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Clinical implications of new findings in HIV basic research

Manish Sagar

Brigham and Women's Hospital, Harvard Medical School, 65 Landsdowne Street, Room 447,
Cambridge, MA 02139, USA, Tel.: +1 617 768 8372; Fax: +1 617 768 8738;
msagar@partners.org

Abstract

HIV has been studied extensively over the past 25 years. Insights into the different stages of the virus' replication cycle and its interaction with host-cell proteins have led to the development of an armamentarium of effective antiretroviral medications. These antiviral drugs have dramatically changed the prognosis for HIV-infected subjects from an inevitable march towards death to a chronic disease with a potentially normal lifespan. Even with these successes, there is a continuing need to provide new drugs, especially those effective against drug-resistant viruses, to devise optimal strategies to prevent adverse events from either immunosuppression or the antiretroviral medications, and to develop treatments aimed at eliminating virus replication in the absence of antiviral drugs. In this review, how these important issues are being addressed will be highlighted, emphasizing clinical implications from some recent basic science studies and demonstrating how they could change the face of HIV therapeutics over the next 5–10 years.

Keywords

CCR5; CD4; envelope; HIV; host-cell factor; integration; nuclease; recombinase; treatment initiation; viral envelope

In 1983–1984, HIV was discovered as the causative agent for the immunosuppression that led to atypical infections in previously healthy adults [1,2]. Over the ensuing 25 years, HIV is arguably the most studied and most well understood virus in human history. In the relatively brief period since its discovery, we have gained a rapid understanding about the virus' replication cycle, its requirement for host-cell factors, its pathogenesis and its epidemiology. However, this accumulated knowledge has not led to an effective vaccine or a prophylactic microbicide that can reduce HIV-1 acquisition [3,4]. Therefore, the number of HIV-infected individuals in the world increases annually, which continues to place a great premium on the clinical care of patients with HIV. New basic science studies have provided novel insights about HIV and have direct consequences for treatment strategies. Although, the results from the majority of basic science investigations do not have immediate clinical applications, implications of some recent studies may be important for HIV clinical care over the next 5–10 years. In this review, I will summarize some recent important findings from basic science HIV studies and attempt to describe their potential impact on future clinical practice.

Search for new antiviral targets

Various aspects of the HIV replication cycle (reviewed in [5]) have been exploited to control viral production. Currently, there are 23 different antiretroviral drugs that retard HIV

replication by interfering at five different stages of the virus replication cycle. In order to enter a cell, the HIV envelope glycoprotein attaches to the CD4 host-cell receptor and then undergoes a conformational change prior to interacting with a coreceptor, primarily CCR5. This sequential interaction between the HIV envelope and host-cell receptors is necessary prior to host-cell entry. A CCR5 antagonist prevents HIV from binding to the CCR5 coreceptor. Viral entry is also blocked by a fusion inhibitor, which prevents the required conformational changes of the viral envelope glycoprotein prior to the melding of the viral and host-cell membranes. In turn, this blocks the viral RNA from reaching the cytoplasm. Reverse transcriptase inhibitors inhibit the transcription of the viral RNA into DNA, which halts the relay of the virus' genetic information within the cell. Integrase inhibitors block HIV reverse-transcribed DNA from being integrated into the host-cell chromosomes, eliminating viral protein production and progeny RNA production. Finally, protease inhibitors block a viral cleaving enzyme whose action is required to produce structural and nonstructural components of a mature virion.

Although antiretroviral therapy has been highly successful in decreasing the level of HIV replication within an infected subject, the effectiveness of these drugs is often compromised by the presence of drug-resistant variants. *In vivo* resistance has been documented against all classes of the currently used anti-retrovirals (reviewed in [6]). In addition, mutations that limit the effectiveness of one drug often lead to resistance against other drugs within the same antiretroviral class owing to generalized cross-resistance. Furthermore, individual HIV variants can be resistant to multiple antiretroviral drug classes simultaneously. Generalized cross-resistance and the presence of multiple drug-resistant variants can present daunting challenges in designing an effective treatment regimen that suppresses viral replication to below the level of detection in commercial viral load assays. Along with optimizing clinical monitoring and improving adherence, the burgeoning clinical problem of HIV drug resistance will require the development of newer drugs that target previously unexploited viral replication pathways.

