Advances in detection and monitoring of plasma viremia in HIV-infected individuals receiving antiretroviral therapy

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Purpose of review
This review will describe advances in detection and results of monitoring persistent viremia in patients on long-term suppressive therapy. In addition, the review explores the usefulness of these methods in determining the effectiveness of new HIV-1 eradication strategies in purging persistent HIV-1 reservoirs.

Recent findings
Quantification of plasma HIV-1 RNA levels remains essential for determining the success of combination antiretroviral therapy (cART) in treated patients. Recently, several new platforms with improved sensitivity for quantifying HIV-1 RNA have been developed and the application of these assays has revealed that low-level viremia persists in patients on suppressive therapy. In addition, new technological advances such as digital PCR have been proposed to increase the sensitivity of measuring and characterizing persistent HIV-1 viremia. The application of these assays will be important in determining the effectiveness of future HIV-1 eradication strategies.

Summary
The level of HIV-1 RNA in patient plasma remains an important marker for determining the success of cART. New sensitive assays have found that HIV-1 persists in the plasma of patients on suppressive therapy that may have implications for the clinical management of this disease and strategies for eliminating HIV-1 infection.

Keywords
eradication strategies, HIV-1 monitoring, persistent viremia, plasma HIV-1 RNA

INTRODUCTION

Recent observational studies have shown that combination antiretroviral therapy (cART) reduces the probability of HIV-1 transmission and provides clinical benefits to those on therapy [1▪▪]. As a result, new treatment guidelines for patient care recommend offering cART to patients immediately upon diagnosis of HIV infection regardless of their $CD4^+$ cell count [2▪]. In addition, early initiation of cART has been associated with lower levels of cell-associated HIV-1 DNA and lower transient viral set point after treatment interruption [3,4▪,5]. With these new guidelines, monitoring the levels of HIV-1 RNA in patient plasma after initiation of cART has become essential and more prevalent [2▪,6–8]. When treatment is initiated, HIV-1 RNA levels should be monitored every 3 months and plasma HIV-1 RNA should decrease to less than 50 copies/ml by 24 weeks regardless of prior treatment experience [9]. New third-generation real-time HIV-1 RNA assays have a lower limit of quantification to 40 or 20 copies/ml and report qualitative detection below these levels in patients receiving effective therapy [10,11]. Current antiretroviral therapy effectively suppresses but does not eradicate HIV-1 infection. During cART, reduction of HIV-1 RNA levels to less than 50 copies/ml is frequently achieved; however, persistent low-level viremia has been detected in plasma and cells of HIV-infected patients using these third-generation HIV-1 RNA assays and other research-based

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Curr Opin HIV AIDS 2013, 8:000–000
DOI:10.1097/COH.0b013e32835d80af

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KEY POINTS

- Quantification of plasma HIV-1 RNA levels is essential during cART.
- HIV-1 RNA persists in the plasma of patients on long-term suppressive therapy albeit at low levels.
- The source of persistent viremia is currently unknown.
- Reactivation of latently infected $CD4^+$ T cells contributes to persistent viremia during suppressive therapy.
- Eradication strategies should include reactivation of latent HIV-1 reservoirs and monitoring of persistent viremia.

ultrasensitive assays [12–17]. The source and significance of this persistent virus is currently unknown but recent studies show that detectable low-level HIV-1 RNA of less than 50 copies/ml can result in viral rebound albeit the lower the level of persistent viremia the less likely a viral rebound will occur [18,19]. Therefore, monitoring persistent virus is not only crucial for determining the effectiveness of cART but will be essential for monitoring the success of future eradication strategies for curing HIV-1 infection. This review will discuss the sensitivity and accuracy of new third-generation HIV-1 RNA assays for detecting and monitoring persistent viremia in patients initiating and maintaining cART. The findings of research-based ultrasensitive assays that measure HIV RNA down to a single copy will be described. Because of the fact that new agents that reduce HIV latency are currently in clinical trials, this review will describe new technologies and methods being developed for quantifying persistent viremia in patients on long-term therapy, which may ultimately be used for assessing the effectiveness of these new HIV-1 eradication strategies.

