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Significance and Clinical Management of Persistent Low-Level Viremia and Very-Low-Level Viremia in HIV-1-Infected Patients

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A goal of HIV therapy is to sustain suppression of the plasma viral load below the detection limits of clinical assays. However, widely followed treatment guidelines diverge in their interpretation and recommended management of persistent viremia of low magnitude, reflecting the limited evidence base for this common clinical finding. Here, we review the incidence, risk factors, and potential consequences of low-level HIV viremia (LLV; defined in this review as a viremia level of 50 to 500 copies/ml) and very-low-level viremia (VLLV; defined as a viremia level of <50 copies/ml detected by clinical assays that have quantification cutoffs of <50 copies/ml). Using this framework, we discuss practical issues related to the diagnosis and management of patients experiencing persistent LLV and VLLV. Compared to viral suppression at <50 or 40 copies/ml, persistent LLV is associated with increased risk of antiretroviral drug resistance and overt virologic failure. Higher immune activation and HIV transmission may be additional undesirable consequences in this population. It is uncertain whether LLV of <200 copies/ml confers independent risks, as this level of viremia may reflect assay-dependent artifacts or biologically meaningful events during suppression. Resistance genotyping should be considered in patients with persistent LLV when feasible, and treatment should be modified if resistance is detected. There is a dearth of clinical evidence to guide management when genotyping is not feasible. Increased availability of genotypic assays for samples with viral loads of <400 copies/ml is needed.

Combination antiretroviral therapy (cART) is the cornerstone of HIV-1 treatment. The goal of cART is to maximize the inhibition of infectious virion production in order to curtail viral evolution, reverse immunologic decline, restore health, and prevent HIV-1 transmission. The suppression of plasma HIV-1 RNA concentration (plasma viral load [pVL]) below the quantification limits of clinically accessible assays is a widely accepted indicator of successful cART (1–3). Transient increases in pVL are common in patients on "successful" cART (4, 5), but most of these patients harbor plasma HIV-1 RNA that can be quantified using more sensitive assays (6, 7). The sensitivities of clinical assays available to diagnose low-level viremia (LLV) and very-low-level viremia (VLLV) have outpaced the understanding of their clinical consequences and access to reliable resistance assays necessary to optimize treatment decisions. As such, both LLV and VLLV are now routinely encountered, yet optimal management is unresolved. There is a need to synthesize emerging literature regarding clinical, virologic, and immunologic consequences of persistent LLV and VLLV, two conditions that routinely challenge clinicians caring for HIV-1-infected patients.

MATERIALS AND METHODS

Data sources. We performed a comprehensive query of PubMed, Medline, and Google Scholar from database inception to 31 August 2013. The search terms used included "low-level viremia," "very-low-level viremia," "residual viremia," or "viral blip" alone or in combination with "HIV," "resistance," "rebound," or "plasma viral load." Language use was not limited to English. Additional references were obtained from the reference lists in the articles identified using this search method. Finally, relevant abstracts and reports from meetings from 2011 to 2013 were queried and included only if they related to previously published work.

Definition of terms and scope of analysis. Our definitions of terms are included in Table 1. Of note, we defined LLV as an HIV-1 plasma viral load of 50 to 500 copies per ml (cpm), although others have included viremia of up to 1,000 cpm in this category. In addition, definitions of virologic failure and viral rebound are varied in the literature on HIV. Where necessary, we highlight here alternate definitions used by authors in the context of particularly informative studies.

The purpose of this review was to examine the clinical, virologic, and immunologic significance of persistent LLV and VLLV. The clinical characteristics of viral blips have been reviewed (4) and are discussed here only where they help contextualize the management of persistent LLV and VLLV.

RESULTS AND DISCUSSION

Evolution of HIV-1 RNA assays and detection of viremia during cART. The Roche Amplicor HIV-1 monitor test, a platform that utilizes endpoint reverse transcription-PCR (RT-PCR) technology, was the first widely available assay for pVL monitoring in the United States (8). Subsequent methods used branched-chain DNA and nucleic acid sequence-based amplification. In addition, single-copy HIV PCR assays have been developed primarily for research purposes (9). The Roche Amplicor HIV-1 monitor version 1.5 RT-PCR platform, with a lower RNA detection limit of 50 copies per ml (cpm) was the most widely used, and suppression at <50 cpm became a widely accepted target for cART success.

More recent real-time RT-PCR platforms detect PCR products during the exponential phase of RNA amplification. They reduce cross-contamination and report RNA across a wider linear dy-

Received 13 January 2014 Returned for modification 16 February 2014
Accepted 31 March 2014
Published ahead of print 14 April 2014
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doi:10.1128/AAC.00076-14

TABLE 1 Definitions of terms

Term	Definition
Low-level viremia	HIV-1 plasma viral load of 50–500 cpm[a]
Viral blip	An episode of low-level viremia that is preceded and followed by suppression below the quantification limit of the assay
Persistent low-level viremia	At least two consecutive episodes of low-level viremia
Very-low-level viremia	HIV-1 plasma viral load of <50 cpm detected by clinical assays with quantification cutoffs of <50 cpm
Residual viremia	Cryptic viremia during cART that is often 1–10 cpm and unaffected by treatment intensification (6, 7, 29, 42, 66, 109–111)
Virological failure	HIV-1 plasma viral load of >1,000 cpm after previously attaining a plasma viral load of <1,000 cpm[b]
Low-level viral rebound	At least one HIV plasma viral load of 50–500 cpm in a patient who previously attained viral suppression to <50 cpm
High-level viral rebound	At least one HIV plasma viral load of 500–1,000 cpm in a patient who previously attained viral suppression to <50 cpm
Viral rebound with HIV resistance	The development of new HIV drug resistance mutations in the context of at least one HIV plasma viral load of >50 cpm

[a] Some authors have defined low-level viremia with an upper plasma viral load of 1,000 copies per ml (cpm).
[b] There is significant heterogeneity in the definition of virological failure across guidelines (see Table 5).

namic range of detection (10). The two most widely available real-time RT-PCR assays have reported RNA quantification limits of 20 cpm (Roche Cobas Amplicor TaqMan assay version 2.0) and 40 cpm (Abbott RealTi*me* RT-PCR assay) and can qualitatively detect HIV-1 RNA below these quantification limits (11, 12). Real-time RT-PCR assays have increased sensitivity at low pVL, revealing a pVL of >50 cpm in up to 15% of samples with a pVL of <50 cpm using traditional endpoint RT-PCR testing (13–15). Differences between endpoint and real-time RT-PCR testing may be more pronounced in non-B HIV subtypes (16, 17). Though different real-time RT-PCR testing platforms show general agreement and correlation at >50 cpm, there is quite poor correlation at the lower limits of assay detection (14). In addition, the intra-assay variabilities of these assays are greatly increased at low pVL (18). The results of HIV pVL assays can be affected by specimen processing and handling, including the types of collection tubes used (19, 20).

Kinetics of HIV-1 decay and viremia during cART. HIV-1 decay during cART occurs in distinct phases (Fig. 1) (6, 21, 22). Though most patients achieve viral suppression at <50 cpm during the second phase of viral decay, a key observation of successful cART is HIV-1 persistence (23–28). Using a single-copy HIV-1 RNA assay, Maldarelli et al. (7) observed that >80% of individuals had stable HIV-1 viremia after 60 weeks of cART, with a median pVL of 3.1 cpm (range, 1 to 49 cpm). Two current hypotheses explain the source for residual viremia. The first proposes that detectable residual viremia is due to the stable periodic release of HIV from latently infected cells, possibly due to antigenic stimulation (6). The second posits that LLV represents the product of ongoing viral replication, presumably from sanctuary sites, which are poorly targeted by cART (29). It is possible that both mechanisms contribute to residual viremia.

Frequency of LLV and VLLV. LLV is common during cART, although actual rates vary across studies (ranging from 18 to 34%) due to differences in study designs, population, and pVL assay used (4, 5, 30–41). Virtually all of these studies included viral blips, and only a few provided estimates of LLV persistence after viral suppression (4, 5, 30, 32).

Most first episodes of LLV (70 to 82%) in large cohort studies of patients with initial suppression were blips, and 18 to 24% became persistent LLV. A minority (6 to 9%) of patients with initial LLV progressed to higher degrees of viremia (4, 5, 30–32). Thus, approximately 4% to 8% of the total population that achieved an initial pVL of <50 cpm in the studies subsequently developed persistent LLV of <500 cpm. The frequency of persistent LLV in patients without recent viral suppression is likely to have wider variance, depending on several factors, including the cART regimen and duration of ART.

Recognition of VLLV is on the rise given the progressive decrease in the reported quantification limits of clinical assays. The precise incidence rates of VLLV are, however, poorly characterized, as studies have differed in terms of the pVL assay used, as well as patient population characteristics, such as type and duration of cART (7, 42, 43).

Risk factors. Several potential risk factors for low-level viremia and very-low-level viremia have been investigated. No one factor appears determinative in all cases, and it is likely that most cases are multifactorial.

Conflicting evidence on stage of HIV infection prior to cART initiation. Hypothetically, a more advanced HIV stage prior to treatment initiation should increase the risk for viral rebound during cART because of a greater diversity of HIV-1 quasispecies, more extensive viral reservoir, and exhaustion of immune effector responses. However, studies have produced mixed results. In one study, patients diagnosed and treated during primary HIV infection had 2-fold-lower rates of LLV compared to those who initiated treatment in the context of chronic HIV infection (44). Among 1,158 patients receiving their first cART in two randomized clinical trials, pretreatment pVL of ≥6 $\log_{10}$ cpm was associated with a 2.2-fold-increased risk of persistent viremia of >50 and <1,000 cpm (45). In contrast, other investigators observed no association between pretreatment pVL and risk of persistent viremia of >50 cpm (4, 46).

In several studies, having VLLV compared to suppression below the assay detection limit (which varied between studies) was associated with higher pre-cART pVL (43, 47, 48), larger amount of proviral HIV DNA (47), lower CD4 cell count (48), and more advanced CDC stage (49).

VLLV is a risk factor for rebound of >50 cpm, but the significance is unclear. Studies that evaluated the risk of viral rebound among patients with detectable viremia below the quantification limits of conventional assays differed in their results (Table 2). Most of the studies demonstrated an increased risk of viral rebound of >50 cpm among patients with detected viremia below the limits of conventional assays (43, 48, 50–52), while two did not reveal an association (42, 49). In one of the studies, Doyle et al.

