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How to best measure HIV reservoirs?

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Abstract

Purpose of review—The persistence of HIV within infected CD4⁺ T cells is a major obstacle to eradication, and assessment of the strategies to reduce HIV reservoirs is one of the major challenges. Measuring HIV reservoirs accurately will be necessary to assess those strategies. The objective of this review is to present the most recent studies that may help to define the best markers to measure HIV reservoirs.

Recent findings—Recent findings have shown that multiple assays can be used to quantify the different analytes that reflect the HIV reservoirs. They have provided new insights, but lack of standardization has made cross-comparisons of data difficult. No single best assay for measuring HIV reservoirs has been identified and these assays often address different questions, such as the size of the reservoirs, the composition of the reservoirs, or the capacity of latent reservoirs to produce virus. A consensus on what values reflect robust conclusions will have to wait for the generation of additional results.

Summary—In conclusion, there is a compelling need for investigators to optimize assays and share protocol reagents and specimens to permit the validation, comparison, and standardization of techniques. There is an important need for validated, high-throughput, sensitive, and accurate assays that can detect changes in HIV reservoir size in order to assess the impact of candidate therapies.

Keywords

HIV latency; markers of HIV reservoirs; quantification

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Conflicts of interest

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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 (p. 250).

INTRODUCTION

The accurate and precise measurement of HIV-1 reservoirs is critical for the process to reliably assess the efficacy of candidate treatments that are aimed at reducing these reservoirs, and for either demonstrating a 'functional' cure or a sterilizing cure (i.e. elimination of all infected cells) [1]. The standard virus marker in clinical practice has been the measurement of plasma HIV-RNA, with the objective of antiretroviral therapy (ART) to reduce viral load as much as possible. With effective ART, the latent reservoir resembles an 'iceberg' with a difficult to discern mass of latently infected cells that persists during ART and that can reignite high levels of ongoing replication following ART withdrawal. Characteristics of HIV reservoirs differ between patients, disease stages, and the interval between date of infection and initiation of ART; nevertheless, the size of the reservoir in each patient does not appreciably change during effective ART. Optimizing assays to measure latent reservoirs will be necessary to assess strategies for cure, as well as in studies of treatment intensification [2], simplification, interruption, or switch of ART [3, 4]. The current effort to develop drugs aimed at eradicating or reducing HIV reservoirs is one of the challenges for the next decade [5, 6]; however, there is no consensus on the assays that can best measure these reservoirs. There are several questions to address and several ways to explore reservoirs. No single best marker of HIV reservoirs has been identified, and markers often address different questions such as the size of the reservoirs, the composition of the reservoirs, or the capacity of latent reservoirs to produce virus.

The objective of this review is to describe the different markers that have been proposed and used by different groups, and then to consider the merits of these various approaches.

The HIV reservoir is widely dispersed

One of the difficulties in defining and measuring the HIV reservoir is that latently infected cells are disseminated throughout the body and are concentrated differentially in different lymphoid organs and in other tissues [7, 8]. Blood is the most accessible organ, but not a truly representative medium for measuring the latent reservoir in the whole body. For example, the concentration of latently infected CD4⁺ lymphocytes and their activation status is higher in the gut than the blood [7]. In untreated patients, this high level of virus replication and lymphocyte activation results in a disproportionately high level of lymphocyte death and epithelial damage in the gastrointestinal tract, facilitating bacterial translocation and systemic immune activation [9]. In animal models, the levels of total simian immunodeficiency virus (SIV)-DNA measured in blood and tissues showed that the gut is the most infected tissue [10]. In ART-treated macaques, the highest levels of SIV-DNA and SIV-RNA in comparison with blood were in lymphoid tissues, particularly the spleen, lymph nodes, and gastrointestinal tract [11■].

The reservoir is complex

Most infected cells are memory CD4⁺ T cells; however, different T-cell subsets have varying levels of latent infection in different patients according to the stage of the disease and the timing of initiation of ART. HIV-DNA can be detected in all CD4⁺ T-cell subsets, including naive, central memory, transitional memory, and effector memory cells [12, 13, 14■■]. Each of those T-cell subsets contains replication-competent HIV that can be induced ex vivo to produce virus [15, 16]. Residual virus has also been detected in tissue macrophages and microglial cells during ART [17]; however, the durability of this reservoir has not been well characterized.

