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HIV reservoirs as obstacles and opportunities for an HIV cure

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The persistence of HIV reservoirs remains a formidable obstacle to achieving sustained virologic remission in HIV-infected individuals after antiretroviral therapy (ART) is discontinued, even if plasma viremia has been successfully suppressed for prolonged periods of time. Numerous approaches aimed at eradicating the virus, as well as maintaining its prolonged suppression in the absence of ART, have had little success. A better understanding of the pathophysiologic nature of HIV reservoirs and the impact of various interventions on their persistence is essential for the development of successful therapeutic strategies against HIV or the long-term control of infection. Here, we discuss the persistent HIV reservoir as a barrier to cure as well as the current therapeutic strategies aimed at eliminating or controlling the virus in the absence of ART.

One of the most remarkable achievements of modern biomedical research has been the discovery and widespread implementation of antiretroviral therapy (ART) for the treatment of human immunodeficiency virus (HIV) infection. Since the introduction of highly effective multidrug combination therapy for HIV infection in 1996, ART has averted millions of AIDS-related deaths worldwide[1] and has dramatically increased the life expectancy of HIV-infected individuals[2,3]. Despite these extraordinary successes, it has become clear that the vast majority, if not all, HIV-infected individuals who are receiving clinically effective ART will need to remain on continuous and uninterrupted therapy for the remainder of their lives. This is mainly due to the persistence of HIV reservoirs, which accounts for the relatively rapid plasma viral rebound that has been observed in virtually all HIV-infected individuals who have discontinued ART, including those whose plasma viremia had been successfully suppressed for years[4]. This has led to the conclusion that with the currently available ART regimens, HIV-infected individuals will require life-long therapy. Such therapy poses many challenges, however, including difficulties associated with adherence to drug regimens, the potential for the emergence of resistant virus and cumulative toxicities from long-term therapy, as well as economic and logistical considerations. To address these concerns, the HIV scientific community has begun to explore a number of novel therapeutic options, with the ultimate goal of achieving sustained virologic suppression and preventing plasma viral rebound upon discontinuation of ART[5]. The focus of these curative strategies has been far-reaching and varied, with the most common approaches being to eliminate the persistent viral reservoir, render CD4$^+$ T cells resistant to HIV or enhance host immunity against HIV. Although the prospect of eradicating HIV has received substantial attention in recent years, many challenges remain. In this Perspective, we discuss the persistent HIV reservoir as a major obstacle to eradicating the virus as well as the various therapeutic interventions aimed at achieving a cure.

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HIV reservoirs are a major obstacle to viral eradication

The first indication that true eradication of HIV in infected individuals would be extremely difficult came in 1997, with the publication of three independent studies that demonstrated the persistence of a latent and inducible viral reservoir in all study subjects, despite the fact that ART had maintained their plasma viremia below the limit of detection for a few years[6–8]. A longitudinal study subsequently confirmed the prolonged persistence of the latent HIV reservoir in individuals receiving clinically effective ART[9], further dimming the prospect for eradication of the virus by antiretroviral drugs alone. Although the precise source(s) of re-emerging plasma viremia has not been fully elucidated, it is widely believed that latently infected, resting CD4$^+$ T cells and other viral reservoirs directly or indirectly contribute to the rapid viral rebound that typically occurs within 2 weeks after the cessation of ART[4]. Multiple mechanisms are thought to be involved in the maintenance and persistence of HIV reservoirs in infected individuals receiving ART. These include intrinsic stability of latently infected, resting CD4$^+$ T cells[9]; periodic homeostatic proliferation of cells that sustain the latent viral reservoir[10]; replenishment of infected cells via residual viral replication[11]; inadequate penetration of antiretroviral drugs into tissues where the virus replicates[12]; and the persistence of HIV in sanctuary sites located in various tissue compartments[13]. Of note, recent extensive molecular analyses of the distribution of viral integration sites in the genome of infected individuals receiving ART has provided important insight into a potential mechanism for the persistence of HIV proviral DNA[14–16]. These studies revealed evidence for clonal expansion of integrated HIV DNA in CD4$^+$ T cells[14–16] and demonstrated that some of these proviral clones are clustered within or near genes associated with human cancer and the growth and division of cells[14,15]. These findings suggest that the location of viral integration in the human genome could potentially affect the expansion and persistence of HIV reservoirs. However, it is important to note that the integrated HIV DNA analyzed may or may not be replication competent. Considering that the vast majority

