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HIV-1 Latency and Eradication: Past, Present and Future

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Abstract

It is well established that antiretroviral therapy (ART), while highly effective in controlling HIV replication, cannot eliminate virus from the body. Therefore, the majority of HIV-1-infected individuals remain at risk for developing AIDS due to persistence of infected reservoir cells serving as a source of virus re-emergence. Several reservoirs containing replication competent HIV-1 have been identified, most notably CD4+ T cells. Cells of the myeloid lineage, which are the first line of defense against pathogens and participate in HIV dissemination into sanctuary organs, also serve as cellular reservoirs of HIV-1. In latently infected resting CD4+ T cells, the integrated copies of proviral DNA remain in a dormant state, yet possess the ability to produce replication competent virus after cellular activation. Studies have demonstrated that modification of chromatin structure plays a role in establishing persistence, in part suggesting that latency is, controlled epigenetically. Current efforts to eradicate HIV-1 from this cell population focus primarily on a “shock and kill” approach through cellular reactivation to trigger elimination of virus producing cells by cytolysis or host immune responses. However, studies revealed several limitations to this approach that require more investigation to assess its clinical application. Recent advances in gene editing technology prompted use of this approach for inactivating integrated proviral DNA in the genome of latently infected cells. This technology, which requires a detailed understanding of the viral genetics and robust delivery, may serve as a powerful strategy to eliminate the latent reservoir in the host leading to a sterile cure of AIDS.

Introduction

Approximately thirty-seven million people are living with HIV-1 infection, and two million new infections were reported in 2014 worldwide [1]. With the introduction of anti-retroviral therapy (ART) that is highly effective in suppressing HIV replication in vivo, there has been a significant reduction in morbidity and mortality associated with this infection [2].

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Conflict of Interests

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However, to date, only fifteen million people living with HIV have access to ART [1]. Unfortunately, long-term ART also has significant adverse effects such as drug toxicities, incomplete immune reconstitution, and residual immune activation/inflammation that enhance the risk of selected co-morbidities [3–5]. Recent studies have shown a substantial shift in the subtypes of lymphoma observed in HIV-infected patients treated with ART [6]. Also, long-term treatment with ART of HIV-1 infected patients worldwide is unsustainable. Therefore, there is an urgent need for discovering a cure for HIV-1 infection.

The resurgence of HIV-1 in patients, relatively soon after the discontinuation of ART, suggests the presence of long-lived viral reservoir(s) that are resistant to ART [7]. Persistence of HIV-1 in patients under suppressive ART is due to the latent cellular reservoirs in circulation as well as anatomical sanctuaries such as the gut-associated lymphoid tissue (GALT), central nervous system (CNS) and bone marrow hematopoietic progenitor cells [8–14]. Studies thus far have demonstrated that the most important cellular reservoir of latent HIV-1 provirus is the resting CD4+ T cells. Estimates suggest that the latently infected CD4+ T cell reservoir may take as long as 60 years to decay naturally [15]. Furthermore, the doses of ART effective in suppressing peripheral viremia may not be sufficient to target HIV-1 that crosses the blood-brain barrier (BBB), and, therefore, may not prevent the establishment of latency. Thus, the existence of a CNS viral reservoir is envisioned, wherein, HIV-1 can exist in perivascular macrophages, microglia, and astrocytes [9, 10 16]. In addition, recent studies have shown HIV-1 in alveolar macrophages in patients on ART with undetectable plasma viral loads [17]. Thus, the lung is a potential reservoir for the virus. The establishment and maintenance of HIV-1 latency are a combination of multifactorial mechanisms [see reviews, 18–21]. Therefore, purging the viral reservoir will likely require a combination of pharmacological agents with unique mechanisms of action with no adverse associated toxicity to effectively target all latent virus, both in circulation and at various anatomical sites. In summary, the primary challenge in the field is how to purge and kill the latent reservoirs from resting CD4+ T cells and other sanctuaries and thus effectively eliminate infected cells. At the same time, new approaches can be developed using gene editing strategies to eradicate completely the HIV-1 genome from infected cells in whole animal models and then in the clinical setting. Success in this approach, indeed, requires an understanding of the viral genetic variations and robust delivery of the editing materials to latently infected cells to these circulating cells and anatomical reservoirs.