Various species of HIV-DNA have been found in these cell types at frequencies that exceed cells with replication-competent virus that can be readily induced. One recent study

suggested that the number of resting CD4⁺ T cells with defective (or non-induced) proviruses may be, on average, 300-fold higher than the number that can produce replication-competent virus following cellular activation [18]. The different species of HIV-DNA include integrated HIV-DNA (provirus) and unintegrated forms such as the linear preintegration complex, and 1-long terminal repeat (LTR) and 2-LTR circles. The provirus is integrated at various sites and most infected CD4⁺ cells contain one HIV-DNA molecule [19[■]]. How the location of the integration site and the state of the chromatin near the provirus modulate inducibility are not well understood. Similarly, the detection of residual HIV transcription during effective ART could reflect either true latent status or a sub-optimal antiviral suppression [20].

The reservoir is highly dynamic

The establishment of the HIV reservoir starts very early at the time of primary infection, when there is cell death and high turnover associated with rounds of new cellular infections [14[■], 21, 22]. During the chronic phase of HIV infection, the dynamics of the latent reservoir is complex and evolving. Just as CD4⁺ T cells are being renewed from the thymus and bone marrow, being activated and maturing, and dying as a result of antigen exposure and virus replication, latently infected CD4⁺ lymphocytes are in a similar flux. The long life and homeostatic proliferative capacity of memory cells almost certainly accounts for the stability of the latent reservoir in patients, even after long-term ART.

THE IMPORTANCE OF SENSITIVE, SPECIFIC, REPRODUCIBLE, AND PRECISE ASSAYS TO QUANTIFY HIV RESERVOIRS

Because most latent reservoirs are widely, but not homogeneously, distributed in the body, measurement of the reservoir must take into account the specimens, the analytes, and the assays.

The specimen

Blood cells may or may not reflect other bodily tissues; however, blood HIV-RNA is universally accepted as a good reflection of virus replication in the body. Other tissues are not as easily sampled. Leukapheresis can generate large numbers of cells to increase the measurable reservoir and permit the more extensive examination of T-cell subsets, but this procedure is not as readily performed as simple phlebotomy.

The analytes and assays

Multiple markers of HIV reservoirs have been proposed. In a recent review [23], advantages and drawbacks were presented and discussed, showing that each marker has a different purpose and provides different information to answer different questions.

The limiting dilution co-culture assay that approximates the number of infected resting CD4⁺ T cells in patients has been considered to represent the 'gold-standard' for measuring latently infected cells, because it detects a true threat for recrudescence of replication after withdrawal of ART. Unfortunately, this technique is labor intensive, expensive, slow, and imprecise [24, 25]. Moreover, it measures only the tip of the 'iceberg', as most latently infected cells will not be available in any collected specimen and its sensitivity may not detect all replication-competent latently infected cells in a specimen.

Total cell-associated HIV-DNA is a marker to estimate the frequency of infected cells per million in peripheral blood mononuclear cells (PBMC) or CD4⁺ T cells [26]. It includes measurement by realtime PCR of both integrated and unintegrated HIV-DNA forms in latently and productively infected cells [27, 28]. This marker could be applied in whole

blood, resting or purified CD4⁺ T cells, lymphocyte subsets, a cell pellet, and in tissue biopsies as demonstrated in human and primate studies. Moreover, a new technology has been recently applied to the quantification of total HIV-DNA and 2-LTR circles [29]. Droplet digital PCR brings the advantage of quantification in absolute values. Validation of this assay compared with a real-time *pol* PCR shows it to be more sensitive and substantially more precise [Strain *et al.* (in press)]. Moreover, in comparison with an Alu-gag PCR assay for integrated HIV-DNA, droplet digital PCR was highly correlated in patients who had been effectively treated with ART, as linear, unintegrated HIV-DNA forms have a short half-life and unintegrated LTR circle forms represent less than 10% of total HIV-DNA [Ericksson *et al.* (in press)].

