SYSTEMATIC REVIEW ARTICLE

A Review of Current Strategies Towards the Elimination of Latent HIV-1 and Subsequent HIV-1 Cure

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Abstract: **Background**: During the past 35 years, highly effective ART has saved the lives of millions of people worldwide by suppressing viruses to undetectable levels. However, this does not translate to the absence of viruses in the body as HIV persists in latent reservoirs. Indeed, rebounded HIV has been recently observed in the Mississippi and California infants previously thought to have been cured. Hence, much remains to be learned about HIV latency, and the search for the best strategy to eliminate the reservoir is the direction current research is taking. A systems-level approach that fully recapitulates the dynamics and complexity of HIV-1 latency *In vivo* and is applicable in human therapy is prudent for HIV eradication to be more feasible.

Objectives: The main barriers preventing the cure of HIV with antiretroviral therapy have been identified, progress has been made in the understanding of the therapeutic targets to which potentially eradicating drugs could be directed, integrative strategies have been proposed, and clinical trials with various alternatives are underway. The aim of this review is to provide an update on the main advances in HIV eradication, with particular emphasis on the obstacles and the different strategies proposed. The core challenges of each strategy are highlighted and the most promising strategy and new research avenues in HIV eradication strategies are proposed.

Methods: A systematic literature search of all English-language articles published between 2015 and 2019, was conducted using MEDLINE (PubMed) and Google scholar. Where available, medical subject headings (MeSH) were used as search terms and included: *HIV, HIV latency, HIV reservoir, latency reactivation, and HIV cure*. Additional search terms consisted of *suppression, persistence, establishment, generation, and formation*. A total of 250 articles were found using the above search terms. Out of these, 89 relevant articles related to HIV-1 latency establishment and eradication strategies were collected and reviewed, with no limitation of study design. Additional studies (commonly referenced and/or older and more recent articles of significance) were selected from bibliographies and references listed in the primary resources.

Results: In general, when exploring the literature, there are four main strategies heavily researched that provide promising strategies to the elimination of latent HIV: Haematopoietic Stem-Cell Transplantation, Shock and Kill Strategy, Gene-specific transcriptional activation using RNA-guided CRISPR-Cas9 system, and Block and Lock strategy. Most of the studies of these strategies are applicable *in vitro*, leaving many questions about the extent to which, or if any, these strategies are applicable to complex picture *In vivo*. However, the success of these strategies at least shows, in part, that HIV-1 can be cured, though some strategies are too invasive and expensive to become a standard of care for all HIV-infected patients.

Conclusion: Recent advances hold promise for the ultimate cure of HIV infection. A systems-level approach that fully recapitulates the dynamics and complexity of HIV-1 latency *In vivo* and applicable in human therapy is prudent for HIV eradication to be more feasible. Future studies aimed at achieving a prolonged HIV remission state are more likely to be successful if they focus on a combination strategy, including the block and kill, and stem cell approaches. These strategies propose a functional cure with minimal toxicity for patients. It is believed that the cure of HIV infection will be attained in the short term if a strategy based on purging the reservoirs is complemented with an aggressive HAART strategy.

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1. INTRODUCTION

The major hurdle towards a cure for HIV are stable cellular reservoirs established by the virus upon infection, which remain inactive, undetectable, and inaccessible to antiretroviral medication [1]. Latent reservoirs are established early, during initial infection stages, before the virus appears in circulation where it infects CD4+ cells [2]. Other immune cell types, such as macrophages, dendritic cells, langerhans cells, B cells, and granulocytes [3], found in the circulating blood are also infected and act as reservoirs [4]. These latently infected cells, harboring replication of competent proviral DNA that can produce infectious viruses upon stimulation, necessitate strict therapeutic adherence for a lifetime in HIV patients [5]. Even though the prescription of combination Antiretroviral therapy (cART) has improved the life expectancy of HIV patients, the long-term usage of these drugs has been associated with neurotoxicity [6], along with accelerated neural aging where there is a deterioration of connectivity, processing, and association areas in the brain [7]. Furthermore, some patients have presented with liver injury after continued usage [8] while others presented with renal tubular impairment [9]. Despite the toxicity of the medication, it must be utilized for a lifetime to lessen the effects of the virus in the body, hence, there is an urgent need for a functional cure.

