 study,⁴ participants who achieved a viral load of less than 20 copies/mL had a 10-fold reduction in risk of virologic failure compared with those who did not. Once virologic suppression is achieved, sustaining suppression is critical for preventing the emergence of drug-resistant virus and subsequent virologic failure.

Maintaining virologic suppression depends on a number of variables including regimen potency, adherence, and tolerability. When patients interrupt therapy or when the potency of a regimen is reduced, abrupt elevations in HIV RNA levels are observed.⁵⁻⁷ How-

Context In HIV-infected patients having virologic suppression (plasma HIV RNA <50 copies/mL) with antiretroviral therapy, intermittent episodes of low-level viremia have been correlated with slower decay rates of latently infected cells and increased levels of viral evolution, but the clinical significance of these episodes is unknown.

Objective To determine if HIV-infected patients with intermittent viremia have a higher risk of virologic failure (confirmed HIV RNA >200 copies/mL).

Design and Setting Retrospective analysis of subjects in well-characterized cohorts, the AIDS Clinical Trials Group (ACTG) 343 trial of induction-maintenance therapy (August 1997 to November 1998) and the Merck 035 trial (ongoing since March 1995).

Patients Two hundred forty-one ACTG 343 patients, of whom 101 received triple-drug therapy throughout the study, and a small group of 13 patients from Merck 035 having virologic suppression after 6 months of indinavir-zidovudine-lamivudine.

Main Outcome Measures Association of intermittent viremia (plasma HIV RNA >50 copies/mL with a subsequent measure <50 copies/mL) with virologic failure (2 consecutive plasma HIV RNA measures >200 copies/mL) in both study groups; evidence of drug resistance in 7 patients from the small (n=13) study group with long-term follow-up.

Results Intermittent viremia occurred in 96 (40%) of the 241 ACTG 343 patients of whom 32 (13%) had 2 consecutive HIV RNA values >50 copies/mL during the median 84 weeks of observation (median duration of observation after first intermittent viremia episode was 46 weeks). Of the 101 individuals receiving triple-drug therapy throughout, 29% had intermittent viremia; the proportion of episodes occurring during the maintenance period was 64% for the entire cohort and 68% for the group not receiving triple-drug therapy throughout vs 55% for those who did ($P=.25$). Intermittent viremia did not predict virologic failure: 10 (10.4%) of 96 patients with and 20 (13.8%) of 145 patients without intermittent viremia had virologic failure (relative risk, 0.76; 95% confidence interval [CI], 0.29-1.72). In a Cox proportional hazards model, the risk for virologic failure was not significantly greater in the ACTG 343 patients with intermittent viremia (hazard ratio, 1.28; 95% CI, 0.59-2.79). Median viral load in 10 ACTG 343 patients assessed between 24 and 60 weeks of therapy using an ultrasensitive 2.5-copies/mL detection level assay was 23 copies/mL in those with intermittent viremia vs <2.5 copies/mL in those without ($P=.15$). Intermittent viremia occurred in 6 of 13 patients from the small study group assessed after 76 to 260 weeks of therapy (using the 2.5-copies/mL detection level assay) and was associated with a higher steady state of viral replication ($P=.03$), but not virologic failure over 4.5 years of observation. Viral DNA sequences from 7 patients did not show evolution of drug resistance.

Conclusions Intermittent viremia occurred frequently and was associated with higher levels of replication (Merck 035), but was not associated with virologic failure in patients receiving initial combination therapy of indinavir-zidovudine-lamivudine (ACTG 343 and Merck 035). In this population, treatment changes may not be necessary to maintain long-term virologic suppression with low-level or intermittent viremia.

JAMA. 2001;286:171-179

www.jama.com

See also pp 196 and 224.

ever, transient smaller elevations in HIV RNA levels are also frequently observed in patients who maintain HIV RNA levels of less than 200 copies/mL

Author Affiliations and Financial Disclosures are listed at the end of this article.

Corresponding Author and Reprints: Diane V. Havlir, MD, University of California, San Diego, 150 W Washington, Suite 100, San Diego, CA 92103 (e-mail: dhavlir@ucsd.edu).

with potent ART.^{8,9} These transient episodes have been described as intermittent viremia or *blips*. In some patients, increases of HIV RNA levels above a threshold of detection may represent assay variation. In other patients, blips may result from laboratory errors in specimen processing, identification, or reporting. Alternatively, the pattern of intermittent viremia may represent less effective viral suppression. Evidence that patients with intermittent viremia have higher levels of viral replication can be found in several small studies that reported increased evolution of the HIV envelope and polymerase genes, slower decay of the latently infected pool, and emergence of subpopulations of drug-resistant virus.⁸⁻¹⁰

While the HIV RNA nadir resulting from ART has been established as an important predictor of long-term virologic suppression, the effects of intermittent viremia on rates of viral suppression have not been established. It is important to understand the relationship between intermittent viremia and virologic failure. If intermittent viremia is associated with virologic failure due to drug-resistant populations, early therapy switches or intensification could potentially improve long-term rates of viral suppression. On the other hand, if intermittent viremia is not predictive of virologic failure, then patients may be able to maintain regimens for longer periods and avoid premature changes of therapy.

To determine the prevalence and predictive value of intermittent viremia, we examined data from the clinical trials of AIDS [acquired immunodeficiency syndrome] Clinical Trials Group (ACTG) 343 (241 patients) and Merck 035 (13 patients). In both trials, patients were treated with indinavir-zidovudine-lamivudine and HIV RNA plasma levels were measured with an assay having a lower limit of detection of 50 copies/mL. Because therapeutic changes were predicated on confirmed HIV RNA levels of greater than 200 copies/mL, we were able to examine the natural history of intermittent and low-level viremia in these pa-

tients. In addition, we used an HIV RNA assay with a limit of detection of 2.5 copies/mL to determine whether quantifiable, steady-state HIV RNA levels differed among patients based on intermittent viremia status. Viral load and drug resistance have been monitored in the Merck 035 cohort for 5 years, providing insight into the long-term consequences of intermittent viremia.