All infectious latent viruses must result from integrated HIV-DNA, although not all integrated HIV-DNA represents inducible replication-competent provirus. The best described and characterized assay for integrated HIV-DNA utilizes Alu-gag PCR [30–32]. This technique is very cumbersome as the assay requires, 42 PCR reactions per sample, because the nonspecificity of the gag primer requires endpoint dilution of the assay to confer specificity.

The quantification of 2-LTR circles has been proposed to be a surrogate marker of ongoing viral replication and recent cycles of replication, although it is highly controversial whether 2-LTR circular forms do in fact have a short half-life [33]. The sensitivity of these in-house techniques is different between studies, making comparisons difficult [34]. Interestingly, in the case of raltegravir treatment, a spike in 2-LTR circle numbers was reported, a result that is expected from this class of drug [2, 35]. The application of droplet digital PCR to the measurement of 2-LTR circles provides a more precise and sensitive assay for this analyte [Strain *et al.* (in press)].

Expression of intracellular HIV-RNA is a marker that has been used to demonstrate that drugs, such as histone deacetylase inhibitors, can induce latently infected cells from patients who are suppressed on ART to make HIV transcripts [36]. The quantification of multiple spliced, singly spliced and unspliced HIV-RNA has been also proposed as a marker of productive cells [20, 34]; however, whether some transcription is consistent with the latent status or represents ongoing low-level replication remains to be determined.

Residual low-level viremia in patients receiving efficient ART is a marker of reservoirs that contain HIV proviruses, mainly CD4⁺ memory long-lived cells [35]. There is a correlation between the level of residual plasma viremia and the HIV-DNA blood level [27]. The HIV-RNA single copy assay (SCA) is an ultrasensitive method to quantify HIV-RNA in plasma, under the limit of conventional assays [37–40].

All these assays have provided new insights, but lack of standardization has made cross-comparisons of the data difficult. No commercial development is currently in progress, so assays are not well standardized. None has been commercialized with United States Food and Drug Administration or European Economic Community approval. Moreover, different standards are used to calibrate measurements. An initial effort to exchange both specimens among laboratories and assays to provide some basis for comparisons was recently made [Ericksson *et al.* (in press)]. Efforts to share protocols and reagents and efforts to compare assays would clearly benefit the field.

WHAT DO WE KNOW IN DIFFERENT CLINICAL SITUATIONS?

The literature on HIV reservoirs most frequently uses total cell-associated HIV-DNA. There is more HIV-DNA available for measurement than for the other analytes. In addition, quantitative PCR assays are easier to perform than SCA assays for plasma viremia or

infectivity assays. This technique is well standardized because it is routinely used in the context of HIV diagnosis in babies, for whom good sensitivity, specificity, and reproducibility are particularly important [41]. The Agence Nationale de Recherches sur le Sida et les Hépatites virales (ANRS) technique has been largely used for the cohorts of naive patients in the acute or chronic phases of infection [21, 23, 42–44], showing the strong predictive value of this marker for progression to AIDS and death, independent of HIV-RNA and CD4⁺ count. A meta-analysis of seven cohorts confirmed that there is some evidence that HIV-DNA might have a better predictive value than plasma HIV-RNA [45[■]]. These results also suggest that total HIV-DNA in blood could reflect the whole level of HIV reservoirs in each patient.

Studies on HIV reservoirs have been particularly informative in elite controllers, those patients who spontaneously control HIV viremia and represent a model for functional cure. The study of these individuals provides direct evidence that they harbor low levels of total HIV-DNA (lower than 2 log₁₀ copies/million PBMC) [46–48, 49].

Among treated patients, there are now consistent results showing that early treatment initiation is a key point to reduce HIV reservoirs while protecting the immune system [40], [Le *et al.* (in press), Hocqueloux *et al.* (in press)], and that the impact of treatment during primary infection facilitates long-term control of HIV reservoirs [22]. A high level of total HIV-DNA predicts a shorter time to rebound after treatment interruption, and is associated with a higher risk of viral rebound during ART [3].

