Perspective

Scientific Rationale for Antiretroviral Therapy in 2005: Viral Reservoirs and Resistance Evolution

Hope for a cure for HIV-1 infection was dampened by the discovery of a latent form of the virus that persists in resting CD4+ cells. This reservoir of latently HIV-infected resting memory T cells represents an archive of viral genotypes produced in an individual from the onset of infection. Entry into the reservoir is stopped with suppressive antiretroviral therapy, but the archived viruses are capable of reinitiating active infections, are released continuously from this reservoir, and can cause viral rebound if antiretroviral therapy is stopped. Studies of residual low-level viremia (<50 HIV RNA copies/mL of plasma) in the setting of effective antiretroviral therapy indicate that such viremia is largely caused by activation of the latently infected cells and the release of virus from this and other stable reservoirs. These studies support the notion that the stability of the latent reservoir is consistent with the long life span of resting memory T cells, rather than reflecting rounds of active replication under suppressive antiretroviral therapy that replenish the reservoir. There may be other stable reservoirs in addition to the resting T-cell pool, further complicating the problem of eradication. This article summarizes a presentation on viral reservoirs and viral evolution by Robert F. Siliciano, MD, PhD, at the International AIDS Society–USA course in New York in March 2005

It has become clear that there are 2 distinct sources of viremia in HIV-infected individuals and that understanding the differences between them is important for making correct decisions about antiretroviral therapy. In an untreated patient, most of the plasma virus is produced by active rounds of replication in permissive cells, such as activated CD4+ cells; this is an extremely dynamic process, with many new cells being infected each day, replacing those killed by infection. In each newly infected cell, the error-prone process of reverse transcription of the HIV genome is occurring, generating mutations that can lead to drug resistance. Indeed, in a patient with a plasma HIV RNA level of 30,000 copies/mL, every possible mutation in the entire HIV genome arises on a daily basis. In the presence of a selective advantage—for example, suboptimal antiretroviral therapy—preferential replication of resistant mutants will result in a outgrowth of a drug-resistant viral population.

Whereas active cycles of replication produce most of the steady-state viremia in an untreated patient, some of the virus in plasma is released by stable reservoirs of HIV. One of these stable reservoirs consists of CD4+ cells infected at some time in the past that carry a latent form of the virus. With appropriate immune stimulation, these cells can subsequently become activated and produce virus. Although the amount of virus produced by the activation of these latently infected cells is quantitatively insignificant, this latent reservoir is nevertheless clinically important for 3 reasons: first, it renders HIV infection intrinsically incurable with current antiretroviral therapy alone; second, it helps explain what is happening virologically in patients who are doing well on antiretroviral therapy; and third, it helps explain the unique mode of evolution of HIV in which all of the major variants that have arisen during the course of an infection persist indefinitely.

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Establishment of Stable Latent Reservoir

The establishment of a reservoir of latently infected CD4+ cells is a result of the normal physiology of the immune system. Most of the T cells in the body are in a resting state; approximately half are naive cells (cells that have not yet responded to any foreign antigen), and the remainder are memory cells (cells that have previously responded to some antigen). The cells circulate throughout the lymphoid tissues until they encounter an antigen that they recognize, after which they undergo blast transformation, proliferate, and carry out their functions. Some of the cells survive and revert back to a resting state as long-lived memory T cells, cells that allow the host to respond to the same antigen again in the future.

In HIV infection, the virus replicates preferentially in activated CD4+ cells and tends to kill them very quickly. However, some of the activated cells can become infected as they are in the process of reverting back to a resting state, resulting in a stably integrated viral genome in a long-lived memory T cell. Conditions for HIV gene expression are unfavorable in resting cells; for example, host transcription factors such as nuclear factor-κß and nuclear factor of activated T cells, which are necessary for high-level HIV gene expression, are excluded from the nucleus in resting CD4+ cells. The result of infection in this case is a stably integrated but transcriptionally silent form of the

virus in a cell that is designed to live a long time: a perfect recipe for viral persistence. If these cells are reactivated in the future, they can begin to produce virus.

