Clinical Infectious Diseases Advance Access published January 11, 2012

Plasma HIV-1 RNA Detection Below 50 Copies/mL and Risk of Virologic Rebound in Patients Receiving Highly Active Antiretroviral Therapy

Tomas Doyle,[1,3] Colette Smith,[4] Paola Vitiello,[3] Valentina Cambiano,[4] Margaret Johnson,[2,5] Andrew Owen,[6] Andrew N. Phillips,[4] and Anna Maria Geretti[1,3,7]

Departments of [1]Virology, and [2]HIV Medicine, Royal Free Hampstead NHS Trust; Departments of [3]Virology; [4]Infection & Population Health, and [5]HIV Medicine, UCL Medical School, London; [6]Department of Molecular and Clinical Pharmacology, and [7]Institute of Infection and Global Health, University of Liverpool, United Kingdom

(See the Editorial Commentary by Gandhi and Deeks, on pages 738–40.)

Background. Plasma human immunodeficiency virus type 1 (HIV-1) RNA suppression <50 copies/mL is regarded as the optimal outcome of highly active antiretroviral therapy (HAART). Current viral load (VL) assays show increased sensitivity, but the significance of RNA detection <50 copies/mL is unclear.

Methods. This study investigated the virologic outcomes of 1247 patients with VL <50 copies/mL at an arbitrary time point during HAART (= T0), according to whether the actual, unreported $_{T0}$VL was 40–49 copies/mL, RNA detected <40 copies/mL (RNA^+), or RNA not detected (RNA^-), as measured by the Abbott Real Time assay. Predictors of rebound >50 and >400 copies/mL over 12 months following T0 were analyzed with Cox proportional hazards models incorporating the $_{T0}$VL and demographic and clinical data.

Results. Rebound rates >50 copies/mL were 34.2% for $_{T0}$VL 40–49 copies/mL, 11.3% for RNA^+, and 4.0% for RNA^-; rebound rates >400 copies/mL were 13.0%, 3.8%, and 1.2%, respectively. The adjusted hazard ratios for rebound >50 copies/mL were 4.67 (95% confidence interval, 2.91–7.47; $P < .0001$) and 1.97 (1.25–3.11; $P < .0001$) with $_{T0}$VL 40–49 copies/mL and RNA^+, respectively, relative to RNA^-, and 6.91 (2.90–16.47; $P < .0001$) and 2.88 (1.24–6.69; $P < .0001$), respectively, for rebound >400 copies/mL. The association was independent of adherence levels.

Conclusions. In treated patients monitored by RealTime, a VL of 40–49 copies/mL and, to a lesser extent, RNA detection <40 copies/mL predict rebound >50 and >400 copies/mL independently of other recognized determinants. The goal of HAART may need to be revised to a lower cutoff than 50 copies/mL.

Guidelines have traditionally recommended sustained viral load (VL) suppression <50 copies/mL as the optimal outcome of highly active antiretroviral therapy (HAART) [1]. Recent guidance has recommended suppression below the detection limit of commercial assays [2, 3], but also increased the threshold for virologic failure to 200 copies/mL [3]. Although the target level of suppression was initially defined by the technical properties of the assay, clinical trials and observational studies have shown that maintaining a VL <50 copies/mL predicts long-term virologic success and immunologic and clinical benefits [4–6]. In many treated patients, human immunodeficiency virus type 1 (HIV-1) RNA remains detectable in plasma below the cutoff of 50 copies/mL—establishing residual viremia that in some populations appears to plateau at 3–10 copies/mL and is not responsive to HAART intensification [7–14].

In recent years, new commercial assays have been introduced based on real-time polymerase chain reaction that have a lower limit of quantification of 20 or 40 copies/mL and can also report qualitative RNA detection below these thresholds [15]. Although the assays have now entered routine practice, the clinical significance of RNA detection <50 copies/mL during HAART is

Received 4 August 2011; accepted 4 October 2011.

Correspondence: Anna Maria Geretti, MD, MSc, PhD, FRCPath, Institute of Infection and Global Health, University of Liverpool, Liverpool L69 3BX, UK (a.geretti@ucl.ac.uk).

Clinical Infectious Diseases 2012;54(5):729–37

DOI: 10.1093/cid/cir936

unknown. In particular, it is unclear whether VL values that fall between the proposed plateau of residual viremia and the 50 copies/mL cutoff should be regarded as an indication that the potency and tolerability of the regimen, adherence, drug resistance, and pharmacokinetics should be reviewed. Our center introduced the RealTime HIV-1 VL assay (Abbott Molecular, Maidenhead, UK) in 2006. The assay quantifies the viral load over the range of 40–10 000 000 copies/mL (1 mL input) and reports qualitative RNA detection <40 copies/mL. The manufacturer indicates detection of 30, 20, and 10 RNA copies/mL in 96%, 88%, and 68% of cases, respectively. Following the introduction of the assay in our laboratory, VL levels of 40–49 copies/mL and qualitative RNA-detected results were reported as <50 copies/mL. The actual results were not released to the treating physician. Here, we investigated the virologic outcomes of treated patients showing a VL <50 copies/mL at an arbitrarily selected time point during HAART (= T0) according to whether the actual, unreported $_{T0}$VL result 40–49 copies/mL, RNA detected <40 copies/mL, or RNA not detected.