Host factors involved in HIV-1 latency maintenance

Histone deacetylation is one of the predominant mechanisms involved in repression of HIV-1 transcription in the maintenance of HIV-1 latency. Elucidation of the mechanisms involved in histone deacetylases (HDACs) mediated repression of HIV-1 latency demonstrates recruitment of HDAC1, 2 and 3 by cellular transcription factors. Binding of cellular factors such as, c-promoter-binding factor (CBF-1), NF-κB p50 homodimer, Ying-Yang 1 (YY1), late SV40 factor (LSF), COUP-TF-interacting protein (CTIP2), c-myc and Sp1 are among some of the potent transcription factors that recruit HDAC1 to the HIV-1 promoter [22–27]. CTIP2 and Sp1 have also been shown to promote recruitment of HDAC2 [23]. In addition, RBF-2 (USF1/2-TFII-I) promotes HDAC3 binding to HIV-1 LTR [28].

Reversible histone methylation also contributes to the establishment of HIV-1 latency in CD4+ T cells and cells of myeloid lineage. Studies in several cell models and peripheral blood mononuclear cells (PBMC) isolated from HIV-1-infected patients have shown the involvement of histone lysine methyltransferases (HKMT) Suv39H1 and HP1 gamma in tri-methylation of histone H3 lysine (H3K9me3) in silencing of HIV-1 [29]. A similar phenomenon is also seen in HIV-1 infected microglia cells that result in the formation of heterochromatin, and ultimately HIV-1 silencing [30]. G9a, a histone methyltransferase (HMT), has been shown to promote HIV-1 latency in ACH2 and OM-10.1 cells by governing H3K9me2 and formation of repressive chromatin [31]. Earlier studies have demonstrated that HKMT enhancer of zeste homolog 2 (EZH2) is also recruited at the silenced 5'LTR in latently infected Jurkat T-cell lines to increase local H3K9me3 level that is rapidly displaced following proviral reactivation [32].

Current strategies for elimination of latent reservoir "Shock and Kill"

The major barrier to elimination of latent reservoirs of HIV-1 is multifactorial. Latently infected cells are long-lived, immunologically invisible, may undergo homeostatic proliferation, and are refractory to combinatorial antiretroviral therapy (cART). Current pharmacological efforts are designed towards a 'shock and kill' [33] approach to eradicate viral reservoirs (Fig. 1). The strategy, in a simplistic view, is to reactivate viral transcription with various latency-reversing agents (LRA) that will result in the death of the productively infected cells by the virus itself, or by the host immune system, and circumvention of new infection by cART. There are several LRA that have been extensively investigated, most notably HDAC inhibitors. In vitro and ex vivo studies have shown that there is significant variability in the potency of HDAC inhibitors (HDACis) in activation of latent reservoirs. Furthermore, there are limited studies on the efficacy of these HDACis in purging the latent HIV-1 reservoir in individuals on cART. Here, we highlight the findings observed with HDAC that belong to four major structural families.

A. Short-chain aliphatic acids, e.g. Valproic acid (VPA). VPA is an FDA-approved anti-epileptic agent that inhibits class I and II HDACs [34]. It reactivates latent HIV in cell line models of latency that include U1 and J-LAT [35]. VPA also reactivates latent HIV in primary CD4+ T-cell model (ex vivo) of HIV [35]. In a proof-of-concept study, VPA was effective in reducing the frequency of latent infection in three out of four patients on ART [36]. However, VPA failed in reducing latent reservoir size in subsequent clinical trials in HIV-1 infected individuals [37, 38].

B. Hydroxamic acids, e.g. Trichostatin A (TSA), Suberoylanilide hydroxamic acid (SAHA), and Panobinostat (LBH-589). TSA has been shown to reactivate latent HIV-1 in cell line models U1, ACH2, J49 and OM 10.1, and in CD4 T+ cells isolated from patients on ART [39, 40]. SAHA, an FDA approved HDACi named Vorinostat as been shown to be effective in HIV-1 reactivation in J89, ACH-2, U1 and J-LAT [37, 41, 42] and reactivation of latent HIV-1 in primary CD4+ T cell and CD4 T+ cells isolated from patients on ART [39, 42]. Panobinostat has been shown to induce HIV-1 expression in latently infected cell lines (ACH2 and U1),

and in a latent primary CD4+ T-cell infection model with greater potency than other HDAC inhibitors, including givinostat, belinostat, SAHA, and VPA. [39]. Subsequently, in a recent clinical trial Panobinostat was shown to effectively disrupted HIV latency in vivo [43].