THERAPEUTIC MONITORING OF PLASMA HIV-1 RNA LEVELS

Three general methods are currently used for monitoring the success of cART in HIV-1 infected patients: standard endpoint PCR assays with a detection limit of 400 copies/ml; ultrasensitive endpoint PCR assays with a detection limit of 50 copies/ml; and real-time PCR assays with lower limits of detection ranging from 20 to 48 copies/ml. Cross-platform comparisons have found good correlation between the endpoint PCR assays and the real-time PCR assays, but the new third-generation real-time methodologies, Roche Taqman v2.0 and Abbott RealTime 0.6 ml, have broader reportable ranges and are the most sensitive with lower detection limits of 20 and 40 copies/ml, respectively [20–26]. Additional comparative studies have shown better detection rates of citrated versus EDTA plasma control samples containing 25–50 copies/ml by these new real-time assays [27]. This may be due to EDTA chelating the manganese in the PCR mix after RNA extraction that can affect the efficiency of the PCR reaction. However, these third-generation real-time PCR assays were very specific and no evidence of carryover contamination leading to false positives was detected [27]. Overall, the lower detection limits of the new real-time assays may result in increased measurements of viral blips or transient detectable viral RNA and the impact of these measurements on clinical studies will have to be evaluated [28]. The increased occurrence of measurable low-level HIV-1 RNA by these more sensitive real-time assays should be carefully interpreted. HIV-1 treatment is not curative; rather, the virus persists and is merely suppressed during therapy. Although low-level viral RNA has been found to be a predictor of subsequent suboptimal virological control, it may not lead to virological failure or require a regimen change [29,30].

LEVELS OF PERSISTENT VIREMIA IN PATIENTS ON LONG-TERM SUPPRESSIVE THERAPY AS DETERMINED BY THE SINGLE-COPY ASSAY

Application of experimental assays with sensitivities in the range of 10 copies/ml has demonstrated that many patients with viremia suppressed to less than 50 copies/ml have persistently detectable HIV-1 RNA in plasma [31,32]. To investigate the source of residual viremia and to quantify plasma HIV-1 RNA below 50 copies/ml, a more sensitive and precise assay than had previously existed was developed. This assay uses larger plasma sample volumes (7 ml), improved nucleic acid isolation and purification techniques, and real-time RT-PCR to accurately quantify HIV-1 in plasma samples to 1 copy/ml with a limit of detection of one copy in 3.8 ml of plasma, making it 20–50 times more sensitive than currently approved commercial assays [15]. Because the assay has a detection limit of 1 RNA copy it is known as the single copy assay (SCA). To control for recovery of HIV-1, each plasma sample is spiked with 30 000 copies of an internal virion standard derived from an unrelated retrovirus, the replication competent avian sarcoma-leukosis retrovirus vector RCAS BP(A) [15].

SCA gave virtually identical results when compared with a standard (bDNA) assay on samples from

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patients initiating triple therapy, but revealed the presence of low-level viremia that persisted long after viremia became undetectable by the standard bDNA assay [33]. Additional studies using this assay have shown that 77% of samples from patients with plasma HIV-1 RNA consistently below 50 copies/ml had quantifiable viremia [15,33]. Notably, in one study of 40 patients, persistent viremia was evident even after 7 years of therapy and all the patients had at least one sample with detectable viremia over the course of those 7 years [34]. The level of viremia correlated with pretherapy plasma HIV-1 RNA but not with the specific treatment regimen. Furthermore, a nonlinear mixed effects model revealed a biphasic decline in plasma RNA levels occurring over weeks 60–384: an initial phase of decay with a half-life of 39 weeks and a subsequent phase with no perceptible decay where levels of plasma HIV RNA remained stable ranging from 1–5 copies/ml or less with an overall median viral load of 3 copies/ml [34]. The level of pretherapy viremia extrapolated for each phase of decay was significantly correlated with total baseline viremia for each patient ($R^2 = 0.27$, $P = 0.001$ and $R^2 = 0.19$, $P < 0.005$, respectively). These findings support a biological link between the extent of overall baseline viral infection and the infection of long-lived reservoirs. The clinical significance of low-level viremia, especially between 1 and 10 copies/ml, in patients on long-term suppressive therapy is currently not established. However, these data suggest that there is a constant source of viremia in patients on long-term suppressive therapy.

