What Do We Need to Do to Cure HIV Infection?

Finding a cure for HIV infection requires methods to stop ongoing viral replication, to identify all reservoirs in which nonreplicating HIV persists, and to eliminate each of these reservoirs. Current antiretroviral therapy largely stops ongoing viral replication. This is a reflection of the extremely high antiviral activity of some classes of antiretroviral drugs as revealed in a novel index, the inhibitory potential, which incorporates the slope parameter of the dose-response curve. This index may aid in the rational selection of fully suppressive therapy. At least 2 stable reservoirs of latently infected cells have been identified, and attempts are under way to identify compounds that selectively reactivate latent HIV and allow elimination of these reservoirs. This article summarizes a presentation made by Robert F. Siliciano, MD, PhD, at the International AIDS Society—USA continuing medical education program held in Atlanta in March 2010.

The cure of HIV infection requires actions on 3 broad fronts: stopping ongoing viral replication, identifying all reservoirs in which nonreplicating HIV persists, and eliminating each of these reservoirs. Recent advances have been made in each of these areas and give reason for optimism that a cure may eventually be feasible.

Viral Dynamics

The topic of eradication is best understood in the context of viral dynamics. Early studies of HIV dynamics during antiretroviral treatment showed that the level of plasma virus declines rapidly, with the logarithm of the viral load declining linearly over time. This decay can be explained by a simple mathematical model in which uninfected cells interact with free virus and become infected at a rate that depends on the concentrations of cells and virus. Because available antiretroviral drugs work by blocking new infection of susceptible cells (not by blocking virus production in cells that are already infected), the decay rate of plasma virus is determined by the lifespan of previously infected cells.

The initial decay rate is very rapid (Figure 1), indicating that the cells producing most of the plasma virus (eg, activated CD4+ cells) turn over very quickly. This reflects a scenario in which infection is extremely dynamic, and the half-life of most of these virus-producing cells is approximately 1 day. Thus, the virus present in plasma on a given day was produced by cells that became infected the previous day and that would be dead by the following day.

With effective antiretroviral therapy, the initial rapid decay is followed after several days by a second decay phase characterized by a more gradual decline in plasma virus. This behavior is consistent with a model in which a second population of infected cells with a longer lifespan produces a much smaller amount of virus. As shown in Figure 1, the second-phase decay indicates that these cells have a half-life of approximately 2 weeks. At the time of these initial observations more than 10 years ago, extrapolation of this second decay rate out to zero residual infected cells suggested that eradication of the infection in an individual patient could occur in 2 years to 5 years after initiation of therapy.

Figure 1. Typical decay phases for plasma virus during antiretroviral therapy (for a hypothetical patient). First and second phases exhibit a precipitous decline and more gradual decline, respectively, after the initiation of antiretroviral therapy. The presence of a latent reservoir predicts a third phase, in which viremia predominantly remains below the limit of quantification but may be revealed by occasional “blips” of detectable virus.

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then returned to quiescence. Naïve and memory CD4+ cells circulate through the lymphoid organs. On encountering an antigen, they undergo blast transformation, enlarging and proliferating, and begin to carry out their effector functions in the immune response.

At the conclusion of the immune response, most of these cells die, but some survive and return to a quiescent state as long-lived memory T cells (thus permitting a subsequent immune response to the same type of antigen in the future). In HIV infection, replication of the virus in activated CD4+ cells results in rapid cell death. However, some of the activated cells can become infected during their return to a quiescent state, resulting in an integrated form of the viral genome in a memory cell that is designed to live for years, if not decades. When these cells return to quiescence, HIV is “deactivated,” providing a perfect scenario for persistence. Essentially, the virus persists simply as information in the resting cell, avoiding detection by the immune system and remaining unaffected by antiretroviral drugs.

This reservoir of latently infected cells is present in all individuals with HIV infection in an extremely small population of CD4 cells (approximately 1 cell per million) with an extremely slow decay rate. Measurements taken at Johns Hopkins University from a large number of patients receiving antiretroviral therapy and with plasma HIV RNA levels maintained below the limit of quantification (50 copies/mL) indicated that clearance of a reservoir of 10⁴ latently infected cells would require more than 75 years.