C. Benzamides, e.g. Entinostat. Entinostat is effective in inducing HIV expression in the latently infected cell lines ACH2 and J-Lat as well as in latently infected chemokine ligand 19 (CCL19)-treated primary CD4+T cells. [44].

D. Cyclic tetrapeptides and depsipeptide, e.g. romidepsin. Romidepsin (RMD) has been shown to be a potent inducer of HIV-1 in an in vitro T-cell model of HIV latency compared with vorinostat and other HDACis in clinical development including panobinostat [45]. Also, RMD induced HIV-1 in both resting and memory CD4+ T cells isolated from HIV-infected patients on suppressive cART [46]. In a recent proof-of-concept phase Ib/IIa trial, RMD significantly reversed HIV-1 latency in vivo without blunting T cell-mediated immune responses [47].

In addition to the HDAC inhibitors, several other agents have been shown to have the ability to reactivate the HIV-1 genome. For example, histone methyltransferase (HKMT) inhibitors such as the Suv39H1 inhibitor chaetocin [48–50], G9a inhibitor BIX01294 [31], and EZH2 inhibitor DZNep [32] have been effective in stimulating latent HIV-1 expression and replication in cell line models or primary CD4+ T-cell model. Another potential reactivation mechanism is through induction of NF-κB, which binds to specific sites on integrated HIV-1 LTR [51] in latently infected cells and thereby promotes transcription. Prostratin, a PKC agonist, is effective in activating latent virus in patient-derived CD4+ T cells (ex vivo), and J-Lat cell lines by inducing NFkB [52, 53]. Similarly, Bryostatin-2, a PKC agonist also reactivates latency in cell line models and CD4+T-cells [54–57]. Indeed, one can predict the universal impact of these LRAs on cellular genes and their toxicity to HIV-1 negative cells. More recently, to achieve HIV-1 targeted specificity for reactivating latent HIV-1, several gene-editing strategies have been modified, by recruiting target specific transcriptional activators such as VP64 [58, 59]

HIV-1 latency and eradication in humanized mouse models

Studies have shown that HIV-1-infected BLT, hu-Rag2−/−γ(c)−/− and NSG mice can develop latent infection as a consequence of virus integration, and is inducible and replication competent [60–66]. These mice models have been used to investigate HIV latency and persistence during therapy, and in the evaluation of pharmacologic agents intended to eliminate the latent reservoir of HIV. Studies have shown that prostatin, a non-tumor-promoting phorbol ester efficiently reactivated HIV expression from latently infected cells generated in the SCID-hu mouse [67]. Using the SCID-hu model, it has been shown that IL-7 induces substantial expression of latent HIV while having minimal effects on the cell phenotype [68]. In a recent study using HIV-1 infected NSG humanized mice with undetected basal levels of viral replication, a dramatic increase in HIV-1 RNA in plasma, lung and brain tissues were observed following ionizing radiation-induced cellular stress [69].

Limitations of LRA and strategies to improve efficacy

SAHA has been used to purge HIV-1 latency both *in vitro* and *in vivo*. However, SAHA was shown to increase significantly the susceptibility of CD4+ T cells to infection by HIV-1 in a dose- and time-dependent manner [70]. This was found to be dependent on the efficiency of post entry viral events [70]. A recent study using transcriptomic and proteomic profiling has shown that SAHA modulates the expression of a number of genes and proteins involved in HIV-1 transcriptional regulation, however, some of these genes and proteins appear to be inhibitory with respect to HIV reactivation [71]. In another independent study, SAHA modulated, in a dose-responsive manner, a number of genes in CD4 T cells that could negatively influence HIV reactivation from latency [72].

Among the PKC activators, prostratin and bryostatin are not suitable for use *in vivo* due to limited availability and major side effects. However, SAHA has been used as a synergistic agent *in vitro* with prostratin and bryostatin [43, 73]. Therefore, it is essential to identify the counter-regulatory effects of other potential LRAs such as Romidepsin [46] and Panobinostat [42] that have better potencies than SAHA in induction of HIV-1 before advancement into clinical trials specifically for reactivation of latent HIV-1 in CNS and GALT where the availability of therapeutic levels of drugs is a major concern for successful eradication of the latent reservoir.