Currently available antiretrovirals target four distinct viral proteins (reverse transcriptase, protease, integrase and the envelope glycoprotein), and one host receptor (CCR5). Besides disrupting these viral proteins, interfering with interactions among vif–APOBEC3G, vpr–tetherin, p6^{gag}–ESCRT pathway, p24^{gag}–TRIM5 α and/or integrase–LEDGF may also be useful in limiting viral replication of multiple drug-resistant HIV. Recent summaries have outlined the HIV therapy potential of these novel targets [7,8] and, therefore, they will not be discussed further in this review. Host-cell targets, as opposed to virus proteins, may provide a greater variety and opportunity for decreasing HIV replication. Generally, a host factor's influence on viral pathogenesis has been defined by either *in vitro* experiments or genetic association studies of a specific candidate gene. For instance, CCR5 receptor polymorphisms, such as a 32 base-pair deletion, which restricts both HIV acquisition and replication, were identified through genome association studies [9–11]. The influence of polymorphisms in specific genes or particular human leukocyte antigens (HLAs) on the level of virus replication within a host have also been determined by examining differences among subjects with high versus low levels of plasma HIV [12,13]. Recently, genome-wide association studies (GWAS) have been used to identify single nucleotide polymorphisms that affect the level of HIV replication, without the *a priori* selection of candidate genes [14,15]. However, GWAS require that associations surpass a high statistical barrier to be considered significant, which potentially means that true host-cell polymorphisms that attenuate virus pathogenesis may be missed. In addition, GWAS identify associations between specific host-genome polymorphisms and a disease phenotype; they do not delineate a specific gene or a potential biological mechanism [16]. Furthermore, genetic association studies are limited in identifying genomic variation associated with a distinct disease phenotype, and thus, it remains likely that a large number of host-cell factors

involved in HIV replication have not been identified. Recently, three independent groups have used a systematic approach to decipher the majority of host-cell factors important during HIV replication [17–19]. siRNA was used to examine the affect of eliminating a protein's expression on HIV replication. These effects were examined for a large number of individual proteins using a siRNA library with a high-throughput screen (Figure 1). These studies identified hundreds of cellular targets that were previously not known to influence HIV replication and whose knockdown did not confer obvious short-term cellular toxicity. Thus, these host-cell factors are prime candidates for future exploration as potential novel targets for HIV drug development.

Such siRNA screens have a number of important implications for future therapeutics relevant to HIV, as well as other microbes. First, they demonstrate the amazing power of the siRNA screening strategy. Although this methodology was applied to describe cellular factors important in HIV replication, the siRNA screen can be used to find host-cell factors important in the replication of other viral and microbial pathogens. These siRNA screens found more than ten times the number of cellular factors compared with those previously identified from all GWAS, candidate gene genetic association studies and *in vitro* studies combined. Second, it is important to note that HIV is dependent on the identified factors, and thus, polymorphisms within these cellular genes may influence the level of plasma virus circulating in different hosts. As plasma virus levels are highly associated with the progression of HIV infection to AIDS and death [20,21], these studies have opened new avenues of research into understanding the diverse spectrum of HIV plasma copy numbers and disease progression observed among different HIV-infected subjects. Most importantly, the newly identified host-cell proteins may represent excellent drug targets to interrupt the HIV replication cycle. In fact, one study reported cellular factors that can be easily targeted with already existing compounds [19]. One potential advantage of targeting host-cell proteins, as opposed to viral genes, is that cellular genes are highly stable, and thus, there is a relatively lower likelihood of developing resistance. Although *in vivo* resistance against CCR5 inhibitors has been documented [22], resistance among cellular targets may arise more slowly and less frequently compared with drugs that inhibit viral gene products. However, one important drawback of targeting cellular proteins, is that it could potentially lead to a higher level of toxicity. The siRNA screens, which knockdown proteins for a brief period of time, cannot provide any indication of the level of toxicity that will potentially accrue from interfering with the normal function of a host-cell protein for a prolonged time. Prior to drug development against some of the identified host-cell factors, other important caveats also need to be considered. By combining all the potential HIV-dependent cellular factors identified in the three screens, it can be estimated that approximately 10% of the known human proteins potentially influence HIV replication. This is a daunting level of viral reliance on host-cell proteins. It is likely that a large number of associations between the identified host-cell factors and HIV replication represent indirect, rather than direct, interactions; these will need to be deciphered in detailed *in vitro* studies. In addition, among the three siRNA screens, there was a limited overlap (less than 10%) among the putative genes that influence HIV replication. The wide divergence among the identified HIV-dependent factors suggests that specific aspects of each strategy, such as the virus, the target cells and the component of the HIV replication cycle assayed, greatly influence the output from the siRNA screens. Finally, it should be noted that none of the three studies used primary target cells, such as peripheral blood mononuclear cells or natural infection conditions. Thus, a majority of the identified host-cell factors may not be relevant for *in vivo* HIV replication but rather be an artifact of the different *in vitro* screening methodologies. In summary, these studies identified host-cell factors that can serve as important future sites for inhibiting HIV replication, but further detailed studies will need to be done prior to selecting and pursuing an appropriate target for drug development.