METHODS

Study Population

In June to September 2008, the laboratory database was searched for treated patients with VL of 40–49 copies/mL, with RNA detection <40 copies/mL (RNA^+), or with RNA not detected (RNA^-). Patients were recruited consecutively, regardless of line and duration of treatment, until the number of observations estimated to be required to power the study on the basis of pilot data were achieved. On the basis of this $_{T0}$VL result, patients were selected that had at least 12 months of follow-up after T0 to evaluate their risk of rebound >50 and >400 copies/mL. Patients who modified their treatment due to toxicity were retained in the analysis. During follow-up, patients underwent VL monitoring with the same assay at intervals of 12–16 weeks. In a subanalysis, VL results obtained at T0 were compared with those obtained at the subsequent VL measurement (referred to as T1) to analyze the effect of confirmed viremia on virologic outcomes. Because guidelines recommend that VL suppression <50 copies/mL should be achieved within 16–24 weeks of starting HAART [1], we also determined the time to VL suppression <50 copies/mL, <40 copies/mL, and RNA^- in a subset of patients starting first-line therapy. Most patients were receiving care at the Royal Free Hospital (RFH), and for these patients, the demographic and clinical data were obtained from the clinic database. The remaining patients were receiving care at other centers, and for these patients, only the duration of VL suppression <50 copies/mL prior to T0 was available.

Evaluation of Adherence and Plasma Drug Levels

For RFH patients, adherence was estimated at T0 on the basis of the proportion of days covered by pharmacy drug prescription records for at least 3 drugs in the previous 6 months, as described elsewhere [16, 17]. Assessment of adherence was not repeated during follow-up. Efavirenz plasma concentrations were measured in stored $_{T0}$VL samples by high-performance liquid chromatography.

Resistance Testing

In patients who experienced rebound >400 copies/mL, stored plasma samples were retrieved for resistance testing, as described elsewhere [18]. Major resistance-associated mutations (RAMs) were analyzed according to the International AIDS Society mutation list (December 2009).

Statistical Analysis

For the main analyses, patients were assigned to 3 groups according to the $_{T0}$VL (40–49 copies/mL, RNA^+, or RNA^-). The characteristics of the 3 groups at T0 were compared using the Kruskal–Wallis test for continuous outcomes and χ^2 or Fisher's exact tests for categorical outcomes.

All patients were followed up from T0 for at least 12 months, with follow-up censored at 18 months. Rebound during follow-up was defined primarily as either 2 consecutive VL measurements >50 copies/mL (or a single VL >50 copies/mL if this was the last available) or the equivalent definition using a cutoff of 400 copies/mL. The date of the first of these 2 consecutive VL measurements >50 or >400 copies/mL was defined as the date of rebound, and treatment changes were ignored. In sensitivity analyses, rebound was also defined as a single VL >50 copies/mL (without the requirement for confirmation). Finally, a modified definition of rebound was considered in the RFH population defined as the first of (1) 2 consecutive VL>400 copies/mL, (2) a single VL>400 copies/mL if this was the last available measurement, or (3) a single VL >400 copies/mL followed by a treatment change before the next VL measurement. Differences in time to rebound according to the $_{T0}$VL were assessed by the log-rank test. Factors associated with rebound were identified in Cox proportional hazards models. First, the estimate was calculated among all available patients, and adjusted for the length of time that the individual had maintained a VL <50 copies/mL prior to T0. In the RFH population, the estimate was adjusted for all available variables. Global *P* values were calculated using change in deviance by comparing nested models.

In a subanalysis, the Cox proportional hazards model was fitted using the combined $_{T0}$VL and $_{T1}$VL measurements, and the analysis of factors associated with rebound >400 copies/mL was repeated, including T0/T1 status as a variable. Because we were interested in the predictive value of a confirmed detectable $_{T0}$VL, the T0/T1 status was classified into 3 groups: RNA^- at T0, RNA 40–49 copies/mL or RNA^+ at T0 and RNA^- at T1, and RNA 40–49 copies/mL or RNA^+ at T0 and any RNA detected <400 copies/mL at T1.

Efavirenz levels according to the $_{T0}$VL group were compared by the Mann–Whitney test; the proportion of patients with levels above or below the therapeutic threshold of 1000 ng/mL was compared by the χ^2 test. Efavirenz levels were not incorporated into the Cox proportional hazards models investigating factors associated with rebound due to the small number of observations.

In the resistance analysis, the prevalence of RAMs at the time of rebound >400 copies/mL in the different $_{T0}$VL group was compared by the Fisher exact test. The analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, NC); *P* values were 2-sided, and $P < .05$ was considered to be statistically significant.

RESULTS

Study Population

The population comprised 1247 patients with $_{T0}$VL 40–49 copies/mL (n = 240; 19.2%), RNA$^+$ (n = 507; 40.7%), or RNA$^-$ (n = 500; 40.1%; Table 1). Overall, 891 of 1247 (71.5%) patients were from the RFH and had demographic and clinical data available, including adherence estimations. There were several differences in the characteristics of the study population at T0. Patients with RNA$^-$ had longer duration of HAART and suppression <50 copies/mL prior to T0, a marginally lower pre-HAART viral load, and higher CD4 lymphocyte cell counts, and were more likely to be of white ethnicity and on nonnucleoside reverse-transcriptase inhibitor (NNRTI)–based therapy. To determine the time to VL <50 copies/mL, <40 copies/mL, or RNA$^-$, we performed a retrospective analysis among 78 patients who started first-line tenofovir/emtricitabine plus either efavirenz (n = 38) or ritonavir-boosted lopinavir (n = 40). The median (95% confidence interval [CI]) time to suppression <50 copies/mL, <40 copies/mL, and to RNA$^-$ was 4.1 (3.3–5.1), 4.4 (3.7–5.4), and 6.2 (5.4–7.2) months, respectively. There was no significant difference between the 2 treatment regimens in the time to RNA$^-$ ($P = .93$).