HIV-1 latency and Cytotoxic T lymphocyte (CTL)

Cytotoxic T lymphocyte (CTL) response contributes to the control of HIV-1 infection in vivo [74, 75]. However, it is unknown whether virus-specific immune mechanisms, including CTLs, can eliminate infected cells in ART-treated patients after reactivation of HIV-1 in latently infected cells. A recent study demonstrated that there is a predominance of CTL-resistant viruses in the latent reservoir that poses a significant challenge in the ‘shock and kill’ approach for viral eradication [76]. A recent study also showed that treatment with HDACis, such as romidepsin and panobinostat, to reactivate the latent reservoir had an adverse impact on CTL-mediated IFN-γ production, and elimination of HIV-infected or peptide-pulsed target cells [77]. More recently, a new strategy that activates and lyses latently infected CD4+ T-cells with HIV-1 has been developed by two independent research teams [78, 79]. This strategy includes the development of a novel bi-specific immunomodulatory protein that combines the broad recognition of HIV-1 Env with binding to the T-cell activation glycoprotein, CD3. Known as Dual-Affinity- Re- Targeting (DARTs) molecules, this therapy seems to be effective at targeting latent and activated cells for killing in *ex vivo* experiments [79, 80].

Therapeutic approaches for efficient delivery of LRA and ART to anatomical sanctuaries

One of the major hurdles that limit the efficacy of LRAs and ART in the “shock and kill” approach is the penetration and availability of these drugs at therapeutic levels in viral sanctuaries of latency. To circumvent these limitations, micro- to nanoformulated ART referred to as “NanoArt” has been developed [81, 82]. The strategies involved the use of macrophage-based nanoparticle for ART delivery [83, 84], magnetic nanoparticles [85], and folic acid receptor-based nanoparticle [86]. A recent study has shown that a polymer-based pluronic nanocarrier containing anti-HIV drug called efavirenz can efficiently target

microfold cells of GALT and inhibit HIV-1 infection [87]. Furthermore, it was also shown that SAHA could be packaged into nanoparticles in conjunction with an antiretroviral drug, tenofovir. The therapeutic potential of this type of nanoparticle has been shown to reactivate and kill HIV-1 across the BBB [82].

Role of genetic variation and quasispecies

While initial infection with HIV has been described as occurring from a small number of transmitter or founder viral strains, genetic diversity of the viral genome occurs rapidly as a consequence of continued replication in multiple cell types with an error prone polymerase (reverse transcriptase), producing numerous and distinct viral quasispecies. These quasispecies accumulate within the patient as the disease progresses, and this accumulation is thought to continue at low-levels even in the presence of suppressive ART. Continued variation has been confirmed in the Drexel Medicine CARES cohort by longitudinal sampling of the LTR (Fig. 2) and by others [88].

Furthermore, the variations within the HIV genome can vary substantially from one tissue or cell type to another, thereby creating compartments. Compartmentalization of HIV has been well established in the literature with compartments described in the brain, cerebrospinal fluid, gut (especially gut associated lymphoid tissue or GALT), lung, liver, and genital tract. The mechanism(s) behind compartmentalization have not been fully elucidated, however, differential pressure from the immune system, cell type differences within these different compartments, accessibility of the compartment to ART, as well as co-infections that may preferentially affect particular compartments may all play a role [89].

Early in HIV infection, the brain is seeded with virus due to increased permeability of the BBB because of a number of factors including increased release of proinflammatory cytokines, exogenous viral proteins including Tat and gp120, infection of perivascular macrophages and microglial cells, and possible infection of brain microvascular endothelial cells and astrocytes. The brain as a viral reservoir with distinct viral genetics is an area that has been heavily researched especially with relation to neurocognitive impairment [reviewed in 90]. Studies have shown that both the brain and the CSF contain distinctly different viral quasispecies as compared to peripheral blood and are therefore distinct compartments [91–97]. Not only were brain sequences found to be distinctly different from those found in the peripheral blood, but also different from those found in other compartments, such as lymphoid tissue [91, 98–100]. In fact, there is even evidence for regional compartmentalization of the virus within the brain of infected individuals based on variable viral replication as measured by viral loads as well as looking at the distinct viral variants [101–104]. Even though it has been shown that the brain contains replication competent, integrated virus and autonomous replication can occur [105], it is unclear what role the brain plays in systemic viral reseeding, although studies utilizing viral sequencing have shown that the meninges harbors virus from both the brain and peripheral tissues suggesting that HIV is capable of migrating out of the brain [78, 90].