The existence of the stable reservoir predicts a third phase in the decay curve for viremia, representing the turnover of these latently infected cells (Figure 1). Every day, a small proportion of the quiescent cells harboring latent HIV-1 become activated. Although the virus produced by the activated cells will not infect other cells if antiretroviral treatment is completely blocking ongoing viral replication, the virus will be released into the plasma and can still be detected by sensitive assays that measure concentrations below 50 copies/mL. Evidence of this phenomenon has come from investigation of the “blips” of detectable virus that are commonly observed in patients with otherwise suppressed replication through antiretroviral therapy. All treated patients with full suppression actually have a low-level, steady-state viremia below 50 copies/mL, typically around 1 copy/mL (Palmer et al, Proc Natl Acad Sci USA, 2008). The isolated, low-level blips of detectable virus may thus reflect biological and statistical variation in the level of this residual viremia.

Although this finding can be taken as evidence of continuous activation of a proportion of the latently infected resting CD4+ cell reservoir, it was initially widely thought to indicate that ongoing rounds of low-level replication were occurring despite drug treatment. Such a scenario would indicate the possibility of emergence of drug-resistant virus and loss of suppressive effect. In trying to ascertain whether the emergence of resistance is in fact detectable in this residual viremia, it is necessary to study samples from patients with good adherence to antiretroviral regimens because of the association of poor adherence with resistance and virologic failure.

Direct analysis of virus in this residual viremia has been attempted—a difficult task given that the typical level of viremia is 1 copy/mL—by cloning and sequencing the virus and comparing it with virus found in resting CD4+ cells. It was found that the sequences from plasma virus and those from the reservoir are intermingled, and in some cases identical, suggesting that at least some of the residual viremia is derived from latently infected cells that have become activated. The sequences of the free plasma virus did not diverge substantially from those in the latent reservoir, and most important, there was no evidence of new drug resistance mutations in the plasma virus. Through frequent sampling, the virus present during low-level blips of detectable plasma virus (eg, below 200 copies/mL) was captured and sequenced and showed no evidence of new drug resistance mutations.

One way to examine the contention that residual viremia is not the result of ongoing cycles of replication is to add a fourth powerful antiretroviral drug to triple-drug therapy for patients with suppression of plasma HIV RNA level to less than 50 copies/mL to determine if the level of viremia is further reduced. In patients receiving an efavirenz-based regimen, average plasma HIV RNA level was measured with an assay capable of detecting vi-

Figure 2. Level of residual viremia before, during, and after intensification of antiretroviral therapy. Each symbol represents data from the same patient. Solid lines indicate median values; open circles and squares indicate below the level of quantification. Based on data from Dinoso et al, Proc Natl Acad Sci USA, 2008.
rus to a level of 1 copy/mL (single-copy assay); results ranged from 1 copy/mL to 10 copies/mL. Intensification of therapy might be expected to drive the level to below the limit of quantification of 1 copy/mL. However, the addition of ritonavir-boosted atazanavir for 8 weeks resulted in no change in viral load during or after intensification.

Additional studies of intensification with ritonavir-boosted lopinavir, efavirenz, or raltegravir have shown no reduction in residual viremia (Figure 2). The finding that therapeutic concentrations of potent intensifying drugs to which the patient’s virus is susceptible have no effect on residual viremia suggests that most or all of this viremia results from the release of virus from stable reservoirs of cells infected before the initiation of antiretroviral therapy. The findings cannot completely rule out the possibility that very low level replication is ongoing somewhere in a patient (ie, at a level below quantification by the single-copy assay), but they do support the notion that stable reservoirs are the greatest barrier to viral eradication.

**Antiretroviral Drug Slope Parameters and the Inhibitory Potential Index**

The idea that antiretroviral therapy can completely block ongoing viral replication is difficult to grasp. One first needs to determine how much replication is occurring in a patient. A way of framing this issue is to ask how many newly infected cells arise in a single viral generation because blocking all new infection events in a generation would leave nothing more to block. The number of cells newly infected in 1 day, which is roughly the time span of a single viral generation, is dependent on viral load. According to the standard model of viral dynamics, in an untreated patient with an average plasma HIV RNA level of 50,000 copies/mL, there are an estimated $10^4$ (1 million, or 6 log) new infection events per day. Thus, given these assumptions, stopping ongoing viral replication requires treatment to block approximately $10^4$ infection events per day.

![Figure 3](image-url) Comparison of standard dose-response curves (left) and log-log dose-response curves (right) for drugs with identical 50% inhibitory concentration ($IC_{50}$) values but different slope values ($m$). Greater antiviral effects are found in drugs with higher slope values at therapeutic drug concentrations. $C_{max}$ indicates maximum plasma concentration; $C_{min}$, minimum plasma concentration. Adapted from Shen et al, *Nat Med*, 2008.