Latently infected cells are present in all infected individuals, at a frequency of approximately 1 per million resting CD4+ cells. Virus in these cells is not eradicated during antiretroviral therapy. This latent reservoir appears to be extremely stable. The best estimates of the decay of the viral population in this latent reservoir, based on studies in patients in whom antiretroviral therapy has reduced plasma HIV RNA levels to below 50 copies/mL for as long as 7 years, indicate a half-life of 44.2 months. Based on these estimates, eradication of HIV from the latent reservoir would require 73.4 years of suppressive therapy.

Role of the Latent Reservoir in Archiving Viral Strains

Actively replicating viruses in the plasma of viremic patients are a complex mixture of majority and minority variants that can be seen as participants in replicative competition. In the latent reservoir, there is a broader group of variants that persist in a manner that does not allow them to engage in such competition. In essence, this reservoir allows viruses to drop out of the competition and persist in a latent form, only to reemerge at a later time. In viremic patients, there is constant entry of viruses of a wide variety of genotypes—including viruses with drug-resistance mutations, if they have arisen—into the reservoir. There is no marked change in reservoir size because the high level of immune activation that occurs with viremia also results in increased emergence of virus from the reservoir as the host cells become activated. With initiation of antiretroviral therapy, entry into the reservoir is reduced. Exit from the reservoir is also reduced, owing to the decreased immune activation that occurs in the setting of effective antiretroviral therapy. Nevertheless, a small number of latently infected cells become activated each day and release virus, which can result in viral rebound if antiretroviral therapy is stopped.

Thus the latent reservoir is an archive of the viral genotypes that have been produced in an individual from the onset of infection. Figure 1 provides an example of the complex mixture of genotypes present in the reservoir. The patient represented received zidovudine beginning in the early 1990s, followed by the addition and substitution of a number of other drugs. After breakthrough viremia occurred in 1998, all drugs then being used were switched to a new 4-drug regimen, and the patient has been doing well on this regimen. The bottom portion of Figure 1 shows results of genotypic analysis of HIV isolates from the resting memory T-cell reservoir in this patient, organized according to presence of resistance mutations. Each horizontal entry represents an independent viral clone.

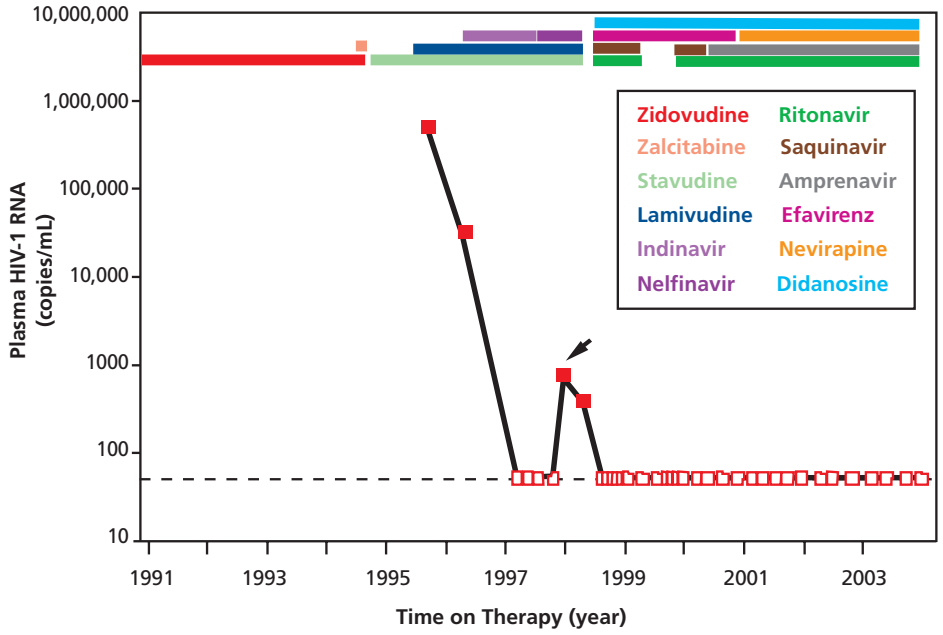
This picture of the types of virus present in the individual differs from that of a routine clinical genotyping of plasma virus, which shows a population average of circulating genotypes. Analysis of individual clones of virus in the latent reservoir reveals a wide variety of genotypes. Wild-type virus is present, and likely represents virus sequestered very early in the patient's infection, since these strains are at a large competitive disadvantage in the context of antiretroviral therapy. Also present are viruses with resistance mutations that reflect exposure to various drugs in the patient's treatment history. All of the viruses in the latent reservoir have the potential to emerge at some point in the future. Wild-type virus, which in the absence of antiretroviral therapy has a competitive advantage against many strains with resistance mutations, reemerges as the dominant plasma virus when patients are taken off failing antiretroviral therapy. The latent reservoir appears to be the source of this reemergence.