METHODS

Study Patients

To address our primary objective of evaluating the predictive value of intermittent viremia for virologic failure, we studied plasma HIV RNA of patients from 2 clinical trials who had achieved virologic suppression levels of less than 200 copies/mL after 6 months of triple-drug therapy. Although the study design and the patient populations differed, patients in both trials were treated with indinavir-zidovudine-lamivudine (or indinavir-stavudine-lamivudine). In ACTG 343, 12% of patients substituted stavudine for zidovudine.

The ACTG 343 study was a randomized trial of induction and maintenance therapy.⁵ At entry, patients were naive to protease inhibitors and lamivudine and had CD4 cell counts greater than 200/ μ L. After achieving viral suppression during 6 months of therapy with indinavir-zidovudine-lamivudine, patients were randomized to indinavir only, to zidovudine plus lamivudine, or to continue receiving all 3 drugs. Patients receiving maintenance therapy (arms with indinavir only or zidovudine plus lamivudine) exhibited higher levels of virologic failure; therefore, the trial was modified. All patients resumed triple-drug therapy and continued to be followed up for evidence of virologic failure with measurement of HIV RNA levels at 4-week intervals. Three hundred nine patients were randomized to the maintenance phase of the trial (FIGURE 1). Twelve patients were excluded from the analysis because less than 2 months of follow-up HIV RNA level data were available. Fifty-six patients who experienced virologic

failure while receiving maintenance therapy were excluded. Thus, 241 patients from ACTG 343 were included in analyses, 101 of whom received triple-drug therapy throughout. The study group was 67% non-Hispanic white and 85% were male. Data on risk factors were not collected. More intensive virologic studies were performed on 10 of the 12 patients who enrolled in this study at University of California, San Diego, 5 of the 10 received triple-drug therapy throughout. All 241 patients achieved HIV RNA nadirs below 50 copies/mL.

In the Merck 035 trial, 97 patients who had previously taken zidovudine and who had baseline CD4 cell counts between 50/ μ L and 400/ μ L were randomized to receive either indinavir alone, zidovudine plus lamivudine, or all 3 drugs.¹¹ Patients could have had previous exposure to didanosine, stavudine, or zalcitabine. All patients received triple-drug therapy when an interim analysis approximately 6 months after the start of the study revealed the superiority of this regimen. Twenty-one patients were enrolled in this study in San Diego. Thirteen of these patients achieved HIV RNA levels below 50 copies/mL and were included in this study. Of the 13 patients, 77% were white; 92% were male; and 77% were in the leading HIV risk category of males having sex with other males. Eight patients received triple-drug therapy initially; 3 patients received zidovudine plus lamivudine; and 2 patients received indinavir only. Plasma HIV RNA levels were measured at 8-week intervals. The original ACTG 343 and Merck 035 studies had institutional review board approval at each of the sites.

Case Definitions of Intermittent Viremia and Virologic Failure

Plasma HIV RNA levels were measured in a central laboratory for both studies using the Amplicor Ultrasensitive assay (Roche Molecular Systems, Nutley, NJ). The label-approved limit of detection of this assay is 50 copies/mL. *Intermittent viremia* was defined as a plasma HIV RNA level greater than 50 copies/mL with a

subsequent value of less than 50 copies/mL without evidence of virologic failure. *Virologic failure* was defined as 2 consecutive HIV RNA levels greater than 200 copies/mL.

Plasma HIV RNA 2.5-Copies/mL Detection-Level Assay

The Amplicor Ultrasensitive assay was adapted to permit detection of 2.5 copies/mL of plasma HIV RNA. Modifications included pelleting virus from 2 mL of plasma at 23 600g at 4°C for 2 hours, addition of half the normal volume of quantitation standard, and resuspension of the RNA pellet in 50 μ L of diluent. The entire 50 μ L of resuspended RNA was used in the reverse transcriptase polymerase chain reaction. Validation experiments were performed using plasma with known viral copy numbers (based on Amplicor assays) diluted with control plasma to achieve final viral RNA concentrations ranging from 100 to 1.25 copies/mL. In total, for validation analysis, plasma virus from 11 patients was measured in 35 separate assays (multiple measurements of a specimen were obtained to evaluate assay reproducibility). The linear correlation of results based on Amplicor assays (on undiluted plasma) and the modified 2.5-copies/mL assay was good with an r^2 of 0.92 ($P = .003$). Coefficients of variation for replicate 2.5 copies/mL assays were 9% to 141% at individual concentrations ranging from 1.25 to 50 copies/mL. At 2.5 copies/mL, the coefficient of variation was 37%. The assay results of 14 out of 15 samples diluted to 2.5 copies/mL yielded values within 2.5-fold of calculated concentrations from Amplicor assay results corrected for dilution. This compares favorably with the manufacturer's specifications (J.K.W., unpublished data, 2000) for interassay variation of replicate Amplicor Monitor (Roche Molecular Systems) or Ultrasensitive assays (2- to 3-fold). All subjects had HIV RNA levels evaluated using the ultrasensitive assay; 10 ACTG 343 and 13 Merck 035 patients (see below) had additional studies performed using the 2.5-copies/mL assay.