The source and dynamics of persistent viremia in treated patients is currently under investigation. Recent studies of treatment intensification with raltegravir have produced conflicting results: no reductions in low-level plasma viremia indicating the source of persistent viremia is long-lived HIV-infected cells and increases in episomal HIV DNA indicating active replication persists in some infected individuals on suppressive therapy [35,36]. These results indicate that low-level viremia could arise from several different sources. These sources may include ongoing replication cycles in cells located in sanctuary sites wherein drug levels are suboptimal, long-lived HIV-infected cells that replicate and produce virus, and/or activation of viral expression from latently infected cell reservoirs [13,33,37–39]. Evidence for the view that long-lived cells are the probable source of persistent viremia includes four studies using a number of different treatment protocols that show no perceptible change in persistent viremia in patients receiving intensified treatment [36,40,41▪,42]. Moreover, genetically homogeneous sub-populations can often be observed in patients under long-term treatment, as well as in the viral population that rebounds during treatment interruption further indicating that persistent viremia arises from long-lived latently infected cells rather than ongoing cycles of replicating virus [43,44]. The role of ongoing HIV-1 replication in tissue compartments and cellular reservoirs, however, remains to be defined as a number of findings suggest that the reservoir is largely established and maintained in tissues, and that the infected cells circulating in blood may not necessarily be representative of the much larger population of infected cells in tissue [45▪▪]. For example, sequences of persistent HIV populations in plasma are often not found in peripheral blood resting memory $CD4^+$ T cells [43,46]. On the contrary, a recent study of two patients found that HIV-1 sequences isolated from virus outgrowth assays of resting memory $CD4^+$ T cells matched the predominant plasma clones [47▪]. These results indicate that persistent viremia is derived in part from reactivation of latent proviruses from resting memory $CD4^+$ T cells.

PLASMA VIREMIA DURING DISRUPTION OF HIV-1 LATENCY IN PATIENTS ON LONG-TERM SUPPRESSIVE THERAPY

One well defined latent HIV-1 reservoir is infected resting memory $CD4^+$ T cells carrying an integrated form of the viral genome [48]. The precise molecular mechanisms contributing to the generation and maintenance of this reservoir, however, remain to be elucidated. The scarcity of such cells, even among those carrying integrated HIV DNA, makes these cells extremely difficult to identify, isolate, and study. However, this latent reservoir is now widely recognized as the major obstacle to HIV-1 eradication [49]. Approaches for the reactivation of HIV-1 from resting memory T cells and the subsequent elimination of these cells include T-cell activating cytokines [50–52], T-cell receptor and T-cell receptor signaling pathway agonists [53–57], histone deacetylase inhibitors [58–60], histone methyltransferase inhibitors [61], DNA methylase inhibitors [62,63], and compounds such as 5-hydroxynaphthalene-1,4-dione [64], and disulfiram [65] that reverse latency through mechanisms that are not yet completely understood. Clinical trials of disulfiram and the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) have been initiated [66▪▪,67▪▪]. In conducting these two different HIV-1 reactivation trials, HIV-infected patients on long-term suppressive therapy with undetectable plasma viremia (by conventional methods) for 6 months to 1 year have been given: 14 daily doses of disulfiram (500 mg) or two separate doses of SAHA (400 mg) given 4–5 weeks apart. To evaluate the effectiveness of these two interventions in reactivating latent