Genotypic markers for predicting outcome

Another area of HIV clinical therapeutics that will require further clarity over the next decade is defining the optimal time for starting treatment in HIV-infected subjects. Current guidelines for initiating antiretroviral therapy in HIV-positive individuals are primarily based upon the incidence of morbidity and mortality associated with existing CD4 T-cell counts and plasma virus levels [23]. For instance, current guidelines recommend HAART for those with a CD4 T-cell count below $350 \mu\text{l}^{-1}$. These guidelines attempt to balance the morbidity and mortality associated with severe immune deficiency against the potential adverse drug effects from a lifelong antiretroviral regimen. Clearly, it would be beneficial to avoid potential adverse drug events in subjects who have minimal risk of HIV morbidity. By contrast, delaying antiretroviral therapy until CD4 T-cell counts or HIV plasma copies reach a specified level may expose some subjects to a higher level of morbidity and mortality. Two recent, retrospective, observational analyses showed that subjects starting antiretroviral regimens early had lower mortality and morbidity compared with individuals who started therapy later in disease [24,25]. A randomized, prospective clinical trial is ongoing to determine the optimal time for starting treatment more definitively [101].

In the absence of definitive data, rational strategies are needed to inform the decision of when to start antiretroviral drugs. For example, after starting an antiretroviral regimen, CD4 T-cell counts fail to rise appropriately in approximately 15–30% of subjects despite good virologic suppression [26–28]. These patients, termed immunological nonresponders, remain at an elevated risk for HIV-related morbidity and mortality even though they have good virologic suppression with their antiretroviral regimen. Ideally, antiretroviral therapy can be delayed in subjects predicted to have a good immunologic response until there is an unacceptable risk from the immunosuppression. Conversely, regardless of current immunologic status, drugs can be administered early to subjects who harbor a high likelihood of having poor CD4 T-cell recovery. However, currently there are no reliable means to predict potential immunologic response among different HIV-infected individuals.

A study from Ahuja and colleagues provides some tests that could help characterize a subject's potential immunologic response prior to initiating therapy [29]. These investigators examined the association between CD4 T-cell reconstitution after therapy and previously identified genetic markers known to influence HIV plasma levels. Specifically, they surveyed *CCR5* polymorphisms and *CCL3L1* gene copy numbers. The majority of HIV variants require the *CCR5* receptor to enter cells, and the *CCL3L1* gene encodes the major natural ligand for this receptor. Various *CCR5* receptor polymorphisms have been previously demonstrated to affect HIV copy set point [12]. For instance, HIV acquisition and replication is retarded in people with a 32 base-pair deletion in the *CCR5* gene (*CCR5Δ32*); this natural polymorphism creates a premature stop codon leading to a truncated receptor that HIV cannot utilize for cell entry. In addition, previous studies from these investigators have demonstrated that the number of *CCL3L1* gene copies varies among different people, and high copy numbers of the *CCL3L1* gene lead to lower HIV levels in the plasma and lower susceptibility to HIV acquisition [30,31]. In a recent study, these investigators demonstrated that these factors also influence immunological recovery after starting antiretroviral therapy. Subjects were separated into a low, moderate and high genetic risk groups (GRGs) based on the presence of beneficial or deleterious *CCR5* polymorphisms and either a high or low number of *CCL3L1* genes. Thus, low GRG individuals harbored beneficial *CCR5* polymorphisms and a high copy number of *CCL3L1*. However, high GRG subjects possessed both unfavorable factors (deleterious *CCR5* polymorphisms and low number of *CCL3L1* genes), while people with moderate risk had a risky genotype in one of the two categories. The authors found that during the first 2 years after initiating antiretroviral treatment, CD4 T-cell recovery was similar in all three GRGs (~five to seven