Resistance Testing

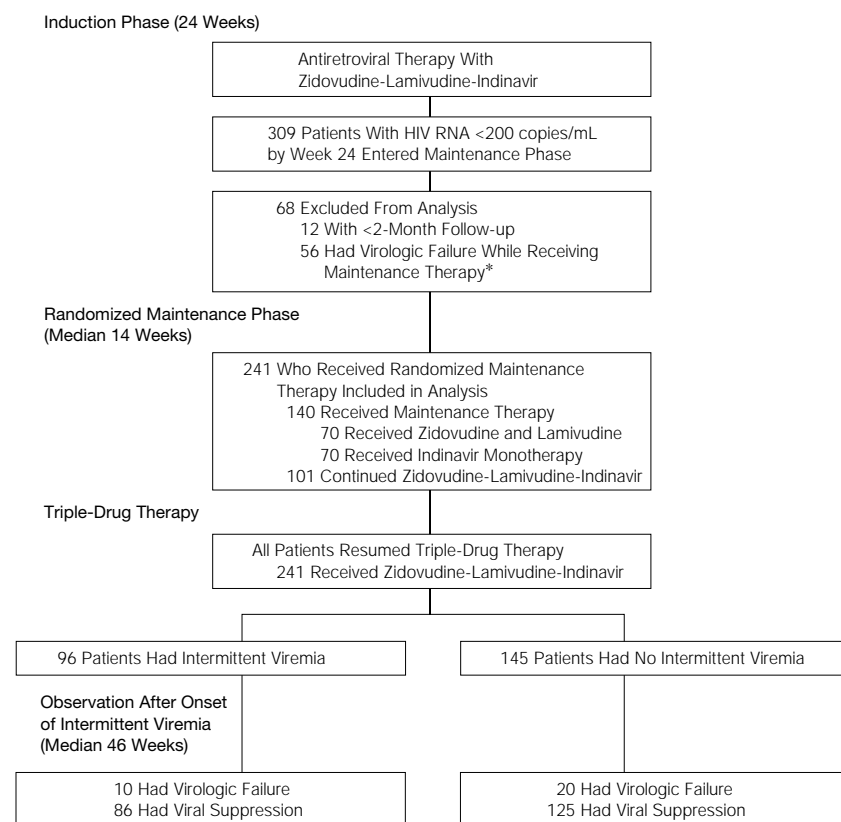
Resistance studies were performed on patients in the Merck 035 cohort at baseline, year 1, and year 5 of treatment. Of the 13 patients in this cohort, 3 patients switched therapy before the 5-year time point. Blood samples were not attainable for 2 patients at year 5. Amplification was not successful in 1 subject, leaving 7 patients available for study.

Baseline and year 1 drug-resistance genotypes were obtained from replicate reverse transcriptase polymerase chain reaction amplification of virus present in patient plasma samples followed by molecular cloning as previously described.¹²

For year 5, population-based sequencing was performed on peripheral blood mononuclear cell samples

using previously described conditions with the following modifications.⁸ Outer primers were RTF1C (5'-TTG-GAAGAAATCTGTTGACTCAG-3') and RTB1C (5'-GGGTCATAATACACTYCATGTACYG GYTCTT-3'). Inner primers were CIPol1 (5'-GGAAGAAAT-CTGTTGACTCAGATTGG-3') and 3RT (5'-ACCCATC-CAAAGGAAT-GGAGGTTCTTTC-3'). Taq platinum (BRL Life Sciences, Gaithersburg, Md) was substituted for Taq polymerase and annealing temperature was 50°C for 1 minute. Polymerase chain reaction products were gel purified, then directly sequenced. Resistance studies were not performed specifically for patients having virologic failure in the ACTG 343 study group for the analyses herein.

Figure 1. Flow Diagram for AIDS Clinical Trials Group 343 Patients



Median duration of observation from time of study entry was 84 weeks; AIDS indicates acquired immunodeficiency syndrome. The asterisk indicates that 51 individuals had virologic failure prior to January 4, 1998, but 5 additional individuals having failure in early January were included because of lag in diffusion of results of the interim analysis.

Statistical Analysis

For analysis of the ACTG 343 cohort, Cox proportional hazards models were used to evaluate risk factors for intermittent viremia when HIV RNA levels were greater than both 50 copies/mL and 200 copies/mL and to evaluate risk factors for virologic failure. Models were fitted for time to intermittent viremia and time to virologic failure. The time to intermittent viremia was defined as the first HIV RNA level greater than 50 copies/mL. Similarly, the time to virologic failure was determined by the first HIV RNA level greater than 200 copies/mL.

Our primary analysis used a Cox proportional hazards model to study the risk of virologic failure over time, with intermittent viremia modeled as a time-dependent covariate. Only the first episode of intermittent viremia was of interest and was included in the inferential analyses. At that point, the subject changed groups for the remainder of the analysis. For a given time point, this model compares the instantaneous risk of virologic failure for patients at risk at that time who had experienced intermittent viremia vs patients at risk at that time who had not experienced intermittent viremia.

The validity of the proportional hazards assumption in the Cox regression model was assessed by the method of Grambsch and Therneau,¹³ indicating validation of the assumption ($P=.64$). The relative risk (RR) is calculated as the Cox proportional hazards model estimate of the hazards ratio, assuming the hazards of virologic failure for the 2 groups are proportional, and is reported as the primary estimate herein.

As a secondary analysis, we compared the risk of virologic failure between patients who did and did not exhibit intermittent viremia at any time point during the study. This analysis is informative because our data suggest that having intermittent viremia is indicative of a higher steady state of viral replication during the entire study period, not just after an episode of intermittent viremia (see below).

Secondary analyses modeled virologic failure as a binary response us-

ing logistic regression. The results of these analyses were consistent with the time-to-event analyses and are not presented here.

The distributions of time to intermittent viremia and time to virologic failure were estimated using the Kaplan-Meier method and comparisons were made using the log-rank test. Patients were classified at time 0 (the end of the ACTG 343 induction phase [following 24 weeks of therapy], which is when patients were eligible to exhibit virologic failure) according to whether they met the definition of intermittent viremia (at any point during the study) or suppressed viremia. Supporting the results of the Cox proportional hazards model with intermittent viremia as a time-dependent covariate, there were no differences between a survival curve estimated using at-risk periods counted after intermittent viremia events (as described above with time 0 at the end of the induction period) and a survival curve estimated using at-risk periods during which intermittent viremia had not yet occurred (P.G., unpublished data, 2000). The proportion of patients with virologic failure in the group of patients with intermittent viremia at any time point during the study was compared with the group without intermittent viremia using the Fisher exact test.