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HIV-1, many different analyses were conducted including measuring plasma HIV-1 RNA levels by SCA. An interim analysis of persistent viremia was measured for 14 patients prior, during, and after treatment with disulfiram (15 separate measurements) and an increase in viremia was measured during disulfiram therapy and after therapy was discontinued (53 and 88% increase, respectively) compared with baseline but these increases in plasma viremia were found to be statistically insignificant [67▪▪]. For the eight patients enrolled in the SAHA study, no significant change in plasma HIV-1 RNA levels were detected 8–24 h after exposure to SAHA compared with baseline, although increased levels of intracellular HIV-1 RNA were measured in resting memory $CD4^+$ T cells [66▪▪]. To date, reactivation of latent HIV-1 RNA from resting memory cells does not translate into statistically significant increases in plasma HIV-1 RNA. These initial results may be due to the very low levels of persistent plasma viremia in patients on long-term therapy thereby limiting the detection of the brief effects of these new reactivation therapies. Another explanation could be that resting memory $CD4^+$ T cells are not the only source of persistent viremia in treated patients.

QUANTIFICATION OF PERSISTENT VIREMIA USING DIGITAL DROPLET PCR METHODOLOGY

In conducting SCA only a limited number of patient samples can be analyzed because each assay run requires two standard curves: one for HIV-1 and one for RCAS [15]. Although the limit of detection of SCA is the most sensitive of all assays to date (0.3 copies/ml in 7 ml plasma), due to the extremely low levels of persistent viremia in the plasma of patients on long-term therapy, new eradication treatment strategies that evoke more than an one log reduction in the size of viral reservoirs contributing to residual viremia may become unmeasurable even by SCA. Digital droplet PCR (ddPCR) is a new technology that quantifies DNA copy number by dividing a 20 μl mixture of sample and PCR reagents into 20 000 droplets, each containing a single molecule and fluorescent-labeled probe. PCR amplification of these droplets to end-point amplification is performed in a conventional thermal cycler [68▪,69]. For droplets that contain template, specific cleavage of TaqMan probes generates a strong fluorescence signal. Positive droplets containing the DNA target are measured by flowing one droplet at a time through a fluorescence reader. This technology provides an absolute measurement of the DNA target without the use of a standard curve. Recent use of ddPCR for the measurement of HIV-1 RNA, wherein HIV RNA transcripts were serially diluted, reverse transcribed and then quantified using ddPCR, found this method had a broad dynamic range from 0.3 to 1×10^5 copies [70]. Due to saturation of the positive droplets, ddPCR was found to be inaccurate above 1×10^5 copies indicating further dilution would be needed to quantify high copy numbers. In addition, ddPCR detected similar HIV-1 RNA levels when compared with SCA in patients with less than 50 copies/ml (mean 2.0-fold difference). However, viral sequence variation in primer/probe binding sites resulted in varying amplitude of positive droplets causing different signal-to-noise ratios and difficulty in setting a threshold for positive/negative droplets. Although this technology will need further improvement, it holds promise for providing absolute quantification of residual plasma HIV-1 RNA equal to or better than current ultrasensitive assays.

CONCLUSION

The level of HIV-1 RNA in patient plasma remains an important marker for determining the success of cART. New sensitive assays have revealed that HIV-1 persists in the plasma of nearly 80% of patients on suppressive therapy. Understanding the source and dynamics of this persistent viremia is essential for developing effective strategies for HIV-1 eradication. In addition, new technologies may improve the quantification of plasma HIV-1 RNA levels during and after latent reservoir reactivation as part of future HIV-1 eradication efforts.

Acknowledgements

The author would like to acknowledge funding from the following sources: The Foundation for AIDS Research (amfAR: ARCHE funding); The Swedish International Development Agency (SWE-2009–046); The Swedish Research Council (K2011–56X-21749–016); and The US National Institutes of Health grant AI071713.

Conflicts of interest

The author has no conflicts of interest in writing this article.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. Cohen MS, Chen YQ, McCauley M, *et al.* Prevention of HIV-1 infection with ▪▪ early antiretroviral therapy. N Engl J Med 2011; 365:493–505.
This in-depth clinical study revealed that suppressive antiretroviral therapy reduces the risk of HIV transmission in HIV-discordant couples.