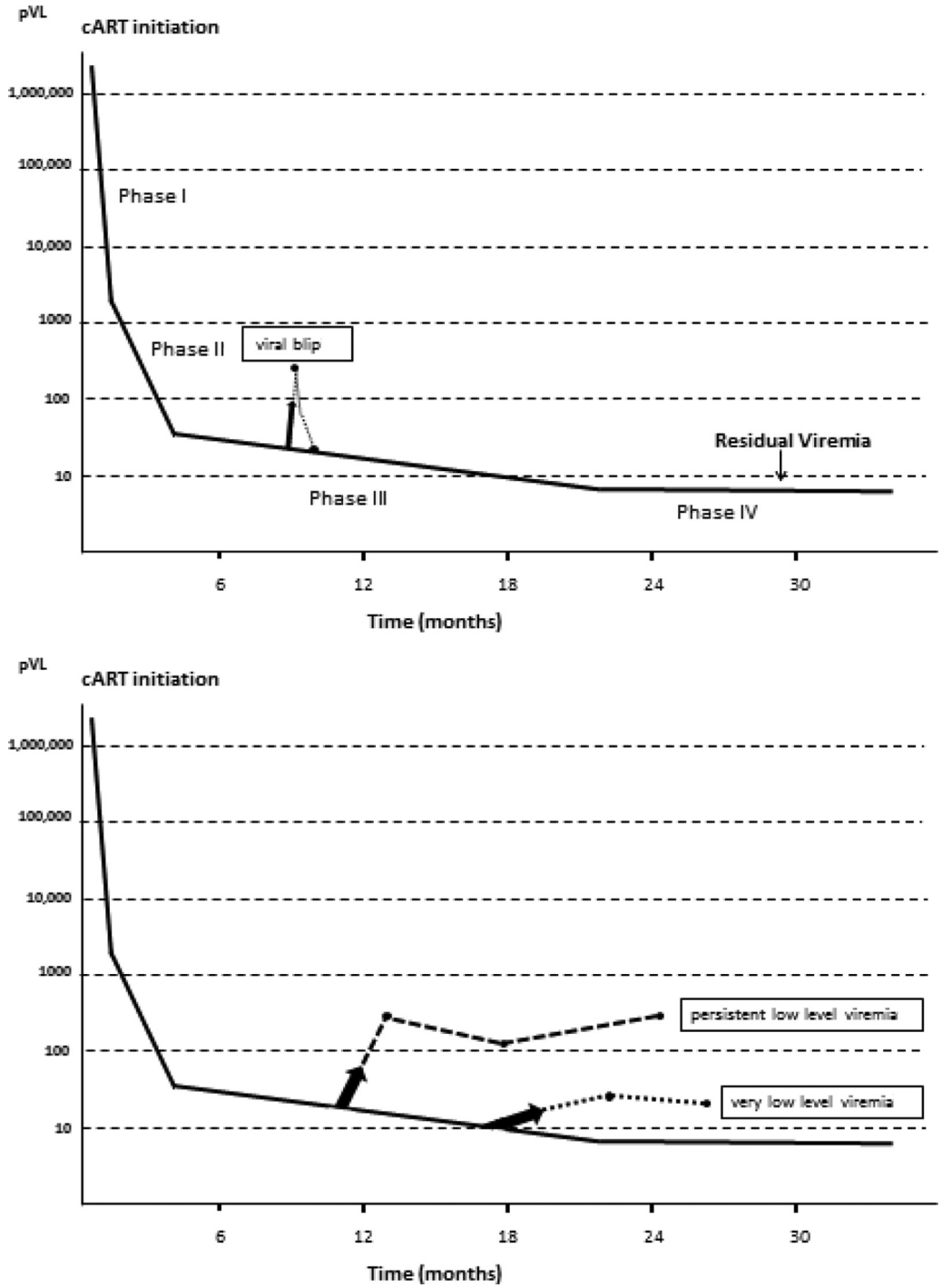

FIG 1 Graphs of pVL over time by phase (top) and viremia level (LLV and VLLV) (bottom). Some data are from Palmer et al. (6). Persistent low-level viremia and very-low-level viremia may also occur prior to having achieved pVL suppression to <50 cpm. cART, combination antiretroviral therapy; pVL, plasma viral load; cpm, copies/ml; at phase I, the half-life ($t_{1/2}$) is 1.5 days; at phase II, the $t_{1/2}$ is 2.5 weeks; at phase III, the $t_{1/2}$ is 39 weeks. The viral blip dynamics are as described by Di Mascio et al. (108), including a 2-phase decay lasting up to 3 weeks.

(48) stratified patients based on the Abbott RealTi*me* assay into pVLs of 40 to 49 cpm, <40 cpm with RNA detected, and <40 cpm with no RNA detected. They found that compared to individuals with a pVL of <40 cpm and no detected RNA, having viremia of 40 to 49 cpm increased the risk of rebound to >50 cpm by 4.67-fold, while having detectable RNA at <40 cpm increased the risk by 1.97-fold. The risks of rebound to >400 cpm were increased by 6.91-fold and 2.88-fold, respectively. Others have reported increased risks of rebound to >50, >200, and >400 but importantly, not >1,000 cpm or higher (50). The majority of the rebounds of >200 cpm were blips, and resistance rarely emerged (50), clouding the significance of these events.

TABLE 2 Literature examining risk of HIV-1 virologic rebound with very-low-level viremia

Study authors, yr (reference)	No. with LLV/total no. in study (%)[a]	Follow-up time	pVL assay	Definitions[b]	Association between VLLV and subsequent rebound/failure?	Rebound parameters[c]
Doyle et al., 2012 (48)	1,247 with pVL of <50 cpm were subdivided into three VL groups: 40–49 cpm, 19%; $<40_{RNA+}$, 41%; $<40_{RNA-}$, 40%	12–18 mo	Abbott RealTi*me* HIV-1 pVL assay	Residual viremia, 40–49 cpm, <40 cpm_{RNA+}; rebound, >50 cpm and >400 cpm	Yes	Rebound >50 cpm by pVL category: <40 cpm_{RNA-} = 4.0% (reference); <40 cpm_{RNA+} = 11.3% (HR, 1.97; 95% CI, 1.25–3.11; $P < 0.0001$); 40–49 cpm = 34.2% (HR, 4.67; 95% CI, 2.91–7.47; $P < 0.0001$) Rebound >400 cpm by pVL category: <40 cpm_{RNA-} = 1.2% (reference); <40 cpm_{RNA+} = 3.8% (HR, 2.88; 95% CI, 1.24–6.69; $P < 0.0001$); 40–49 cpm = 13% (HR, 6.91; 95% CI, 2.90–16.47; $P < 0.0001$)
Maggiolo et al., 2012 (43)	346/1,214 (28.5)	378 days (primary endpoint was 4 mo)	Versant kinetic PCR (kPCR) molecular system (Siemens)	Residual viremia, 3–50 cpm; failure subcategorized as broad, >50 cpm × 2 or restricted, >200 cpm × 2	Yes	Risk of failure (2 definitions) between those with pVL consistently <3 cpm vs those with pVL 3–50 cpm: broad, 0.4% vs 3.2% (OR, 7.52; 95% CI, 3.8–15.0; $P < .0001$), or restricted, 0.4% vs 2.0% (OR, 4.64; 95% CI, 2.2–9.7; $P < 0.0001$)
Henrich et al., 2012 (50)	182/778 (23)	22 mo	Roche AmpliPrep/ Cobas TaqMan HIV-1, version 1.0	Residual viremia, <48 cpm, RNA signal detected; rebound, confirmed >50, >200, >400, or >1,000 cpm	Yes	Rebound: >50 cpm (HR, 3.02; CI, 2.12–4.30; $P < 0.05$); >200 cpm (HR, 1.83; 95% CI, 1.05–3.19; $P < 0.05$); >400 cpm (HR, 1.31; 95% CI, 1.03–3.47); >1,000 cpm, NS
Widdrington et al., 2011 (51)	69/139 (49.6)	36 mo	Roche AmpliPrep/ Cobas TaqMan HIV-1, version 1.0	Residual viremia, <40 cpm, RNA signal detected; blip, >50 cpm on only one occasion; transient rebound, >50 cpm on two or more occasions but returning to <50 cpm; failure, persistent >50 cpm or cART change	Yes	Failure, 11.6% vs 4.3%, $P = 0.122$; higher risk of transient rebound among those with residual viremia (17.4% vs 4.5%, $P = 0.015$)
Álvarez-Estévez et al., 2013 (52)	156/290 (54)	12 mo	Roche AmpliPrep/ Cobas TaqMan HIV-1, version 2.0	Residual viremia, 20–49 cpm; failure, two consecutive pVL >50 cpm, >400 cpm	Yes	VLLV, 20–39 cpm, rebound in 20.2%; 40–49 cpm, rebound in 24.2%; $P < 0.001$ in comparison to pVL <20 cpm
Gianotti et al., 2012 (42)	293/739 (40)	49 wk	Versant kinetic PCR molecular system (kPCR) (Siemens)	Residual viremia, 1–49 cpm; rebound, two consecutive >50 cpm	No	2% vs 0.9% in those with <1 HIV RNA copy/ml ($P = 0.231$)
Charpentier et al., 2012 (49)	38/656 (5.8)	NR[d]	Roche AmpliPrep/ Cobas TaqMan HIV-1, version 2.0	Residual viremia, at least two 20–50 cpm during 1 yr of follow-up; blip ratio, no. of pVL >50 cpm/no. of pVL determinations; failure, two consecutive >50 cpm	No	8% (20–50 cpm) vs 4% (<20 cpm); $P = 0.32$; no difference in blip ratio

[a] LLV, low-level viremia; pVL, plasma viral load; RNA+, RNA detected; RNA−, no RNA detected.
[b] cpm, copies per ml; cART, combination antiretroviral therapy.
[c] HR, hazard ratio; CI, confidence interval; OR, odds ratio; NS, not statistically significant; VLLV, very-low-level viremia.
[d] NR, not reported.

Viral blips of low amplitude are unlikely to lead to persistent LLV. Several heterogeneous studies have examined the risk of virologic failure among patients experiencing viral blips, using different cutoffs to define virologic failure (Table 3). One of these studies evaluated a broad range of blips and found an increased risk of viral rebound (two consecutive pVLs of >50 cpm or a single pVL of >1,000 cpm) among patients with viral blips of 500 to 999 cpm, but not among those with viral blips of 50 to 500 cpm (34). Another study that specifically evaluated sustained viral rebound at 50 to 400 cpm found no association with a prior blip (14). The four studies that found an association between viral blips and subsequent virologic failure included either a pVL of ≥400 cpm in the definitions of viral blip (34, 53, 54) or considered a single blip of >500 cpm to be virologic failure (5). Thus, studies to date agree that the risk of subsequent virologic failure (including persistent viremia in the LLV range) is not significantly increased among patients with isolated low-amplitude viral blips (<400 or <500 cpm). The risk in patients with higher-amplitude blips is less clear.

It is unknown whether associations exist between viral blips and subsequent VLLV, though the findings of two studies support this relationship (42, 49).

Adherence. Elucidating the relationships between adherence and persistent LLV or VLLV is a challenge because adherence is difficult to measure accurately, and the consequences of imperfect adherence vary depending on the pattern of missed doses and the cART regimen. Further, adherence studies have addressed viral rebound in general and not persistent LLV specifically. Some studies have linked viral rebound to adherence (40, 54), but this has not been consistently reported (33, 37, 48, 55). Given the well-established relationship between lapses in adherence and overt virologic failure, it is prudent to exclude suboptimal adherence in all patients with persistent LLV or VLLV while recognizing that adherence lapses will not be detected in some patients.

Antiretroviral regimen. Some antiretroviral combinations or strategies may predispose a patient to LLV, particularly in patients with high baseline pVL (56). Thus, triple-nucleoside reverse transcriptase inhibitor (NRTI) cART and boosted protease inhibitor (PI) monotherapy regimens have been associated with failure with relatively high rates of LLV (57, 58). Also, LLV was common among failures of raltegravir plus darunavir-ritonavir in a preliminary study (59), though this result has not been confirmed.

Regimens containing a nonnucleoside reverse transcriptase inhibitor (NNRTI) plus two NRTIs have been shown to suppress pVL to lower HIV-1 RNA copy numbers than those with ritonavir-boosted PI plus two NRTIs (32, 42, 43, 48, 51, 60, 61). Since rebound to >50 cpm is more likely in those with VLLV than those in whom no viremia was detected, as discussed above, it has been hypothesized that recipients of PI-based regimens will be more prone to viral rebound and, by extension, persistent LLV. This is not proven, however, as a few studies have demonstrated a higher risk (4, 45, 57), but the majority found no difference between PI- and NNRTI-based regimens (5, 30, 31, 38, 50). Results demonstrating increased rebound risk with PIs may reflect clinician preference for prescribing PI-based cART to patients perceived to be at risk for poor adherence, or it may be influenced by defining LLV as a pVL of >50 cpm after 6 months, since PI-based regimens exhibit a slower decay of plasma virus than NNRTI-based regimens (4, 31, 45, 57).

It has been postulated that the mechanism of antiretroviral activity may influence the frequency of LLV. In recent tissue culture experiments, maraviroc and other entry inhibitors (enfuvirtide and AMD3100) were associated with a virus redistribution phenomenon and higher extracellular HIV-1 RNA count than those with efavirenz and raltegravir, which act intracellularly (62). Differences in the penetration of antiretrovirals (ARVs) into cellular and tissue reservoirs of HIV-1 is yet another potential though unproven pathway by which ARVs may have a differential predilection for LLV (63–65).