One of the current alternative approaches would be to aim for remission or functional cure, as recently demonstrated in rare patients who have been treated early at the time of primary infection and have long-term virologic control after treatment interruption [50[■]]. Such posttreatment controllers represent a model of functional cure as they have a very small HIV reservoir (total HIV-DNA less than 2 log₁₀ copies/million PBMC), although they do not have the protective human leukocyte antigen (HLA) alleles such as HLA-B57 and -B27 [50[■], 51–53]. At present, there are very few studies showing the impact of various ART regimens on HIV reservoirs. Is there any difference between the five and three drug regimens? Could the level of HIV-DNA blood level provide a marker in clinical practice for different therapeutic strategies? Do we need to systematically measure all analytes, or just total HIV-DNA, integrated and unintegrated forms [54–56]?

Lastly, within protocols aiming at a total eradication, no assay will have the sensitivity of one infectious unit in the body. Thus, the ultimate test of eradication will be interruption of ART without recrudescence infection, as has been demonstrated in the ‘Berlin patient’ after bone marrow transplantation [57].

CONCLUSION

In order to assess the true impact of a candidate regimen to reduce the HIV reservoir, assays are required that are sufficiently sensitive, specific, and precise so that a reduction in the reservoir is statistically robust enough to be believed. One challenge is biological. The measurable reservoir in a clinical specimen is near the level of detection and does not reflect the ‘iceberg’ effect or the heterogeneity of the reservoir in the whole body. The other challenge is technical. Assays need to be validated for sensitivity, specificity, reproducibility, precision, and accuracy to be able to depend on their results to predict a change with an intervention with confidence. Few described assays have been able to do this [30] [Strain *et al.* (in press)]. A consensus on what endpoints reflect robust conclusions will have to wait for the generation of additional data. It will be also important to select the patients, based on size of the HIV reservoir, who are most appropriate for candidate

interventions to reduce HIV reservoirs. Patients with larger reservoirs provide an easier substrate for measuring an effect. It will be harder to measure the reservoirs in patients treated shortly after infection; however, these patients will be more amenable to cure because of their smaller reservoir and better immune status. There is a compelling need for investigators to share protocols, reagents and specimens to permit the validation, comparison, and standardization of techniques. There is an important need for validated, high-throughput, sensitive, and accurate assays that can detect changes in HIV reservoir size to assess the impact of candidate therapies. Ultimately, however, the proof of a cure will require a properly designed study that withdraws ART.

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REFERENCES AND RECOMMENDED READING

1. Richman DD, Margolis DM, Delaney M, et al. The challenge of finding a cure for HIV infection. *Science*. 2009; 323:1304–1307. [PubMed: 19265012]
2. 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. [PubMed: 20228817]
3. Piketty C, Weiss L, Assoumou L, et al. A high HIV DNA level in PBMCs at antiretroviral treatment interruption predicts a shorter time to treatment resumption, independently of the CD4 nadir. *J Med Virol*. 2010; 82:1819–1828. [PubMed: 20872707]
4. Lam YM, McBride KL, Amin J, et al. Switching virally suppressed, treatment-experienced patients to a raltegravir-containing regimen does not alter levels of HIV-1 DNA. *PLoS One*. 2012; 7:e31990. [PubMed: 22396745]
5. Trono D, Van Lint C, Rouzioux C, et al. HIV persistence and the prospect of long-term drug-free remissions for HIV-infected individuals. *Science*. 2010; 329:174–180. [PubMed: 20616270]
6. Richman DD. Introduction: challenges to finding a cure for HIV infection. *Curr Opin HIV AIDS*. 2011; 6:1–3. [PubMed: 21242886]
7. 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. [PubMed: 20827162]
8. Zeng M, Southern PJ, Reilly CS, et al. Lymphoid tissue damage in HIV-1 infection depletes naive T cells and limits T cell reconstitution after antiretroviral therapy. *PLoS Pathog*. 2012; 8:e1002437. [PubMed: 22241988]
9. Brenchley JM, Schacker TW, Ruff LE, et al. CD4⁺ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med*. 2004; 200:749–759. [PubMed: 15365096]
10. Mattapallil JJ, Douek DC, Hill B, et al. Massive infection and loss of memory CD4⁺ T cells in multiple tissues during acute SIV infection. *Nature*. 2005; 434:1093–1097. [PubMed: 15793563]
11. North TW, Higgins J, Deere JD, et al. Viral sanctuaries during highly active antiretroviral therapy in a nonhuman primate model for AIDS. *J Virol*. 2010; 84:2913–2922. This study is the first comprehensive analysis of the tissue distribution of the RT-SHIV reservoir in HAART-treated macaques. [PubMed: 20032180]
12. Chomont N, El-Far M, Ancuta P, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med*. 2009; 15:893–900. [PubMed: 19543283]
13. Descours B, Avettand-Fenoel V, Blanc C, et al. Immune responses driven by protective human leukocyte antigen alleles from long-term nonprogressors are associated with low HIV reservoir in central memory CD4 T cells. *Clin Infect Dis*. 2012; 54:1495–1503. [PubMed: 22441653]
14. Ganesan A, Chattopadhyay PK, Brodie TM, et al. Immunologic and virologic events in early HIV infection predict subsequent rate of progression. *J Infect Dis*. 2010; 201:272–284. The strength of this study is the comprehensive evaluation of T-cell subsets at early time of infection