Rebound After T0

The time to rebound according to the $_{T0}$VL and each of the 4 definitions of rebound is shown in Figure 1. In the primary analyses, 211 patients experienced confirmed (or last available measurement; n = 56) rebound >50 copies/mL, and 80 patients experienced confirmed (or last available measurement; n = 21) rebound >400 copies/mL. Rebound rates differed significantly according to the $_{T0}$VL. After 12 months, the Kaplan–Meier estimates of the probability (95% CI) of confirmed (or last available) rebound >50 copies/mL were 34.2% (28.1%–40.3%), 11.3% (8.5%–14.0%), and 4.0% (2.3%–5.7%) for the $_{T0}$VL groups 40–49 copies/mL, RNA$^+$, and RNA$^-$, respectively ($P < .0001$ for all). The estimates for the >400 copies/mL cut-off were 13% (8.6%–17.3%), 3.8% (2.1%–5.4%), and 1.2% (0.2%–2.2%) for the $_{T0}$VL groups 40–49 copies/mL, RNA$^+$, and RNA$^-$, respectively ($P < .0001$ for all).

Predictors of Rebound

In the univariable analysis, predictors of confirmed (or last available) rebound >50 copies/mL in the whole population comprised the $_{T0}$VL group and the length of suppression <50 copies/mL prior to T0; additional predictors identified in the RFH population were the time since starting HAART, CD4 cell count and HAART regimen at T0, age, ethnicity, risk group, and adherence levels (Table 2). In the multivariable analysis, the $_{T0}$VL group and the length of suppression <50 copies/mL prior to T0 (and for the RFH population, the HAART regimen) were the only independent predictors of rebound (Table 2). Overall, rebound >50 copies/mL was least likely in patients with $_{T0}$VL RNA$^-$, those with longer duration of suppression <50 copies/mL prior to T0, and those receiving NNRTI-based HAART. The observations were similar in the analysis of confirmed (or last available) rebound >400 copies/mL, showing that the $_{T0}$VL group and the length of VL suppression <50 copies/mL prior to T0 were strong independent predictors of rebound (Table 3).

In a subanalysis, we analyzed the VL measurement obtained at the next time point after T0, referred to as $_{T1}$VL. This followed T0 by a median (range) of 12 (8–17) weeks, 15 (12–19) weeks, and 16 (12–18) weeks for the $_{T0}$VL groups 40–49 copies/mL, RNA$^+$, and RNA$^-$, respectively. In the Cox proportional hazards model, relative to patients with RNA$^-$ at T0 (n = 485), the adjusted hazard ratios (HRs; 95% CI) for confirmed (or last available) rebound >400 copies/mL were 1.52 (0.37–6.22) for RNA 40–49 copies/mL or RNA$^+$ at T0 and RNA$^-$ at T1 (n = 335), and 10.42 (3.36–32.33) for RNA 40–49 copies/mL or RNA$^+$ at T0 and any RNA detection <400 copies/mL at T1 (n = 180) ($P < .0001$) (Table 4). The results were consistent when analyzing the RFH population alone (data not shown).

Next, we investigated T0 efavirenz plasma levels among 186 RFH patients receiving efavirenz-based HAART, comprising 90 patients with $_{T0}$VL either 40–49 copies/mL or RNA$^+$ and 96 patients with RNA$^-$. We limited the analysis to patients on efavirenz due to its stable pharmacokinetics and bedtime dosing. In the 2 groups, the median efavirenz levels at T0 were 1484 ng/mL (interquartile range [IQR], 1054–2272 ng/mL) and 1593 ng/mL (IQR, 1047–2323 ng/mL), respectively ($P = .48$); levels were above the recommended threshold of 1000 ng/mL in 70 of 90 (77.8%) and 74 of 96 (77.1%) patients, respectively ($P = 1.00$; Figure 2). The median levels by $_{T0}$VL group were 1666 ng/mL (IQR, 1278–2449 ng/mL) for 40–49 copies/mL, 1339 ng/mL (IQR, 984–2041 ng/mL) for RNA$^+$, and 1593 ng/mL (1047–2323 ng/mL) for RNA$^-$ ($P = .11$; Kruskal–Wallis test).

Table 1. Characteristics of the Population at Study Entry, Defined as Time Zero

Characteristics	$_{T0}$VL[a]			*P*
	40–49	RNA$^+$	RNA$^-$	
Whole population, No.	240	507	500	
Years of VL <50 copies/mL pre-T0	0.2 (0.0–0.6)	1.3 (0.4–2.8)	2.8 (1.2–4.5)	<.0001
Month of follow-up post-T0	14 (1–33)	14 (2–21)	14 (4–17)	.69
Number of VL measurements post-T0	4 (1–13)	4 (1–13)	4 (1–11)	<.0001
RFH patients, No.[b]	164	352	375	
Years of VL <50 copies/mL pre-T0, median (IQR)	0.2 (0.0–1.0)	1.9 (0.5–3.2)	3.2 (1.9–4.7)	<.0001
Months of follow-up post-T0, median (IQR)	14 (12–31)	14 (12–21)	14 (4–17)	.12
Number of VL measurements post-T0, median (IQR)	5 (1–13)	4 (1–13)	4 (1–11)	<.0001
Pre-HAART VL, $\log_{10}$ copies/mL, median (IQR)	5.3 (4.7–5.8)	5.1 (4.6–5.6)	5.0 (4.5–5.6)	.04
Years of HAART, median (IQR)	2.9 (1.1–7.9)	5.8 (2.4–9.8)	6.9 (4.1–10.1)	<.0001
CD4 cell count, cells/mm^3, median (IQR)	405 (268–559)	564 (413–725)	581 (419–794)	<.0001
Age, years, median (IQR)	40 (34–44)	43 (38–48)	43 (38–48)	<.0001
Male, No. (%)	112 (68.3)	267 (75.9)	284 (75.7)	.14
Ethnicity, No. (%)				
White	79 (48.2)	233 (66.2)	253 (67.5)	.0006
Black	67 (40.9)	95 (27.0)	98 (26.1)	
Other	18 (11.0)	24 (6.8)	24 (6.4)	
Risk group, No. (%)				
MSM	75 (45.7)	212 (60.2)	219 (58.4)	.0001
Heterosexual	86 (52.4)	116 (33.0)	131 (34.9)	
Other	3 (1.8)	24 (6.8)	25 (6.7)	
HAART regimen, No. (%)				
NRTI + NNRTI	26 (15.9)	95 (27.0)	147 (39.2)	<.0001
NRTI + PI/r	114 (69.5)	196 (55.7)	165 (44.0)	
NRTI + PI	1 (0.6)	10 (2.8)	6 (1.6)	
NRTI only	3 (1.8)	16 (4.6)	11 (2.9)	
Other[c]	20 (12.2)	35 (9.9)	46 (12.3)	
Adherence (% refilled prescriptions)[d]				
Unavailable	42 (25.6)	38 (10.8)	20 (5.3)	<.0001
≤60%	27 (16.5)	47 (13.4)	40 (10.7)	
>60 to ≤80	20 (12.2)	46 (13.0)	41 (10.9)	
>80 to ≤95	35 (21.3)	76 (21.6)	95 (25.3)	
>95 to ≤99.9	11 (6.7)	53 (15.1)	63 (16.8)	
100	29 (17.7)	92 (26.1)	116 (30.9)	