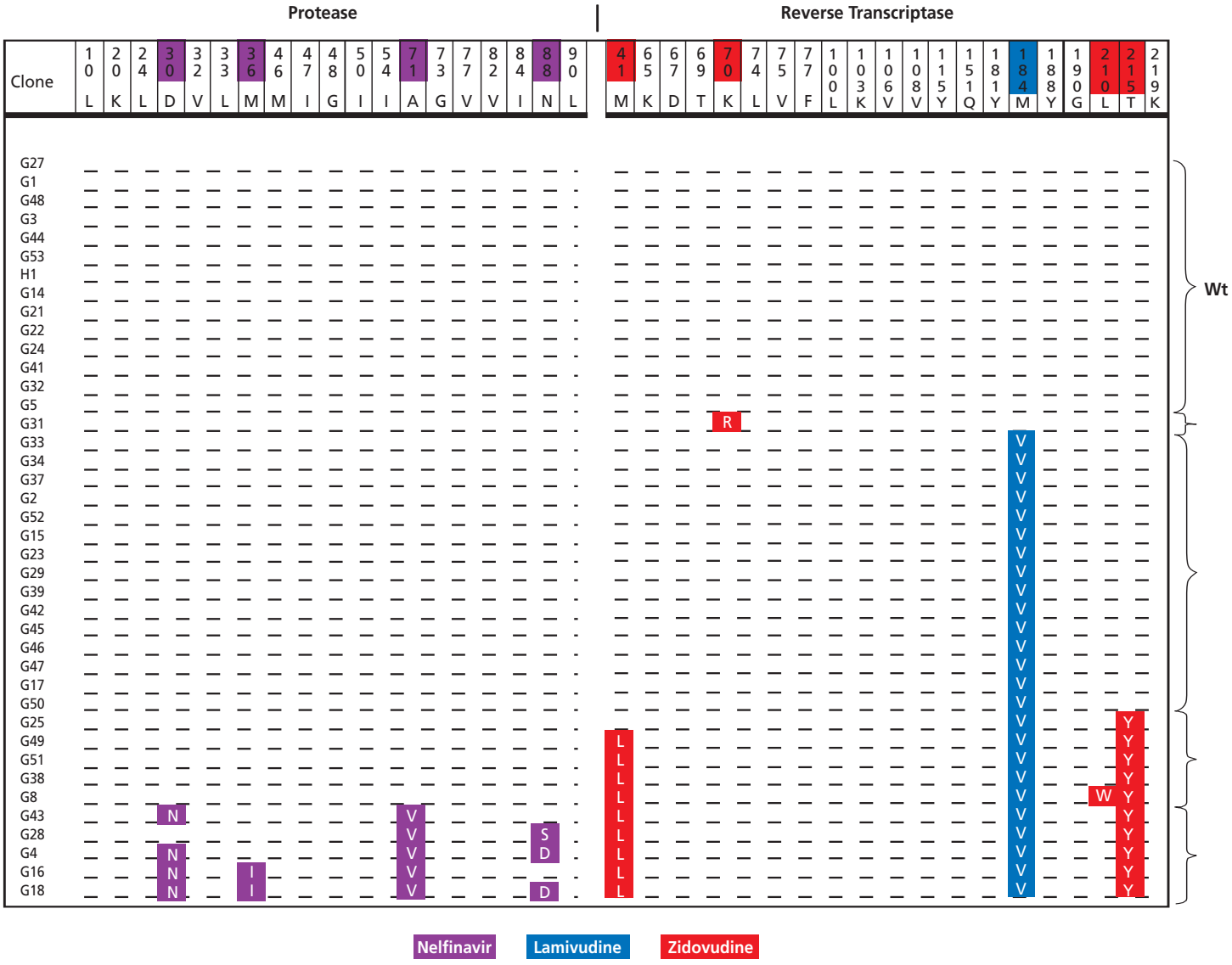
The latent reservoir allows the virus in an individual to evolve in a unique way. With HIV, what occurs is not survival of the fittest, but survival of all major forms that have been generated, and active replication of the forms that are the most fit under the current conditions. This situation needs to be taken into account in strategies for antiretroviral treatment. For example, the use of nevirapine to prevent mother-to-child transmission of HIV in developing countries results in emergence of nevirapine-resistant virus in most mothers after the single dose of nevirapine, with the resistant variants appearing to disappear over time. However, they are likely to be archived in the latent reservoir and will have the potential to reemerge if nevirapine, or other nonnucleoside reverse transcriptase inhibitors with similar resistance profiles, are subsequently used in an antiretroviral regimen. Similarly, the observation that wild-type virus may reemerge as the dominant plasma virus when antiretroviral therapy is stopped in patients in whom therapy is failing contributed to the notion of strategic treatment interruptions to regain viral susceptibility to antiretroviral drugs. However, the resistant strains are likely to have been archived and to reemerge when drugs are restarted. Patients in whom this strategy has been attempted did not have better outcomes as a result of treatment interruption.