A Wei-Johnson test, which does not assume independence of repeat measurements from individuals, was applied to data from the ACTG 343 study to assess whether the level of HIV RNA measured using the 2.5-copies/mL threshold differed between those with intermittent viremia and those with suppressed viremia. For the Merck 035 data, the median HIV RNA level and the proportions of values of less than 2.5 copies/mL for each subject were determined. Each patient had measurements available at different weeks; thus, the determination of proportion of values of less than 2.5 copies/mL. These medians and proportions were compared between patients with intermittent viremia and those with complete viral suppression by using a Wilcoxon rank-sum test.

The primary analysis excluded patients with initial viral suppression who then developed virologic failure during maintenance therapy. These patients were excluded because the intent of the study was to evaluate the predictive value of intermittent viremia for patients receiving triple-drug therapy. These patients were included in a secondary analysis (D. V. H., unpublished data, 2000) that produced similar results except that maintenance therapy was a predictor of virologic failure.

RESULTS

Frequency and Predictors of Intermittent Viremia in ACTG 343

The ACTG 343 study population had a baseline median CD4 cell count of 452/ μ L and HIV RNA plasma level of 4 \log_{10} copies/mL. The median time to the first HIV RNA measurement level of less than 200 copies/mL was 4 weeks in the induction phase. Among the 241 patients included in the analysis, 70 were randomized to zidovudine plus lamivudine, 70 were randomized to indinavir, and 101 were randomized to continue triple-drug therapy. Patients continued taking zidovudine plus lamivudine maintenance therapy or indinavir maintenance therapy for a median of 14 weeks before switching back to triple-drug therapy. The median duration of observation was 84 weeks and the median duration of observation after the onset of intermittent viremia was 46 weeks. The median number of HIV RNA measurements was 17 per patient after the first 24-week induction period (mean of 16.6 for those with intermittent viremia and 16.4 for those without it).

Intermittent viremia occurred frequently in the study population. In 96 (40%) of 241 patients, at least 1 episode of intermittent viremia occurred with an HIV RNA level of greater than 50 copies/mL. Of the 96 patients with episodes of intermittent viremia based on HIV RNA levels greater than 50 copies/mL, the mean and median number of episodes was 1.5 and 1, respectively. In 54 (20%) of 241 patients, at least 1 value measurement was greater than 200 copies/mL. Fifteen percent of

patients (36/241) had more than 1 episode of intermittent viremia (HIV RNA level >50 copies/mL). Thirteen percent of patients (32/241) had 2 consecutive HIV RNA levels greater than 50 copies/mL. One subject had 6 consecutive measurements between 50 and 200 copies/mL but never developed virologic failure.

Predictors of intermittent viremia included randomization to maintenance therapy (arms with indinavir only or zidovudine plus lamivudine) and baseline HIV RNA level (TABLE 1). Forty-two percent (102/241) of ACTG 343 participants had been treated with zidovudine previously, and genotypic resistance mutations were present at codon 215 in 54 (26%) of 208 patients at baseline. Prior zidovudine exposure and baseline zidovudine resistance mutations were not associated with an increased risk of intermittent viremia. No systematic measurements of medication adherence were included in this study to assess whether this variable was associated with intermittent viremia.

Ultrasensitive 2.5-Copies/ μ L Assay and Residual Viral Replication

In samples evaluated with the ultrasensitive assay adapted to permit detection of 2.5 copies/mL of HIV RNA from 10 patients in the ACTG 343 trial who had accessible specimens (median of 4 measurements per patient) at weeks 24, 32, 52, and 60 of therapy (excluding the HIV RNA measurements of >50 copies/mL that led to identification of intermittent viremia), HIV RNA levels were higher in the 3 patients with intermittent viremia compared with the 7 patients with suppressed viremia (FIGURE 2). However, this difference did not reach statistical significance ($P=.15$, Wei-Johnson test). The median HIV RNA level was 23.0 copies/mL in patients with intermittent viremia and less than 2.5 copies/mL in patients with suppressed viremia. In addition, 14 (52%) of 27 samples assayed from patients with suppressed viremia had HIV RNA levels below the 2.5-copies/mL threshold compared with

Table 1. Predictors of Intermittent Viremia in AIDS Clinical Trials Group 343*

Characteristic	RR (95% CI)†	P Value
Baseline CD4 cells/ μ L‡	0.92 (0.83-1.02)	.11
Baseline log HIV RNA copies/mL§	1.41 (1.05-1.88)	.02
Time to HIV RNA <200 copies/mL	1.05 (1.00-1.11)	.06
Prior zidovudine duration	1.00 (0.99-1.01)	.93
Baseline zidovudine resistance mutations		
Codon 41	1.48 (0.74-2.96)	.27
Codon 70	1.25 (0.74-2.10)	.41
Codon 215	1.58 (0.81-3.07)	.18
Randomized to maintenance therapy	1.89 (1.22-2.92)	.004

*AIDS indicates acquired immunodeficiency syndrome; RR, relative risk; CI, confidence interval; and HIV, human immunodeficiency virus.

†RR calculated as a Cox model estimate of the hazard ratio assuming that the hazards of virologic failure of the 2 groups are proportional.

‡Per 100-CD4 cells/ μ L increase.

§Per log HIV RNA copies/mL increase.

||The maintenance therapy regimens were either indinavir alone or zidovudine plus lamivudine.