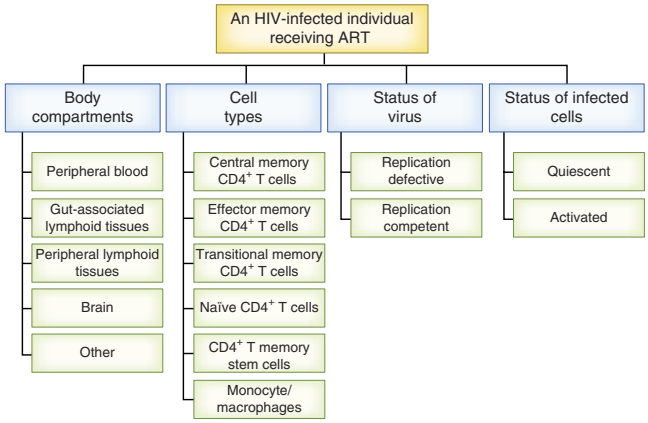

Figure 1 The complex nature of HIV reservoirs in infected individuals receiving ART. The complexity of persistent HIV reservoirs is reflected by the involvement of several tissue compartments and cell types, and the status of the virus and infected cells in infected individuals receiving ART.

of HIV proviral DNA in CD4+ T cells is replication defective[16–18], the implications of these findings remain unclear, especially with regard to the persistence of viral reservoirs that carry infectious HIV, the clinical outcome and future therapeutic strategies aimed at eradicating the virus in infected individuals. Given the complex nature of HIV persistence and the variety of curative strategies that focus on eliminating and/or suppressing viral reservoirs, a better understanding of the pathophysiology of these infected cells in regard to their anatomical location, the effect of tissue environment on viral latency, the cell type(s) involved in latency, and the replication capacity, degree and extent of viral expression *in vivo* is important in the development of therapeutic strategies aimed at curing HIV infection (**Fig. 1**).

Lessons learned from attempts to purge HIV reservoirs

With the recognition that the latent HIV reservoir is a major barrier to cure, several therapeutic strategies targeting such infected cells have been the focus of recent research efforts. Many, if not all, of these efforts are predicated on the assumption that inducing HIV expression from latently infected, resting CD4+ T cells would cause a reduction of the overall viral burden through the death of infected cells due to virus-induced cytopathic effects and/or host immunologic mechanisms, at the same time that virus released from these activated cells would be prevented from infecting other cells by the ongoing ART regimen. Indeed, over 15 years ago, the concept of HIV purging was first tested with powerful immune-activating agents, such as interleukin 2 (IL-2)[19] and anti-CD3 antibody[20], that were administered to infected individuals receiving ART. These studies were based on the consistent observation that cellular activation enhances HIV replication and induces these productively infected CD4+ T cells to die within hours to days[21]. One study demonstrated that the frequency of resting CD4+ T cells carrying replication-competent HIV was significantly lower in infected individuals who received IL-2 plus ART than in those who received ART alone[19]. Nonetheless, discontinuing ART resulted in rapid plasma viral rebound in all study participants[22]. A similar observation was made in individuals who received anti-CD3 antibody[20]. Subsequently, small inhibitory molecules that target histone deacetylases (HDAC) and trigger viral expression without grossly activating the cells were proposed as potential virus purging agents[23,24]. This proposal was based on *in vitro* analyses demonstrating that HDAC inhibitors induced acetylation and remodeling of the chromatin and could, in theory, allow expression of HIV in the latent viral reservoir, resulting in cell death[24]. A first set of studies involved the administration of valproic acid, an HDAC inhibitor that is used for treating epilepsy and bipolar disorders. This approach generated conflicting results[23,25], although a later randomized clinical study concluded that this particular drug failed to decrease the size of the pool of latently infected, resting CD4+ T cells[26]. More recently, clinical studies involving other HDAC inhibitors, including vorinostat[27,28] and panobinostat[29], have consistently shown an increase of the level of cell-associated HIV RNA in resting CD4+ T cells from infected individuals. However, the size of the latent reservoir remained unchanged, indicating that these drugs failed to purge the virus *in vivo*[27–29]. It is important to note that reversing HIV latency, often described as the induction of cell-associated HIV RNA in latently infected, resting CD4+ T cells following administration of HDAC inhibitors, may not necessarily lead to the depletion of such infected cells. In this regard, two *ex vivo* studies had shown that HDAC inhibitors failed to significantly enhance HIV production from the latent viral reservoir, a necessary first step for virus purging and cell death[30,31]. Furthermore, one study demonstrated that HDAC inhibitors may stimulate HIV expression only in a very small fraction of latently infected, resting CD4+ T cells[30], further challenging the ability of these agents to purge HIV *in vivo*. Although HDAC inhibitors alone may not be able to eradicate the latent viral reservoir, it is conceivable that such compounds may lead to expression of HIV peptides or proteins on the cell surface, potentially allowing cytotoxic CD8+ T lymphocytes (CTL) to recognize and clear these infected cells[32]. CTL escape mutations were present in HIV Gag epitopes in the latent viral reservoir of infected individuals who began ART during the chronic phase of infection[33], indicating that additional immunologic approaches that stimulate and expand pre-existing CTLs against common epitopes may be necessary to decrease the frequency of infected cells. However, such approaches are likely to present significant challenges that may require highly effective therapeutic vaccines.