In addition to the brain, CSF, and lymphoid tissue discussed above, the GALT, lung [106], genital tract [107–109], and liver [110] have also been shown to act as compartments that differ from what is seen in the peripheral blood. The role that each of these compartments

plays in disease course, as well as potential reseeding of the periphery, is variable and results can vary between studies. Gut, for example, has been shown to be an important reservoir and compartment in HIV infection. Initial studies showed that the GALT contained distinct quasispecies in different parts of the gut that differed from what was seen in the peripheral blood [13]. Additional studies also suggested that these reservoirs were maintained even in the presence of long-term ART [111], and the levels of T cell restoration and activation differed between different parts of the gut [112]. However, other studies have suggested that if ART is initiated during primary infection there is an absence of HIV-1 evolution [113]. Although it has been suggested that there is active viral replication to a low level in the presence of active, suppressive ART [114], each of these sites requires further study to determine what role exactly these sites play in HIV compartmentalization and reseeding of the periphery as the disease progresses.

The peripheral blood can also be viewed as its own compartment, and is the most widely studied compartment. Within the peripheral blood, resting memory CD4+ T cells constitute the most abundant latently infected cells [115–117]. The abundance of this reservoir as well as the long half-life of these cells is a major barrier to eradication [118, 119]. It has been assumed that the size of this reservoir can be directly assessed by the ability to reactive the resting cells to again produce infectious virus. However, cellular based assays to assess the size of this reservoir indicate the frequency to be 300-fold lower than the frequency of resting CD4+ T cells that harbor proviruses as detectable by PCR [120]. It has been assumed that the non-induced virus is defective, however, it is unclear if it is truly defective or if it is in fact replication competent [121] and simply not inducible through the systems used. Further molecular characterization of these integrated proviruses is necessary to fully understand the nature of this reservoir to be able to effectively develop an eradication strategy. This has implications for infected cells in both the peripheral blood compartment as well as cells in other anatomical sanctuaries.

While a number of studies have been completed looking at the differences and similarities between different potential anatomical sanctuaries or compartments and the peripheral blood, little work has been completed looking at the differences in genetic variability/quasispecies production, susceptibility to long-term ART, ability to reseed the periphery all within the context of the same model (Fig. 2). It is important to understand the potential variability between not only individual sites and the blood, but also between the sites themselves. Each site represents a different compartment and understanding the differences and similarities in the quasispecies, the effects of ART on these quasispecies, as well as the activity of these species is important in the context of developing a sterilizing or functional cure. To date, a sterilizing cure has only been realized in one patient coined the “Berlin” patient, wherein the patient received an allogeneic hematopoietic stem cell transplant in 2007 and to date all tests indicate that he is HIV-free in the absence of ART [122, 123]. This can be compared to the two “Boston” patients who also received allogeneic hematopoietic stem cell transplants. In these patients, they maintained undetectable viral loads in the presence of ART for 4.3 years and 2.6 years, however when ART was removed both patients experienced viral rebounds at 12 weeks and 32 weeks post therapy removal [124–126]. This suggests that long-lived reservoirs remained in both patients and in the absence of therapy began to reseed the patient. However from the viewpoint of the virus, genetics studies have

not been reported that have made any comments as to where the virus may have come from. While it may be very difficult to perform these types of studies in a human, this could be accomplished in the SIV system that was recently published [114] to obtain clues to what reservoirs may reseed the peripheral blood. Therefore, it is important to understand these reservoirs so that an appropriate sterilizing strategy can target these compartments.