Other potential factors. Since HIV-1 decay occurs in distinct phases and viral reservoirs tend to diminish with increasing duration of effective cART, a longer duration of viral suppression should be associated with decreased risk of viral rebound (6, 7, 66). Indeed, a longer duration of suppression at <50 cpm has been associated with a reduced risk of viral rebound, including rebound to >50 cpm (43, 48, 67).

Clinical significance. (i) Antiretroviral resistance increases with persistent LLV. ARV drug resistance is well documented in patients with a viremia level of <1,000 cpm (Table 4), though the reported prevalence varies depending on ART experience, virological suppression/failure, duration of viremia, and treatment regimen (45, 68–75). In one of the largest studies (n = 1,001), genotyping of clinical samples between 1999 and 2006 in the United Kingdom revealed at least one drug resistance mutation (DRM) in 60% and 72% of samples, with pVLs of <300 cpm and 300 to 999 cpm, respectively (70). This high rate of resistance reflected the extensive treatment experience of the population, common use of unboosted PIs in the earlier years of the study, and the tendency of clinicians to request resistance testing in the patients with the highest risk. More recently in British Columbia, Canada, where genotyping is routinely performed on all clinical samples with a pVL of >250 cpm, 38 (19%) of 196 treatment-naive patients without baseline resistance had at least one DRM at their first LLV of 50 to 1,000 cpm. Almost all of the DRM affected NRTIs and/or NNRTIs, and none affected a boosted PI (71). Other investigators have found relatively low risk of PI resistance early during LLV (45, 69). In comparison, integrase strand transfer inhibitor (INSTI) DRM were present in 5 (20%) of 25 patients who failed a dual regimen of ritonavir-boosted darunavir plus raltegravir; all the patients with INSTI resistance had a pVL of <400 cpm at the time of failure, and there were no PI DRM (59).

New DRM can emerge during viremia of <1,000 cpm, including when viremia is <500 cpm (45, 69, 73). In a population with at least three episodes of a pVL of 40 to 500 cpm within 6 months, Delaugerre et al. (68) found at least one new DRM during LLV (median pVL, 134 cpm) in 11/37 (30%) patients followed for a median of 11 months. There is a trend toward greater resistance with higher pVL during LLV (36, 45, 71, 76, 77). In one study, new DRM were detected in 0 of 10 patients with a maximum pVL of 51 to 100 cpm, compared with 5 (38%) of 13, with a pVL of 101 to 200 cpm and 15 (48%) of 31, with a maximum pVL of 200 to 1,000 cpm (45). In the British Columbia cohort, the median pVLs in patients who evolved resistance and those who did not were 472 cpm versus 369 cpm (P = 0.067), respectively (72). While resistance is more likely against NNRTI/NRTIs and INSTIs than against ritonavir-boosted PIs early during LLV, all classes are at risk over time (72). Antiretroviral drug resistance is yet to be adequately characterized in patients with VLLV because of the limitations of current assays.

TABLE 3 Literature examining risk of virologic failure or rebound with detectable plasma HIV viral load by conventional assays

Study type, study authors, yr (reference)	No. with LLV/ total no. in the study (%)[a]	Study period	Follow-up	Assay	Definitions[b]	Risk of virologic failure (compared with viral suppression)?[c]		Risk of immunologic failure?	
						Yes/no	Details	Yes/no	Details
Viral blip									
Havlir et al., 2001 (36)	29/101 (29)	1997–1998	84 wk	Roche UltraSensitive Amplicor HIV-1 monitor assay, version 1.0	LLV, >50 cpm with subsequent pVL <50 cpm; failure, 2 consecutive pVL >200 cpm	No	10.4% vs 13.8% (RR, 0.76; 95% CI, 0.29–1.72)	NR[d]	
Sungkanuparph et al., 2005 (38)	128/380 (34)	1998–2003	23.5 mo	Roche UltraSensitive Amplicor HIV-1 monitor assay, version 1.5	LLV, 50–1,000 cpm preceded and followed by <50 cpm; failure, 2 consecutive >1,000 cpm	No	HR, 1.75; 95% CI, 0.77–3.97; P = 0.183	NR	
Mira et al., 2002 (39)	37/330 (11)	1997–2000	144 wk	Roche Amplicor HIV-1 monitor assay; Roche UltraSensitive Amplicor HIV-1 monitor assay, version 1.0	LLV, 51–1,000 cpm; failure, 2 consecutive pVL >200 cpm	No	8.1% vs 16.9% (P = 0.25)	No	
Sklar et al., 2002 (31)	122/448 (27)	1997–2000	69 wk	Roche UltraSensitive Amplicor HIV-1 monitor assay, version 1.0; bDNA assay (Chiron Corporation)[e]	<50, >50, <50 cpm; failure, "lasting viremia" low level, 50–400 cpm; high level, >400	No	13.1% vs 16% (RR, 0.82; 95% CI, 0.49–1.38; P = NS)	Yes	Lower $CD4^+$ increase at last measurement in those with blips than those with complete suppression
Martinez et al., 2005 (37)	8/43 (19)	NA[f]	18 mo	Roche UltraSensitive Amplicor HIV-1 monitor assay, version 1.5	LLV, pVL >50 cpm preceded and followed by pVL <50 cpm; failure, two consecutive plasma >200	No	No episodes of virologic failure	Yes	Lower $CD4^+$ counts at 12 and 18 mo than those with complete suppression (P = 0.04 and 0.02, respectively)
Nettles et al., 2005 (33)	9/10 (90)	2003–2004	97.5 days	Roche UltraSensitive Amplicor HIV-1 monitor assay, version 1.5	LLV, pVL >50 cpm preceded and followed by pVL <50 cpm	No	No episodes of virologic failure	NR	
Podsadecki et al., 2007 (40)	60/223 (27)	NA	22 mo	NA	LLV, pVL 50–1,000 cpm preceded and followed by pVL <50 cpm; failure, pVL >50 × 2, >200 ×2, >1,000 ×2	No	P > 0.3 for all failure definitions	NR	
García-Gascó et al., 2008 (30)	779/2,720 (28.6)	1999–2006	NA	Bayer Versant bDNA assay, version NA	LLV, pVL 51–500 cpm; failure, pVL >500 cpm	NR	9.1% of those with viral blips developed failure, but no comparison to undetectable pVL	No	
Greub et al., 2002 (5)	155/2,055 (7.5)	1998–1999	17.7 mo	Roche UltraSensitive Amplicor HIV-1 monitor assay, version NA	LLV "blip," isolated pVL 51–500 cpm; failure, >500 cpm	Yes	HR blip, 2.01 (95% CI, 1.51–2.91; P < 0.0001 compared with viral suppression)	NR	
Easterbrook et al., 2002 (53)	122/767 (16)	1996–1998	27.9 mo	Roche UltraSensitive Amplicor HIV-1 monitor assay, version 1.0, 1.5, and then Bayer Versant bDNA assay, version 2.0, 3.0	LLV, pVL ≥400 cpm followed by pVL <400 cpm; failure, sustained pVL >400 cpm	Yes	19.3% vs 7.7% (HR, 3.15; 95% CI, 1.72–5.77; P < 0.001)	Yes	$CD4^+$ gain, 138 vs 224 (P = 0.003)
Masquelier et al., 2005 (54)	20/219 (9)	1998–1999	96 wk	NA	LLV, pVL >500 cpm after preceding pVL <500 cpm; failure, sustained pVL >500 cpm	Yes	25% vs 5% (P = 0.03)	No	
Persistent low-level viremia									
Greub et al., 2002 (5)	155/2,055 (7.5)	1998–1999	17.7 mo	Roche UltraSensitive Amplicor HIV-1 monitor assay, version NA	LLV, "bump," two consecutive 51–500 cpm; failure, >500 cpm	Yes	HR bump, 5.80 (95% CI, 4.26–7.90; P < 0.0001)	NR	
Karlsson et al., 2004 (73)	18/46 (39)	NA	27 mo	bDNA assay (Chiron Corp.)	LLV, >50% of measurements >50, <1,000 cpm; failure, >1,000 cpm	Yes	56% vs 0% (P = 0.002)	No	

Sungkanuparph et al., 2006 (46)	78/362 (27.5)	1999–2003	29.5 mo	Roche UltraSensitive Amplicor HIV-1 monitor assay, version NA	LLV, 51–1,000 cpm for ≥3 mo; failure, 2 consecutive >1,000 cpm	Yes	39.7% vs 9.2% ($P < 0.001$)	NR
Geretti et al., 2008 (4)	85/1,386 (6)	1996–2005	2.2 yr	Roche UltraSensitive Amplicor HIV-1 monitor assay, version 1.0, 1.5	LLV, 50–400 cpm for ≥2 consecutive measurements; failure, 2 consecutive >400 cpm or >400 × 1 and ARV change	Yes	14% vs 5% (95% CI, 2.29–7.73)	NR
Boillat-Blanco et al., 2012 (57)	179 vs 5,389 controls (HIV RNA <20 cpm)	2000–2010	48 wk	NA	LLV, 21–400 cpm for ≥3 measurements in a row; controls, 3 consecutive measurements <20 cpm; failure, >400 cpm	Yes	12% vs 0% among those with pVL 21–49 cpm	NR
Laprise et al., 2013 (78)	165/1,357 (12)	1999–2012	5 yr	Bayer Versant bDNA assay, version 3.0; Abbott RealTi*me* HIV-1 assay	LLV, >1 pVL 50–999 for ≥6, 9, or 12 mo; failure, >1,000 cpm	Yes	Compared to viral suppression (failure rate of 6.6%); pLLV, 50–199 cpm for ≥6 mo, 22.7% (95% CI, 14.9–33.6); pLLV, 201–499 cpm for ≥6 mo, 24.2% (95% CI, 14.5–38.6); pLLV, 500–999 cpm for ≥12 mo, 58.9% (95% CI, 43.1–75.2)	NR

[a] LLV, low-level viremia.
[b] cpm, copies per ml; pVL, plasma viral load.
[c] RR, relative risk; CI, confidence interval; HR, hazard ratio; NS, not significant; pLLV, persistent low-level viremia.
[d] NR, not reported.
[e] bDNA, branched DNA.
[f] NA, not available.

(ii) Virologic failure increases with persistent LLV. There is consistent evidence of increased risk of virologic failure in patients with persistent viremia of 50 to 1,000 cpm (Table 3). Some of the studies demonstrated an increased risk in patients with persistent viremia of ≤500 cpm (4, 5, 58, 78), including after only two consecutive pVLs of 51 to 500 cpm (5), and when persistent viremia was 50 to 199 cpm for 6 months (78). The increased risk of virologic failure may be explained by drug resistance during LLV. Among 1,965 patients experiencing persistent viremia of <1,000 cpm, 30% had regimen-compromising DRM, and 5% had <1 active drug in their regimen (79). The genotype susceptibility score predicted virologic failure in a linear fashion even when the pVL was 50 to 250 cpm.

(iii) Immunologic alteration, inflammation, and microbial translocation. There does not appear to be a clinically meaningful impact of either VLLV or persistent viremia of <400 cpm on the $CD4^+$ T-cell trajectory (26, 31, 39, 45, 47, 49, 51, 80), while a few studies have signaled that the trajectory may be affected by viremia of >400 cpm (31, 37, 53).

Biomarkers of immune activation, inflammation, and microbial translocation remain elevated in virologically suppressed HIV-infected patients compared to HIV-negative individuals (81–84) and may be a pathway to noninfectious morbidities (85, 86). The cellular markers of immune activation appear to remain elevated during persistent LLV. A study ($n = 33$) found that patients with a pVL 50 to 1,000 cpm in >50% of the measurements had higher levels of $CD8^+$ T-cell activation than those of the group with a sustained pVL of <50 cpm (73). In a study of 832 patients on cART for ≥96 weeks, the mean CD8 activation was 1.9% higher among those with LLV of 50 to 200 cpm than those with consistent viral suppression at <50 cpm ($P = 0.016$) (87). Another study that stratified 101 patients into consistent pVL of ≤20 cpm versus pVL of >20 cpm at least once (median, 81 cpm) during 24 months of follow-up reported an association between higher pVL and increase in activated $CD8^+$ $CD38^+$ and $CD8^+$ HLA-DR^+ cells (88). Whereas there is no evidence of an association between viral blips and markers of immune activation, one small study demonstrated higher levels of soluble immune activation markers during periods of detectable residual viremia of <20 cpm (89, 90).