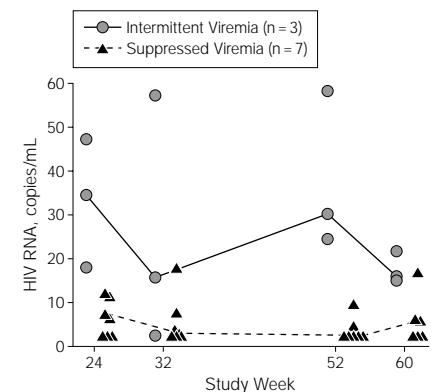
1 (8%) of 12 samples tested from the patients with intermittent viremia. One patient receiving triple-drug therapy had an episode of intermittent viremia during the maintenance phase, 1 receiving monotherapy had an episode after the maintenance phase, and 1 receiving dual therapy had an episode during the maintenance phase.

In samples evaluated with the ultrasensitive (2.5-copies/mL detection level) assay from 13 patients in the Merck 035 cohort after 76 to 260 weeks of therapy (median of 8 measurements per patient), HIV RNA levels similarly were higher in the patients with intermittent viremia (FIGURE 3). The median of HIV RNA levels in the 6 patients with intermittent viremia was 5.5 copies/mL compared with less than 2.5 copies/mL in the 7 patients with suppressed viremia ($P=.03$, Wilcoxon rank sum test). Patients with intermittent viremia had a median of 27% of their values at levels of less than 2.5 copies/mL, compared with a median of 56% of values at levels of less than 2.5 copies/mL for patients with complete viral suppression ($P=.04$, Wilcoxon rank-sum test). All patients had at least 1 sample with detectable viremia and all episodes of intermittent viremia occurred during receipt of triple-drug therapy.

Drug-Resistance Mutations

Drug-resistance studies were performed in 7 of the Merck 035 patients, of which 2 exhibited intermittent vire-

Figure 2. Median HIV RNA Levels Using the 2.5-Copies/mL Detection-Level Assay in a Subset of 10 AIDS Clinical Trials Group 343 Patients



Median HIV RNA levels were higher in patients with intermittent viremia (23 copies/mL) vs suppressed viremia (<2.5 copies/mL) ($P=.15$, Wei-Johnson test). Nine patients had 4 measurements and 1 had 3 measurements; the subject with a missing measurement was in the group without intermittent viremia. Mean number of measurements was 4 for those with intermittent viremia and 3.86 for those without; the median was 4 for both groups.

mia. All 7 patients had 1 or more nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations at baseline (41L, 44D, 65R, 67N, 69N, 70R, 74I/V, 75T, 118I, 215Y/F, 219Q) consistent with prior NRTI experience. None had mutations at codon 184 at baseline. The 1 subject who had M184V (lamivudine resistance) detected in the year 5 sequence had received zidovudine plus lamivudine initially; this patient had intermittent viremia. The M184V mutation had been identified in

plasma after the first year of nonsuppressive therapy and before suppression of viremia was achieved with the triple-drug regimen. Two other patients so treated did not have the M184V mutation present in peripheral blood mononuclear cell provirus at year 5 despite its presence in plasma virus after 1 year of nonsuppressive therapy with zidovudine plus lamivudine. In 4 patients, 1 or more NRTI-resistance mutations present at baseline were not identified in year 5 specimens. Thus, neither new zidovudine-resistance mutations nor the signature codon 184 lamivudine-

resistance mutation were observed in patients after 5 years with the exception of an individual who initially received only zidovudine plus lamivudine.

Relationship of Intermittent Viremia to Virologic Failure

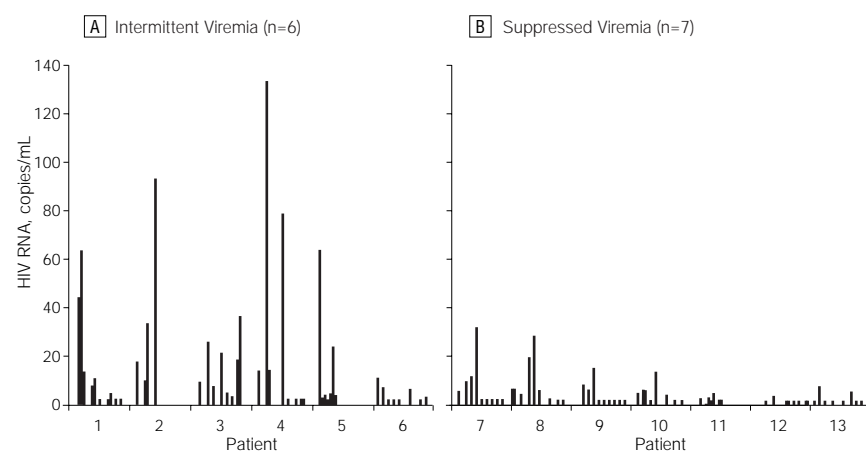
In the ACTG 343 study, intermittent viremia did not predict further virologic failure. In a Cox proportional hazards model with intermittent viremia modeled as a time-dependent covariate, the risk for virologic failure was not significantly higher in patients with intermittent viremia, with a hazard ratio esti-

mate of 1.28 (95% confidence interval [CI], 0.59-2.79; $P=.53$) (TABLE 2). Similarly, when patients were classified according to the intermittent viremia status during any time point in the study, the risk of virologic failure from the time of randomization until the end of follow-up was not different between patients (FIGURE 4). With this classification, 10 (10.4%) of 96 patients with intermittent viremia compared with 20 (13.8%) of 145 patients with continuous viral suppression failed virologically, with an RR of 0.76 (95% CI, 0.29-1.72; $P=.55$, Fisher exact test). When the case definition of intermittent viremia was changed to use a virologic threshold of HIV RNA greater than 200 copies/mL rather than HIV RNA greater than 50 copies/mL, the intermittent viremia covariate was not a significant predictor of virologic failure (RR, 1.48; 95% CI, 0.56-3.93; $P=.43$; Table 2).