Gene editing strategies for eliminating HIV-1

Similar to other retroviruses, after infection, HIV-1 proviral DNA permanently integrates into the genome of its target cells and presents a strong challenge for curing AIDS. Currently, combination ART is effective at reducing viral loads but it doesn't reduce the source of the infection, which is the stable, integrated provirus in the cellular genome. While the shock and kill strategies discussed above can reactivate and cause eradication of a portion of this virus, largely the inducible, replication competent virus, it has been proven over many studies that it can not reactivate all proviral DNA, including hypermutated and defective deleted forms which are two among many that exists in the latent reservoir, and this in turn is potentially its Achilles heal. In recent years, several novel systems for targeting endogenous genes have been developed including homing endonucleases (HE) or meganucleases, zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN) and most recently clustered regularly interspaced short palindromic repeats (CRISPR)-associated system 9 (Cas9) proteins [127–132], which utilize site-specific, double-strand DNA break (DSB)-mediated DNA repair mechanisms. These DSB-mediated genome editing techniques have enabled genetic studies including targeted gene deletion, insertion, or modification that were previously difficult to perform, and are currently being explored as novel therapeutic approaches. While all three are still being developed, the ZFN and TALEN approaches have limitations with respect to complicated design and size of the molecules necessary to carry out the editing. However, the CRISPR/Cas9 technology, in particular, has captured the attention of many investigators due to its simplicity, precision, and versatility. In addition, the CRISPR/cas9 technology is the only one of the three that has been shown to successfully excise the entire HIV-1 genome from cells. We recently modified this system to enable recognition of specific HIV-1 LTR sequences while excluding sequences that might trigger host off-target effects (Fig. 3). Using this modified system, for the first time, we were able to demonstrate complete excision of a 9.7 kb DNA fragment corresponding to HIV-1 proviral DNA incorporated in various chromosomes of the latently infected cells with no off-target effects [133, 134]. Furthermore, these studies demonstrated that CRISPR/Cas9 could protect uninfected cells against HIV-1 infection, suggesting that this technology can be refined to provide a specific and efficacious prophylactic and therapeutic approach against HIV/AIDS. Several other studies describe utilization of CRISPR and ZFN to introduce InDels and large deletions of the HIV-1 provirus from the host genome [132, 135–137]. TALEN, ZFN and Cas9 have also been used experimentally for efficient disruption of cellular genes that are important for HIV-1 infection including CCR5 and CXCR4 [138–144]. The gene editing strategy for eliminating HIV-1 co-receptors has also entered clinical phase. By using ZFN strategy for targeting CCR5 [141, 145, 146], it was shown that in autologous CD4+ T cells from a small cohort of patients, the viral load remained undetectable at the time of treatment [147]. Given the ease and rapidity of Cas9/gRNA development, it is expected that CRISPR/Cas9 will soon enter clinical trials for the

treatment of AIDS. However, there are several important issues that deserve close attention. For example, the CRISPR/Cas9 gene editing strategy utilizes multiplexes of gRNAs that can be designed to be broadly recognized by all HIV-1 isolates that have been characterized to date. However, due to the presence of HIV-1 quasispecies, it is important to determine the most conserved regions of the LTR quasispecies and use this information to develop a personalized therapeutic strategy that effectively eliminates HIV-1 DNA from the patient's genome. While CD4+ T cells are recognized as a site of latency and serve as a critical reservoir for virus during ART, there are several studies that have ascribed a role for other cell types, including macrophages, astrocytes, brain-derived microglia, as well as lymph nodes of gastric origin, in hosting HIV-1 in a latent state and may therefore serve as additional reservoirs. Another important issue related to the delivery of the gene editing apparatus that is comprised of Cas9 endonuclease and the gRNAs. This can be accomplished by gene therapy approach utilizing a suitable and efficient vector that robustly delivers the genes expressing both Cas9 and gRNAs. In this respect, several viral vectors including lentivirus, adenovirus, and adeno-associated virus have been employed for delivery of Cas9 and gRNAs to various human cell lines [148–150]. However, its employment in the clinic with respect to efficiency, specificity and safety remains to be seen. On the other hand, a broad range of non-viral vehicles including nanomolecules, lipid-based or polymer-based such as polyethylamine and polylysine, which have the ability to carry its cargo at the protein, RNA and DNA levels [for review see 151]. Further, by decorating the surface of the nanoparticles with specific targeting ligands, targeted nanoparticles can be developed for cell type specific delivery of the payload. These issues are of critical importance to precisely target all of the HIV-1 viral quasispecies within an individual.