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2. Thompson MA, Aberg JA, Hoy JF, *et al.* Antiretroviral treatment of adult HIV
■ infection: 2012 recommendations of the International Antiviral Society-USA panel. JAMA 2012; 308:387–402.

This article describes the current treatment guidelines for HIV-infected individuals and that treatment should be initiated regardless of $CD4^+$ cell count.

3. Archin NM, Vaidya NK, Kuruc JD, *et al.* Immediate antiviral therapy appears to restrict resting CD4+ cell HIV-1 infection without accelerating the decay of latent infection. Proc Natl Acad Sci U S A 2012; 109:9523–9528.
4. Gianella S, von Wyl V, Fischer M, *et al.* Effect of early antiretroviral therapy
■ during primary HIV-1 infection on cell-associated HIV-1 DNA and plasma HIV-1 RNA. Antivir Ther 2011; 16:535–545.

This study describes the benefits of initiating HIV-1 therapy during primary infection include decreases in cell-associated HIV-1 DNA and plasma HIV-1 RNA setpoint.

5. Wyl V, Gianella S, Fischer M, *et al.* Early antiretroviral therapy during primary HIV-1 infection results in a transient reduction of the viral setpoint upon treatment interruption. PLoS One 2011; 6:e27463.
6. Hughes MD, Johnson VA, Hirsch MS, *et al.* Monitoring plasma HIV-1 RNA levels in addition to CD4+ lymphocyte count improves assessment of antiretroviral therapeutic response. ACTG 241 Protocol Virology Substudy Team. Ann Intern Med 1997; 126:929–938.
7. Mellors JW, Munoz A, Giorgi JV, *et al.* Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med 1997; 126:946–954.
8. Mellors JW, Rinaldo CR Jr, Gupta P, *et al.* Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 1996; 272:1167–1170.
9. Sayana S, Javanbakht M, Weinstein M, Khanlou H. Clinical impact and cost of laboratory monitoring need review even in resource-rich setting. J Acquir Immune Defic Syndr 2011; 56:e97–e98.
10. de Boer MG, Wessels E, Claas EC, Kroon FP. Potential influence of more-sensitive HIV-1 load detection by the new Roche Cobas AmpliPrep/Cobas TaqMan version 2.0 assay on clinical management of HIV-positive pregnant women. J Clin Microbiol 2010; 48:4301–4302.
11. Gueudin M, Plantier JC, Lemee V, *et al.* Evaluation of the Roche Cobas TaqMan and Abbott RealTime extraction-quantification systems for HIV-1 subtypes. J Acquir Immune Defic Syndr 2007; 44:500–505.
12. 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; 282:1627–1632.
13. Havlir DV, Koelsch KK, Strain MC, *et al.* Predictors of residual viremia in HIV-infected patients successfully treated with efavirenz and lamivudine plus either tenofovir or stavudine. J Infect Dis 2005; 191:1164–1168.
14. 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; 77:11212–11219.
15. Palmer S, Wiegand AP, Maldarelli F, *et al.* New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. J Clin Microbiol 2003; 41:4531–4536.
16. Ramratnam B, Ribeiro R, He T, *et al.* Intensification of antiretroviral therapy accelerates the decay of the HIV-1 latent reservoir and decreases, but does not eliminate, ongoing virus replication. J Acquir Immune Defic Syndr 2004; 35:33–37.
17. Smit E, Bhattacharya S, Osman H, Taylor S. Increased frequency of HIV-1 viral load blip rate observed after switching from Roche Cobas Amplicor to Cobas Taqman assay. J Acquir Immune Defic Syndr 2009; 51:364–365.
18. Doyle T, Smith C, Vitiello P, *et al.* Plasma HIV-1 RNA detection below 50 copies/ml and risk of virologic rebound in patients receiving highly active antiretroviral therapy. Clin Infect Dis 2012; 54:724–732.
19. Maggiolo F, Callegaro A, Cologni G, *et al.* Ultrasensitive assessment of residual low-level HIV viremia in HAART-treated patients and risk of virological failure. J Acquir Immune Defic Syndr 2012; 60:473–482.