in a large well characterized cohort of patients. This study confirms that relatively high rates of HIV infection could be detected in naive T cells in the first months of HIV infection. [PubMed: 20001854]

15. Reuse S, Calao M, Kabeya K, et al. Synergistic activation of HIV-1 expression by deacetylase inhibitors and prostratin: implications for treatment of latent infection. *PloS One*. 2009; 4:e6093. [PubMed: 19564922]
16. 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. [PubMed: 22555163]
17. Cobos-Jiménez V, Booiman T, Hamann J, Kootstra NA. Macrophages and HIV-1. *Curr Opin HIV AIDS*. 2011; 6:385–390. [PubMed: 21734564]
18. Eriksson S, Graf EH, Dahl V, et al. Comparative Analysis of Measures of Viral Reservoirs in HIV-1 Eradication Studies. *PloS Pathog*. 2013; 9:e1003174. [PubMed: 23459007]
19. Josefsson L, King MS, Makitalo B, et al. Majority of CD4⁺ T cells from peripheral blood of HIV-1-infected individuals contain only one HIV DNA molecule. *Proc Natl Acad Sci USA*. 2011; 108:11199–11204. This study is important in the context of HIV reservoirs studies as it demonstrates that the majority of infected CD4⁺ T cells contain one HIV-DNA molecule. [PubMed: 21690402]
20. Schmid A, Gianella S, von Wyl V, et al. Profound depletion of HIV-1 transcription in patients initiating antiretroviral therapy during acute infection. *PloS One*. 2010; 5:e13310. [PubMed: 20967271]
21. Ghosn J, Deveau C, Chaix ML, et al. Despite being highly diverse, immunovirological status strongly correlates with clinical symptoms during primary HIV-1 infection: a cross-sectional study based on 674 patients enrolled in the ANRS CO 06 PRIMO cohort. *J Antimicrob Chemother*. 2010; 65:741–748. [PubMed: 20167586]
22. Strain MC, Little SJ, Daar ES, et al. Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. *J Infect Dis*. 2005; 191:1410–1418. [PubMed: 15809898]
23. Lewin SR, Rouzioux C. HIV cure and eradication: how will we get from the laboratory to effective clinical trials? *AIDS*. 2011; 25:885–897. [PubMed: 21422987]
24. Eisele E, Siliciano RF. Redefining the viral reservoirs that prevent HIV-1 eradication. *Immunity*. 2012; 37:377–388. [PubMed: 22999944]
25. Vallejo A, Gutierrez C, Hernandez-Novoa B, et al. The effect of intensification with raltegravir on the HIV-1 reservoir of latently infected memory CD4 T cells in suppressed patients. *AIDS*. 2012; 26:1885–1894. [PubMed: 22992577]
26. Avettand-Fenoel V, Boufassa F, Galimand J, et al. HIV-1 DNA for the measurement of the HIV reservoir is predictive of disease progression in seroconverters whatever the mode of result expression is. *J Clin Virol*. 2008; 42:399–404. [PubMed: 18455956]
27. Chun TW, Murray D, Justement JS, et al. Relationship between residual plasma viremia and the size of HIV proviral DNA reservoirs in infected individuals receiving effective antiretroviral therapy. *J Infect Dis*. 2011; 204:135–138. [PubMed: 21628667]
28. Blazkova J, Chun TW, Belay BW, et al. Effect of histone deacetylase inhibitors on HIV production in latently infected, resting CD4(+) T cells from infected individuals receiving effective antiretroviral therapy. *J Infect Dis*. 2012; 206:765–769. [PubMed: 22732922]
29. Henrich TJ, Gallien S, Li JZ, et al. Low-level detection and quantitation of cellular HIV-1 DNA and 2-LTR circles using droplet digital PCR. *J Virol Methods*. 2012; 186:68–72. [PubMed: 22974526]
30. O'Doherty U, Swiggard WJ, Jeyakumar D, et al. A sensitive, quantitative assay for human immunodeficiency virus type 1 integration. *J Virol*. 2002; 76:10942–10950. [PubMed: 12368337]
31. Liszewski MK, Yu JJ, O'Doherty U. Detecting HIV-1 integration by repetitive-sampling Alu-gag PCR. *Methods*. 2009; 47:254–260. [PubMed: 19195495]
32. Yu JJ, Wu TL, Liszewski MK, et al. A more precise HIV integration assay designed to detect small differences finds lower levels of integrated DNA in HAART treated patients. *Virology*. 2008; 379:78–86. [PubMed: 18649912]