Recently, HIV research has focused on strategies to eliminate latent HIV. This is important if the UNAID objective of eliminating HIV in the globe by 2030 is to be realized. Though coupled with a myriad of challenges, three recent isolated instances of a cure for HIV infection have fueled the notion that a cure for HIV is possible; the "Berlin patient" [10], the French VISCONTI cohort [11], and the Mississippi and California babies [12]. These developments in HIV eradication strategies provide renewed hope in advancing efforts toward a functional cure. On the horizon is research concentrated in multiple separate but potentially complementary domains including, viral transcript editing, gene editing, shock and kill, block and lock, stem cell transplant, and gene-specific transcriptional activation. The most studied strategy, "shock and kill" involves reactivation of latent proviruses with a variation of Latent Reversing Agents (LRAs), followed by the elimination of the cells that express the viral proteins through cytopathic effects or immune-mediated processes. [13] It describes the dominant model currently used in the search for a cure for HIV-1 infection. Gene-specific transcriptional activation uses RNA-guided CRISPR-Cas9 system [14]. The "block and lock" or "deep latency" creates a permanent latency which aims to create a permanent nonproductive state of infection and suppress viral reactivation through transcriptional and post-transcriptional gene silencing of the virus [15]. Bone marrow transplantation replaces blood cells with CCR5 mutated stem cells from the donor [16]. These strategies that help develop a safe and effective cure for HIV are varied and wide in range due to the diversity in the tissues and cells that make up different pools of reservoirs and the abundance of the molecular mechanisms that contribute to the persistence of HIV [4]. Here, these current strategies are reviewed for HIV eradication, promises, challenges, and to describe relevant new work towards HIV elimination.

2. MATERIALS AND METHODS

A systematic literature search of all English language articles published in online journals from 2015 to 2019 was performed using Google Scholar and PubMed. The search was carried out using keywords *HIV, HIV latency, HIV reservoir, latency reactivation, and HIV cure.* Additional search terms consisted of *suppression, persistence, establishment, generation, and formation.* All the relevant articles related to HIV-1 latency establishment, elimination, and reversal were collected and reviewed, with no limitation of study design. A total of 250 articles were collected and reviewed, covering different cure strategies. In addition to the papers identified in the primary search, reference lists of included articles were analyzed for additional references related to the topic. Articles, which did not fit the objectives of the study, as well as those that did not fit into the time frame of the study, were excluded from the review. Finally, the study utilized information from 89 primary articles, which contained data from clinical and laboratory research work, and 38 secondary articles.

3. RESULTS

3.1. General Overview of The Strategies and Their Importance

Out of the 89 articles reviewed, 71% were based on the shock and kill strategy Table (**1**). Data from these articles show that the shock and kill strategy is potentially scalable and cost-effective to administer to individuals. The shock and kill strategy uses LRAs to increase HIV transcription, protein expression, and virion production [13, 17-19].

Though promising, there is a need for clinical trials to understand the potential synergy, antagonism or toxicity of all the classes of LRAs and the effects of these molecules on non-infected cells. The molecular target compounds that have been studied for reversal of latent reservoir have multiple and varied genetic implications resulting in diverse patient responses. Generally, this strategy seems to be a nonexclusive combination of therapies tailor made for each patient. For example, a potent latent reversing agent can be used in combination with an apoptosis promoter and an immune booster to bring about change in the viral rebound rate.

Ten percent (10%) of articles covered the Transplantation approach in HIV patients and showed that remission is possible but only if all the variables align. Transplantation approach uses bone marrow transplant to repopulate the hematopoietic system with HIV-resistant cells [20-22]. This strategy is not scalable because it is rare to find a matching bone marrow donor who also exhibits CCR5Δ32/Δ32 mutation, while the risk of allograft rejection is always eminent. Gene editing discussed in the articles reviewed indicates that it is not yet ready to be applied on a large scale because of the potential off-target effects, which may induce important gene mutations and chromosomal translocations that can have deleterious effects.

At 8.5%, block and lock strategy was the least researched. This strategy is aimed at permanently suppressing the virus after discontinuation of cART [2, 20, 23]. The achievements of this strategy indicate that it's possible to permanently silence the virus. Time will tell whether lock and block strategy can help us achieve the ultimate goal of eliminating HIV by the year 2030.

3.2. The Strategies

3.2.1. The Shock and Kill Strategy

Shock and Kill is a therapeutic approach in which drugs are used to activate dormant infected cells with the aim of reducing the size of latent reservoirs of the virus [13]. Activation of viral transcription, viral protein production, and release of HIV particles using Latency reversing agents (LRAs), subsequently triggering cytolysis or elimination by ARVs is the approach of this strategy [24]. Depending on their mode of action, these compounds can target different stages of gene expression. They can act as chromatin modulators, or transcription activators, or transcriptional elongation controllers or be involved in post transcription control.

3.2.1.1. Chromatin Modulation

Chromatin modulation is important in the regulation of transcription due to its' effect on nucleosome stability and the subsequent access to DNA, which is important in latency reversal [25]. Example of compounds in this category are shown in Table (**1**). These compounds have been shown to induce HIV-1 expression in latently infected T cells, monocytes, and in resting CD4+ T cells isolated from HIV-1-infected patients [4, 26]. They target histone deacetylases and acetyltransferases, enzymes involved in the regulation of DNA expression [27]. Several of these agents, including Vorinostat [28, 29], Panobinostat [17, 29, 30], and Romidepsin [1, 31, 32] have been studied in clinical trials Table (**1**). Vorinostat activates latent HIV-1 in patients' cells ex vivo as well as reactivate latent HIV-1 *In vivo* in patients [28, 33]. Furthermore, Panobinostat has been shown to reactivate latent HIV in patients *in vivo* [18]. Despite the activation of latent virus by Panobinostat, no reduction in the size of the latent HIV reservoir has been observed in patients

[18, 32]. Romidepsin, a potent, bicyclic class 1 inhibitor, has been shown to induce viral expression in proviruses with highly similar or identical genetic backgrounds [31] and also, when combined with therapeutic HIV immunization, showed reduced HIV DNA expression [34]. The anti-alcoholism drug, disulfiram, activates the Akt signaling pathway showing effective latency reactivation *in vivo*, but this has failed to show an effect on patient cells [35, 36].