Abbreviations: HAART, highly active antiretroviral therapy; IQR, interquartile range; MSM, men who have sex with men; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, unboosted protease inhibitor; PI/r, ritonavir-boosted protease inhibitor; RFH, Royal Free Hospital; T0, time zero (arbitrarily selected time point during HAART); VL, viral load.

[a] $_{T0}$VL: First viral load measurement prior to the start of follow-up, taken at an arbitrary time point during HAART and grouped into 40–49 copies/mL (40–49), RNA detected <40 copies/mL (RNA$^+$), and RNA not detected (RNA$^-$).

[b] RFH patients: individuals with demographic and clinical data from the Royal Free Hospital.

[c] The most commonly prescribed antiretrovirals comprised tenofovir (65.9%, 61.9%, and 58.7% in the $_{T0}$VL groups 40–49 copies/mL, RNA$^+$, and RNA$^-$, respectively), emtricitabine (43.9%, 42.6%, 42.7%), lamivudine (34.8%, 34.7%, 37.3%) and abacavir (25.6%, 32.1%, 29.6%) for the NRTIs; efavirenz (16.5%, 19.6%, 28.0%) for the NNRTIs; and ritonavir-boosted lopinavir (48.8%, 39.5%, 30.4%), saquinavir (20.1%, 14.8%, 18.1%), and atazanavir (17.1%, 15.1%, 16.5%) for the PIs. Other regimens: NRTI + NNRTI + PI/r (n = 7, 12, and 15 in the 3 $_{T0}$VL groups, respectively), and other PI/r-based combinations (n = 13, 23, and 31).

[d] Adherence was estimated on the basis of the proportion of days covered by drug prescription refill pharmacy data for at least 3 drugs in the previous 6 months.

Drug Resistance at Rebound

Stored plasma samples were retrieved from 68 of 80 patients with rebound >400 copies/mL for resistance testing at rebound. The median interval between the first VL measurement >400 copies/mL and the resistance test was 5.5 weeks (IQR, 2–8.5 weeks). Among 63 test results, proportions showing ≥1 major RAM were 12 of 35 (34.3%), 9 of 21 (42.9%), and 2 of 7 (28.6%) with $_{T0}$VL groups 40–49 copies/mL, RNA$^+$, and RNA$^-$, respectively ($P = .75$). The breakdown by drug class was nucleoside/nucleotide reverse-transcriptase inhibitors (NRTIs), 18 of