Setting attainable goals

The high-profile case report, published in 2009, that described an American HIV-infected patient in Germany (referred to as the Berlin patient) who may have completely eliminated the virus from his body[34] has given the HIV and AIDS scientific community hope that the virus could be completely eliminated in other infected individuals. This single case of an individual having been cured of HIV infection, along with a number of related scientific advances in the subsequent years, have led to an unprecedented mobilization of resources and efforts dedicated to HIV cure research. In broad terms, it is important to define what one means by a 'cure' of HIV infection. If an HIV cure is defined by the indefinite or permanent absence of plasma viral rebound after discontinuation of ART, then one can consider at least two approaches to this goal. The first approach seeks to completely eliminate all traces of HIV in an infected individual, ultimately leading to permanent virologic remission in the absence of ART (this is often referred to as a 'sterilizing cure'). The second approach seeks to achieve what is defined as 'sustained virologic remission' characterized by replication-competent virus remaining in the body but not causing clinically significant replication or rebound of detectable plasma viremia in the absence of ART. This state of sustained virologic remission could potentially be maintained by enhancement of the host's endogenous HIV-specific immune response and/or by intermittent administration of immune-mediating agents to infected individuals before or after the discontinuation of ART. In this regard, it is important for scientists and clinicians engaged in cure research to set attainable goals when designing and implementing clinical trials that test therapeutic strategies

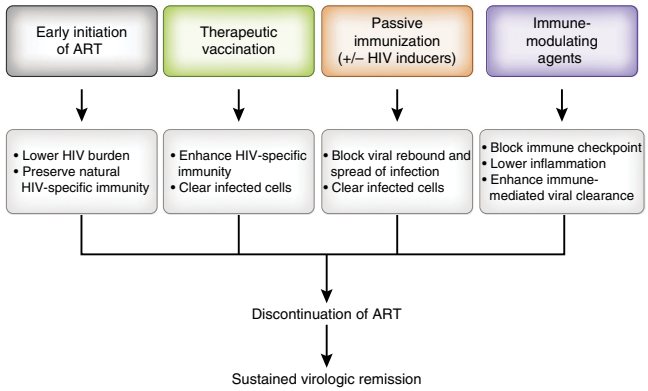

Figure 2 Path to achieving sustained virologic remission in infected individuals following discontinuation of ART. Early initiation of ART alone may not be sufficient for achieving sustained virologic remission; however, by significantly reducing the size of the persistent HIV reservoir and preserving host immune responses against HIV, early intervention could lead to remission in infected individuals by enhancing or complementing other curative strategies, such as therapeutic vaccination, passive immunization and immune-modulating agents.