Histone Methyltransferases (HMT) inhibitors work by inhibiting enzymes that transfer a methyl group from S-adenosyl-L-methionine (SAM) to nucleic acids, histones, and non-histone proteins. Methylation of histones is an irreversible process because histones and methyl-lysines have the same half-lives and this promotes the reversal of latency [37]. Moreover, DNA methylation at the HIV-1 50 –LTR has been observed in the latent reservoir of patients with undetectable levels of virus compared to those isolated from patients with viremia. This was also observed in PMBCs, thereby legitimizing DNA methylation inhibitors as a mode of targeting reservoirs [38, 39]. For example, Chaetocin has been shown to increase latent HIV expression without significant T cell activation in Jurkat T cells. Another example is 5-aza-2′ deoxycytidine, which has been shown to inhibit DNA methylation and also prevents the recruitment of methyl-CpG binding domain proteins to the 5′LTR in U1 cells, and J-Lat cell lines [26]. A wide spectrum of inhibitors in this class like DZnep activate latent cell lines but are highly toxic [40]. Though GSK343 has been shown to reduce the HIV provirus in resting cells [41], there has been no significant change in the reactivation of latent cells unless Vorinostat is added to 5-aza-2′ deoxycytidine in which case, antigen production is doubled as compared to exposure one of the inhibitors [41]. This indicates that for HMT inhibitors to work optimally as latency reversing agents, there is a need for combination with HDACis. The main issue that has been raised about molecules in this category is toxicity, a requirement for the use of more than one LRA, and lack of effect when applied to patient cells. More research should focus on non-toxic chromatin modulators, those potent LRA requiring no combination with other molecules as well as those that may be scalable *in vivo*. This analysis is expected to provide insight into further research of optimized designs for new classes of more potent LRAs.

Table 1. Agents that target the latent HIV-1 reservoirs.

LRA	Class	Mode of Action	Experimental Model	Outcome	References
Vorinostat	HDACi	Histone deacetylase enzymes	*Ex vivo* infected primary CD4+ T cells	• Up-regulated PTEFb by CDK9 T-loop phosphorylation and reversed latency. • Has a long-term impact on host gene transcription. • Induces HIV MHC: peptide as well as Env antigen expression on the cell surface of latently infected cells. • Has synergy with GSK343 and EZH2/EZH1 inhibitor. • Increases transcription from all proviruses	[25, 33, 57, 81-83]

(Table 1) contd....

LRA	Class	Mode of Action	Experimental Model	Outcome	References
Panobinostat	HDACi	Histone deacetylase enzymes	*In vivo* aviremic participants	• Induced plasma Viremia • Up-regulated PTEFb by CDK9 T-loop phosphorylation and reversed latency. • Has antagonistic effect when combined with Ingenol • Induces apoptosis but does not induce expression of CD69 • Increases transcription from all proviruses • Disrupts HIV latency *In vivo* but does not reduce the number of latently infected cells	[18, 30, 57, 80, 84]
Romidepsin	HDACi	Histone deacetylase enzymes	*In vivo* aviremic participants	• Failed to induce T-loop phosphorylation or reactivate the latent virus. • Induced high frequency of cells expressing HIV-1 RNA • Capable of impacting all memory cells • Non-selectively induced transcription from proviruses • No reduction in the cells harboring total HIV-1 DNA • Combination with Vacc-4x did not result in a change in HIV-1RNA and DNA diversity	[30, 31, 33, 34, 85, 86]
Disulfiram	HDACi	Akt signaling pathway	*In vivo* aviremic participants	• Resulted in an increase in cell-associated unspliced HIV RNA • Does not induce MATR3 levels from quiescent PBLs.	[35, 58, 87]
Chaetocin	HMTi	Histone Methyltransferase enzymes	Jurkat T cells	• Is highly toxic • Did not efficiently activate Cells expressing shRNA PromA, 143, or both	[88, 89]
DZnep	HMTi	Histone Methyltransferase enzymes	Cell Lines	• Did not activate cells expressing shRNA PromA, 143, or both. • Is highly toxic.	[88, 90]
GSK343	HMTi	Histone Methyltransferase enzymes	Resting Cell Lines	• Has mechanical synergistic effect with Vorinostat.	[71]
5-aza-2′ deoxycytidine	HMTi	Prevents the recruitment of methyl-CpG binding domain proteins	UI cells and J-Lat cell lines	• Reactivated HIV-1 expression in latent cells	[91, 92]
I-Bet & I-bet151	BETi	Allows Tat-mediated recruitment of P-TEFb	J-Lat cell lines	• I-BET led to the reactivation of 61% of the patient cell cultures while I-BET151 led to 50%.	[4, 30, 64]
UMB-136	BETi	Inhibit bromodomain containing proteins	J-lat cell clones Monocytes Primary CD4+ T cell JQ1	• Reactivates HIV-1 in multiple cell models of HIV-1 latency with better efficiency than either JQ1 or UMB-32. • Enhances the effects Prostratin and bryostatin-1	[19, 93]
OTX015	BETi	Allows Tat-mediated recruitment of P-TEFb	CD4+ T cells from aviremic participants	• More potent when used in combination with prostratin. • Has minimal toxicity but is less potent	[63]
JQ1	BETi	P-TEFb agonist	Primary CD4+ T cells	• Reactivated HIV-1 very poorly • Combinations with either bryostatin, prostratin or ingenol led to the highest synergistic increases in the percentage of GFP-positive cells • More potent than OTX015 • Increases chromatin accessibility by altering nuc-1 positioning. • Unable to induce MATR3 levels from quiescent PBLs	[19, 48, 57, 58, 62-64, 90]
Bryostatin	PKC activators	PKC pathway	J-Lat 9.2 cells	• Did not induce a significant viral reactivation. • Combinations with JQ1 led to the highest synergistic increases in the percentage of GFP-positive cells. • Combination with PEP005 did not induce viral reactivation. • Did not activate Cells expressing shRNA PromA, 143	[1, 30, 48, 64, 88]