Future considerations

Figure 4 highlights several notable discoveries and events that have occurred since the first cases of AIDS appeared in 1981. After 34 years we have reached the point of being able to identify, characterize and manipulate the integrated proviral DNA in the latently infected cells in the laboratory setting. Clearly, gene editing strategy with its ability to completely eliminate the HIV-1 genome from the latently infected human CD4+ T cells as well as the other cell types harboring HIV-1 proviral DNA has brought new hope that AIDS may be a curable disease. However, there are several challenges that need to be met to assess its efficacy in eliminating HIV-1 prior to implementation in the clinic. While at this stage, it is difficult to predict the extent of gene editing delivery and high efficiency of the viral DNA excision by CRISPR/Cas9 in an in vivo system, one may speculate that under ART protection of a fraction of the cells from HIV-1 infection and death could have dramatic functional consequences in the infected subject, once the ART is interrupted after a certain period of time post repeated application of gene editing molecules. Under these circumstances, it is possible that the protected uninfected cells would be free to become activated, promote CD8+ T-cell and B-cell responses, yet remain immune to virus-induced cellular death and/or dysregulation. In such a scenario, even an incomplete transduction could result in complete eradication assuming the promotion of a significant immunologic effect. Such an effect would be analogous to the Berlin patient's donor derived cells (CCR5Δ32) being able to clear infected cells within the transplant recipient. Thus, one may envision that eliminating HIV-1 DNA and diminishing the toxicity associated with the

presence of the viral genome and expression of the viral proteins, even at very low levels, by gene editing strategy can empower the fragile immune system and collaboratively rid the virus from the remaining infected cells in the patient.

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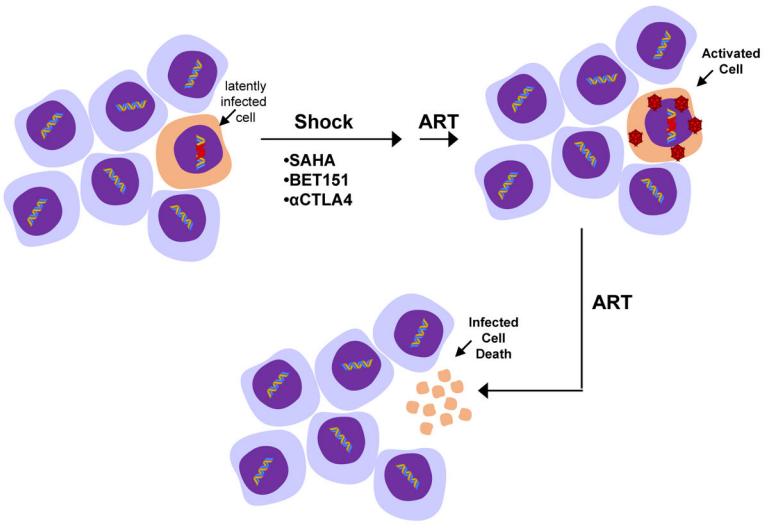

Figure 1.

Shock and kill approach aiming to reactivate latently infected cells by HDAC inhibition including suberoylanilide hydroxamic acid (SAHA), the BET bromodomain protein inhibitor (BET151), and anti-CTLA4 antibody and induce subsequent cell death due to viral toxicity and/or host immune defense while HIV-1 replication is inhibited by ART.

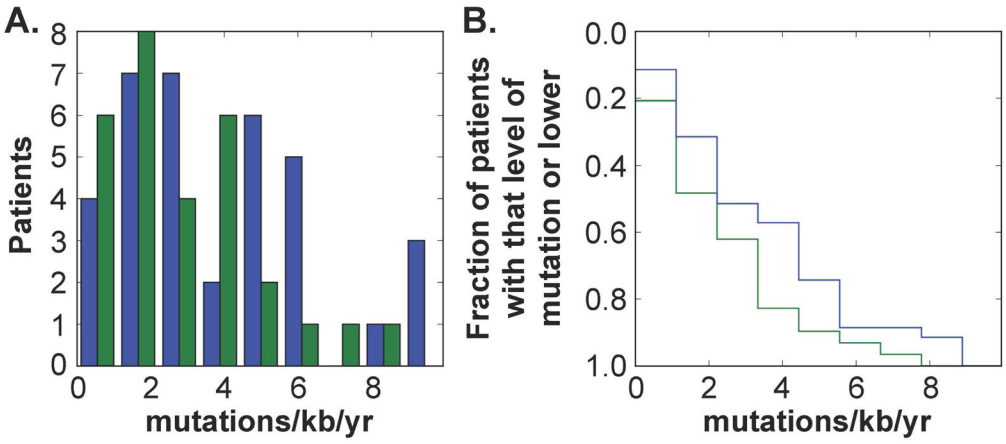

Figure 2.