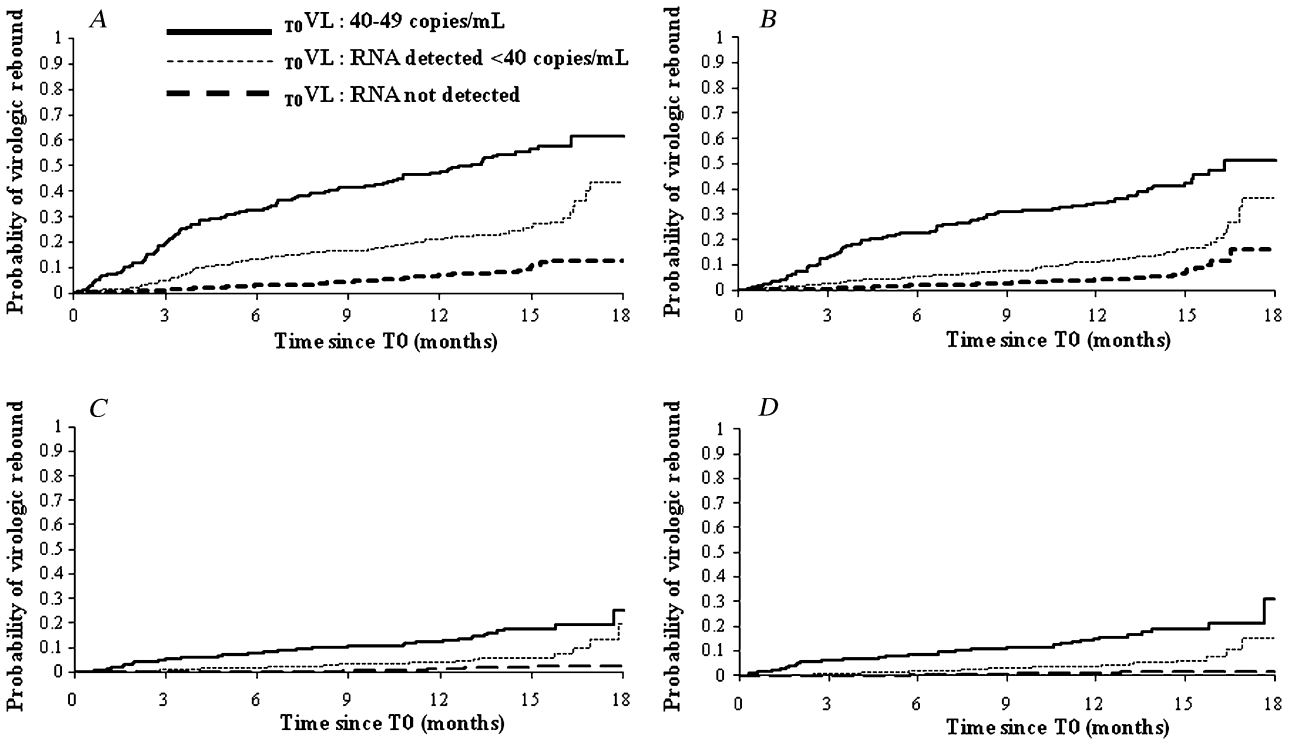

Figure 1. Time to virologic rebound according to the T0 viral load (VL) and 4 definitions (*A*–*D*) of rebound. *A*, Single viral load >50 copies/mL. *B*, confirmed or last available viral load >50 copies/mL. *C*, confirmed or last available viral load >400 copies/mL. *D*, confirmed or last available viral load >400 copies/mL or single viral load >400 copies/mL followed by a treatment change. Whole population (*A*–*C*); Royal Free Hospital population (*D*). $P < .0001$ for all analyses (log rank test). Abbreviation: T0, time zero (arbitrarily selected time point during highly active antiretroviral therapy).

23 (78.3%); NNRTIs, 10 of 23 (43.5%); and protease inhibitors (PIs), 5 of 23 (21.7%). Overall, 17 of 23 (73.90%) patients had ≥1 RAM affecting drugs taken at the time of rebound.

DISCUSSION

In patients receiving HAART and showing VL <50 copies/mL, HIV-1 RNA detection below this cutoff predicted a risk of rebound >50 and >400 copies/mL during follow-up. The effect was independent of other recognized determinants of rebound, including adherence levels. Although further supportive evidence is desirable, the findings indicate that the goal of HAART may need to be revised to a lower cutoff than 50 copies/mL.

Using sensitive testing methods, HIV-1 RNA can be detected in plasma in a large proportion (>80%) of patients receiving HAART and showing a VL <50 copies/mL for many years [7–14]. Studies have shown that HIV-1 RNA levels decline to <50 copies/mL within approximately 12 weeks of starting therapy [8, 9]. Once levels are <50 copies/mL, they continue to decline for several months before reaching a plateau at approximately 3–10 copies/mL [7–14]. The source of this residual viremia is much debated [7–14, 19–22]. In recent studies, residual viremia was unresponsive to HAART intensification [11, 13]. Although not excluding ongoing replication in "sanctuary" sites, the lack of response provides support to the hypothesis that residual viremia does not reflect ongoing virus replication, but rather originates from virus reactivation in latently infected cells, with rapid suppression by HAART. The alternative model proposes ongoing virus replication due to suboptimal drug levels, penetration, or activity. It seems plausible that the 2 models may coexist. In a previous study, abacavir intensification of a 2-drug regimen of efavirenz and indinavir in patients with RNA levels below 50 copies/mL and above 2.5 copies/mL lowered the levels to <2.5 copies/mL [23]. Conversely, RNA levels have been seen to increase before rebounding >50 copies/mL in patients simplifying triple HAART to ritonavir-boosted atazanavir [24]. HIV-1 genetic evolution has also been observed with RNA level above 6.5 copies/mL [12]. These findings suggest that there is a threshold level of viremia <50 copies/mL that is indicative of ongoing virus replication. Our data are consistent with this view, and although they fall short of precisely defining a cutoff, the observed relevance of detecting RNA <40 copies/mL suggests that the threshold may be even lower than 40 copies/mL.

Table 2. Cox Proportional Hazards Regression Analyses of Predictors of Confirmed (or Last Available) Virologic Rebound >50 Copies/mL

	Univariable Results			Multivariable Results		
Whole Population	HR	95% CI	*P*	HR	95% CI	*P*
$_{T0}$VL group			<.0001			<.0001
40–49	7.76	5.23–11.53		4.67	2.91–7.47	
RNA^+	2.47	1.64–3.70		1.97	1.25–3.11	
$^-RNA^-$	1.00	…		1.00	…	
Length VL <50 copies/mL pre-T0, per year longer	0.62	0.55–0.70		0.74	0.65–0.83	
RFH patients						
$_{T0}$VL group						
40–49	8.77	5.37–14.32	<.0001	4.68	2.40–9.12	<.0001
RNA^+	2.61	1.57–4.33		2.33	1.26–4.31	
RNA^-	1.00	…		1.00	…	
Length VL <50 copies/mL pre-T0, per year longer	0.64	0.56–0.72	<.0001	0.79	0.69–0.91	.0005
Pre-HAART VL, per 1 $\log_{10}$ copies/mL higher	1.13	0.89–1.43	.32	1.04	0.80–1.33	.79
Time since starting HAART, per year longer	0.94	0.90–0.98	.003	1.06	0.99–1.15	.10
CD4 count, per 100 cells/mm^3 higher	0.82	0.77–0.89	<.0001	0.92	0.84–1.00	.06
Age, per 10 years older	0.70	0.57–0.86	.0005	0.80	0.61–1.04	.09
Sex						
Male	0.69	0.49–0.99	.05	0.81	0.45–1.45	.47
Female	1.00	…		1.00	…	
Ethnicity						
White	1.00	…	<.0001	1.00	…	.11
Black	2.31	1.62–3.28		1.91	1.00–3.63	
Other	2.09	1.19–3.66		1.50	0.75–2.98	
Risk group						
MSM	1.00	…	.02	1.00	…	.36
Heterosexual	1.66	1.18–2.34		0.83	0.41–1.70	
Other	1.29	0.62–2.68		1.70	0.63–4.64	
HAART regimen						
NNRTI-based	0.27	0.16–0.45	<.0001	0.40	0.21–0.77	.002
Other	0.75	0.47–1.19		1.40	0.79–2.48	
PI-based	1.00	…		1.00	…	
Adherence (% refilled prescriptions)						
Unavailable	1.40	0.89–2.21	.0008	0.59	0.32–1.10	.23
≤95%	1.00	…		1.00	…	
>95%	0.56	0.38–0.83		0.87	0.54–1.39	

Abbreviations: CI, confidence interval; HAART, highly active antiretroviral therapy; HR, hazard ratio; MSM, men who have sex with men; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RFH, Royal Free Hospital; T0, time zero (arbitrarily selected time point during HAART); VL, viral load.