cells per month). Importantly however, subjects in the low GRG demonstrated continued CD4 T-cell increases (~0.5 to two cells per month) after the first 2 years. By contrast, moderate and high GRG individuals demonstrated no significant additional CD4 T-cell increases after the initial 2 years of antiretroviral therapy. Interestingly, this lack of continued CD4 T-cell rise was evident even in individuals with complete virologic suppression. In the study, the authors noted that low risk GRG individuals also had lower CD4 T-cell depletion during the antiretroviral-free treatment period, as compared with subjects with a moderate or high GRG, although the level of CD4 T-cell counts at the time of anti-retroviral initiation did not significantly affect the subsequent CD4 T-cell recovery.

This study has a number of important implications for HIV clinical care. First, these results suggest that *CCR5* genotypes and *CCL3L1* gene copy numbers can be used to stratify a subjects' risk of CD4 T-cell depletion over the course of HIV disease. In turn, clinicians might be able to use these genetic markers to personalize the initiation of antiretroviral therapy for each subject as opposed to following standard guidelines (Figure 2). Thus, by ascertaining a subject's GRG, clinicians may be more judicious in their use of antiretroviral therapy, which could both decrease HIV-related morbidity and reduce antiretroviral-associated adverse events. An example of such personalized medicine is already used in clinical HIV practice, where HLA alleles are identified prior to starting abacavir, a nucleoside analog reverse transcriptase inhibitor, to avoid a potentially fatal hypersensitivity reaction [32]. However, prior to the use of these genetic markers to inform prospective clinical decisions, further studies will be needed to independently validate these findings and assess the generalizability of these results. Specifically, future investigations will need to determine whether the results are valid when examined in cohorts with a minority number of subjects of European-American descent. A second important implication from this study is that the *CCR5-CCL3L1* axis has an important influence on CD4 T-cell homeostasis. From this study, it remains unclear whether the genetic factors affect CD4 T-cell production, destruction and/or peripheral distribution. Regardless, owing to the potential importance of the *CCR5-CCL3L1* axis on peripheral CD4 T-cell counts, clinical studies underway are examining a *CCR5* antagonist's ability to augment CD4 T-cell recovery among virologically suppressed subjects with poor immunological recovery [102]. It should be noted that these studies will need to demonstrate that *CCR5* antagonists provide clinical benefit (i.e., reduce mortality and morbidity) and not just raise CD4 counts in immunological non-responders. Other immunotherapeutic compounds, such as IL-2 and IL-7 raise peripheral CD4 T-cell counts in lymphopenic subjects, but recently completed, large, randomized Phase III trials demonstrated that IL-2 administration does not decrease death or morbidity events [33–35].

Novel strategies for 'curing' HIV

Another outstanding question in the field of HIV-1 therapeutics has been whether HIV-1 can be eradicated so that an infected subject does not have to take lifelong antiretroviral therapy. Early studies that intensively measured HIV copies and used mathematical modeling suggested that there were different phases of viral decay, and these phases were potentially related to the lifespan of different infected cells [36]. Demonstration of the existence of long-lived latently infected cells suggested that HIV eradication, if possible, would take decades of effective suppressive therapy [37,38]. One potential possibility that has been preliminarily explored is to intensify treatment to suppress all virus replication even in the latently infected reservoir. However, preliminary results suggest that this strategy does not significantly impact the low level of viremia in patients on a successful antiretroviral regimen [39]. This implies that either drugs cannot reach cells in the latent reservoir or that the low level of virus seen in some subjects with virological suppression is not due to ongoing virus replication. Other strategies were aimed at activating the latently infected cells to produce virus so that the infected cells could then be attacked by the host immune system

and targeted with anti-retroviral drugs. Compounds, such as histone deacetylases, hexamethylbisacetamide, prostratin and IL-7, disrupt latency *in vitro* through a variety of molecular mechanisms, including chromatin remodeling and recruitment of transcriptional activation/elongation factors (reviewed in [40]). However, to date, trials examining these compounds have failed to show any significant clinical or virological benefit (reviewed in [41]).