Finally, we evaluated whether intermittent viremia predicted failure in the 101 patients who received triple-drug therapy for the entire study. Of note, the proportion of episodes of intermittent viremia occurring during the maintenance period did not significantly differ between patients who received triple-drug therapy throughout the study (55%) and those who received maintenance therapy (68%) ($P=.25$, Fisher exact test; 64% for the entire cohort). Twenty-nine (29%) of the 101 patients receiving triple-drug therapy throughout the study exhibited intermittent viremia. Virologic failure occurred in 3 (10%) of 29 patients with intermittent viremia and 10 (14%) of 72 patients without intermittent viremia. When the Cox proportional hazards analysis was repeated for these 101 patients, the risk of virologic failure was not increased in patients with vs those without intermittent viremia (RR, 1.37; 95% CI, 0.36-5.12; $P=.64$).

In the patients evaluated in the Merck 035 study, neither the 6 patients with intermittent viremia nor the 7 patients with complete viral suppression exhibited viral failure. The median duration of observation in these patients was 4.5 years.

Figure 3. Human Immunodeficiency Virus (HIV) RNA Levels Using the 2.5-Copies/mL Detection-Level Assay in 13 Merck 035 Patients Between 76 and 260 Weeks of Therapy



The bars represent HIV RNA level determinations using the 2.5-copies/mL assay on stored sequential specimens for each patient. Patients with intermittent viremia had higher median (5.5 copies/mL) HIV RNA levels compared with those with suppressed viremia (<2.5 copies/mL) ($P=.03$, Wilcoxon rank-sum test). Patients with intermittent viremia had a median of 27% of their values with less than 2.5 copies/mL compared with a median of 56% for patients with complete viral suppression ($P=.04$, Wilcoxon rank-sum test).

Table 2. Predictors of Virologic Failure in AIDS Clinical Trials Group 343*

Characteristic	RR (95% CI)†	P Value
Intermittent viremia‡		
>50 copies/mL	1.28 (0.59-2.79)	.53
>200 copies/mL	1.48 (0.56-3.93)	.43
Baseline CD4 cells/ μ L	0.86 (0.70-1.05)	.14
Baseline log HIV RNA copies/mL	1.98 (1.13-3.47)	.02
Prior zidovudine duration	1.00 (0.98-1.02)	.91
Baseline zidovudine resistance mutations		
Codon 41	0.67 (0.16-2.82)	.59
Codon 70	0.54 (0.16-1.80)	.31
Codon 215	0.70 (0.16-3.01)	.64

*AIDS indicates acquired immunodeficiency syndrome; RR, relative risk; CI, confidence interval; and HIV, human immunodeficiency virus.

†RR calculated as a Cox model estimate of the hazard ratio, assuming that the hazards of virological failure of the 2 groups are proportional.

‡Modeled as a time-dependent covariate.

Intermittent Viremia and ACTG 343 Study End Points

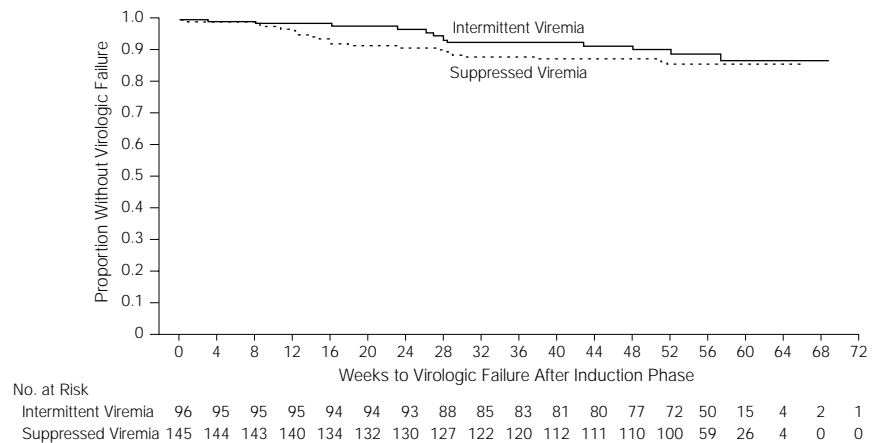
The case definitions of virologic suppression and virologic failure used in clinical trials have been largely dictated by the sensitivity of available assays. The definition of virologic failure in the ACTG 343 study was 2 consecutive measurements of HIV RNA levels greater than 200 copies/mL. If virologic failure had been defined by 2 HIV RNA measurements of greater than 50 copies/mL, an additional 32 end points would have occurred in the study. Eleven end points would have been captured at an earlier time point with this more stringent definition.

COMMENT

In patients treated with indinavir-zidovudine-lamivudine who achieved virologic suppression, intermittent viremia was a frequent event that did not predict subsequent virologic failure. Intermittent viremia was defined by an arbitrary threshold of 50 copies/mL dictated by the sensitivity of the HIV RNA assay. By applying a more sensitive assay, we demonstrated that patients sustaining levels of HIV RNA less than 50 copies/mL have varying levels of ongoing viral replication. Blips of HIV RNA above 50 copies/mL represented fluctuations around a higher steady state of replication (Merck 035) compared with patients who did not exhibit this pattern. The HIV RNA levels obtained at times near episodes of intermittent viremia were consistently higher than in patients without intermittent viremia, arguing against laboratory error as the explanation for the frequent occurrence of intermittent viremia.