aimed at curing HIV by one of these two approaches. The approaches are very different from one another with respect to feasibility, safety, cost, likelihood of success and scalability. For obvious reasons, therapeutic strategies aimed at completely eradicating the viral reservoirs represent the most definitive and compelling approach. However, attempts to achieve a sterilizing cure are most likely to be associated with significant risks to patients and formidable challenges in terms of science and logistics. Such was the case with the Berlin patient. Multiple research groups have made exhaustive attempts to detect any residual HIV in the plasma, peripheral blood and tissues specimens isolated from this patient[35]. With the exception of a blip of transient and extremely low-level plasma viremia, HIV has not been detected thus far in the Berlin patient[35]. Two critical factors are thought to have contributed to complete eradication of HIV in this individual. First, repeated rounds of chemotherapy followed by stem cell transplantation, as well as the development of graft-versus-host-disease, likely eliminated a substantial percentage of his persistently infected $CD4^+$ T cells and other potential viral reservoirs. Second, the transplanted donor cells lacked CCR5 expression (as a result of the *CCR5*-Δ32 mutation), making the newly populated $CD4^+$ T cells resistant to HIV infection and thereby preventing the regeneration of HIV reservoirs[34]. Although this series of interventions and treatments may have led to eradication of HIV in this patient, such procedures carry high risks and pose significant challenges, such as the identification of HLA-matched donors who are homozygous for *CCR5*-Δ32, extensive clinical follow-up and variability in outcome. Also, this specific approach, although it is put forth as a 'proof of concept' for an HIV cure, is suitable only for people who require stem cell transplantation for a condition other than HIV infection. In this regard, a number of studies[36,37] were subsequently conducted in an attempt to recapitulate the success achieved with the Berlin patient by using *CCR5*-32 stem cell transplantation following chemotherapy for underlying malignancies in HIV-infected patients. However, the curative outcome observed in the Berlin patient has thus far not been repeated. Instead, the outcomes have included several deaths due to patients' underlying medical conditions unrelated to HIV status[37]. In another study, two subjects received allogeneic stem cell transplantation from donors who were wild type for CCR5. Despite an initial period (3–8 months) of undetectable HIV plasma viremia after discontinuation of ART, viremia later returned, requiring reinitiation of ART[38]. The mechanism(s) associated with relatively long periods of aviremia (months rather than days to weeks) in these latter individuals are unclear, although some possibilities include a profound depletion of viral reservoirs by chemotherapy and stem cell transplantation and/or by graft-versus-host-disease and suppression of HIV replication by immunosuppressive drugs. The persistent and quiescent nature of the latent viral reservoir may also have contributed to the prolonged periods of aviremia. Clearly, stem cell transplantation is not suitable as a curative strategy for broad use among HIV-infected individuals without underlying malignancies.

However, another approach is being pursued that operates on the same principle of replacing susceptible target cells with cells refractory to infection with the virus. The strategy involves gene editing using zinc-finger nucleases that could specifically eliminate or reduce the expression of genes of interest, such as *CCR5* (ref. 39). Autologous cells are targeted *ex vivo* by modification or deletion of the *CCR5* gene (thereby avoiding the need to identify an HLA-matched donor and/or administer immunosuppressive therapy). Genetically modified cells are then propagated *ex vivo* and transfused back into the patient. Preliminary data have shown positive outcomes in terms of repopulation and increases in the peripheral blood and lymphoid tissues of the proportion of modified cells that no longer express CCR5[39]. However, this therapeutic strategy may also ultimately require chemotherapy to eliminate remaining original donor cells that are susceptible to HIV infection, raising a concern as to whether this approach can be safely expanded to the vast majority of HIV-infected individuals receiving ART who do not have malignancies. Other strategies include targeted gene disruption using sequence-specific enzymes that can excise integrated HIV DNA or host genes[40]. These technologies are still in the early discovery phase.

The development of therapeutic strategies aimed at achieving sustained virologic remission is considered to be a more realistic approach to the long-term clinical management of HIV-infected individuals. A rare population of HIV-infected individuals, termed elite controllers, whose plasma viremia is maintained below the limit of detection and whose $CD4^+$ T cell counts are within the normal range without the requirement for ART[41] may represent a model for achieving sustained virologic remission. Multiple studies have demonstrated that HIV reservoirs, and low levels of ongoing viral replication, persist in such individuals[42–44], even though they maintain undetectable levels of plasma viremia for years to decades. The suppression of viral replication in these individuals involves $CD8^+$ T cells[45]. Thus, these examples of natural immunologic control of HIV suggest that complete eradication of HIV reservoirs may not be necessary to achieve sustained virologic remission. It is anticipated that multiple strategies aimed at preventing and/or minimizing the establishment and size of persistent HIV reservoirs may be needed for this approach to succeed (see below). Examples of such strategies include early initiation of ART, eliminating infected $CD4^+$ T cells with therapeutic agents that target the virus and/or infected cells, and harnessing anti-HIV immune responses using therapeutic vaccines or immunotherapies (**Fig. 2**).