33. Sharkey M, Babic DZ, Greenough T, et al. Episomal viral cDNAs identify a reservoir that fuels viral rebound after treatment interruption and that contributes to treatment failure. *PLoS Pathog.* 2011; 7:e1001303. [PubMed: 21383975]
34. Lewin SR, Murray JM, Solomon A, et al. Virologic determinants of success after structured treatment interruptions of antiretrovirals in acute HIV-1 infection. *J Acquir Immune Defic Syndr.* 2008; 47:140–147. [PubMed: 18300365]
35. Murray JM, McBride K, Boesecke C, et al. Integrated HIV DNA accumulates prior to treatment while episomal HIV DNA records ongoing transmission afterwards. *AIDS.* 2012; 26:543–550. [PubMed: 22410637]
36. 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. [PubMed: 22837004]
37. 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. [PubMed: 20711481]
38. 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. [PubMed: 14532178]
39. Palmer S, Maldarelli F, Wiegand A, et al. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc Natl Acad Sci USA.* 2008; 105:3879–3884. [PubMed: 18332425]
40. Zheng L, Bosch RJ, Chan ES, et al. Predictors of residual viremia in patients on long-term suppressive antiretroviral therapy. *Antivir Ther.* 2012; 20
41. Avettand-Fenoel V, Chaix ML, Blanche S, et al. LTR real-time PCR for HIV-1 DNA quantitation in blood cells for early diagnosis in infants born to seropositive mothers treated in HAART area (ANRS CO 01). *J Med Virol.* 2009; 81:217–223. [PubMed: 19107966]
42. Rouzioux C, Hubert JB, Burgard M, et al. Early levels of HIV-1 DNA in peripheral blood mononuclear cells are predictive of disease progression independently of HIV-1 RNA levels and CD4+T cell counts. *J Infect Dis.* 2005; 192:46–55. [PubMed: 15942893]
43. Goujard C, Bonarek M, Meyer L, et al. CD4 cell count and HIV DNA level are independent predictors of disease progression after primary HIV type 1 infection in untreated patients. *Clin Infect Dis.* 2006; 42:709–715. [PubMed: 16447119]
44. Minga AK, Anglaret X, d' Aquin Toni T, et al. HIV-1 DNA in peripheral blood mononuclear cells is strongly associated with HIV-1 disease progression in recently infected West African adults. *J Acquir Immune Defic Syndr.* 2008; 48:350–354. [PubMed: 18545149]
45. Tsiara CG, Nikolopoulos GK, Bagos PG, et al. Impact of HIV type 1 DNA levels on spontaneous disease progression: a meta-analysis. *AIDS Res Hum Retroviruses.* 2012; 28:366–373. This meta-analysis of six studies involving 1074 patients definitely confirms that total HIV-DNA was a strong predictive marker for progression to AIDS (Risk ratio = 3.01). [PubMed: 21877926]
46. Julg B, Pereyra F, Buzon MJ, et al. Infrequent recovery of HIV from but robust exogenous infection of activated CD4(+) T cells in HIV elite controllers. *Clin Infect Dis.* 2010; 51:233–238. [PubMed: 20550452]
47. Graf EH, Mexas AM, Yu JJ, et al. Elite suppressors harbor low levels of integrated HIV DNA and high levels of 2-LTR circular HIV DNA compared to HIV+ patients on and off HAART. *PLoS Pathog.* 2011; 7:e1001300. [PubMed: 21383972]
48. Autran B, Descours B, Avettand-Fenoel V, Rouzioux C. Elite controllers as a model of functional cure. *Curr Opin HIV AIDS.* 2011; 6:181–187. [PubMed: 21460722]
49. Lambotte O, Boufassa F, Madec Y, et al. HIV controllers: a homogeneous group of HIV-1-infected patients with spontaneous control of viral replication. *Clin Infect Dis.* 2005; 41:1053–1056. [PubMed: 16142675]
50. Hocqueloux L, Prazuck T, Avettand-Fenoel V, et al. Long-term immunovirologic control following antiretroviral therapy interruption in patients treated at the time of primary HIV-1 infection. *AIDS.* 2010; 24:1598–1601. This study presents the first group of posttreatment controllers, presenting a long-term viral control after interruption of a treatment initiated at