Two recent studies present different strategies for potentially eradicating the virus and avoiding lifelong antiretroviral therapy. One promising avenue is to eliminate the CCR5 receptor so that HIV cannot initiate infection, owing to its inability to enter a cell. This scenario was recently explored in one successful, virologically suppressed HIV-infected subject who had a bone marrow transplant [42]. This patient developed leukemia for which he underwent bone marrow ablation, and he was subsequently grafted with cells from a donor who harbored a homozygous *CCR5*Δ32 mutation. After successful reconstitution of the immune system with the *CCR5*Δ32 graft, even in the absence of antiretroviral therapy, HIV could not be detected in this subject's blood, bone marrow or GI tract. Interestingly, viral sequence data prior to transplant suggested that this subject harbored viruses that could use a coreceptor other than CCR5, but no viruses have emerged after the engraftment. Although, it is unlikely that the patient's HIV has been eradicated, the virus cannot replicate in the new target cells and, thus, virological suppression has been achieved without using antiretroviral drugs. A number of groups are exploring whether similar strategies can be used to eliminate virus production. One group has developed *CCR5*-directed zinc finger nucleases (ZFNs) [43]. ZFNs are DNA-binding compounds that permanently disrupt the targeted gene. The *CCR5*-directed ZFN deletes a portion of the *CCR5* gene by eliminating intervening DNA between two sequence-specific binding sites and, thus, it causes an irreversible disruption in CCR5 expression (Figure 3). Essentially, the *CCR5*-directed ZFN replicates the *CCR5*Δ32 phenotype. This group treated CD4 T-cells *ex vivo* with the *CCR5*-targeted ZFN and reintroduced them into a mouse model for HIV. They demonstrated that the treated cells had a survival advantage compared with nontreated cells, and treatment leads to preserved T-cell counts and retards HIV replication. Potentially, elimination and replacement of all CCR5-expressing cells would decrease the opportunity for the virus to replicate within the infected host, which would obviate the need for antiretroviral therapy. Similar ideas to eliminate CCR5 expression are being explored using other strategies, such as RNAi and antibodies, although these pathways may not lead to permanent disruption [44,45]. All CCR5 elimination strategies are in early clinical development, but they hold real promise for eliminating the need for lifelong anti-retroviral therapy. However, these strategies have a number of caveats. First, autologous transfusion of *ex vivo* cells treated with *CCR5*-targeting ZFNs is not feasible for the majority of subjects, especially those in developing nations. Second, there is a real concern that elimination of the CCR5 receptor will lead to the emergence of viruses that use other coreceptors, such as CXCR4, which have been associated with faster disease progression [46,47]. Finally, there is some suggestion that the CCR5 receptor serves an, as yet undefined, important immunologic function, since individuals with a *CCR5*Δ32 mutation are at a higher risk for serious infectious consequences from flaviviruses, such as West Nile virus and tick-borne encephalitis virus [48,49]. Thus, eliminating HIV replication by preventing CCR5 expression may produce a host of other infectious complications.

Another potential strategy to eradicate HIV and avoid lifelong antiretroviral therapy has been to target integrated HIV. Once HIV integrates into a host-cell chromosome, it exists as a provirus flanked by long terminal repeat (LTR) sequences. Thus, a quiescent infected cell is essentially invisible to the immune system and antiretroviral drugs, and these cells retain the potential to produce virions upon activation. One promising method to target the latently infected cells is to remove the integrated viral genome. Towards achieving this goal,

researchers have modified a Cre–LoxP system to remove integrated HIV genomes. Cre–LoxP is a recombination system that has been extensively used in mouse genetics, especially to create knockout strains (reviewed in [50]). Cre recombinase removes intervening DNA flanked by loxP sequences [51]. LTR sequences share approximately 50% homology with loxP and, as expected, given this low level of sequence similarity, Cre recombinase cannot remove a HIV genome flanked by LTRs. Directed protein evolution was used to develop a recombinase, termed Tre, that removed sequences flanked by a specific HIV LTR. These investigators demonstrated that Tre could be used to remove integrated sequences from *ex vivo* human cells infected with HIV (Figure 4). This study is the first documentation of the ‘holy grail’ of HIV therapy, targeting the generally believed irreversible integration event of HIV pathogenesis. However, prior to clinical use a large number of hurdles will need to be cleared. For instance, Tre recombinase cannot target the wide variety of HIV LTR sequences present in naturally circulating viruses. Furthermore, the potential adverse effects of a drug with the ability to recombine the genome are immense and will need to be carefully documented prior to clinical use. Finally, it remains unclear whether Tre can both reach and affect latently infected cells.