Less than half of the patients who experienced rebound >400 copies/mL showed RAMs, which suggests poor adherence as a major driver of rebound. Resistance is only 1 of the possible negative outcomes of rebound. Previous studies have shown an association between VL >400 copies/mL during HAART and mortality [25]. Measuring adherence is complex, and all methodologies have shortcomings. We previously validated a measure of adherence based on pharmacy prescription records [16, 17]. We showed that calculating the proportion of days covered by a prescription is a simple way of estimating adherence and predicting virologic failure. In this study, adherence was not associated with rebound in adjusted analyses, indicating that the viral load was a more powerful predictor. We also measured efavirenz plasma levels in the $_{T0}$VL sample as a possible indicator of adherence. We detected no significant differences in drug levels between groups. It should be noted that, given the retrospective nature of the study, the timing of the last HAART dose relative to the collection of the $_{T0}$VL sample was not available. Efavirenz levels, however, are not expected to fluctuate considerably.

We found that a single VL measurement had a strong predictive value for rebound. In clinical practice, it is recommended

Table 3. Cox Proportional Hazards Regression Analyses of Predictors of Confirmed (or Last Available) Virologic Rebound >400 Copies/mL

	Univariable Results			Multivariable Results		
Whole Population	HR	95% CI	*P*	HR	95% CI	*P*
$_{T0}$VL group						
40–49	8.64	4.30–17.35	<.0001	6.91	2.90–16.47	<.0001
RNA^{+}	3.01	1.48–6.15		2.88	1.24–6.69	
RNA^{-}	1.00	…		1.00	…	
Length VL <50 copies/mL pre-T0, per year longer	0.67	0.56–0.80	<.0001	0.82	0.69–0.97	.02
RFH patients						
$_{T0}$VL group						
40–49	13.92	5.39–35.94	<.0001	10.71	3.30–34.81	<.0001
RNA^{+}	4.16	1.56–11.10		3.78	1.23–11.59	
RNA^{-}	1.00	…		1.00	…	
Length VL <50 copies/mL pre-T0, per year longer	0.70	0.58–0.84	<.0001	0.88	0.72–1.06	.15
Pre-HAART VL, per 1 $\log_{10}$ copies/mL higher	0.79	0.59–1.06	.13	0.74	0.52–1.05	.10
Time since starting HAART, per year longer	0.95	0.89–1.01	.10	1.14	1.02–1.27	.03
CD4 cell count, per 100 cells/mm^3 higher	0.88	0.79–0.99	.02	1.00	0.87–1.15	.97
Age, per 10 years older	0.72	0.52–1.00	.05	1.07	0.71–1.62	.74
Sex						
Male	0.69	0.39–1.21	.21	1.49	0.65–3.38	.35
Female	1.00	…		1.00	…	
Ethnicity						
White	1.00	…	.002	1.00	…	.17
Black	2.73	1.57–4.77		2.40	0.91–6.36	
Other	1.90	0.72–5.00		1.85	0.59–5.83	
Risk group						
MSM	1.00	…	.03	1.00	…	.63
Hetero	2.07	1.19–3.61		1.36	0.48–3.85	
Other	1.86	0.64–5.41		2.07	0.50–8.60	
HAART regimen						
NNRTI-based	0.32	0.14–0.71	.007	0.46	0.17–1.23	.23
Other	0.80	0.39–1.66		0.99	0.40–2.46	
PI-based	1.00	…		1.00	…	
Adherence (% refilled prescriptions)						
Unavailable	1.32	0.65–2.68	.009	0.99	0.39–2.47	.99
≤95%	1.00	…		1.00	…	
>95%	0.43	0.22–0.83		0.96	0.45–2.07	

Abbreviations: CI, confidence interval; HAART, highly active antiretroviral therapy; HR, hazard ratio; MSM, men who have sex with men; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RFH, Royal Free Hospital; T0, time zero (arbitrarily selected time point during HAART); VL, viral load.

that a detectable VL result during HAART should be confirmed in a subsequent sample prior to making management decisions [1]. In this study, the risk of rebound was associated with confirmed RNA detection in the subsequent VL measurement. However, it should be noted that the next available VL test was performed approximately 12–16 weeks after T0, whereas prompt confirmation of viremia is generally sought in clinical practice. Further studies are required to determine the relevance of seeking immediate confirmation.

Our analysis included patients regardless of treatment duration and was adjusted for this factor. Longer duration of suppression <50 copies/mL prior to T0 was associated with a reduced risk of rebound, in line with previous reports [26–28]. To apply our results to clinical practice, one should take into account that the median times to RNA levels <50 copies/mL, <40 copies/mL, and to RNA^{-} were 4.1, 4.4, and 6.2 months, respectively. This, however, does not detract from the strong independent predictive value of the $_{T0}$VL for the risk of virologic rebound. Although we did not observe significant differences between efavirenz-treated and lopinavir/ritonavir-treated patients, this analysis should be repeated with a larger data set, preferably within a randomized study. The composition of the

Table 4. Cox Proportional Hazards Model Investigating the Effect of Confirmed Viremia on the Risk of Viral Load Rebound >400 Copies/mL

	Univariable			Multivariable[a]		
VL at T0 and T1	HR	95% CI	*P*	HR	95% CI	*P*
$_{T0}$VL RNA$^-$	1	…	<.001	1	…	<.0001
$_{TO}$VL 40–49 copies/mL or RNA$^+$ $_{T1}$VL RNA$^-$	1.80	0.55–5.92		1.52	0.37–6.22	
$_{TO}$VL 40–49 copies/mL or RNA$^+$ $_{T1}$VL, any RNA detected <400 copies/mL	13.39	5.23–34.23		10.42	3.36–32.33	

[a] Adjusted for the variables indicated in Table 3.