20. Berger A, Scherzed L, Sturmer M, *et al.* Evaluation of the Cobas AmpliPrep/Cobas Amplicor HIV-1 Monitor Ultrasensitive Test: comparison with the Cobas Amplicor HIV-1 Monitor test (manual specimen preparation). J Clin Virol 2002; 25 (Suppl 3):S103–S107.
21. Bourlet T, Signori-Schmuck A, Roche L, *et al.* HIV-1 load comparison using four commercial real-time assays. J Clin Microbiol 2011; 49:292–297.
22. Crump JA, Scott LE, Msuya E, *et al.* Evaluation of the Abbott m2000rt RealTime HIV-1 assay with manual sample preparation compared with the ROCHE COBAS AmpliPrep/AMPLICOR HIV-1 MONITOR v1.5 using specimens from East Africa. J Virol Methods 2009; 162:218–222.
23. Erali M, Hillyard DR. Evaluation of the ultrasensitive Roche Amplicor HIV-1 monitor assay for quantitation of human immunodeficiency virus type 1 RNA. J Clin Microbiol 1999; 37:792–795.
24. Glaubitz J, Sizmann D, Simon CO, *et al.* Accuracy to 2nd International HIV-1 RNA WHO Standard: assessment of three generations of quantitative HIV-1 RNA nucleic acid amplification tests. J Clin Virol 2011; 50:119–124.
25. Holguin A, Aracil B, Alvarez A, *et al.* Prevalence of human immunodeficiency virus type 1 (HIV-1) non-B subtypes in foreigners living in Madrid, Spain, and comparison of the performances of the AMPLICOR HIV-1 MONITOR version 1.0 and the new automated version 1.5. J Clin Microbiol 2001; 39:1850–1854.
26. Schumacher W, Frick E, Kauselmann M, *et al.* Fully automated quantification of human immunodeficiency virus (HIV) type 1 RNA in human plasma by the COBAS AmpliPrep/COBAS TaqMan system. J Clin Virol 2007; 38:304–312.
27. Jennings C, Harty B, Granger S, *et al.* Cross-platform analysis of HIV-1 RNA data generated by a multicenter assay validation study with wide geographic representation. J Clin Microbiol 2012; 50:2737–2747.
28. Lee PK, Kieffer TL, Siliciano RF, Nettles RE. HIV-1 viral load blips are of limited clinical significance. J Antimicrob Chemother 2006; 57:803–805.
29. Grennan JT, Loutfy MR, Su D, *et al.* Magnitude of virologic blips is associated with a higher risk for virologic rebound in HIV-infected individuals: a recurrent events analysis. J Infect Dis 2012; 205:1230–1238.
30. Widdrington J, Payne B, Medhi M, *et al.* The significance of very low-level viraemia detected by sensitive viral load assays in HIV infected patients on HAART. J Infect 2011; 62:87–92.
31. Havlir DV, Bassett R, Levitan D, *et al.* Prevalence and predictive value of intermittent viremia with combination hiv therapy. Jama 2001; 286:171–179.
32. 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; 78:968–979.
33. 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.
34. 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 U S A 2008; 105:3879–3884.
35. Buzon MJ, Massanella M, Llibre JM, *et al.* HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. Nat Med 2010; 16:460–465.
36. Dinoso JB, Kim SA, Wiegand AM, *et al.* Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. PNAS 2009; 106:9403–9408.
37. Gunthard HF, Havlir DV, Fiscus S, *et al.* Residual human immunodeficiency virus (HIV) Type 1 RNA and DNA in lymph nodes and HIV RNA in genital secretions and in cerebrospinal fluid after suppression of viremia for 2 years. J Infect Dis 2001; 183:1318–1327.
38. Pantaleo G, Graziosi C, Fauci AS. New concepts in the immunopathogenesis of human immunodeficiency virus infection. N Engl J Med 1993; 328:327–335.
39. Veazey RS, Lackner AA. The gastrointestinal tract and the pathogenesis of AIDS. AIDS 1998; 12:S35–S42.
40. Gandhi, RT, Zheng, L, Bosch, RJ, *et al.* The effect of raltegravir intensification on low-level residual viremia in HIV-infected patients on antiretroviral therapy: a randomized controlled trial. PLoS Med 2010; 7:e1000321. doi:10.1371/journal.pmed.1000321.
41. Hatano H, Hayes TL, Dahl V, *et al.* A randomized, controlled trial of raltegravir
■ intensification in antiretroviral-treated, HIV-infected patients with a suboptimal CD4+ T cell response. J Infect Dis 2011; 203:960–968.