The magnitude of HIV RNA suppression as a predictor of virologic failure has been examined in numerous studies. Patients who do not achieve a nadir of HIV RNA below 50 copies/mL have an increased risk for failure.^{3,4,14-17} Any measurable HIV RNA value has also been associated with greater risk for failure.¹⁸ We found that intermittent HIV RNA values above 50 copies/mL were not associated with increased risk for failure. The

Figure 4. Time to Virologic Failure in AIDS Clinical Trials Group 343 (ACTG) Patients With Intermittent and Suppressed Viremia



Estimated probability of sustaining viral suppression starting with the time point at which virologic suppression was achieved in ACTG 343. Patients were classified at time 0 (the end of the ACTG 343 induction phase [following 24 weeks of therapy] when patients were eligible to exhibit virologic failure) according to whether they met the definition of intermittent viremia (at any time point during the study) or suppressed viremia. There was no significant difference in the distributions of the probability curves ($P = .38$, log-rank test). The values at the far right of the figure are slightly different from those provided in the text because the figure represents an estimation of the probability of remaining free of virologic failure that accounts for the amount of follow-up time for each patient (the proportions reported in the text represent estimates of binomial failure probabilities that do not account for differences in follow-up time among patients). AIDS indicates acquired immunodeficiency syndrome.

difference between our findings and prior studies can be explained by differences in the patient populations. Studies reporting increased risk for detectable HIV RNA included patients regardless of whether they achieved virologic nadirs below 50 copies/mL.^{3,4,14-18} Patients who did not achieve virologic nadir below 50 copies/mL have only partial viral suppression and are known to exhibit virologic failure more frequently. We exclusively studied patients who achieved HIV RNA nadirs below 50 copies/mL. Our results thus are not in conflict with these prior studies, but rather extend our understanding of predictors of virologic failure by distinguishing the groups of patients who receive potent ART who established initial viral suppression below 50 copies/mL.

Many factors may have contributed to varying steady-state levels of viral replication observed in our patients. Suboptimal regimen potency, higher baseline HIV RNA levels, and the presence of baseline drug resistance have been associated with reduced rates of viral suppression.^{5-7,15,19-21} Adherence, antiretro-

viral pharmacokinetics, vaccine administration, and polymorphisms in chemokine receptor genes also influence viral suppression.²²⁻²⁵ In patients with less effective viral suppression (ie, intermittent viremia), viral evolution and drug resistance are demonstrable. In an earlier study of the Merck 035 patients,⁸ evolution in the envelope gene was detectable in patients with but not in those without intermittent viremia after 2 years of treatment. Three of these patients with intermittent viremia were included in a study by Martinez-Picado et al,¹⁰ which used assays to determine drug-resistance mutations using clonal analyses. New, low-frequency NRTI and protease inhibitor-resistance mutations were detected after 2 years of therapy in all 3 patients. However, despite evolution and drug-resistance mutations, these patients exhibited neither new NRTI-resistance mutations nor virologic failure after 5 years of therapy in our analysis.

The most direct explanation of these findings is based on our current understanding of viral dynamics, namely

that the number of HIV replication cycles is insufficient to overcome the evolutionary requirements for the selection and fixation of a predominantly drug-resistant population.²⁶ A threshold level of residual replication (perhaps as reflected by viral loads in the 50-200 copies/mL range) may exist below which viral adaptive mechanisms are rendered ineffective. Such mechanisms may include the generation of large numbers of variants with every replicative cycle and the coinfection of cells by genotypically distinct virus at sufficiently high frequency to permit advantageous recombination events.²⁶⁻²⁹ Moreover, many protease inhibitors require compensatory mutations to overcome reductions in viral fitness associated with early drug-resistant breakthroughs, thus delaying the appearance of majority drug-resistant populations. Grossman et al^{30,31} have proposed that viral replication may be more accurately viewed as focal replicative bursts, described as proximal activation and transmission. Residual replication under long-term highly active ART may continue in small bursts that are spatially and temporally discontinuous at levels insufficient for effective viral adaptation.

Host-immune responses provide another explanation for the absence of a correlation between intermittent viremia and virologic failure. Higher levels of HIV antigens in patients with intermittent viremia may stimulate immune responses, which prevent escape of subpopulations of drug-resistant virus. While early studies suggested that HIV-specific immune responses could be restored only in treatment of acutely infected patients, emerging data suggest that chronically infected patients can also develop and maintain HIV-specific responses.³²

The results of this analysis raise several issues surrounding current management strategies. Clinicians are advised to administer therapy that sustains HIV RNA levels below the threshold of detection to reduce the opportunity for resistant virus to emerge. But virologic suppression or undetectability is rela-

tive to the sensitivity of the assay. In both clinical trials and clinical practice, the assay-detection threshold commonly used has decreased from 400 copies/mL to 50 copies/mL. Current guidelines thus imply that any detectable or confirmed HIV RNA level above 50 copies/mL should trigger therapy intensification or modification, assuming effective treatment options are available.^{1,2}

These recommendations warrant reconsideration for several reasons. First, nearly all patients receiving potent ART, including those who reach a nadir of 50 copies/mL, have detectable HIV RNA levels when using even more sensitive assays.³³⁻³⁶ Second, if blips or low-level intermittent viremia are not associated with greater rates of virologic failure for as long as 4.5 years, then it may not be necessary to switch or intensify therapy until patients exhibit higher levels. Unnecessary regimen switching may result in disruption of a patient's medication routine, toxic effects from new drugs, and premature discarding of useful drugs.

The results of this study are also relevant to the design of clinical trials. If achieving HIV RNA levels of less than 50 copies/mL is associated with more sustained viral suppression, it is logical to use this threshold as a marker for success. It is less clear, however, how virologic failure should be defined. The data presented here argue that a definition of 2 consecutive HIV RNA values greater than 50 copies/mL is too stringent a definition of virologic failure. Thirty-two patients in the ACTG 343 trial would have been classified as having virologic failure who otherwise did not meet the study definition as confirmed HIV RNA values greater than 200 copies/mL. Using a definition of HIV RNA level greater than 50 copies/mL as evidence of virologic failure, 11 additional patients would have met the criteria for virologic failure at an earlier time point. This issue is particularly relevant for trials comparing different therapy sequences in which protocols mandate switching regimens after reaching an end point of virologic failure. Required, premature switching of treatment regimens may

produce underestimates of their clinical durability and utility.