Studies of Residual Viremia in Effective Antiretroviral Therapy

With the initiation of antiretroviral therapy, viremia is reduced from the original setpoint of replication in an individual to some point below the assay detection limit of 50 RNA copies/mL. Whether there is residual active viral replication under such therapy remains controversial. However, even if it were possible for antiretroviral therapy to be 100 percent effective in preventing new infection of susceptible cells from the moment treatment was initiated, there would still be residual low-level viremia, detectable

Figure **1.** Right: Antiretroviral treatment and plasma HIV RNA level over time in one patient. Arrow indicates period of breakthrough. Bottom: Results of recent clonal genotypic analysis of HIV in the latent reservoir of the same patient showing persistence of archival wild-type viruses (Wt) and viruses with drug resistance mutations (indicated as colored boxes: purple, nelfinavir; red, zidovudine; dark blue, lamivudine).





only with special research assays. This residual viremia results from the release of virus from latent reservoirs. This level of viremia can be termed the "release point," representing the amount of virus released from the reservoir without any additional cycles of replication. This point represents the best that can ever be expected from antiretroviral therapy in terms of reduction in viremia. Determining how close current antiretroviral therapy is to achieving reduction to this release point is important because any residual replication can generate drug-resistant variants and replenish the latent reservoirs. In a recent study, phylogenetic analyses of plasma virus and virus from resting memory T cells were performed in patients who had been doing well on antiretroviral therapy for an average of 3 years (Nettles et al, *JAMA,* 2005) Plasma and reservoir samples were taken at baseline; plasma samples were then taken every other day for 3 months and follow-up samples from the latent reservoir were obtained at regular intervals. As shown in Figure 2, the genetic sequences of the plasma and reservoir viruses are intermingled and often identical, consistent with the idea that the source of the plasma virus in the residual viremia observed in the patient is predominantly the infected resting T-cell pool. No resistance mutations to the drugs in the patient's antiretroviral regimen were observed, despite the fact that a single mutation could produce high-level resistance for 2 of the drugs in the regimen.

Such findings suggest that residual viremia in patients on potent antiretroviral therapy may be due to the release of virus from the latent reservoir and that such viremia can occur for prolonged periods without viral evolution (including resistance mutations). In addition, such findings support the notion that lifelong control of HIV infection is possible if highly active regimens can be maintained, and if suboptimal treatment—and consequent emergence of resistant virus—can be avoided.