How well do current antiretroviral drugs reach this $6 \log_{10}$ per day target? Classic dose-response curves do not reveal a $\log_{10}$ value for the extent that antiretroviral drugs reduce new infection events. Rather, they show changes in infection on a linear scale (as percent of control) according to increasing drug concentration, and they allow derivation of the 50% inhibitory concentration ($IC_{50}$) of a drug. However, this linear depiction of the inhibition of viral replication is incongruous with the fact that replication is exponential.

Another problem with the standard plots is that the effect of the dose-response curve steepness or slope is obscured. Antiretroviral drugs are used at concentrations well above their $IC_{50}$ values, and on standard plots, differences in slope parameter do not appear to make much difference in antiviral effect within the therapeutic concentration range. However, conversion of the y-axis of the dose-response curve to a logarithmic scale provides a more accurate representation of the degree of inhibition and of differences in inhibition for drugs with identical $IC_{50}$ values but different slope parameters. Such a conversion reveals a dramatic divergence in the magnitude of inhibition within the range of clinically relevant therapeutic concentrations. Drugs that have a higher slope value produce inhibition that is orders of magnitude greater than drugs with lower slope values (Figure 3) (Shen et al, *Nat Med*, 2008), indicating that the dose-response curve slope is a major determinant of antiviral activity. The slope parameter is analogous to the Hill coefficient, which is a measure of cooperativity in the binding of several drug molecules to a multivalent receptor. Because nucleoside analogue reverse transcriptase inhibitors (NRTIs), nonnucleoside analogue reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) all bind to single

![Figure 4](image-url) Example of a difference in the instantaneous inhibitory potential (IIP) between drugs with identical 50% inhibitory concentration ($IC_{50}$) values but different slope values ($m$). $C_{max}$ indicates maximum plasma concentration; $C_{min}$, minimum plasma concentration. Adapted from Shen et al, *Nat Med*, 2008.
Figure 5. Instantaneous inhibitory potential (IIP) values at maximum plasma concentration (C\text{max}) varies by more than 10 log_{10} for drugs in different antiretroviral classes. Single asterisk indicates investigational drug; double asterisk indicates no longer on the market. FI indicates fusion inhibitor; INSTI, integrase strand transfer inhibitor; NNRTI, nonnucleoside analogue reverse transcriptase inhibitor; nRTI, nucleoside analogue reverse transcriptase inhibitor; PI, protease inhibitor. Adapted from Shen et al, Nat Med, 2008.

The different classes of antiretroviral drugs were investigated to determine whether the slope values differed from the expected value of 1. The slope was 1 for all nRTIs, approximately 1.7 for NNRTIs, 2 to 4.5 for PIs, 1.7 for entry inhibitors, and 1.5 structurally diverse integrase strand transfer inhibitors (INSTIs). Mechanistically, these results make sense when it is considered that for the 2 drug classes that show a slope of 1, the nRTIs and the INSTIs, the target is an enzyme-nucleic acid complex for which there is only 1 relevant copy per virus. In contrast, the NNRTIs and PIs target viral enzymes, of which numerous copies participate in the relevant step in the virus life cycle. This form of intermolecular cooperativity is manifest in a steep dose-response curve.

To describe the differences in antiviral activity that result from differences in slope value, Shen and colleagues developed the instantaneous inhibitory potential (IIP) index, which is the logarithmic decrease in a single round of infection caused by a drug at a clinically relevant concentration (such as the maximum plasma concentration, C\text{max}). The formula for IIP includes the slope parameter and allows for an estimation of the degree of inhibition achievable at clinically relevant drug concentrations. For example, in an in vitro infection in which there are 1 million infected cells in the absence of a drug, the presence of a C\text{max} concentration of a drug with a slope of 1 and a C\text{max} value that is 100-fold greater than the IC\text{50} value will result in a 2 log_{10} reduction in infection events (IIP = 2), leaving 10^4 infected cells from that single generation (Figure 4) (Shen et al, Nat Med, 2008). For another drug with the same IC\text{50} value but a slope of 3, there is a 6 log_{10} reduction in infectivity (IIP = 6), which should result in absence or near absence of infected cells. The steep slope of the dose-response curve results in these “extra” logarithmic reductions that are not apparent on a linear scale or in conventional assays but that are essential in reducing ongoing replication to zero.