(Table 1) contd....

LRA	Class	Mode of Action	Experimental Model	Outcome	References
Prostratin	PKC activator	PKC pathway	J-Lat cells Primary cells of aviremic participants	• Combinations with JQ1 led to the highest synergistic increases in the percentage of GFP-positive cells. • Has synergy with OTX015 and Toll-like receptor 8 (TLR8) agonist resulting in greater reversal of latency.	[30, 32, 63, 64]
PEP005	NF-KB signaling Pathway	Induction of the NF-KB Pathway	Primary CD4+ T cells	• Has an effect on other T cells but has no significant effect on TEM cells • Upregulates CD69 levels but does not induce apoptosis • Bryostatin-1 combination did not induce HIV latency reactivation • Combination with JQ1 increased HIV-1 RNA than by itself • Unable to induce MATR3 levels from quiescent PBLs • Upregulates of CD69 and reactivates latent virus • Combination with Birinapant reactivated and eliminated the HIV latent cells but with minimal viral production • More potent than Vorinostat and JQ1 • Has little cellular toxicity	[47, 48, 57, 84]
PMA	Phorbol ester	Mimic DAG	J-Lat cells	• Has similar capacity as PEP005 to reactivate latent HIV both *in vitro* and *ex vivo* • Induces NF-κB nuclear translocation and activation through the PKC pathway	[4, 47]
Maraviroc	CCR5 antagonist	NF-kB pathway	*In vivo* Aviremic Participants	• Is efficient in reactivating X4 and R5-tropic HIV-1 • Activates latent virus transcription through the activation of NF-κB as a result of binding CCR5. • The combination with Bryostatin-1 was antagonistic	[49, 50]
GS9620, MGN1703 & TL-R2/7 agonist	TLR agonists	Toll like receptors	CD4+ T cells	• Reactivate HIV by activation of plasmacytoid DCs (pDCs) and NK cells • GS-9620 is unable to reactivate latent HIV directly in CD4+ T cells, while the dual TLR2/7 agonists have the ability to do so • Dual TLR2/7 agonists preserve the ability to promote TNF-α and to induce TNF-induced viral reactivation to levels similar to GS-9620 and superior to Pam2CSK4. • Induce production of IL-22 which has antiviral responses • GS-9620 also activated HIV-specific T cells and enhanced antibody-mediated clearance of HIV-infected cells. • GS-9620 increased CD8 and CD4 T cell function • MGN1703 induced strong antiviral innate immune responses, enhanced HIV-1 transcription, and boosted NK cell-mediated suppression of HIV-1 infection in autologous CD4^{+} T cells.	[25, 41, 52, 53]
SBI-0637142 & LCL161	Smac mimetics	Non-canonical NF-kB pathway	In vitro J-lat cells	• Enhanced HIV-1 replication and decreased BIRC2 protein levels and resulted in the stabilization of NIK. • Combination with either Panobinostat or Vorinostat, reactivated latent provirus synergistically	[94]
Birinapant	Smac mimetics	Non-canonical NF-kB pathway	In vitro J-lat cells ACH-2 cells U1 cells.	• Had a minor effect on HIV-1 reactivation in the ACH-2 cells and U1 cells. • Induced apoptosis rather than HIV-1 reactivation. • Combination with PEP005 reactivated and eliminated the HIV latent cells but with minimal viral production	[57]

(Table 1) contd....