Although the above results suggest

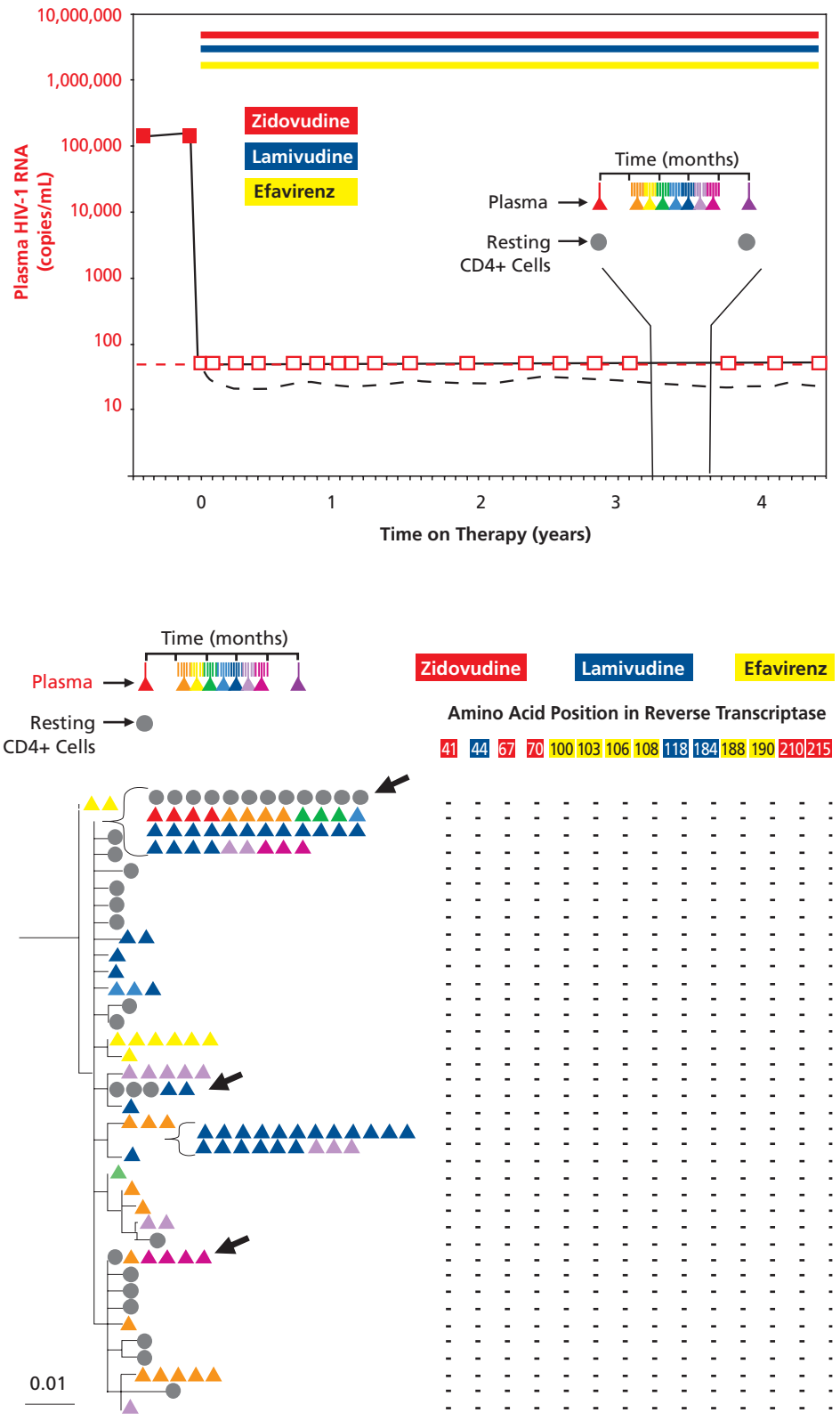


Figure **2.** Top: Treatment course, plasma viral load, and times of sampling of plasma and resting T-cell latent reservoir viruses obtained for phylogenetic analysis. Bottom: Phylogenetic tree showing relationship between plasma and latent-reservoir virus genetic sequences; arrows indicate identical sequences found in both plasma and latent reservoir virus.