The IIP values for current antiretroviral drugs at C\text{max} are shown in Figure 5. The values range from 1 to 4 for nRTIs, as high as 6 for NNRTIs, and from 2 to 10 for PIs, with lower values observed for entry inhibitors and INSTIs. It is striking that some PIs can produce a 9 log_{10} to 10 log_{10} (1 billion-fold) reduction in a single round of replication.

Figure 6 shows the average IIP values (IIP_{\text{avg}}, representing the average IIP values over the dosing interval) for current antiretroviral drugs against a shaded bar that indicates the estimated range of infection events that must be inhibited to achieve a full and immediate halt to viral replication in the typical untreated patient (5 log_{10} to 7.5 log_{10} reduction in infection events). Only ritonavir-boosted darunavir appears to have an IIP_{\text{avg}} value sufficient as a single drug to prevent all infection events. Otherwise, combinations of drugs are needed to achieve the approximate 6 log_{10} reduction target.

These findings suggest that antiretroviral therapy can indeed produce a complete or near-complete arrest in ongoing replication. The findings also explain why regimens containing a PI or NNRTI are the most effective: these 2 classes have an extraordinary ability to inhibit viral replication. The findings also may explain why combinations of nRTIs have proven inferior in clinical trials. The unique case of the integrase inhibitors is discussed below.

Two of the drugs with the highest inhibitory potential, indinavir and saquinavir, are no longer widely used, reflecting one of the drawbacks of a high slope value. A high slope value indicates that a small increase in drug concentration results in a dramatic increase in inhibition. By the same token, a small decrease in drug concentration results in a dramatic decrease in inhibition. Thus, for drugs with a high slope value and a short half-life, inhibitory potential declines dramatically, even during the dosing interval. In fact, the rate at which inhibitory potential decreases after the previous dose is proportional to the slope over the half-life. Drugs with a longer half-life can maintain a high level of inhibition for long periods after the previous dose. The drugs that maintain the highest inhibitory potential 24 hours after the previous dose—ritonavir-boosted darunavir, efavirenz, and ritonavir-boosted atazanavir—have done very well in comparative clinical trials. In fact, sustained inhibitory potential measured in this way does a good job of predicting the outcome of comparative clinical trials, although the index was not developed for this purpose and many other factors are involved (Shen et al, Nat Med, 2008).
Attempts are under way to define inhibitory potential for combinations of drugs that would take into account synergy and antagonism as well as the effects of drug resistance mutations, rendering it possible to select a regimen with the greatest inhibitory potential for patients on an individualized basis. The integrase inhibitors, which have low slope values and only modest inhibitor potential, have done well in clinical trials. One explanation, supported by work from Dr Siliciano’s group, is that these drugs synergize in a highly favorable fashion with all other classes of antiretroviral drugs. Inhibitory potential alone does not necessarily correlate with clinical outcome, however. Many regimens have enough inhibitory potential to control replication. The best regimen is one that not only succeeds in preventing replication but is also the best tolerated because adherence is required to achieve the optimal antiretroviral effect.

**Prospects for Eliminating Viral Reservoirs**

As discussed above, antiretroviral therapy works extremely well in inhibiting viral replication—step 1 in the 3 steps needed to cure HIV infection. For the second step, identifying all reservoirs of nonreplicating HIV, at least 1 reservoir has been identified: the quiescent CD4+ T cells carrying latent HIV-1. Studies of residual viremia have provided evidence of another reservoir in an as-yet-identified cell type. In approximately half of patients studied, residual viremia is dominated by a small number of clones. These clones do not show evidence of sequence evolution and are not found in resting CD4+ T cells (Bailey et al. J Virol. 2006; Tobin et al. J Virol. 2005).

For the final step, elimination of these reservoirs, a strategy that has been pursued is to activate latently infected cells. However, when done nonspecifically, broad activation of T cells results in heightened expression of cytokines and unacceptable toxic effects. Work is ongoing to screen for compounds that might activate latent HIV without causing global T-cell activation. Although identifying compounds that reanimate latent HIV in primary resting CD4+ T cells is surprisingly easy in vitro, whether any of these compounds is safe enough for use in humans remains to be seen. Overall, these results suggest that the identification and elimination of stable reservoirs is a feasible goal of HIV therapeutics.

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