Some markers of inflammation and/or endothelial activation have also been studied (91–95). In the subgroup with a pVL of <400 cpm in one study, the levels of von Willebrand factor (vWF) remained higher than in the HIV-negative controls and correlated with levels of interleukin-6 (IL-6) (91). In another study of 122 patients with LLV (1 to 500 copies/ml) on stable cART, there was a positive correlation between pVL and IL-6, with a pVL threshold value for significantly increased IL-6 of 31 cpm ($P = 0.023$) (92). In contrast, a recent study that explored associations of inflammatory biomarkers with several pVL strata found no correlations of pVL with C-reactive protein (CRP), IL-6, or fibrinogen (94). Microbial translocation, as measured based on bacterial 16S rRNA genes, was higher in individuals with LLV (20 to 200 cpm) on cART than in those with pVL suppression (<20 cpm) (93). More studies are needed to fully understand the impact of persistent LLV or VLLV on immune activation, inflammation, and microbial translocation.

(iv) Morbidity and mortality. Two recent studies found no association between LLV (<400 cpm) and AIDS progression and/or overall mortality (94, 95). These results should be inter-

TABLE 4 Studies assessing HIV-1 drug resistance in the context of low-level viremia

Study authors, yr (reference)	No. of subjects[a]	Patient population[b]	Pre-LLV genotype available?[c]	LLV definition[d]	Median pVL (cpm) during LLV	DRM (no. resistant/total no. [%]); specific DRM (*n*)[e]	Comment(s)
Nettles et al., 2004 (69)	21	81% were treatment experienced	No	50–400 cpm for ≥3 mo, ≥2 consecutive measurements	Resistance, 64; no resistance, 89	9/21 (43); RT DRM: 8/21 T215Y, M184V, M41L, K65R, K70R, K103N, Y115F, V179D, M230L, Y188C, V75A; PR DRM: 3/21 L10I, M36I, I47V, A71V, G73T, I84V, L90M, I93L, V82A, D30N, N88D	Median LLV duration, 11 mo
Karlsson et al., 2004 (73)	15	NNRTI- or PI-based cART for ≥1 yr	No	50–1,000 cpm; persistent (>50% episodes LLV)	NR[f]	NR; increased phenotypic drug resistance was detected in most patients ($P = 0.06$ for abacavir and $P = 0.05$ for PI)	Resistance measured at first two observation points
Tobin et al., 2005 (74)	14	Children with perinatal HIV transmission; NNRTI- or PI-based cART; 11/14 had prior mono or dual-NRTI therapy	Yes	50–400 cpm preceded by ≥1 pVL <50 cpm and followed by ≥50 cpm	72	2/11 (18); RT: 2/2 G190E K70R, K103N, Y181C, M184V, T215F; PR: 1/2 L101, I54V, V82A	LLV viral sequences grouped with virus from early stage of infection in 8/11 subjects
Mackie et al., 2010 (70)	1,001	Patients could be on any ARV combination	No	<1,000 cpm	NR	pVL <300 cpm, 270/449 (60); pVL 300–999 cpm: 399/552 (72); highest representation, RT DRM: K65R, M184V, and pathway 2 TAM; K103N, Y181C, G190A; PR DRM: D30N, M46I, V82A	Genotyping was done in routine clinical setting; results likely affected by clinician selection bias; median no. of DRM: pVL <300 cpm, 3; pVL 301–999 cpm, 4
Taiwo et al., 2011 (45)	54	Patients on their first cART (NRTI, EFV, LPV) in randomized clinical trials (ACTG 5142 and ACTG 5095)	Yes	50–1,000 cpm at ≥2 time points over 24-week period	282	20/54 (37); RT DRM: M184I/V (14), K103N (9), and M230L (3), Y181C (1), Y188C (1), M30L/M (1), K101E (1), V106I (1), K70R (1),Y115F (1), L74V (1), D67D/M (1), P225H/P (1); PR DRM: D30N (1)	Compared pretreatment DRM to those identified during LLV, not necessarily substitutions that emerged between onset of LLV and end of LLV; mutations increased with increasing pVL: 51–100 cpm, 0/10 (0%); 101–200 cpm, 5/13 (38%); 200–1,000 cpm, 15/31 (48%)
Gallien et al., 2011 (35)	39	Patients randomized to RAL-based cART after initial suppression on an enfuvirtide-based cART	Yes (baseline proviral DNA)	At least one episode of pVL of 50–500 cpm	100	3/39 (8); INSTI DRM: N155H (2/3), P145S (1/3)	In short-term follow-up, the two patients with N155H had pVL <200 cpm while the patient with P145S was suppressed to <50 cpm
Delaugerre et al., 2012 (68)	37	92% were treatment experienced	Yes	40–500 cpm on ≥3 occasions within ≥6 mo	134	11/37 (30); RT DRM: 7/37 (M184V/I, D67N, T215Y, L74V, L103N, K219E); PR DRM: 4/37 (G48V, L63P, A71V, V77I, V82T, I84V, L101I, L33V/F, L76V, I47V, I53L, L10F); INSTI DRM: 2/37 (T97A, N155H, T97A, Y143C, G163R)	Median LLV duration, 11 mo; 10 of the 11 patients with new DRM during persistent LLV had at least one DRM at onset of LLV
Li et al., 2012 (72)	18	89% were treatment experienced	Yes	50–1,000 cpm, at ≥2 time points	267	8/18 (44); RT DRM: 6/8 (D30N, M41L, M46I, D67N, K70R, K219Q Y115F, Y188C, Y181C); PR DRM: 5/8 (L10R/F, A71V, L90 M, I54 M)	By multivariate analysis, longer elapsed time with LLV and fewer active ARVs associated with increased risk of resistance accumulation
Gonzalez-Serna et al., 2013 (71)	212	ART-naive patients with initial viral suppression on cART	Yes	50–1,000 cpm without prior viral blip	374	38/196 (19); most common substitutions were M184V/I (9.4% V, 3.3% I), K103N (5.2%), T215 revertants (3.8%), M41L (3.3%)	Success at genotyping was 75% at pVL 50–249 cpm, 89% at PVL 250–499 cpm, 92% at 500–749 cpm, and 90% at 750–999 cpm; 196 (92%) had no baseline resistance

[a] With available genotype.
[b] NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; cART, combination antiretroviral therapy; NRTI, nucleos(t)ide reverse transcriptase inhibitor; ARV, antiretroviral; EFV, efavirenz; LPV, lopinavir-ritonavir; RAL, raltegravir.
[c] LLV, low-level viremia.
[d] cpm, copies per ml; pVL, plasma HIV-1 viral load.
[e] DRM, drug resistance mutation; PR, protease; RT, reverse transcriptase; INSTI, integrase strand transfer inhibitor; TAM, thymidine analogue mutations.
[f] NR, not reported.

TABLE 5 Summary of guidelines for management of HIV viremia

Guideline category	DHHS guidelines, 2013 (2)[b]	BHIVA[c] guidelines, 2012 (3, 112)	WHO guidelines, 2013 (113)	Italian consensus statement, 2009 (114, 115)	Spanish guidelines; GESIDA[d] and Spanish Secretariat for the National Plan on AIDS consensus document, 2012 (116)	Guidelines for antiretroviral treatment of HIV from the International AIDS Society–USA Panel, 2012 (117)	EACS[e] guidelines, 2012 (1)
Definition of optimal viral suppression	pVL, <20–75 cpm, depending on the assay used	Below the limits of detection of commercially available assays	Below the limits of detection of commercially available assays	pVL, undetectable, defined as <50 cpm	Below the limits of detection of commercially available assays	pVL, <50 cpm by 24 wk on ART	pVL, ≤50 cpm
Definition of LLV[a]	pVL, 48–200 cpm	Detectable pVL, <400 cpm over a sustained period of time (duration of time not defined)	pVL, 50–1,000 cpm (duration of time not defined)	Not defined	Not defined	Sustained elevation of pVL, 50–200 cpm	Though not defined as LLV, pVL 50–500 cpm warrants further investigation prior to defining as virologic failure
Definition of blips	Transiently detectable pVL at <400 cpm	A single VL 50–400 cpm preceded and followed by an undetectable VL	Transiently detectable pVL, 50–1,000 cpm	Not defined	Transiently detectable pVL, 50–200 cpm	Not defined	Not defined
Definition of virologic failure	Confirmed pVL >200 cpm	Failure to achieve a pVL <50 cpm 6 mo after commencing ART, or pVL >400 cpm on two consecutive occasions following viral suppression to <50 cpm	Two consecutive pVL >1,000 cpm within a 3-mo interval after ≥6 mo on ARV	Confirmed virologic rebound (defined as pVL >50 cpm) in patients with at least two previous undetectable pVL (<50 cpm) measurements, or the presence two consecutive measurements of pVL >50 cpm following 24 wk of ART	Detectable pVL (>50 cpm) after 24 wk of ART, or two consecutive detectable levels after having been undetectable (<50 cpm)	Confirmed detectable pVL >200 cpm after virologic suppression	Confirmed plasma HIV RNA of >50 cpm 6 mo after starting therapy
Management of LLV	(i) For persistent pVL of 200–1,000 cpm, resistance testing should be performed if possible; (ii) consider treatment change if resistance mutation detected; (iii) if no resistance mutation detected, suspect nonadherence; consider resuming prior ART regimen and repeating resistance testing in 2–4 wk	Prompt treatment change is recommended, especially in the absence of a boosted PI	Management defined only for virologic failure: (i) if pVL >1,000 cpm, assess adherence and retest in 3–6 mo; (ii) if pVL <1,000 cpm, resume current regimen; if >1,000 cpm on retest, change regimen	Management defined only for virologic failure; treatment change recommended if criteria for virologic failure are met	Management defined only for virologic failure; treatment change recommended if criteria for virologic failure are met; if possible, obtaining a genotype resistance panel is recommended prior to medication change	Management defined only for virologic failure; prompt change to a new regimen with consideration of contributory factors	If pVL >50 and <500–1,000 cpm: (i) confirm adherence; (ii) check pVL 1–2 mo later; (iii) if pVL is confirmed >500 cpm, change regimen as soon as possible for goal of virologic suppression to <400 copies/ml after 3 mo and <50 cpm after 6 mo; perform resistance testing if possible

[a] LLV, low-level viremia.
[b] pVL, plasma viral load; cpm, copies per ml; DHHS, Department of Health and Human Services.
[c] BHIVA, British HIV Association.
[d] GESIDA, Grupo de Estudio de SIDA.
[e] EACS, European AIDS Clinical Society.

preted with caution, because any impact on morbidity and mortality is likely to be modest and require a long period of follow-up and a large number of participants in order to be detected. Other investigators have observed a correlation between cumulative plasma HIV-1 exposure over time and both AIDS and non-AIDS morbidity and mortality (96), and they speculated that these effects may be due to the sequelae of increased systemic immune activation and inflammation (82, 97). Several studies discussed above suggest the potential for alteration of immune activation and inflammation during periods of LLV. However, a large cohort study found no increased risk of non-AIDS disease associated with LLV compared to that associated with viral suppression, although 75% of LLV episodes consisted of viral blips only (95).

(v) HIV transmission. pVL correlates with HIV RNA concentration in genital secretions and is one of the best predictors of HIV transmission risk (98). Viral transmission risk is considered minimal (i.e., approaching zero) in patients with consistently undetectable plasma HIV levels (99, 100). However, a recent study reported the presence of HIV RNA in seminal fluid (135 to 2,365 cpm) in 6.6% of patients at time points when plasma HIV-1 RNA was not detectable (101). Others have also recovered both free and cell-associated HIV RNA from genital secretions of individuals with undetectable HIV in plasma (102), possibly reflecting the limited penetration of antiretroviral drugs into the genital compartment (102–104).