that there can be residual viremia without viral evolution in patients on potent antiretroviral therapy who have viral loads reduced to below assay detection limits, many patients experience transient elevations of viremia into the detectable range (blips). The source of virus in the blips remains unclear. Blips are common, having been detected in 10% to 40% of patients doing well on antiretroviral therapy, and available evidence has suggested that they do not necessarily predict treatment failure. However, there has been concern that these blips may represent viral evolution through active replication and may thus result in the increased emergence of resistance to ongoing regimens. Much of the data on these transient elevations are from studies using relatively infrequent sampling. The frequent sampling performed as part of the above-mentioned study provided an opportunity to more closely evaluate characteristics of the viral blips. Blips were detected in the majority of patients examined; they were low in magnitude, typically at an HIV RNA level below 200 copies/mL, and brief in duration, generally resolving within 2 to 3 days. These increases were more common in some patients than in others but showed no correlation with concurrently measured plasma levels of antiretroviral drugs or with intercurrent illness or vaccinations.

Genotyping showed that no new resistance mutations appeared during or after the blips, with any resistance mutations found in the samples having been present in patients at baseline. These findings are consistent with the notion that viral blips represent normal biologic variation or statistical variation around a mean setpoint value that is below 50 copies/mL. However, blips that are greater than 200 copies/mL or that are reproducible on sequential testing are a greater cause for concern in terms of the potential for ongoing active replication and generation of resistant mutants.

With regard to stable viral reservoirs, it is possible that reservoirs other than the resting T-cells exist. Such other stable reservoirs would further complicate the problem of HIV eradication.

Conclusion

Both ongoing replication and viral release from stable reservoirs contribute to viremia in infected individuals. Antiretroviral therapy largely stops ongoing replication. The low-level viremia that continues despite antiretroviral therapy and the (smaller-magnitude) viral blips that occur in many patients do not depend on the presence of new drug resistance mutations, resulting from rounds of active replication; they may instead largely reflect viral release from stable reservoirs. Most blips may represent normal biologic and statistic variation around mean HIV RNA levels of below 50 copies/mL, rather than clinically significant elevations in viremia. However, blips that are greater than 200 copies/mL or reproducible on independent or sequential testing are a greater cause for concern.

Findings in the studies of viremia in patients with plasma HIV RNA levels below 50 copies/mL indicate that there can be viremia without viral evolution and thus suggest that lifelong control of viral replication is possible.

Presented in March 2005. First draft prepared from transcripts by Matthew Stenger. Reviewed and updated by Dr Siliciano in August 2005.

Financial Disclosure: Dr Siliciano has no affiliations with commercial organizations that may have interests related to the content of this article

Suggested Reading

Chun TW, Carruth L, Finzi D, et al. Quantification of latent tissue reservoirs and total body fat viral load in HIV-1 infection [see comments]. *Nature*. 1997;387: 183-188.

Chun TW, Stuyver L, Mizell SB, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci USA*. 1997;94:13193-13197.

Deeks SG, Wrin T, Liegler T, et al. Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. *N Engl J Med*. 2001;344:472-480.

Dornadula G, Zhang H, VanUitert B, et al. Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. *JAMA*. 1999;282: 1627-1632.

Finzi D, Blankson J, Siliciano JD, et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med*. 1999;5:512-517.

Finzi D, Hermankova M, Pierson T, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science*. 1997;278:1295-1300.

Hermankova M, Ray SC, Ruff C, et al. HIV-1 drug resistance profiles in children and adults with viral load of < 50 copies/ml receiving combination therapy. *JAMA*. 2001;286:196-207.

Lassen K, Han Y, Zhou Y, Siliciano J, Siliciano RF. The multifactorial nature of HIV-1 latency. *Trends Mol Med*. 2004;10:525-531.

Nettles RE, Kieffer TL, Kwon P, et al. Intermittent HIV-1 viremia (Blips) and drug resistance in patients receiving HAART. *JAMA*. 2005;293:817-829.

Siliciano JD, Kajdas J, Finzi D, et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat Med*. 2003;9:727-728.

Wong JK, Hezareh M, Gunthard HF, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science*. 1997;278:1291-1295.

Top HIV Med. 2005;13(3):96-100.