This study shows that treatment intensification does not have any effect on persistent viremia suggesting that effective highly active antiretroviral therapy completely inhibits viral replication. Similar results are described in references [36,40,41■,42].

42. McMahon D, Jones J, Wiegand A, *et al.* Short-course raltegravir intensification does not reduce persistent low-level viremia in patients with HIV-1 suppression during receipt of combination antiretroviral therapy. Clin Infect Dis 2010; 50:912–919.
43. 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; 80:6441–6457.
44. Joos B, Fischer M, Kuster H, *et al.* HIV rebounds from latently infected cells, rather than from continuing low-level replication. Proc Natl Acad Sci U S A 2008; 105:16725–16730.
45. Yukl SA, Shergill AK, McQuaid K, *et al.* Effect of raltegravir-containing
■■ intensification on HIV burden and T-cell activation in multiple gut sites of HIV-positive adults on suppressive antiretroviral therapy. AIDS 2010; 24:2451–2460.

This study revealed unspliced HIV-1 RNA decrease in gut $CD4^+$ cells during treatment intensification with raltegravir indicating suppressive antiretroviral therapy may not prevent on-going replication in the gut and possibly other tissue compartments.

46. Brennan TP, Woods JO, Sedaghat AR, *et al.* 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; 83:8470–8481.
47. Anderson JA, Archin NM, Ince W, *et al.* Clonal sequences recovered from
■ plasma from patients with residual HIV-1 viremia and on intensified antiretroviral therapy are identical to replicating viral RNAs recovered from circulating resting CD4+ T cells. J Virol 2011; 85:5220–5223.

This study of two patients on suppressive therapy found that sequences isolated by viral outgrowth assays of resting $CD4^+$ T cells matched clonal sequences in the plasma. This is consistent with the hypothesis that persistent viremia arises in part from reactivation of latently infected $CD4^+$ T cells.

48. Chun TW, Finzi D, Margolick J, *et al.* In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. Nat Med 1995; 1:1284–1290.
49. Richman DD, Margolis DM, Delaney M, *et al.* The challenge of finding a cure for HIV infection. Science 2009; 323:1304–1307.
50. Nunnari G, Argyris E, Fang J, *et al.* Inhibition of HIV-1 replication by caffeine and caffeine-related methylxanthines. Virology 2005; 335:177–184.

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51. Scripture-Adams DD, Brooks DG, Korin YD, Zack JA. Interleukin-7 induces expression of latent human immunodeficiency virus type 1 with minimal effects on T-cell phenotype. J Virol 2002; 76:13077–13082.

52. Wang FX, Xu Y, Sullivan J, *et al.* IL-7 is a potent and proviral strain-specific inducer of latent HIV-1 cellular reservoirs of infected individuals on virally suppressive HAART. J Clin Invest 2005; 115:128–137.

53. Brooks DG, Arlen PA, Gao L, *et al.* Identification of T cell-signaling pathways that stimulate latent HIV in primary cells. Proc Natl Acad Sci U S A 2003; 100:12955–12960.

54. Brooks DG, Hamer DH, Arlen PA, *et al.* Molecular characterization, reactivation, and depletion of latent HIV. Immunity 2003; 19:413–423.