It is expected that viral shedding into infectious body fluid occurs during episodes of persistent LLV. Consistent with this, maternal-to-fetal HIV transmission is decreased to approximately 1% (not eliminated) when pVL declines to <1,000 cpm (105), and sexual transmission can still occur when pVL is <1,000 cpm (106). Accordingly, the risk of HIV transmission is likely to be lower during persistent LLV than during untreated HIV but higher than in patients with consistently undetectable plasma HIV. The transmission risk during VLLV is unknown but expected to be exceedingly low.

Management. There is a dearth of clinical trials on the management of LLV; hence, guidelines often reflect expert opinion. In addition, management strategies necessarily reflect available resources that differ by region. As such, the following discussion primarily relates to the management of LLV in non-resource-poor settings.

Persistent LLV. The definitions of virologic failure and recommended management approaches vary between guidelines (Table 5). Notably, virologic failure is defined in the Department of Health and Human Services (DHHS) guidelines as a confirmed pVL of >200 cpm, which is a higher threshold than the 50 cpm cutoff adopted by other guidelines in Europe and North America. The cutoff of 200 cpm is supported by studies showing that viremia of 51 and 200 cpm in some patients may represent biological variation around a mean value of <50 cpm and/or assay effects (2, 33). The potential for resistance and increased virologic failure with persistent viremia of <200 cpm has yielded consideration of a lower cutoff (50 cpm), but this remains controversial (48, 50). Adherence should be evaluated and strengthened as needed in all patients with LLV.

Broadly, the management of persistent LLV should be conceptualized as occurring with or without resistance data. Genotype-guided treatment modification in patients with persistent LLV can improve viral suppression rates (79, 107), but a challenge in clinical practice is that genotyping is less available and/or successful for the lower end of the LLV range. In the United States, FDA-approved genotypic assays have a pVL cutoff of ≥1,000 cpm, but several commercial laboratories will perform genotypic testing on samples with much lower pVLs. Independent laboratories can undertake in-house genotyping in lieu of commercial tests. The genotyping success rate in one study was 75% for pVLs of 50 to 249 cpm and ~90% for pVLs of 250 to 499 cpm (71). Critically, despite the potential genotyping success at the lower strata of LLV, there is a lack of consensus regarding the role of genotype-guided management of patients with a pVL of <200 cpm.

If genotyping is not available or is attempted but unsuccessful, the options to the clinician are to continue the current regimen, repeat the genotyping attempt in a few weeks, or modify empirical treatment. These options have not been compared in randomized clinical trials, and widely followed guidelines offer different recommendations (see Table 5). The clinician should consider the patient's treatment history, known drug resistance, resistance barriers of the constituent antiretroviral drugs, duration of persistent LLV, and pVL trajectory. The clinical course of each patient should be considered as well, since different approaches may be indicated for patients with stable clinical status and CD4 counts compared to those with deteriorating indices. Empirical antiretroviral modification among patients with LLV of <200 cpm is controversial. A potential pitfall of empirical regimen modification is that some patients may erroneously discontinue antiretroviral drugs to which there is no resistance or retain/initiate drugs to which there is unrecognized resistance.

VLLV. The management of VLLV is a newly appreciated concern. There are no prospective studies to inform best practices, and no consensus exists that VLLV requires treatment intensification or cART change. Given the high interassay variability near the detection limits of real-time PCR platforms, any repeat testing should be done using the same assay.

Conclusions. Both resistance and virologic failure are more common in patients with persistent LLV than in those with sustained viral suppression at <50 cpm. We encourage additional studies of the prevalence, causes, clinical significance, and management of LLV and VLLV to fill gaps in the evidence base for patient care. There is a need to harmonize treatment guidelines for managing persistent LLV, particularly for viremia of 50 to 200 cpm. There is also a need to increase the availability of assays to genotype samples with a pVL of <400 copies/ml.

ACKNOWLEDGMENTS

B.T. has served as a consultant for ViiV Healthcare, Gilead Sciences, Pfizer, and GlaxoSmithKline. J.Z.L. has served as a consultant for Quest Diagnostics, SeraCare Life Sciences, and TherapyEdge. P.R.H. has served as a consultant for Merck, ViiV, Quest Diagnostics, and Selah Genomics. P.R. and S.K. declare no conflicts of interest.

REFERENCES

1. **European AIDS Clinical Society.** 2013. European guidelines for treatment of HIV infected adults in Europe, version 6.1. European AIDS Clinical Society, Brussels, Belgium.
2. **Panel on Antiretroviral Guidelines for Adults and Adolescents.** 2013. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services Panel on Antiretroviral Guidelines for Adults and Adolescents, Rockville, MD. http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf.
3. **Writing Group, Williams I, Churchill D, Anderson J, Boffito M, Bower M, Cairns G, Cwynarski K, Edwards S, Fidler S, Fisher M,**

Freedman A, Geretti AM, Gilleece Y, Horne R, Johnson M, Khoo S, Leen C, Marshall N, Nelson M, Orkin C, Paton N, Phillips A, Post F, Pozniak A, Sabin C, Trevelion R, Ustianowski A, Walsh J, Waters L, Wilkins E, Winston A, Youle M. 2012. British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy. HIV Med. **13:**S1–S85. http://dx.doi.org/10.1111/hiv.12119.

4. **Geretti AM, Smith C, Haberl A, Garcia-Diaz A, Nebbia G, Johnson M, Phillips A, Staszewski S.** 2008. Determinants of virological failure after successful viral load suppression in first-line highly active antiretroviral therapy. Antivir. Ther. **169:**927–936.
5. **Greub G, Cozzi-Lepri A, Ledergerber B, Staszewski S, Perrin L, Miller V, Francioli P, Furrer H, Battegay M, Vernazza P, Bernasconi E, Günthard HF, Hirschel B, Phillips AN, Telenti A, Frankfurt HIV Clinic Cohort and Swiss HIV Cohort Study.** 2002. Intermittent and sustained low-level HIV viral rebound in patients receiving potent antiretroviral therapy. AIDS **16:**1967–1969. http://dx.doi.org/10.1097/00002030-200209270-00017.
6. **Palmer S, Maldarelli F, Wiegand A, Bernstein B, Hanna GJ, Brun SC, Kempf DJ, Mellors JW, Coffin JM, King MS.** 2008. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. Proc. Natl. Acad. Sci. U. S. A. **105:**3879–3884. http://dx.doi.org/10.1073/pnas.0800050105.
7. **Maldarelli F, Palmer S, King MS, Wiegand A, Polis MA, Mican J, Kovacs JA, Davey RT, Rock-Kress D, Dewar R, Liu S, Metcalf JA, Rehm C, Brun SC, Hanna GJ, Kempf DJ, Coffin JM, Mellors JW.** 2007. ART suppresses plasma HIV-1 RNA to a stable set point predicted by pretherapy viremia. PLoS Pathog. **3:**e46. http://dx.doi.org/10.1371/journal.ppat.0030046.
8. **Roche Molecular Diagnostics.** 2013. Timeline of PCR and Roche. Roche Molecular Diagnostics, Pleasanton, CA. http://molecular.roche.com/About/pcr/Pages/PCRTimeline.aspx.
9. **Palmer S.** 2013. Advances in detection and monitoring of plasma viremia in HIV-infected individuals receiving antiretroviral therapy. Curr. Opin. HIV AIDS **8:**87–92. http://dx.doi.org/10.1097/COH.0b013e32835d80af.
10. **Cobb BR, Vaks JE, Do T, Vilchez RA.** 2011. Evolution in the sensitivity of quantitative HIV-1 viral load tests. J. Clin. Virol. **52:**S77–S82. http://dx.doi.org/10.1016/j.jcv.2011.09.015.
11. **Roche.** 2013. Cobas AmpliPrep/Cobas TaqMan HIV-1 Test, v2.0. Roche Molecular Diagnostics, Pleasanton, CA. http://molecular.roche.com/assays/Pages/COBASAmpliPrepCOBASTaqManHIV-1Testv20.aspx.
12. **Abbott Molecular.** 2013. Abbott RealTi*me* HIV-1 package insert. Abbott Molecular, Abbott Park, IL. http://www.abbottmolecular.com/static/cms_workspace/pdfs/US/51-602146R6.pdf.
13. **Lima V, Harrigan R, Montaner JS.** 2009. Increased reporting of detectable plasma HIV-1 RNA levels at the critical threshold of 50 copies per milliliter with the TaqMan assay in comparison to the Amplicor assay. J. Acquir. Immune Defic. Syndr. **51:**3–6. http://dx.doi.org/10.1097/QAI.0b013e31819e721b.
14. **Karasi JC, Dziezuk F, Quennery L, Förster S, Reischl U, Colucci G, Schoener D, Seguin-Devaux C, Schmit JC.** 2011. High correlation between the Roche Cobas AmpliPrep/Cobas TaqMan HIV-1, v2.0 and the Abbott m2000 RealTi*me* HIV-1 assays for quantification of viral load in HIV-1 B and non-B subtypes. J. Clin. Virol. **52:**181–186. http://dx.doi.org/10.1016/j.jcv.2011.07.002.
15. **Garrett NJ, Apea V, Nori A, Ushiro-Lumb I, Oliver AR, Baily G, Clark DA.** 2012. Comparison of the rate and size of HIV-1 viral load blips with Roche Cobas TaqMan HIV-1 versions 1.0 and 2.0 and implications for patient management. J. Clin. Virol. **53:**354–355. http://dx.doi.org/10.1016/j.jcv.2011.12.024.
16. **Wirden M, Soulie C, Valantin M.** 2011. Sequential HIV RNA resistance test results are more informative than proviral DNA genotyping in cases of suppressed or residual viremia, abstr 613. 18th Conference on Retroviruses and Opportunistic Infections, Boston, MA, 27 February to 2 March 2011.
17. **Xu S, Song A, Nie J, Li X, Meng S, Zhang C, Wang Y.** 2012. Comparison between the automated Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 test version 2.0 assay and its version 1 and Nuclisens HIV-1 EasyQ version 2.0 assays when measuring diverse HIV-1 genotypes in China. J. Clin. Virol. **53:**33–37. http://dx.doi.org/10.1016/j.jcv.2011.10.001.
18. **Ruelle J, Debaisieux L, Vancutsem E, De Bel A, Delforge ML, Piérard D, Goubau P.** 2012. HIV-1 low-level viraemia assessed with 3 commercial real-time PCR assays show high variability. BMC Infect. Dis. **12:**100. http://dx.doi.org/10.1186/1471-2334-12-100.
19. **Kran AB, Jonassen TØ, Sannes M, Jakobsen K, Lind A, Maeland A, Holberg-Petersen M.** 2009. Overestimation of human immunodeficiency virus type 1 load caused by the presence of cells in plasma from plasma preparation tubes. J. Clin. Microbiol. **47:**2170–2174. http://dx.doi.org/10.1128/JCM.00519-09.
20. **Stosor V, Palella FJ, Jr, Berzins B, Till M, Leake A, Chmiel JS, Murphy RL.** 2005. Transient viremia in HIV-infected patients and use of plasma preparation tubes. Clin. Infect. Dis. **41:**1671–1674. http://dx.doi.org/10.1086/498025.
21. **Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M.** 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature **373:**123–126. http://dx.doi.org/10.1038/373123a0.
22. **Perelson AS, Essunger P, Cao Y, Vesanen M, Hurley A, Saksela K, Markowitz M, Ho DD.** 1997. Decay characteristics of HIV-1-infected compartments during combination therapy. Nature **387:**188–191. http://dx.doi.org/10.1038/387188a0.
23. **Sedaghat AR, Siliciano RF, Wilke CO.** 2008. Low-level HIV-1 replication and the dynamics of the resting CD4+ T cell reservoir for HIV-1 in the setting of HAART. BMC Infect. Dis. **8:**1–14. http://dx.doi.org/10.1186/1471-2334-8-1.
24. **Gilmore JB, Kelleher AD, Cooper DA, Murray JM.** 2013. Explaining the determinants of first phase HIV decay dynamics through the effects of stage-dependent drug action. PLoS Comput. Biol. **9:**e1002971. http://dx.doi.org/10.1371/journal.pcbi.1002971.
25. **Andrade A, Rosenkranz SL, Cillo AR, Lu D, Daar ES, Jacobson JM, Lederman M, Acosta EP, Campbell T, Feinberg J, Flexner C, Mellors JW, Kuritzkes DR, AIDS Clinical Trials Group A5248 Team.** 2013. Three distinct phases of HIV-1 RNA decay in treatment-naive patients receiving raltegravir-based antiretroviral therapy: ACTG A5248. J. Infect. Dis. **208:**884–891. http://dx.doi.org/10.1093/infdis/jit272.
26. **Palmer S, Wiegand AP, Maldarelli F, Bazmi H, Mican JM, Polis M, Dewar RL, Planta A, Liu S, Metcalf JA, Mellors JW, Coffin JM.** 2003. New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. J. Clin. Microbiol. **41:**4531–4536. http://dx.doi.org/10.1128/JCM.41.10.4531-4536.2003.
27. **Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margolick JB, Kovacs C, Gange SJ, Siliciano RF.** 2003. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting $CD4^+$ T cells. Nat. Med. **9:**727–728. http://dx.doi.org/10.1038/nm880.
28. **Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, Pierson T, Smith K, Lisziewicz J, Lori F, Flexner C, Quinn TC, Chaisson RE, Rosenberg E, Walker B, Gange S, Gallant J, Siliciano RF.** 1999. Latent infection of $CD4^+$ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nat. Med. **5:**512–517. http://dx.doi.org/10.1038/8394.
29. **Yukl SA, Gianella S, Sinclair E, Epling L, Li Q, Duan L, Choi AL, Girling V, Ho T, Li P, Fujimoto K, Lampiris H, Hare CB, Pandori M, Haase AT, Günthard HF, Fischer M, Shergill AK, McQuaid K, Havlir DV, Wong JK.** 2010. Differences in HIV burden and immune activation within the gut of HIV-positive patients receiving suppressive antiretroviral therapy. J. Infect. Dis. **202:**1553–1561. http://dx.doi.org/10.1086/656722.
30. **García-Gascó P, Maida I, Blanco F, Barreiro P, Martín-Carbonero L, Vispo E, González-Lahoz J, Soriano V.** 2008. Episodes of low-level viral rebound in HIV-infected patients on antiretroviral therapy: frequency, predictors and outcome. J. Antimicrob. Chemother. **61:**699–704. http://dx.doi.org/10.1093/jac/dkm516.
31. **Sklar PA, Ward DJ, Baker RK, Wood KC, Gafoor Z, Alzola CF, Moorman AC, Holmberg SD, HIV Outpatient Study (HOPS) Investigators.** 2002. Prevalence and clinical correlates of HIV viremia ('blips') in patients with previous suppression below the limits of quantification. AIDS **16:**2035–2041. http://dx.doi.org/10.1097/00002030-200210180-00008.
32. **van Sighem A, Zhang S, Reiss P, Gras L, van der Ende M, Kroon F, Prins J, de Wolf F.** 2008. Immunologic, virologic, and clinical consequences of episodes of transient viremia during suppressive combination antiretroviral therapy. J. Acquir. Immune Defic. Syndr. **48:**104–108. http://dx.doi.org/10.1097/QAI.0b013e31816a1d4f.
33. **Nettles RE, Kieffer TL, Kwon P, Monie D, Han Y, Parsons T, Cofran-**