Minimizing the size of HIV reservoirs via early initiation of ART

Well over a decade ago it was shown that HIV latency is established within days of an individual's acquiring the virus[46]. The rapid establishment of HIV reservoirs during the acute-early phase of infection was also recently shown in a controlled animal study[47]. Given that the vast majority of HIV-infected individuals do not begin ART during the acute or early phase of infection, either because they are unaware of their status, therapy is not available in certain resource-poor

countries, and/or they are unwilling to start therapy for a variety of reasons, it is highly unlikely that blocking the initial seeding and establishment of persistent viral reservoirs by ART alone is a realistic approach to eradicating HIV at the population level. Nonetheless, comparison of cohorts of individuals who began ART early after infection to those who delayed therapy indicates that the HIV reservoirs of the former decay at faster rates[48,49] and their immunity against HIV is better preserved[50], suggesting that initiation of ART during the acute-early phase of infection could be a crucial first step toward achieving sustained virologic remission through the addition of other interventions. A study of an HIV-infected infant who began ART shortly after birth[51] and another describing a small subset of early-treated adults who showed spontaneous virologic control of the virus after discontinuation of ART[52] have highlighted a potential impact of early initiation of ART on the feasibility of achieving an HIV cure. The first case described an infant (commonly called the 'Mississippi baby') who was born to an HIV-infected mother and who began ART within 30 h of birth[51]. The infant successfully showed suppression of plasma viremia to below the limit of detection and continued ART for 18 months, at which point she was lost to follow-up and discontinued medications. Remarkably, she remained aviremic for more than 2 years without receiving ART, raising hopes that she might have completed eradicated HIV. Unfortunately, her plasma viremia ultimately rebounded to above 10,000 copies/ml and ART was reinitiated[53]. Although many factors may have contributed to this unprecedented duration (over 2 years) of viral suppression in the absence of antiretroviral drugs, it is believed that the extremely early initiation of ART, by minimizing the size of the persistent HIV reservoir, played a key role in the protracted period of aviremia in the absence of ART. It is also probable that the latent viral reservoir is less prone to spontaneous reactivation and/or the spread of infection is less efficient in the context of the immature immune system of an infant or child. In the second example, a small subset of HIV-infected adults began ART during the acute-early phase of infection and subsequently controlled their plasma viremia for prolonged periods of time after discontinuation of therapy[52]. Considering that plasma viremia typically rebounds within a few weeks after the cessation of ART in the majority of HIV-infected individuals who initiate therapy during the chronic phase of infection[4], this finding, though based on a small study that needs to be confirmed in large, randomized trials, highlights potential benefits of, and reasons for, promoting the early initiation of ART. It is highly likely that early initiation of ART alone may not be sufficient to achieve sustained virologic remission for prolonged periods of time in a majority of individuals; however, it could succeed in combination with other curative strategies, such as therapeutic vaccines and other immunotherapies (**Fig. 2**). Future studies should focus on delineating the impact of the size of HIV reservoirs and the timing and duration of ART on the kinetics of plasma viral rebound. Furthermore, the identification of genetic, immunologic and virologic factors that may contribute to long-term suppression of viral replication in infected individuals who begin ART during the acute-early phase of infection should also be investigated.