LRA	Class	Mode of Action	Experimental Model	Outcome	References
AZD5582	Smac mimetics	Non-canonical NF-kB pathway	Jurkat reporter cell line model Invitro	• Enhanced HIV-1 replication while altering a few host genes. • Induced continued virus production in the blood when monkeys were still receiving daily antiretroviral therapy	[42, 95]
Tat-R5M4	Tat vaccine	Viruses in resting cells	*In vivo* Aviremic Participants	• Increased the production of HIV-1 viral particles • Combination with Vorinostat showed 76% activation efficiency • Has little toxicity and does not alter the physiological function of major organs.	[10]
Nivolumab	PD-1 blocker	PD-1Production	*In vivo* Aviremic Participants	• Did not significantly increase vision production • Significantly decrease in latent infection	[75, 76]
BMS-936559	PD-L1 Blocker	PD-L1 production	PMBCs	• Virus activation responses are infrequent, variable, and generally not reproducible	[76]

3.2.1.2. Transcription Activators

Transcription activators are used either alone or in combination with the chromatin modulators. In one study, AZD5582 has been shown to activate the transcription factor NF-κB, a major instigator of HIV-1-gene expression [42]. AZD5582 was tested in two animal models; 'humanized' mice that were infected with HIV; and rhesus macaques infected with the simian immunodeficiency virus (SIV). The treatment led to marked increases in the levels of viral RNA in CD4$^+$ T cells and a substantial rise in virus levels in the blood, indicating that transcription of the virus had been activated. In another study [43], N-803, which has been previously shown [44] to activate HIV-1 transcription *in vitro,* was used. Like Nixon *et al.*, Mc Brien *et al.* found that their treatment caused substantial increases in virus levels in the blood, and in viral RNA in cells from various tissues. Though not optimized for use in humans, these results suggest that pharmacological activation of the non-canonical NF-κB pathway could be an attractive way to trigger HIV-1-gene expression.

Transcription activators, such as protein kinase C (PKC) activators, including bryostatin, ingenol, and phorbol esters, have been used in activating the provirus through NF-κB and AP-1 signal transduction [17]. Low levels of NF-κB and the disruption of positive transcription elongation factor b (P-TEFb) signaling has been associated with latent reservoirs in HIV, hence has been identified as a target for transcription activation [45]. In HIV latency cell models, prostratin and byrostain-1 have been reported to reduce cell survival and activation of pro-inflammatory cytokines important in viral clearance [17]. Phorbol esters like PMA have been shown to reverse latency in vitro when used in combination with either Ionomycin or JQ1 or HDACis [46, 47]. The synergistic effects of these molecules raise the provocative possibility that the best strategies for targeting viral-reservoir cells involve a mix of immune interventions. In a number of studies [47, 48], PKC agonists, combined with other inhibitors, showed modest results. However, ingenol-3-angelate (PEP005) caused marked reactivation of HIV latency and had lower toxicity as compared to PMA [47]. This observation places PEP005 as one of the promising LRA that would require further research to validate its effects. The use of these molecules, therefore, comes with an intrinsic risk of toxic off-target effects. The toxicity seems to be acceptable in animal models, with most showing no clinical side effects. However, much more stringent safety standards must be met in human clinical trials. Additionally, although these compounds were found to be effective in disrupting HIV latency in vitro [47, 48], their clinical uptake has not been carried out because of associated toxicity and possible carcinogenesis [17]. The only compound in this category, which has shown to have promising results in a clinical setting, is the CCR5 antagonist known as Maraviroc [49]. This compound functions by targeting the NF-κB pathway through phosphorylation *in vitro*, resulting in increased transcription in resting CD4+ T cells when administered to HIV infected patients [50]. Nevertheless, the combination of Maraviroc and Bryostatin-1 has shown minimal results because Bryostatin-1 reduces CCR5 expression levels [49]. The second class of transcription activators is the Toll-like receptor agonists. These are compounds that target a family of receptors involved in the initiation of the immune response, allowing expressing cells to recognize pathogens, triggering responses for their elimination, and the development of long-term memory [51]. TLR7 (GS-9620) and TLR9 agonist (MGN1703) are able to induce HIV expression and HIV-Specific Immunity by activation of plasmacytoid DCs, NK cells, and the secretion of soluble factors in an IFN-α–mediated process [52, 53]. A dual combination of TLR2/7 agonists has produced better results at reactivating latent reservoir directly in CD4+ T cells, promoting TNF-α and the TNF-induced viral production [54]. In addition, αCD40, HIV5pep vaccine co-administered with the poly (I: C)(TLR3 agonist) adjuvant *in vivo* in humanized mice model of persistent HIV-1 infection induced immune responses and reduced HIV-1 reservoirs [55]. It has been shown that a combination of prostratin and TLR8 agonist reversed HIV latency in primary cells of HIV-infected patients [56]. It is evident from these studies that TLRs, if rightly combined, will lead to better results. Hence the challenge is striking the right combination.