ART therapy reduces mutation rate of the LTR by an average of 1.1 mutations per Kb per year. A) A histogram of the average mutation rate per patient while naive to therapy (blue) and after virologic control on cART (green). B) A cumulative normalized histogram of the same data in A) showing the fraction of patients with a mutation rate. Fourty two non-drug using patients that currently have undetectable viral load and at least 5 years in the study were selected. from the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort. The LTR region was PCR amplified from genomic DNA isolated from PBMCs and phylogenetic trees were constructed to estimate the time varying mutation rate of the virus.

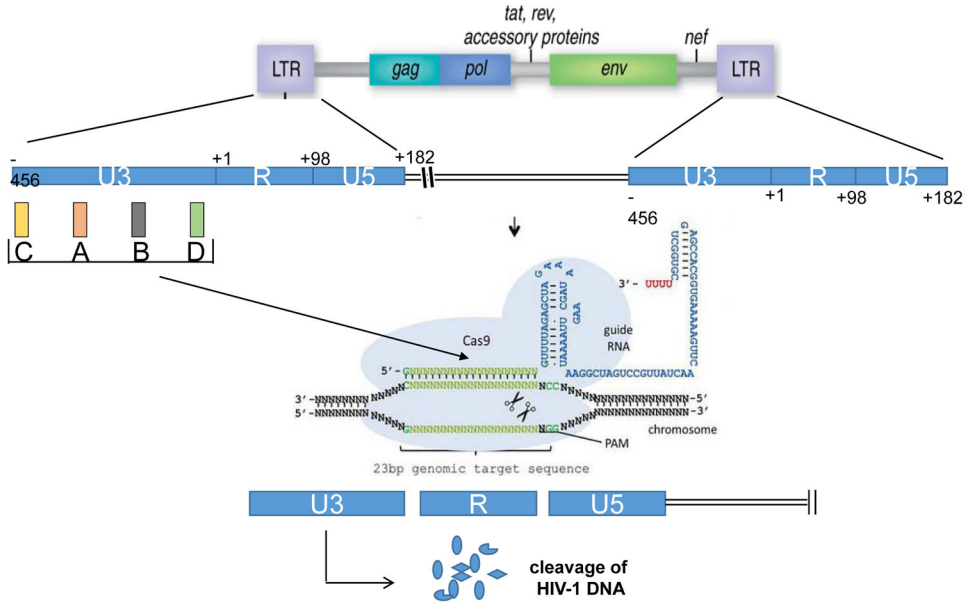

Figure 3.

Strategy for CRISPR/Cas9 mediated cleavage of the HIV-1 genome. Guide RNAs (gRNAs) targeting the U3 region of the LTR can recruit Cas9 to the viral DNA sequence integrated in the host chromosome and results in cleavage of the viral DNA at the designated sites and introduces InDel mutations. Cleavage at both the 5′ and 3′ LTRs can lead to removal of the complete coding region of HIV-1 from the host genome and lead to eradication of HIV-1 from the host [133, 134].

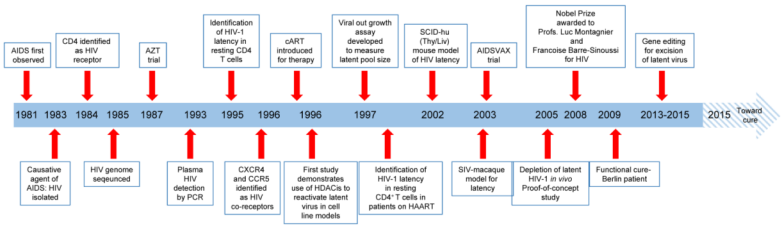

Figure 4.
HIV-1, thirty years of latency: from discovery to excision.