cesco J, Jr, Gallant JE, Quinn TC, Jackson B, Flexner C, Carson K, Ray S, Persaud D, Siliciano RF. 2005. Intermittent HIV-1 viremia (blips) and drug resistance in patients receiving HAART. JAMA **293:**817–829. http://dx.doi.org/10.1001/jama.293.7.817.

34. **Grennan JT, Loutfy MR, Su D, Harrigan PR, Cooper C, Klein M, Machouf N, Montaner JS, Rourke S, Tsoukas C, Hogg B, Raboud J, CANOC Collaboration.** 2012. Magnitude of virologic blips is associated with a higher risk for virologic rebound in HIV-infected individuals: a recurrent events analysis. J. Infect. Dis. **205:**1230–1238. http://dx.doi.org/10.1093/infdis/jis104.
35. **Gallien S, Delaugerre C, Charreau I, Braun J, Boulet T, Barrail-Tran A, de Castro N, Molina JM, Kuritzkes DR.** 2011. Emerging integrase inhibitor resistance mutations in raltegravir-treated HIV-1-infected patients with low-level viremia. AIDS **25:**665–669. http://dx.doi.org/10.1097/QAD.0b013e3283445834.
36. **Havlir DV, Bassett R, Levitan D, Gilbert P, Tebas P, Collier AC, Hirsch MS, Ignacio C, Condra J, Günthard HF, Richman DD, Wong JK.** 2001. Prevalence and predictive value of intermittent viremia with combination HIV therapy. JAMA **286:**171–179. http://dx.doi.org/10.1001/jama.286.2.171.
37. **Martinez V, Marcelin AG, Morini JP, Deleuze J, Krivine A, Gorin I, Yerly S, Perrin L, Peytavin G, Calvez V, Dupin N.** 2005. HIV-1 intermittent viraemia in patients treated by non-nucleoside reverse transcriptase inhibitor based regimen. AIDS **19:**1065–1069. http://dx.doi.org/10.1097/01.aids.0000174453.55627.de.
38. **Sungkanuparph S, Overton ET, Seyfried W, Groger RK, Fraser VJ, Powderly WG.** 2005. Intermittent episodes of detectable HIV viremia in patients receiving nonnucleoside reverse-transcriptase inhibitor-based or protease inhibitor-based highly active antiretroviral therapy regimens are equivalent in incidence and prognosis. Clin. Infect. Dis. **41:**1326–1332. http://dx.doi.org/10.1086/496985.
39. **Mira JA, Macias J, Nogales C, Fernández-Rivera J, García-García JA, Ramos A, Pineda JA.** 2002. Transient rebounds of low-level viraemia among HIV-infected patients under HAART are not associated with virological or immunological failure. Antivir. Ther. **7:**251–256.
40. **Podsadecki TJ, Vrijens BC, Tousset EP, Rode RA, Hanna GJ.** 2007. Decreased adherence to antiretroviral therapy observed prior to transient human immunodeficiency virus type 1 viremia. J. Infect. Dis. **196:**1773–1778. http://dx.doi.org/10.1086/523704.
41. **Macias J, Palomares JC, Mira JA, Torres MJ, García-García JA, Rodríquez JM, Vergera S, Pineda JA.** 2005. Transient rebounds of HIV plasma viremia are associated with the emergence of drug resistance mutations in patients on highly active antiretroviral therapy. J. Infect. **51:**195–200. http://dx.doi.org/10.1016/j.jinf.2004.11.010.
42. **Gianotti N, Galli L, Racca S, Salpietro S, Cossarini F, Spagnuolo V, Barda B, Canducci F, Clementi M, Lazzarin A, Castagna A.** 2012. Residual viraemia does not influence 1 year virological rebound in HIV-infected patients with HIV RNA persistently below 50 copies/ml. J. Antimicrob. Chemother. **67:**213–217. http://dx.doi.org/10.1093/jac/dkr422.
43. **Maggiolo F, Callegaro A, Cologni G, Bernardini C, Velenti D, Gregis G, Quinzan G, Soavi L, Iannotti N, Malfatto E, Leone S.** 2012. Ultrasensitive assessment of residual low-level HIV viremia in HAART-treated patients and risk of virological failure. J. Acquir. Immune Defic. Syndr. **60:**473–482. http://dx.doi.org/10.1097/QAI.0b013e3182567a57.
44. **Di Mascio M, Markowitz M, Louie M, Hurley A, Hogan C, Simon V, Follmann D, Ho DD, Perelson AS.** 2004. Dynamics of intermittent viremia during highly active antiretroviral therapy in patients who initiate therapy during chronic versus acute and early human immunodeficiency virus type 1 infection. J. Virol. **78:**10566–10573. http://dx.doi.org/10.1128/JVI.78.19.10566-10573.2004.
45. **Taiwo B, Gallien S, Aga E, Ribaudo H, Haubrich R, Kuritzkes DR, Eron JJ, Jr.** 2011. Antiretroviral drug resistance in HIV-1-infected patients experiencing persistent low-level viremia during first-line therapy. J. Infect. Dis. **204:**515–520. http://dx.doi.org/10.1093/infdis/jir353.
46. **Sungkanuparph S, Groger RK, Overton ET, Fraser VJ, Powderly WG.** 2006. Persistent low-level viraemia and virological failure in HIV-1-infected patients treated with highly active antiretroviral therapy. HIV Med. **7:**437–441. http://dx.doi.org/10.1111/j.1468-1293.2006.00403.x.
47. **Havlir DV, Koelsch KK, Strain MC, Margot N, Lu B, Ignacio CC, Miller MD, Wong JK, Gilead 903 Study Team.** 2005. Predictors of residual viremia in HIV-infected patients successfully treated with efavirenz and lamivudine plus either tenofovir or stavudine. J. Infect. Dis. **191:**1164–1168. http://dx.doi.org/10.1086/428588.
48. **Doyle T, Smith C, Vitiello P, Cambiano V, Johnson M, Owen A, Phillips AN, Geretti AM.** 2012. Plasma HIV-1 RNA detection below 50 copies/ml and risk of virologic rebound in patients receiving highly active antiretroviral therapy. Clin. Infect. Dis. **54:**724–732. http://dx.doi.org/10.1093/cid/cir936.
49. **Charpentier C, Landman R, Laouénan C, Joly V, Hamet G, Damond F, Brun-Vézinet F, Mentré F, Descamps D, Yeni P.** 2012. Persistent low-level HIV-1 RNA between 20 and 50 copies/ml in antiretroviral-treated patients: associated factors and virological outcome. J. Antimicrob. Chemother. **67:**2231–2235. http://dx.doi.org/10.1093/jac/dks191.
50. **Henrich TJ, Wood BR, Kuritzkes DR.** 2012. Increased risk of virologic rebound in patients on antiviral therapy with a detectable HIV load <48 copies/ml. PLoS One **7:**e50065. http://dx.doi.org/10.1371/journal.pone.0050065.
51. **Widdrington J, Payne B, Medhi M, Valappil M, Schmid ML.** 2011. The significance of very low-level viraemia detected by sensitive viral load assays in HIV infected patients on HAART. J. Infect. **62:**87–92. http://dx.doi.org/10.1016/j.jinf.2010.11.001.
52. **Álvarez-Estévez M, Chueca Porcuna N, Guillot Suay V, Peña Monge A, García García F, Muñoz Medina L, Vinuesa García D, Parra Ruiz J, Hernández-Quero J, García García F.** 2013. Quantification of viral loads lower than 50 copies per milliliter by use of the Cobas AmpliPrep/Cobas TaqMan HIV-1 test, version 2.0, can predict the likelihood of subsequent virological rebound to >50 copies per milliliter. J. Clin. Microbiol. **51:**1555–1557. http://dx.doi.org/10.1128/JCM.00100-13.
53. **Easterbrook PJ, Ives N, Waters A, Mullen J, O'Shea S, Peters B, Gazzard BG.** 2002. The natural history and clinical significance of intermittent viraemia in patients with initial viral suppression to <400 copies/ml. AIDS **16:**1521–1527. http://dx.doi.org/10.1097/00002030-200207260-00009.
54. **Masquelier B, Pereira E, Peytavin G, Descamps D, Reynes J, Verdon R, Fleury H, Garraffo R, Chêne G, Raffi F, Brun-Vézinet F, APROCO/COPILOTE Study Group.** 2005. Intermittent viremia during first-line, protease inhibitors-containing therapy: significance and relationship with drug resistance. J. Clin. Virol. **33:**75–78. http://dx.doi.org/10.1016/j.jcv.2004.11.012.
55. **Miller LG, Golin CE, Liu H, Hays RD, Hua J, Wenger NS, Kaplan AH.** 2004. No evidence of an association between transient HIV viremia ("blips") and lower adherence to the antiretroviral medication regimen. J. Infect. Dis. **189:**1487–1496. http://dx.doi.org/10.1086/382895.
56. **Jones LE, Perelson AS.** 2007. Transient viremia, plasma viral load, and reservoir replenishment in HIV-infected patients on antiretroviral therapy. J. Acquir. Immune Defic. Syndr. **45:**483–493. http://dx.doi.org/10.1097/QAI.0b013e3180654836.
57. **Boillat-Blanco N, Darling K, Schoni-Affolter F.** 2012. Persistent low-level viraemia in HIV-1-infected patients: Swiss HIV Cohort Study, abstr H-1566. 52nd Interscience Conference on Antimicrobials and Chemotherapy. San Francisco, CA, 9 to 12 September 2012.
58. **Bierman WF, van Agtmael MA, Nijhuis M, Danner SA, Boucher CA.** 2009. HIV monotherapy with rotinavir-boosted protease inhibitors: a systematic review. AIDS **23:**279–291. http://dx.doi.org/10.1097/QAD.0b013e32831c54e5.
59. **Taiwo B, Zheng L, Gallien S, Matining RM, Kuritzkes DR, Wilson CC, Berzins BI, Acosta EP, Bastow B, Kim PS, Eron JJ, Jr, ACTG A5262 Team.** 2011. Efficacy of a nucleoside-sparing regimen of darunavir/ritonavir plus raltegravir in treatment-naive HIV-1-infected patients (ACTG A5262). AIDS **25:**2113–2122. http://dx.doi.org/10.1097/QAD.0b013e32834bbaa9.
60. **Cologni G, Soavi L, Leone S, Valenti D, Callegaro A, Gregis G, Suter F, Maggiolo F.** 2010. Low-level residual viremia and risk of virological failure. J. Int. AIDS Soc. **13:**O5. http://dx.doi.org/10.1186/1758-2652-13-S4-O5.
61. **Allavena C, Rodallec A, Sécher S, Reliquet V, Baffoin S, André-Garnier E, Billaud E, Raffi F, Ferré V.** 2013. Evaluation of residual viremia and quantitation of soluble CD14 in a large cohort of HIV-infected adults on a long-term non-nucleoside reverse transcriptase inhibitor-based regimen. Med. Virol. **85:**1878–1882. http://dx.doi.org/10.1002/jmv.23679.
62. **Kramer VG, Schader SM, Oliveira M, Colby-Germinario SP, Donahue DA, Singhroy DN, Tressler R, Sloan RD, Wainberg MA.** 2012. Maraviroc and other HIV-1 entry inhibitors exhibit a class-specific redistribution effect that results in increased extracellular viral load. Antimicrob. Agents Chemother. **56:**4154–4160. http://dx.doi.org/10.1128/AAC.00409-12.
63. **Eisfeld C, Reichelt D, Evers S, Husstedt I.** 2013. CSF penetration by