Abbreviations: CI, confidence interval; HR, hazard ratio; T0, time zero (arbitrarily selected time point during HAART); T1, results obtained at subsequent VL measurement; VL, viral load.

regimen appears to play a significant role in both the $_{T0}$VL result and the subsequent risk of rebound. Patients on NNRTI-based regimens were more prevalent in the RNA$^-$ group and showed a lower risk of rebound. It has been previously observed that, compared with patients on other regimens, those receiving NNRTI-based HAART were less likely to experience viremia between 50 and 400 copies/mL in the first year after achieving <50 copies/mL, and were also less likely to rebound >400 copies/mL [5]. Others have reported a lower risk of low-level viremia in patients receiving NNRTIs (especially nevirapine) compared with other regimens [29, 30]. These observations may reflect different tolerability profiles, which may influence adherence, and different pharmacokinetic properties, which may influence penetration into "sanctuary" sites and modify the consequences of incomplete adherence. In addition, given that NNRTI-based regimens are the recommended first-line therapy in the United Kingdom, patients on these regimens were either less experienced or may have been perceived as being at lesser risk of nonadherence than those given PIs. Controlled studies are needed to confirm the findings.

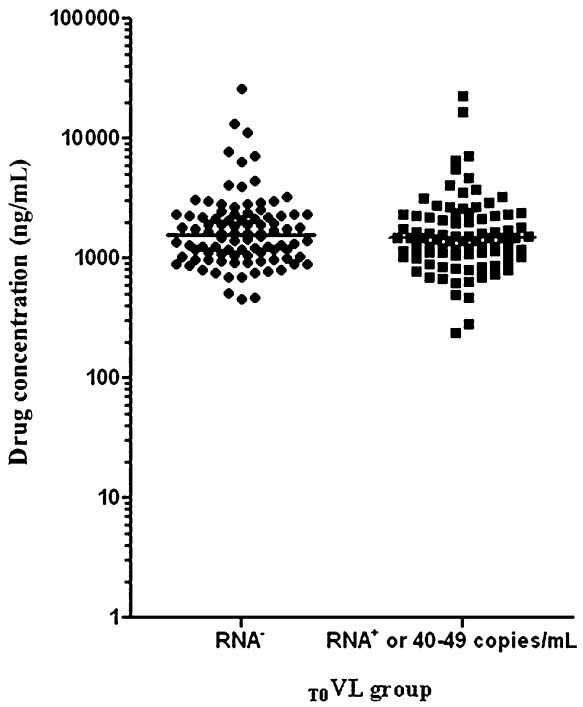

Figure 2. Efavirenz plasma concentration at T0 in patients receiving efavirenz-based highly active antiretroviral therapy (HAART). Lines indicate median values. Abbreviations: T0, time zero (arbitrarily selected time point during HAART), VL, viral load.

Our study has limitations. Demographic and clinical data, including adherence estimations and drug levels, were available for a subset of patients, albeit this was a large and well-defined subset. We were unable to perform further testing to identify a level of viremia below which the risk of virologic rebound was no longer apparent. We were also unable to assess the performance of other commercial VL assays, and must caution against uncritically extrapolating our findings to assays with different detection and reporting ranges. Nonetheless, our study demonstrates that in treated patients monitored with the RealTime assay, a VL <50 but >40 copies/mL, and, to a lesser extent, qualitative RNA detection <40 copies/mL, indicate that treatment efficacy should be reviewed. Further studies, including possibly a controlled trial, are required to support these recommendations.

Notes

Author contributions. T. D. collected the data, performed the analysis, and contributed to the writing of the manuscript; C. S. performed the statistical analysis; P. V. contributed to data collection and the resistance analysis; V. C. performed the adherence estimations; A. O. performed the pharmacology study and contributed to the analysis; M. J. contributed to data collection; A. P. contributed to overall study design and analysis; A. M. G. designed the study, contributed to data collection and analysis, and wrote the manuscript.

Audit project approved by the Royal Free Hospital Ethics Committee.