The two genetic manipulation strategies detailed previously, are not feasible at this time. Successful deletion of *CCR5* sequences and elimination of intervening portions between LTRs will require targeting of diverse cells from various compartments of the body. In addition, the therapies will need to be highly efficient. In other words, ZFNs will need to delete *CCR5* gene sequences from both alleles in a majority of susceptible cells. As *in vivo* cells are often infected with multiple HIVs [52], an effective Tre will require the excision of all integrated HIV sequences. However, it should be noted that gene manipulation therapies for HIV are not completely unrealistic. A recent, randomized Phase II clinical trial demonstrated the feasibility of retroviral transduction of stem cells with anti-HIV ribozyme [53]. Although the virologic results from the study were not necessarily impressive, this study is important since it documented the safety and feasibility of gene transfer therapies in HIV-infected subjects. More randomized gene therapy clinical trials may be pursued as conventional therapeutic strategies against HIV in the future.

Conclusion & future perspective

In this review, exciting results from some recent, basic science studies have been discussed. Clearly, the findings from these publications will not be applied to clinical practice in the next couple of years. However, future work along similar avenues, as detailed in this review, will provide some exciting possibilities for some of the major outstanding issues in HIV patient care. Current antiretroviral therapies are highly effective, but drug resistance is, and will continue to be a big challenge in the future. Developing new antiretroviral therapies that affect novel targets will be an important arm of strategies to combat drug-resistant viruses. When to initiate therapy continues to be one of the central controversies in the care of patients with HIV. Rather than following general guidelines, genetic tests may help personalize the decision of when to initiate antiretroviral therapy to best avoid complications from immunosuppression and adverse drug effects. Finally, in the absence of an effective HIV vaccine or microbicide that prevents acquisition, strategies for controlling HIV without antiretroviral drugs is a grand challenge. Over the next decade, novel gene therapies may provide viable strategies for avoiding lifelong antiretroviral therapy in HIV-1-infected individuals.

Executive summary

Important issues in current HIV clinical care

- The need for new antiretroviral medications that are effective against drug-resistant HIV variants:
 - Silencing RNA screens are being used to identify host-cell factors important during the HIV replication cycle. These cellular factors could be ideal targets for future HIV drugs. As opposed to targeting viral genes, cellular factors are more stable and thus, less likely to engender drug resistance.
- Optimal time to initiate antiretroviral therapy:
 - Starting an antiretroviral regimen while the CD4 count is relatively high is costly and potentially leads to a large number of adverse drug effects from the lifelong therapy. By contrast, beginning treatment when the CD4 count is low leads to an unacceptable risk for opportunistic infections. Assays for specific genetic markers may help discriminate patients who should have antiretroviral therapy started early rather than later in infection.
- Controlling HIV replication without lifelong antiretroviral therapy:
 - Currently, patients must strictly adhere to a lifelong antiretroviral regimen to control HIV replication. Newer strategies aimed at blocking the expression of a functional CCR5 receptor and/or excising the integrated HIV proviral genome may lead to the control of HIV replication in the absence of antiretroviral drugs.

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Figure 1. Identification of host cellular factors important for HIV replication using siRNA
siRNA was used to silence gene expression within a target cell. The effects were individually examined for a large number of different proteins using siRNA libraries in high-throughput screens utilizing separate *in vitro* cultures. These siRNA screens identified a large number of proteins whose lack of expression halted virus infection and replication [17–19].

Figure 2. Flow diagram detailing the potential use of genetic markers in clinical decision making regarding the initiation of antiretroviral therapy.

Figure 3. Halting HIV replication by eliminating the CCR5 receptor

(A) CCR5-directed ZFN (shown as a green oval) bind to specific DNA sequences (shown as smaller ovals of different colors). (B) Binding leads to disruption of the gene and subsequent aberrant transcription, translation and/or protein function. (C) A nonfunctional CCR5 receptor prevents entry of HIV, which requires the CCR5 receptor prior to fusion. ZFN: Zinc finger nucleases.

Figure 4. HIV eradication by eliminating the integrated viral genome

Within an infected cell, such as a latently infected immune cell, the HIV genome (blue rectangle) is flanked by long terminal repeats (black rectangles). Tre recombinase binds to specific sequences within the long terminal repeats. After binding, Tre excises intervening sequences, which renders an infected cell free of proviral DNA.