55. Korin YD, Brooks DG, Brown S, *et al.* Effects of prostratin on T-cell activation and human immunodeficiency virus latency. J Virol 2002; 76:8118–8123.

56. Kulkosky J, Culnan DM, Roman J, *et al.* Prostratin: activation of latent HIV-1 expression suggests a potential inductive adjuvant therapy for HAART. Blood 2001; 98:3006–3015.

57. Williams SA, Chen LF, Kwon H, *et al.* Prostratin antagonizes HIV latency by activating NF-kappaB. J Biol Chem 2004; 279:42008–42017.

58. Archin NM, Espeseth A, Parker D, *et al.* Expression of latent HIV induced by the potent HDAC inhibitor suberoylanilide hydroxamic acid. AIDS Res Hum Retroviruses 2009; 25:207–212.

59. Contreras X, Schweneker M, Chen CS, *et al.* Suberoylanilide hydroxamic acid reactivates HIV from latently infected cells. J Biol Chem 2009; 284:6782–6789.

60. Lehrman G, Hogue IB, Palmer S, *et al.* Depletion of latent HIV-1 infection in vivo: a proof-of-concept study. Lancet 2005; 366:549–555.

61. Bouchat S, Gatot JS, Kabeya K, *et al.* Histone methyltransferase inhibitors induce HIV-1 recovery in resting $CD4^+$ T cells from HIV-1-infected HAART-treated patients. AIDS 2012; 26:1473–1482.

62. Blazkova J, Trejbalova K, Gondois-Rey F, *et al.* CpG methylation controls reactivation of HIV from latency. PLoS Pathogen 2009; 5:e1000554.

63. Kauder SE, Bosque A, Lindqvist A, *et al.* Epigenetic regulation of HIV-1 latency by cytosine methylation. PLoS Pathog 2009; 5:e1000495.

64. Yang HC, Xing S, Shan L, *et al.* Small-molecule screening using a human primary cell model of HIV latency identifies compounds that reverse latency without cellular activation. J Clin Invest 2009; 119:3473–3486.

65. Xing S, Bullen CK, Shroff NS, *et al.* Disulfiram reactivates latent HIV-1 in a Bcl-2-transduced primary CD4+ T cell model without inducing global T cell activation. J Virol 2011; 85:6060–6064.

66. ■■ Archin NM, Liberty AL, Kashuba AD, *et al.* Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. Nature 2012; 487:482–485.

This report is a proof-of-concept study which provided the first evidence that the antilatency activity of vorinostat, an HDAC inhibitor, decreased the levels of HIV RNA in resting $CD4^+$ T cells.

67. ■■ Spivak AM, Andrade A, Hoh R, *et al.* Safety and feasibility of Using disulfiram to enhance HIV transcription among long-term ARV-treated adults: Preliminary results from a pilot study. In: 19th Conference on Retroviruses and Opportunistic Infections, Seattle, WA, 5–8 March 2012. [Abstract 369]

This interim report is a proof-of-concept study that provided the first evidence that the antilatency activity of disulfiram demonstrated a 14% decline in the latent reservoir size and average 4.5-fold increase in viremia.

68. ■ Hindson BJ, Ness KD, Masquelier DA, *et al.* High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Anal Chem 2011; 83:8604–8610.

This article described the digital droplet technique for measuring absolute DNA copy numbers in patient samples.

69. Shen F, Sun B, Kreutz JE, *et al.* Multiplexed quantification of nucleic acids with large dynamic range using multivolume digital RT-PCR on a rotational SlipChip tested with HIV and hepatitis C viral load. J Am Chem Soc 2011; 133:17705–17712.

70. Anderson EM, Wiegand A, Boltz VF, *et al.* Single-copy detection of plasma HIV-1 RNA using droplet digital PCR technology. In: 19th Conference on Retroviruses and Opportunistic Infections, Seattle, WA, 5–8 March 2012. [Abstract V-1002]

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