antiretroviral drugs. CNS Drugs **27:**31–55. http://dx.doi.org/10.1007/s40263-012-0018-x.

64. **Costiniuk CT, Kovacs C, Routy JP, Singer J, Gurunathan S, Sekaly RP, Angel JB.** 2013. Short communication: human immunodeficiency virus rebound in blood and seminal plasma following discontinuation of antiretroviral therapy. AIDS Res. Hum. Retroviruses **29:**266–269. http://dx.doi.org/10.1089/AID.2011.0343.
65. **Chun TW, Murray D, Justement JS, Hallahan CW, Moir S, Kovacs C, Fauci AS.** 2011. Relationship between residual plasma viremia and the size of HIV proviral DNA reservoirs in infected individuals receiving effective antiretroviral therapy. J. Infect. Dis. **204:**135–138. http://dx.doi.org/10.1093/infdis/jir208.
66. **Bonora S, Nicastri E, Calcagno A, Gonzalez de Requena D, D'Ettorre G, Sarmati L, Palmisano L, Vullo V, Di Perri G, Andreoni M.** 2009. Ultrasensitive assessment of residual HIV viremia in HAART-treated patients with persistently undetectable plasma HIV-RNA: a cross-sectional study. J. Med. Virol. **81:**400–405. http://dx.doi.org/10.1002/jmv.21405.
67. **Lima VD, Bangsberg DR, Harrigan PR, Deeks SG, Yip B, Hogg RS, Montaner JS.** 2010. Risk of viral failure declines with duration of suppression on highly active antiretroviral therapy irrespective of adherence level. J. Acquir. Immune Defic. Syndr. **55:**460–465. http://dx.doi.org/10.1097/QAI.0b013e3181f2ac87.
68. **Delaugerre C, Gallien S, Flandre P, Mathez D, Amarsy R, Ferret S, Timsit J, Molina JM, de Truchis P.** 2012. Impact of low-level-viremia on HIV-1 drug-resistance evolution among antiretroviral treated-patients. PLoS One **7:**e36673. http://dx.doi.org/10.1371/journal.pone.0036673.
69. **Nettles RE, Kieffer TL, Simmons RP, Cofrancesco J, Jr, Moore RD, Gallant JE, Persaud D, Siliciano RF.** 2004. Genotypic resistance in HIV-1-infected patients with persistently detectable low-level viremia while receiving highly active antiretroviral therapy. Clin. Infect. Dis. **39:**1030–1037. http://dx.doi.org/10.1086/423388.
70. **Mackie NE, Phillips AN, Kaye S, Booth C, Geretti AM.** 2010. Antiretroviral drug resistance in HIV-1-infected patients with low-level viremia. J. Infect. Dis. **201:**1303–1307. http://dx.doi.org/10.1086/651618.
71. **Gonzalez-Serna A, Woods C, Li J, Harrigan R, Swenson L.** 2013. Low-level HIV viremia and drug resistance, abstr 601. 20th Conference on Retroviruses and Opportunistic Infections. Atlanta, GA, 3 to 6 March 2013.
72. **Li JZ, Gallien S, Do TD, Martin JN, Deeks S, Kuritzkes DR, Hatano H.** 2012. Prevalence and significance of HIV-1 drug resistance mutations among patients on antiretroviral therapy with detectable low-level viremia. Antimicrob. Agents Chemother. **56:**5998–6000. http://dx.doi.org/10.1128/AAC.01217-12.
73. **Karlsson AC, Younger SR, Martin JN, Grossman Z, Sinclair E, Hunt PW, Hagos E, Nixon DF, Deeks SG.** 2004. Immunologic and virologic evolution during periods of intermittent and persistent low-level viremia. AIDS **18:**981–989. http://dx.doi.org/10.1097/00002030-200404300-00005.
74. **Tobin NH, Learn GH, Holte SE, Wang Y, Melvin AJ, McKernan JL, Pawluk DM, Mohan KM, Lewis PF, Mullins JI, Frenkel LM.** 2005. 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. **79:**9625–9634. http://dx.doi.org/10.1128/JVI.79.15.9625-9634.2005.
75. **Prosperi MC, Mackie N, Di Giambenedetto S, Zazzi M, Camacho R, Fanti I, Torti C, Sönnerborg A, Kaiser R, Codoñer FM, Van Laethem K, Bansi L, van de Vijver DA, Geretti AM, De Luca A, SEHERE Consortium.** 2011. Detection of drug resistance mutations at low plasma HIV-1 RNA load in a European multicentre cohort study. J. Antimicrob. Chemother. **66:**1886–1896. http://dx.doi.org/10.1093/jac/dkr171.
76. **Pellegrino D, Brígido L, Souza Cavalcanti J, Paul Ferreira J, Vidal J, Battaggia Gutierrez E.** 2012. High frequency of genotypic resistance in HIV-1-infected patients on highly active antiretroviral therapy with persistent low level viremia. J. Int. AIDS Soc. **15:**18173. http://dx.doi.org/10.7448/IAS.15.6.18173.
77. **von Wyl V, Yerly S, Böni J, Shah C, Cellerai C, Klimkait T, Battegay M, Bernasconi E, Cavassini M, Furrer H, Hirschel B, Vernazza PL, Ledergerber B, Günthard HF, Swiss HIV Cohort Study.** 2012. Incidence of HIV-1 drug resistance among antiretroviral treatment-naive individuals starting modern therapy combinations. Clin. Infect. Dis. **54:**131–140. http://dx.doi.org/10.1093/cid/cir728.
78. **Laprise C, de Pokomandy A, Baril JG, Dufresne S, Trottier H.** 2013. Virologic failure following persistent low-level viremia in a cohort of HIV-positive patients: results from 12 years of observation. Clin. Infect. Dis. **57:**1489–1496. http://dx.doi.org/10.1093/cid/cit529.
79. **Swenson LC, Gonzalez-Serna A, Min JE, Woods CK, Li JZ, Harrigan PR.** 2013. HIV drug resistance occurring during low level viremia is associated with subsequent virologic failure. International Workshop on HIV & Hepatitis Virus Drug Resistance and Curative Strategies. Toronto, Canada, 4 to 8 June 2013.
80. **Lo Re V, III, Gasink L, Kostman JR, Leonard D, Gross R.** 2004. Natural history of patients with low-level HIV viremia on antiretroviral therapy. AIDS Patient Care STDs **18:**436–442. http://dx.doi.org/10.1089/1087291041703692.
81. **Corbeau P, Reynes J.** 2011. Immune reconstitution under antiretroviral therapy: the new challenge in HIV-1 infection. Blood **117:**5582. http://dx.doi.org/10.1182/blood-2010-12-322453.
82. **Neuhaus J, Jacobs DR, Jr, Baker JV, Calmy A, Duprez D, La Rosa A, Kuller LH, Pett SL, Ristola M, Ross MJ, Shlipak MG, Tracy R, Neaton JD.** 2010. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. J. Infect. Dis. **201:**1788–1795. http://dx.doi.org/10.1086/652749.
83. **Lichtfuss GF, Hoy J, Rajasuriar R, Kramski M, Crowe SM, Lewin SR.** 2011. Biomarkers of immune dysfunction following combination antiretroviral therapy for HIV infection. Biomark. Med. **5:**171–186. http://dx.doi.org/10.2217/bmm.11.15.
84. **Klatt NR, Funderburg NT, Brenchley JM.** 2013. Microbial translocation, immune activation, and HIV disease. Trends Microbiol. **21:**6–13. http://dx.doi.org/10.1016/j.tim.2012.09.001.
85. **Sandler NG, Wand H, Rogue A, Law M, Nason MC, Nixon DE, Pedersen C, Ruxrungtham K, Lewin SR, Emery S, Neaton JD, Brenchley JM, Deeks SG, Sereti I, Douek DC, INSIGHT SMART Study Group.** 2011. Plasma levels of soluble CD14 independently predict mortality in HIV infection. J. Infect. Dis. **203:**780–790. http://dx.doi.org/10.1093/infdis/jiq118.
86. **Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, Ledergerber B, Lundgren J, Neuhaus J, Nixon D, Paton NI, Neaton JD, INSIGHT SMART Study Group.** 2008. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med. **5:**e203. http://dx.doi.org/10.1371/journal.pmed.0050203.
87. **Zheng L, Bosch RJ, Chan ES, Read S, Kearney M, Margolis DM, Mellors JW, Eron JJ, Gandhi RT, AIDS Clinical Trials Group (ACTG) A5244 Team.** 2013. Predictors of residual viremia in patients on long-term suppressive antiretroviral therapy. Antivir. Ther. **18:**39–43. http://dx.doi.org/10.3851/IMP2323.
88. **Ostrowski SR, Katzenstein TL, Thim PT, Pedersen BK, Gerstoft J, Ullum H.** 2005. Low-level viremia and proviral DNA impede immune reconstitution in HIV-1-infected patients receiving highly active antiretroviral therapy. J. Infect. Dis. **191:**348–357. http://dx.doi.org/10.1086/427340.
89. **Taiwo B, Hunt PW, Gandhi RT, Ellingson A, McKenna M, Jacobson JM, Gripshover B, Bosch RJ.** 2013. $CD8^+$ T-cell activation in HIV-1-infected patients experiencing transient low-level viremia during antiretroviral therapy. J. Acquir. Immune Defic. Syndr. **63:**101–104. http://dx.doi.org/10.1097/QAI.0b013e3182895af4.
90. **Ostrowski SR, Katzenstein TL, Pedersen BK, Gerstoft J, Ullum H.** 2008. Residual viraemia in HIV-1-infected patients with plasma viral load <or=20 copies/ml is associated with increased blood levels of soluble immune activation markers. Scand. J. Immunol. **68:**652–660. http://dx.doi.org/10.1111/j.1365-3083.2008.02184.x.
91. **de Larrañaga GF, Petroni A, Deluchi G, Alonso BS, Benetucci JA.** 2003. Viral load and disease progression as responsible for endothelial activation and/or injury in human immunodeficiency virus-1-infected patients. Blood Coagul. Fibrinolysis **14:**15–18. http://dx.doi.org/10.1097/00001721-200301000-00004.
92. **Bastard JP, Soulié C, Fellahi S, Haïm-Boukobza S, Simon A, Katlama C, Calvez V, Marcelin AG, Capeau J.** 2012. Circulating interleukin-6 levels correlate with residual HIV viraemia and markers of immune dysfunction in treatment-controlled HIV-infected patients. Antivir. Ther. **17:**915–919. http://dx.doi.org/10.3851/IMP2093.
93. **Reus S, Portilla J, Sánchez-Payá J, Giner L, Francés R, Such J, Boix V, Merino E, Gimeno A.** 2013. Low-level HIV viremia is associated with microbial translocation and inflammation. J. Acquir. Immune Defic. Syndr. **62:**129–134. http://dx.doi.org/10.1097/QAI.0b013e3182745ab0.
94. **Eastburn A, Scherzer R, Zolopa AR, Benson C, Tracy R, Do T,**

Bacchetti P, Shlipak M, Grunfeld C, Tien PC. 2011. Association of low level viremia with inflammation and mortality in HIV-infected adults. PLoS One **6:**e26320. http://dx.doi.org/10.1371/journal.pone.0026320.