Financial support. This work was supported by The Royal Free Hampstead National Health Service (NHS) Trust, which contributed funding toward the performance of resistance testing (R&D fund 07889); and by NEAT (European AIDS Treatment Network), which contributed toward the plasma drug concentration analysis.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Gazzard BG, Anderson J, Babiker A, et al. British HIV Association guidelines for the treatment of HIV-1-infected adults with antiretroviral therapy 2008. HIV Med **2008**; 9:563–608.
2. Thompson MA, Aberg JA, Cahn P, et al. Antiretroviral treatment of adult HIV infection: 2010 recommendations of the International AIDS Society—USA panel. JAMA **2010**; 304:321–33.
3. Panel on Antiretroviral Guidelines for Adult and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. United States Department of Health and Human Sciences **2011**. Available at: http://www.aidsinfo.nih.gov/contentfiles/AdultandAdolescentGL.pdf. Accessed 1 December 2011.
4. Mocroft A, Phillips AN, Gatell J, et al. Normalisation of CD4 counts in patients with HIV-1 infection and maximum virological suppression who are taking combination antiretroviral therapy: an observational cohort study. Lancet **2007**; 370:407–13.
5. Geretti AM, Smith C, Haberl A, et al. Determinants of virological failure after successful viral load suppression in first-line highly active antiretroviral therapy. Antivir Ther **2008**; 13:927–36.
6. Margot NA, Enejosa J, Cheng AK, et al. Development of HIV-1 drug resistance through 144 weeks in antiretroviral-naïve subjects on emtricitabine, tenofovir disoproxil fumarate, and efavirenz compared with lamivudine/zidovudine and efavirenz in study GS-01-934. J Acquir Immune Defic Syndr **2009**; 52:209–21.
7. Dornadula G, Zhang H, VanUitert B, et al. Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. JAMA **1999**; 282:1627–32.
8. Di Mascio M, Dornadula G, Zhang H, et al. In a subset of subjects on highly active antiretroviral therapy, human immunodeficiency virus type 1 RNA in plasma decays from 50 to <5 copies per milliliter, with a half-life of 6 months. J Virol **2003**; 77:2271–5.
9. Maldarelli F, Palmer S, King MS, et al. ART suppresses plasma HIV-1 RNA to a stable set point predicted by pretherapy viremia. PLoS Pathog **2007**; 3:e46.
10. Palmer S, Maldarelli F, Wiegand A, et al. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. Proc Natl Acad Sci USA **2008**; 10:3879–84.
11. Dinoso JB, Kim SY, Wiegand AM, et al. Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. Proc Natl Acad Sci USA **2009**; 106:9403–8.
12. Shiu C, Cunningham CK, Greenough T, et al. Identification of ongoing human immunodeficiency virus type 1 (HIV-1) replication in residual viremia during recombinant HIV-1 poxvirus immunizations in patients with clinically undetectable viral loads on durable suppressive highly active antiretroviral therapy. J Virol **2009**; 83:9731–42.
13. Archin NM, Cheema M, Parker D, et al. Antiretroviral intensification and valproic acid lack sustained effect on residual HIV-1 viremia or resting CD4+ cell infection. PLoS One **2010**; 5:e9390.
14. Bailey JR, Sedaghat AR, Kieffer T, et al. Residual human immunodeficiency virus type 1 viremia in some patients on antiretroviral therapy is dominated by a small number of invariant clones rarely found in circulating $CD4^+$ T cells. J Virol **2006**; 80:6441–57.
15. Labbett W, Garcia-Diaz A, Fox Z, et al. Comparative evaluation of the ExaVirTM Load version 3 reverse transcriptase assay for the measurement of HIV-1 plasma viral load. J Clin Microbiol **2009**; 47:3266–70.
16. Cambiano V, Lampe FC, Rodger AJ, et al. Use of a prescription-based measure of antiretroviral therapy adherence to predict viral rebound in HIV-infected individuals with viral suppression. HIV Med **2010**; 11:216–24.
17. Cambiano V, Lampe FC, Rodger AJ, et al. Long-term trends in adherence to antiretroviral therapy from start of HAART. AIDS **2010**; 24:1153–62.
18. Geretti AM, Fox ZV, Booth CL, et al. Low-frequency K103N strengthens the impact of transmitted drug resistance on virologic responses to first-line efavirenz or nevirapine-based highly active antiretroviral therapy. J Acquir Immune Defic Syndr **2009**; 52:569–73.
19. Persaud D, Siberry GK, Ahonkhai A, et al. Continued production of drug-sensitive human immunodeficiency virus type 1 in children on combination antiretroviral therapy who have undetectable viral loads. J Virol **2004**; 78:968–79.
20. Kieffer TL, Finucane MM, Nettles RE, et al. Genotypic analysis of HIV-1 drug resistance at the limit of detection: virus production without evolution in treated adults with undetectable HIV loads. J Infect Dis **2004**; 189:1452–65.
21. Brennan TP, Woods JO, Sedaghat AR, Siliciano JD, Siliciano RF, Wilke CO. Analysis of human immunodeficiency virus type 1 viremia and provirus in resting $CD4^+$ T cells reveals a novel source of residual viremia in patients on antiretroviral therapy. J Virol **2009**; 83:8470–81.
22. Tobin N H, Learn GH, Holte SE, et al. Evidence that low-level viremias during effective highly active antiretroviral therapy result from two processes: expression of archival virus and replication of virus. J Virol **2005**; 79:9625–34.
23. Havlir DV, Strain MC, Clerici M, et al. Productive infection maintains a dynamic steady state of residual viremia in human immunodeficiency virus type 1-infected persons treated with suppressive antiretroviral therapy for five years. J Virol **2003**; 77:11212–9.
24. Wilkin TJ, McKinnon JE, DiRienzo AG, et al. Regimen simplification to atazanavir-ritonavir alone as maintenance antiretroviral therapy: final 48-week clinical and virologic outcomes. J Infect Dis **2009**; 199: 866–71.
25. Lohse N, Kronborg G, Gerstoft J, et al. Virological control during the first 6–18 months after initiating highly active antiretroviral therapy as a predictor for outcome in HIV-infected patients: a Danish, population-based, 6-year follow-up study. Clin Infect Dis **2006**; 42:136–44.
26. Mocroft A, Ruiz L, Reiss P, et al. Virological rebound after suppression on highly active antiretroviral therapy. AIDS **2003**; 17:1741–51.
27. Benzie AA, Bansi LK, Sabin CA, et al. Increased duration of viral suppression is associated with lower viral rebound rates in patients with previous treatment failures. AIDS **2007**; 21:1423–30.
28. Smith CJ, Phillips AN, Dauer B, et al. Factors associated with viral rebound among highly treatment-experienced HIV-positive patients who have achieved viral suppression. HIV Med **2009**; 10:19–27.
29. Bonora S, Nicastri E, Calcagno A, et al. Ultrasensitive assessment of residual HIV viraemia in HAART-treated patients with persistently undetectable plasma HIV-RNA: a cross-sectional evaluation. J Med Virol **2009**; 81:400–5.
30. Haïm-Boukobza S, Morand-Joubert L, Flandre P, et al. Higher efficacy of nevirapine than efavirenz to achieve HIV-1 plasma viral load below 1 copy/mL. AIDS **2011**; 25:341–4.