primary infection. These patients are different from elite controllers and do not exhibit HLA-protective alleles. [PubMed: 20549847]

51. Goujard C, Girault I, Rouzioux C, et al. HIV-1 control after transient antiretroviral treatment initiated in primary infection: role of patient characteristics and effect of therapy. *Antivir Ther.* 2012; 17:1001–1009. [PubMed: 22865544]
52. Lodi S, Meyer L, Kelleher AD, et al. Immunovirologic control 24 months after interruption of antiretroviral therapy initiated close to HIV seroconversion. *Arch Intern Med.* 2012; 172:1252–1255. [PubMed: 22826124]
53. Saez-Cirion A, Bacchus C, Hocqueloux L, et al. ANRS VISCONTI Study Group. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy. ANRS VISCONTI study 2013. *PLoS Pathog.* 10.1371/journal.ppat.1003211
54. Mexas AM, Graf EH, Pace MJ, et al. Concurrent measures of total and integrated HIV DNA monitor reservoirs and ongoing replication in eradication trials. *AIDS.* 2012; 26:2295–2306. [PubMed: 23014521]
55. Agosto LM, Liszewski MK, Mexas A, et al. Patients on HAART often have an excess of unintegrated HIV DNA: implications for monitoring reservoirs. *Virology.* 2011; 409:46–53. [PubMed: 20970154]
56. d'Ettorre G, Zaffiri L, Ceccarelli G, et al. The role of HIV-DNA testing in clinical practice. *New Microbiol.* 2010; 33:1–11. [PubMed: 20402409]
57. Hutter G, Nowak D, Mossner M, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med.* 2009; 360:692–698. [PubMed: 19213682]

KEY POINTS

- Total cell-associated HIV-DNA is the easiest and most studied marker of HIV reservoirs in large cohorts; it is the only marker that demonstrates the predictive value of blood reservoir level.
- The measurement of low-level viremia in controlled patients is representative of residual replication in the whole body.
- The post-treatment controllers, representing the first cases of ‘functional’ cure, have a very low level of total HIV-DNA.