There are important caveats to the generalizations and implications of our findings. First, two thirds of patients in the ACTG 343 study received a brief period of maintenance therapy and the median follow-up for the trial was 84 weeks. However, even when the analysis was limited to ACTG 343 patients who received triple-drug therapy, findings were consistent. Second, the safety margin or threshold is not precisely defined for continuing or changing the regimen of a patient who experiences low-level viremia. Third, this threshold may be higher or lower for different drug regimens because genetic barriers for the emergence of resistant populations vary due to fitness of early drug-resistant populations.³⁷ Finally, in patients with predominantly drug-resistant viral populations who initiate salvage therapy, low-level viremia may be a harbinger for imminent virologic failure. We did not address issues of adherence or pharmacokinetics in this analysis, and these may be the most important considerations in the decision of when to switch or modify therapy. Unexplained elevations in plasma HIV RNA level should always prompt a careful assessment of patient adherence. All these caveats underscore the importance of additional studies evaluating the clinical significance of low-level viremia and validating virologic-failure thresholds in a variety of clinical settings.

In summary, in patients who achieved virologic suppression with indinavir-zidovudine-lamivudine, intermittent viremia was a frequent occurrence and was associated with higher steady-state levels of viral replication (Merck 035) but was not associated with virologic failure for up to 4.5 years (ACTG 343 and Merck 035). Clinical management options are increased by this knowledge. A higher HIV RNA level that would trigger a therapy change may preserve the number of drugs available for future therapeutic regimens.

Author Affiliations: Departments of Medicine (Drs Havlir, Levitan, Richman, and Wong) and Pathology (Dr Richman and Ms Ignacio), University of Calif-

nia, San Diego, and the San Diego VA Healthcare System; School of Public Health (Mr Bassett and Dr Gilbert) and Department of Medicine, Infectious Diseases Unit (Dr Hirsch), Harvard University, Boston, Mass; Department of Internal Medicine, Washington University, St Louis, Mo (Dr Tebas); Department of Allergy and Infectious Diseases, University of Washington School of Medicine, Seattle (Dr Collier); Merck Research Laboratory, West Point, Pa (Dr Condra); and Department of Medicine, Division of Infectious Diseases and Hospital Epidemiology, University of Zurich, Zurich, Switzerland (Dr Günthard).

Author Contributions: *Study concept and design:* Havlir, Bassett, Tebas, Collier, Hirsch, Richman, Wong. *Acquisition of data:* Havlir, Bassett, Levitan, Collier, Ignacio, Condra, Günthard, Richman, Wong.

Analysis and interpretation of data: Havlir, Bassett, Levitan, Gilbert, Tebas, Collier, Hirsch, Ignacio, Condra, Günthard, Richman, Wong.

Drafting of the manuscript: Havlir, Bassett, Tebas, Hirsch, Wong.

Critical revision of the manuscript for important intellectual content: Havlir, Bassett, Levitan, Gilbert, Tebas, Collier, Hirsch, Ignacio, Condra, Günthard, Richman, Wong.

Statistical expertise: Bassett, Gilbert.

Obtained funding: Richman, Wong.

Administrative, technical, or material support: Collier, Hirsch, Ignacio, Condra, Günthard, Wong.

Study supervision: Havlir, Tebas, Hirsch, Richman, Wong.

Financial Disclosures: Dr Havlir received grants or funding from Merck and GlaxoSmithKline; Dr Tebas received grants or funding (including participation in studies sponsored by companies) or lecture sponsorships from or was a consultant for Abbott, Agouron, Boehringer, Bristol-Myers Squibb, Campbell Foundation, Dupont, Gilead, GlaxoSmithKline, Merck, National Institutes of Health, Triangle, Trimeris, and Virologics; Dr Collier received funding from, was a consultant to, or authored material for Abbott, Agouron, Anor-Med, Gilead Sciences, GlaxoSmithKline, Hoffmann LaRoche, JAMA HIV/AIDS Information Center (Web site), Medscape (Web site), Merck, Pharmacia-Upjohn, The Body (Web site), ViroLogic, and the National Institute of Allergy and Infectious Diseases; Dr Hirsch received grants, funding, or honoraria from or was a consultant or advisor for Bristol-Myers Squibb, GlaxoSmithKline, Merck, Schering Plough, Trimeris, and the National Institute of Allergy and Infectious Diseases; Ms Ignacio received funding from the National Institutes of Health; Dr Günthard holds stocks in Roche

and Pfizer, received assay kits or reagents from Roche Molecular Systems, and received funding from Swiss National Science Foundation and Swiss HIV-Cohort Study; Dr Richman was a consultant for Abbott, Bristol-Myers Squibb, Chiron, Gilead Roche, GlaxoSmithKline, Lilly, Merck, Pfizer, Triangle, ViroLogic, and Visible Genetics; Dr Wong received grant funding from the National Institute of Allergy and Infectious Diseases, Department of Veterans Affairs, and Merck, and participated in continuing medical education programs sponsored by Merck.

Funding/Support: Supported by grants to the AIDS Clinical Trials Group from the National Institute of Allergy and Infectious Diseases (AI43752, AI27670, AI38855, AI38858), the National Institutes of Health (AI29164), and the University of California, San Diego, Center for AIDS Research (AI36214) and the Research Center for AIDS and HIV Infection of the San Diego Veterans Affairs Healthcare System.

Acknowledgment: We are indebted to the participants and investigators of the ACTG 343 and Merck 035 clinical trials. We thank Linda Meixner, RN, Kathy Nuffer, NP, and Gary Dyak, RN, for data and specimen retrieval. We appreciate the technical assistance of Janice Kaping, MS, Diane Greene, BS, Colin Reed, and Bryna Block, BA.

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