95. **Zhang S, van Sighem A, Kesselring A, Gras L, Smit C, Prins JM, Kauffmann R, Richter C, de Wolf F, Reiss P.** 2012. Episodes of HIV viremia and the risk of non-AIDS diseases in patients on suppressive antiretroviral therapy. J. Acquir. Immune Defic. Syndr. **60:**265–272. http://dx.doi.org/10.1097/QAI.0b013e318258c651.
96. **Mugavero MJ, Napravnik S, Cole SR, Eron JJ, Lau B, Crane HM, Kitahata MM, Willig JH, Moore RD, Deeks SG, Saag MS, Centers for AIDS Research Network of Integrated Clinical Systems (CNICS) Cohort Study.** 2011. Viremia copy-years predicts mortality among treatment-naive HIV-infected patients initiating antiretroviral therapy. Clin. Infect. Dis. **53:**927–935. http://dx.doi.org/10.1093/cid/cir526.
97. **El-Sadr WM, Lundgren J, Neaton JD, Gordin F, Abrams D, Arduino RC, Babiker A, Burman W, Clumeck N, Cohen CJ, Cohn D, Cooper D, Darbyshire J, Emery S, Fätkenheuer G, Gazzard B, Grund B, Hoy J, Klingman K, Losso M, Markowitz N, Neuhaus J, Phillips A, Rappoport C.** 2006. $CD4^+$ count-guided interruption of antiretroviral treatment. N. Engl. J. Med. **355:**2283–2296. http://dx.doi.org/10.1056/NEJMoa062360.
98. **Fiore J, Suligoi B, Saracino A, Di Stefano M, Bugarini R, Lepera A, Favia A, Monno L, Angarano G, Pastore G.** 2003. Correlates of HIV-1 shedding in cervicovaginal secretions and effects of antiretroviral therapies. AIDS **17:**2169–2176. http://dx.doi.org/10.1097/00002030-200310170-00004.
99. **Vernazza P, Hirschel B, Bernasconi E, Flepp M.** 2008. HIV-positive individuals not suffering from any other STD and adhering to an effective antiretroviral treatment do not transmit HIV sexually. Bull. Méd. Suisses **89:**165–169. (In French.) http://dx.doi.org/10.1097/QAD.0b013e3282fd167c.
100. **Loutfy MR, Wu W, Letchumanan M, Bondy L, Antoniou T, Margolese S, Zhang Y, Rueda S, McGee F, Peck R, Binder L, Allard P, Rourke SB, Rochon PA.** 2013. Systematic review of HIV transmission between heterosexual serodiscordant couples where the HIV-positive partner is fully suppressed on antiretroviral therapy. PLoS One **8:**e55747. http://dx.doi.org/10.1371/journal.pone.0055747.
101. **Lambert-Niclot S, Tubiana R, Beaudoux C, Lefebvre G, Caby F, Bonmarchand M, Naouri M, Schubert B, Dommergues M, Calvez V, Flandre P, Poirot C, Marcelin AG.** 2012. Detection of HIV-1 RNA in seminal plasma samples from treated patients with undetectable HIV-1 RNA in blood plasma on a 2002–2011 survey. AIDS **26:**971–975. http://dx.doi.org/10.1097/QAD.0b013e328352ae09.
102. **Cu-Uvin S, DeLong AK, Venkatesh KK, Hogan JW, Ingersoll J, Kurpewski J, De Pasquale MP, D'Aquila R, Caliendo AM.** 2010. Genital tract HIV-1 RNA shedding among women with below detectable plasma viral load. AIDS **24:**2489–2497. http://dx.doi.org/10.1097/QAD.0b013e32833e5043.
103. **Kalichman SC, Di Berto G, Eaton L.** 2008. Human immunodeficiency virus viral load in blood plasma and semen: review and implications of empirical findings. Sex. Transm. Dis. **35:**55–60. http://dx.doi.org/10.1097/OLQ.0b013e318141fe9b.
104. **Halfon P, Giorgetti C, Khiri H, Pénaranda G, Terriou P, Porcu-Buisson G, Chabert-Orsini V.** 2010. Semen may harbor HIV despite effective HAART: another piece in the puzzle. PLoS One **5:**e10569. http://dx.doi.org/10.1371/journal.pone.0010569.
105. **Ioannidis JP, Abrams EJ, Ammann A, Bulterys M, Goedert JJ, Gray L, Korber BT, Mayaux MJ, Mofenson LM, Newell ML, Shapiro DE, Teglas JP, Wilfert CM.** 2001. Perinatal transmission of human immunodeficiency virus type 1 by pregnant women with RNA virus loads <1000 copies/ml. J. Infect. Dis. **183:**539. http://dx.doi.org/10.1086/318530.
106. **Hughes JP, Baeten JM, Lingappa JR, Magaret AS, Wald A, de Bruyn G, Kiarie J, Inambao M, Kilembe W, Farquhar C, Celum C, Partners in Prevention HSV/HIV Transmission Study Team.** 2012. Determinants of per-coital-act HIV-1 infectivity among African HIV-1-serodiscordant couples. J. Infect. Dis. **205:**358–365. http://dx.doi.org/10.1093/infdis/jir747.
107. **McConnell MJ, Mier-Mota J, Flor-Parra F, Martinez-Fernandez FJ, Lopez-Cortes LF, Viciana P, Fernandez-Cuenca F, Perez-Romero P.** 2011. Improved viral suppression after treatment optimization in HIV-infected patients with persistent low-level viremia. J. Acquir. Immune Defic. Syndr. **58:**446–449. http://dx.doi.org/10.1097/QAI.0b013e3182364513.
108. **Di Mascio M, Markowitz M, Louie M, Hogan C, Hurley A, Chung C, Ho DD, Perelson AS.** 2003. Viral blip dynamics during highly active antiretroviral therapy. J. Virol. **77:**12165–12172. http://dx.doi.org/10.1128/JVI.77.22.12165-12172.2003.
109. **Hatano H, Delwart EL, Norris PJ, Lee TH, Neilands TB, Kelley CF, Hunt PW, Hoh R, Linnen JM, Martin JN, Busch MP, Deeks SG.** 2010. Evidence of persistent low-level viremia in long-term HAART-suppressed, HIV-infected individuals. AIDS **24:**2535–2539. http://dx.doi.org/10.1097/QAD.0b013e32833dba03.
110. **Gandhi RT, Zheng L, Bosch RJ, Chan ES, Margolis DM, Read S, Kallungal B, Palmer S, Medvik K, Lederman MM, Alatrakchi N, Jacobson JM, Wiegand A, Kearney M, Coffin JM, Mellors JW, Eron JJ, AIDS Clinical Trials Group A5244 Team.** 2010. The effect of raltegravir intensification on low-level residual viremia in HIV-infected patients on antiretroviral therapy: a randomized controlled trial. PLoS Med. **7:**e1000321. http://dx.doi.org/10.1371/journal.pmed.1000321.
111. **Dinoso JB, Kim SY, Wiegand AM, Palmer SE, Gange SJ, Cranmer L, O'Shea A, Callender M, Spivak A, Brennan T, Kearney MF, Proschan MA, Mican JM, Rehm CA, Coffin JM, Mellors JW, Siliciano RF, Maldarelli F.** 2009. Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. Proc. Natl. Acad. Sci. U S A. **106:**9403–9408. http://dx.doi.org/10.1073/pnas.0903107106.
112. **Asboe D, Aitken C, Boffito M, Booth C, Cane P, Fakoya A, Geretti AM, Kelleher P, Mackie N, Muir D, Murphy G, Orkin C, Post F, Rooney G, Sabin C, Sherr L, Smit E, Tong W, Ustianowski A, Valappil M, Walsh J, Williams M, Yirrell D, BHIVA Guidelines Subcommittee.** 2012. British HIV Association guidelines for the routine investigation and monitoring of adult HIV-1-infected individuals 2011. HIV Med. **13:**1–44. http://dx.doi.org/10.1111/j.1468-1293.2011.00971.x.
113. **Bennett DE, Bertagnolio S, Sutherland D, Gilks CF.** 2008. The World Health Organization's global strategy for prevention and assessment of HIV drug resistance. Antivir. Ther. **13**(Suppl 2)**:**S1–S13.
114. **Antinori A, Ammassari A, Torti C, Marconi P, Andreoni M, Angarano G, Bonora S, Castagna A, Cauda R, Clerici M, Monforte Ad, De Luca A, Di Perri G, Galli M, Girardi E, Gori A, Lazzarin A, Lo Caputo S, Mazzotta F, Montella F, Mussini C, Perno CF, Puoti M, Rizzardini G, Rusconi S, Vullo V, Carosi G.** 2009. Italian consensus statement on management of HIV-infected individuals with advanced disease naïve to antiretroviral therapy. Infection **37:**270–282. http://dx.doi.org/10.1007/s15010-008-8134-8.
115. **Carosi G, Torti C, Andreoni M, Angarano G, Antinori A, Bonora S, Borderi M, Castagna A, Castelli F, Cauda R, Chiodo F, D'arminio-Monforte A, De Luca A, Di Perri G, Dianzani F, Filice G, Galli M, Lazzarin A, Maggiolo F, Maserati R, Mazzotta F, Moroni M, Perno CF, Vullo V, Italian HIV Guidelines Working Group.** 2006. Key questions in antiretroviral therapy: Italian Consensus Workshop (2005). J. Antimicrob. Chemother. **57:**1055–1064. http://dx.doi.org/10.1093/jac/dkl113.
116. **Panel de Expertos de Gesida y Plan Nacional Sobre el Sida.** 2012. Consensus document of Gesida and Spanish Secretariat for the National Plan on AIDS (SPNS) regarding combined antiretroviral treatment in adults infected by the human immunodeficiency virus (January 2012). Enferm. Infecc. Microbiol. Clin. **30:**e1–89. (In Spanish.) http://dx.doi.org/10.1016/j.eimc.2012.03.006.
117. **Thompson MA, Aberg JA, Hoy JF, Telenti A, Benson C, Cahn P, Eron JJ, Günthard HF, Hammer SM, Reiss P, Richman DD, Rizzardini G, Thomas DL, Jacobsen DM, Volberding PA.** 2012. Antiretroviral treatment of adult HIV infection: 2012 recommendations of the International Antiviral Society–USA panel. JAMA **308:**387–402. http://dx.doi.org/10.1001/jama.2012.7961.