Immunologic approaches for containment of HIV reservoirs

The development of a preventive HIV vaccine is considered an essential element of the path toward ending the AIDS pandemic[54]. Although a highly effective preventive HIV vaccine does not currently exist, certain vaccine candidates, despite their lack of efficacy in preventing HIV infection, have been shown to elicit varying degrees of HIV-specific immunity in vaccines[54,55]. In addition, there is compelling evidence that the ability of elite controllers to control their plasma viremia spontaneously without ART is mediated, at least in a certain percentage of individuals, by strong host immunologic responses against HIV[41]. In light of these observations, several immune-based therapeutic strategies are currently being considered as part of multiprong approaches for achieving sustained virologic remission in HIV-infected individuals after the discontinuation of ART. Examples of such efforts include the induction of CTLs that could target the persistent viral reservoir and/or suppress HIV viral replication after the cessation of therapy. In this regard, a number of therapeutic vaccines have been tested, although none thus far has led to prolonged periods of viral suppression in infected individuals after discontinuation of ART[56]. However, trials with vaccines that target multiple epitopes thought to be expressed within the persistent HIV reservoir of early-treated individuals who manifest features such as a relatively intact immune system, low overall HIV burden and relatively homogeneous viral quasispecies should be considered. Such approaches could provide important insight into the feasibility of achieving sustained virologic remission. In addition to CTL-based approaches and in the light of recent advances in high-throughput technologies and B cell biology[57], passive immunotherapy with recently identified highly potent and broadly neutralizing HIV-specific antibodies is also being considered. This approach relies on the capacity of monoclonal antibodies to prevent cell-to-cell spread of HIV and to possibly eliminate infected cells via antibody-dependent cell-mediated cytotoxicity and/or antibody-dependent cell-mediated viral inhibition[58–60]. Of note, combining broadly neutralizing HIV-specific antibodies with a cocktail of virus-inducing agents allowed suppression of viral replication in about half of infected humanized mice examined[61], further expanding therapeutic options involving passive transfer of such antibodies. With the added possibility of biochemical modification aimed at extending the serum half-lives of these HIV-neutralizing antibodies, it is conceivable that infrequent (every several months) administration of one or more such antibodies could result in the maintenance of very low or undetectable plasma viremia in infected individuals in the absence of ART. Other immunologic strategies aimed at containing HIV reservoirs and/or achieving sustained virologic remission include the blocking of certain key immunoregulatory molecules, such as PD-1, and the use of immune-modulating agents such as cytokines and inhibitors of cell signaling pathways[62].

What to measure and how long to wait to predict outcome

Comprehensive evaluation of HIV reservoirs in infected individuals undergoing a potentially curative therapy is an important part of determining the effectiveness of interventions *in vivo*. Although plasma viremia remains the most clinically relevant virologic marker, other laboratory assays that are designed to detect cell-associated HIV are crucial to evaluating the impact of various therapies on the persistent viral reservoir, especially in individuals whose plasma viremia is undetectable as a result of ongoing ART. A number of laboratory assays have been designed to measure HIV burden in the peripheral blood and lymphoid tissues of infected individuals[63]. The majority are molecular assays that quantify HIV nucleic acid using polymerase chain reaction (PCR) technologies[63]. However, given that most cell-associated HIV is replication defective[17,18], it is necessary to include additional assay(s) that measure levels of replication-competent virus[63]. For example, for therapeutic interventions, such as HDAC inhibitors, that are intended to purge HIV from the latent viral reservoir, it is important to determine whether such agents can diminish the number of $CD4^+$ T cells carrying replication-competent HIV and not simply monitor levels of cell-associated HIV RNA in the latent viral reservoir. However, it should be noted that a severe

reduction or even an undetectable level of the persistent viral reservoir in infected individuals may not necessarily imply that the virus has been eradicated, even when multiple assays for evaluating viral burden have been performed. In this regard, two aforementioned instances (the Mississippi baby and two adults who underwent heterologous stem cell transplantation)[38,51] have taught us a valuable lesson. In all three of these HIV-infected individuals, levels of HIV, as measured by several different laboratory assays, were undetectable before and for prolonged periods after cessation of ART. Yet all three eventually experienced HIV plasma viral rebound. These findings suggest that the likelihood of and/or the time to plasma viral rebound in HIV-infected individuals who have undergone an attempt at curative therapy cannot be predicted accurately with current assays for assessing viral reservoirs. Of note, it is conceivable that $CD4^+$ T cells carrying replication-defective HIV proviral DNA could persist for prolonged periods of time, even in an infected individual who may have actually achieved a cure. Such a scenario could complicate the laboratory evaluation of the outcomes of curative interventions. Clearly, the enigma of the HIV reservoir needs further exploration.

Conclusions

The research toward a cure for HIV conducted over the past decade has yielded extraordinary insight into the pathogenesis of HIV disease and has been the source of both excitement and disappointment. Despite these recent scientific advances and the increased funding from both public and private investments that have certainly advanced ongoing efforts aimed at developing therapeutic strategies toward an HIV cure, it remains uncertain whether the development of simple, safe and scalable curative interventions is realistic, at least in the foreseeable future. The goal of an HIV cure for at least in a proportion of HIV-infected individuals is potentially attainable; however, it has proven to be one of the most challenging aspects of all HIV research. Clearly, any possibility of a cure requires strong and sustained efforts in order to better understand the establishment, composition, maintenance and renewal of the extraordinarily enigmatic HIV reservoir.

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The authors declare no competing financial interests.

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