A novel class of transcription activators currently under study is the Small molecule antagonists known as Smac mimetics. These compounds have been utilized therapeutically to target the non-canonical NF-κB activation pathway. The combination of LRAs (*e.g.* PEP005) and Birinapant, a

smac mimetic that induces apoptosis, minimized secondary HIV-1 Infection in vitro and eliminated reactivated cells [57]. Using a Jurkat reporter cell line model of latency, Sampey *et al.*, in 2018, showed that AZD5582 has single agent latency reversal activity while altering a few selected genes [58]. Even though these compounds are multifunctional, they have been shown to have fewer effects than other LRAs because there is no host cell activation and minimal host transcription changes. Toxicity, possible carcinogenesis, and lack of potency, when used alone, have been the major shortcomings of transcription activators. Research on a combination that would possibly produce the maximum effect and minimal toxicity or carcinogenic, is the direction researchers need to concentrate if a breakthrough is to be realized in these transcription activators.

3.2.1.3. Transcriptional Elongation

Transcriptional elongation control is very important because the continuation of the viral cycle depends on the removal of the transcriptional elongation block and Tat's role as a specific activator is a prerequisite for this to occur [38]. Inefficient transcriptional elongation is a major contributor to latency reservoirs; hence compounds targeting this process are needed for reversal [59]. Among the compounds that target this process are the Bromodomain and extra terminal domain inhibitors (BETis), a class of LRAs that target the bromodomain extra-terminal (BET) family of proteins, the epigenetic readers of lysine acetylation [60]. These drugs have been reported to activate HIV-1 transcription but with conflicting results. I-Bet and I-Bet151 release Bromodomain-containing protein 4 from the 5′LTR and allow Tat-mediated recruitment of P-TEFb to the 5′LTR in J-Lat cell lines, primary CD4+ T cells [26]. Another study has shown that UMB-136 is capable of reversing latent HIV-1 in several J-Lat cell clones as well as in monocytes and primary CD4+ T cell model [19]. Though JQ1 was shown to have no effect in inducing proviruses [26], in another study, JQ1, UMB-136, and OTX015 have been shown to induce HIV mRNA production through the binding with BRD4 and release of P-TEFb [61, 62]. OTX015 treatment resulted in elongated transcripts and increased viral expression in resting CD4+ T cells in patients on ART and was more potent when co-administered with Prostatin [63]. UMB-136, on the other hand, is better than JQ1 in enhancing HIV-1transcription and has been shown to enhance the activities of PKC agonists in CD8-depleted PBMCs, containing latent viral reservoirs [19]. Several other studies have shown that BETis work optimum in reversing latency when combined with protein kinase C (PKC) agonists [46, 64]. These studies suggest that BETis have therapeutic potential in terms of latency reversal, but they are still novel and further investigations are necessary.

Tat derivatives have been suggested as a tool to reverse latency [14, 19, 38, 65, 66]. Tat creates a positive transcriptional feedback loop by recruiting cofactors, such as super elongation complex (SEC) pushing for HIV-1 transcriptional activation [61, 67]. Phosphorylation processes carried out by Tat recruit PTEFb, activate RNA polymerase II, which carries on the elongation process [68, 69]. For example, Tat-R5M4, a synthetic derivative of Tat, has been shown to activate diverse viruses found in resting CD4+ T cells isolated from HIV-1 patients undergoing ARV treatment with less toxicity [70]. The introduction of exosomal, Tat-activated HIV-1 in primary, resting CD4+ T cells from virally suppressed patients resulted in the release of replication competent HIV-1 and heightened the activity of other LRAs [71]. Tat used as a vaccine in a recent study resulted in a significant increase in T cell, B cell, NK cell, CD4+ and CD8+ cell levels, and a decrease in immune activation and effector memory cells [72]. Though showing some promising results, there is a lack of consensus on the effect of transcriptional elongation molecules on latent HIV. Furthermore, it should be appreciated that molecules in this category are less toxic and still novel, and hence further investigations are needed. More research on these molecules would clear the controversial findings and probably provide a clear therapeutic potential of these molecules in latent HIV.

3.2.1.4. Immune Checkpoint Inhibitors

Immune checkpoint inhibitors are another class of compounds, which are under study, that have been implicated in the transcriptional process of HIV-1 and may disrupt latency [73]. The principle behind the usage of these compounds in latency reversal is that they are expressed by exhausted T cells. They act as regulators of T lymphocyte immune response to pathogens, and have been implicated in apoptosis and often serve as biomarkers for disease progression. It has been observed that there is an upregulation of immune checkpoint molecules like PD-1, CTLA4, TIM3, and LAG3 on CD4+ and CD8+ T cells in patients who are not on ARTs [74]. One study showed that the administration of anti-PD-1 to an HIV-infected individual on ART resulted in a significant increase in HIV RNA, and consequently, reversal of HIV latency [75]. On the contrary, *ex vivo* exposure to BM-S-936559, a PD-1 blocker, did not consistently increase HIV-1 expression in PMBCs [76]. *In vivo* blockade of PD-L1 increased proliferation capacity and cytokine production in HIV-specific CD8+ and CD4+ T cells [77], while blocking LAG3 *ex vivo-enhanced* proliferation of HIV-1 infected T cell. Immune checkpoint inhibitors have been used in cancer research and treatment. It will be interesting to further characterize these molecules for their role in HIV disease pathogenesis and as potential contributors to the reversal of HIV-1 latency endeavor.

3.2.1.5. Post-Transcriptional Modification

Compounds targeting post-transcriptional modification are another class, which are currently being studied because, for full activation of latent proviruses, post-transcriptional modification restrictions need to be overcome [78]. These restrictions contribute to the formation of reservoirs because they reduce the expression of viral proteins [79]. So far, in Jurkat cell models and in PBLs, it has been shown that MA-TR3 has a role in the post-transcriptional regulation of HIV-1 latency [78]. RNA surveillance proteins UPF1, UPF2, and SMG6 have a role in the post transcription process;

hence their regulation can be a point of target for the eradication latent reservoirs [80]. The administration of these LRAs alone has not yielded a lot of results in terms of the eradication of HIV reservoirs. Probably, lack of potency of these LRAs, insufficient apoptosis, and increased mutations that evade virus or immune-mediated cytolysis might be the reason. Future research should consider different post-transcriptional mechanisms as druggable targets for a combined approach of more potent latency reversal. Overall, an important issue to be addressed in future research is the development of a more effective approach that would result in full viral reactivation from all latently-infected cells composing the reservoirs.

4. HAEMATOPOIETIC STEM-CELL TRANSPLANTATION

Infection with the human immunodeficiency virus type 1 (HIV-1) requires the presence of a CD4 receptor and a chemokine receptor, principally chemokine receptor 5 (C-CR5). Homozygosity for a 32-bp deletion in the CCR5 allele provides resistance against HIV variants that interact with the CCR5 co-receptor [101]. In this strategy, a matching donor that naturally lacks the essential HIV entry receptor CCR5 is used. The criteria of matching the graft and host are based on the human leukocyte antigen (HLA) system and 5 gene loci (A, B, C, DRB, DRQ) with 2 allelic variants each (=10 alleles total) [102]. So far, there have only been two successful attempts in this strategy. A Caucasian male infected with HIV-1 underwent allogeneic hematopoietic stem-cell transplantation (HSCT) for Hodgkin's lymphoma using cells from a CCR5Δ32/Δ32 donor in the London case [10, 103]. This result is similar to that of the Berlin patient who achieved remission 11 years prior [104]. The Berlin case involved transplantation of stem cells from a donor who was homozygous for CCR5 delta32 to a patient with acute myeloid leukemia and HIV-1 infection. The patient remained without viral rebound 20 months after transplantation and discontinuation of antiretroviral therapy [102]. In this patient, bone marrow transplantation led to complete chimerism, and the patient's peripheral-blood monocytes changed from a heterozygous to a homozygous genotype regarding the CCR5 delta32 allele. The main difference between these cases is that the London patient achieved full remission after single transplantation, whereas the Berlin patient experienced a relapse of cancer and had to undergo further chemotherapy before a second transplant (Gupta *et al.*, 2019). The London study shows that HIV remission can be achieved in a less toxic manner, with reduced intensity drug regimens. Furthermore, single CCR5Δ32/Δ32 transplantation is sufficient and total body irradiation is not needed; this is attested to by the fact that the London patient was still negative 28 months after the treatment. Viral load in semen was undetectable and HIV-1 DNA by ddPCR assay was negative in rectum, caecum, and sigmoid colon and terminal ileum tissue samples at 22 months [103]. However, Lymph-node tissue was positive for the long terminal repeats indicating remnants of the virus that would not be viable to replicate [103]. The absence of rebound after ART interrup-

tion has been attributed to the reduction in the latent reservoir after transplantation and in the fraction of the host CCR5 cells, which serve as targets for the virus [21]. These two cases demonstrate that sustained HIV remission is possible if successful stem cell transplantation is carried out. Other reported cases of bone marrow transplantation not receiving CCR5 mutant donors resulted in viral rebounds weeks after ART interruption despite a considerable reduction of HIV reservoir in the patients [105-107]. Despite the success, this strategy has been noted as risky, or highly morbid procedure that takes time and may fail as viruses that enter the cells via the CXCR4 co-receptor can continue the infection [108]. There is also a considerable graft-versus-host effect as well as the problem of finding a matching donor with CCR5Δ32/Δ32 cells [103].

5. GENE-SPECIFIC TRANSCRIPTIONAL ACTIVATION

Clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR associated protein 9 (Cas9) system is an engineered genome editing method, which utilizes RNA to target DNa [68]. There are two main strategies to target an HIV infection by CRISPR/Cas9 technology; targeting host genes or targeting viral DNA [84]. In targeting the host chemokine receptor CCR5, CRISPR/Cas9 introduces deletion at both alleles in induced pluripotent stem cells, and CRISPR/Cas9 has been used to target CXCR4 receptor [84]. Another study [109] similarly demonstrated that various guide RNA (gRNA) combinations targeting both CXCR4 and CCR5, using CRISPR-sgRNAs-Cas9, could induce editing of CXCR4 and CCR5 genes in various cell lines and in CD4+ T cells. CRISPR-Cas9 system has been used to activate viral gene expression in cell line models of HIV-1 latency [110]. Liao *et al.*, in 2015, has shown that CRISPR/Cas9 can disrupt the genome of silent cells infected with HIV-1 and it can serve as a defense against new infections when engineered into pluripotent stems cells that show resistance after differentiation [111]. This has been confirmed further by the activation of viral gene expression in cell line models of HIV-1 latency and the enhancement of latent HIV-1 transcription by the CRISPR/Cas9 when combined with LRAs [110]. In Jurkat cells containing HIV-1 proviral DNA, treatment with Cas9 and gRNAs targeted to 10 different viral regions, including tat and rev genes have resulted in efficient mutation and a considerable reduction in viral load [112]. Validating this, another study showed that guide RNAs (gRNAs) could be used to induce SaCas9 to disrupt the latent HIV-1 provirus and suppress HIV-1 proviral reactivation [22, 86]. These studies have demonstrated that there are different targets in the viral genome that can be used to reverse latency in HIV-1 infected cells. Further studies in this area are needed in order for the best targets to be identified.

6. BLOCK AND LOCK STRATEGY

Block and lock is a strategy that is in the inceptive period and proposes to serve as a functional cure for HIV-1.

Table 2. Agents that prevent latency reversal.

Agent	Mode of Action	Experimental Model	Outcome	References
siRNAs & shRNAs	Transcriptional gene silencing	J-Lat 9.2 cells	• Expression of siRNAs 143 and shRNAs Prom A • Made Cells resistant to viral reactivation by: TNF, SAHA, SAHA/TNF, Bryostatin/TNF, DZNep, and Chaetocin.	[88, 96]
Didehydro-cortistatin A	Epigenetic silencing	CD4+ T cells from aviremic participants & BLT mouse model	• Prevents viral rebound after therapy interruption. • Combination of dCA and ART decreases chromatin accessibility at the LTR, reducing transcriptional competence of latent HIV-1 genomes • Inhibits viral reactivation upon CD3/CD28 or Prostratin stimulation of latently infected CD4$^+$ T cells	[23, 97]
Sudemycin D6	Inhibitors of SF3B1	Jurkat T cells & differentiated THP-1 cells	• Abrogated the production of all HIV splice forms • Suppressed HIV reactivation, irrespective of the latency-reversing agent used.	[98]
Dual inhibitors Torin1 and pp242	Inhibition for mTORC1 and mTORC2	CD4+ T cells from aviremic participants	• Prevented HIV reactivation from latency • Dual inhibitors for mTORC1 and mTORC2 are more potent against HIV than a more specific mTORC1-specific inhibitor such as Rapamycin.	[7]
pyrimidin-7-amine, biphenylcarboxamide& Benzohydrazide	Affect protein stability	PBMCs	• Suppressed HIV-1 gene expression by preventing accumulation of two key HIV-1 regulatory factors, Tat and Rev.	[99]
ABX464	Affect mRNA transport	PBMCs & humanized mice	• Efficiently blocked virus replication in a dose-dependent manner	[100]

Molecules that have been used to achieve this strategy are shown in Table (**2**). This strategy aims to allow HIV-1-positive patients to attain viral remission without taking ART, which is similar to those observed in elite controllers who are noted to have high CD4+ T cells, low viral load, and a robust immune [67]. The theory behind this strategy is that the virus could be stably disabled if its genome and chromatin could be silenced such that patients no longer need to be on ARVs. The aim of this strategy is not to eliminate the virus but to suspend it in place so that there is a functional cure. One-way of going about this is through transcription silencing in proviruses [20]. Small interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs) can be used as tools to target conserved regions of HIV-1 genome, resulting in gene silencing [113]. In J-Lat 9.2 cells, a model of HIV-1 latency, expressing shRNAs PromA, 143, PromA/143, and treated with LRAs, the virus remained inactive and their chromatin compact [88]. This study showed that shRNAs could make proviruses resistant to subsequent activation. Similarly, siRNA named si143, which targets the tandem NF-κB motifs within viral 5′LTR has been shown to induce transcriptional gene silencing [96]. SiRNAs and shRNAs have great promise due to their specificity, potency, and their adaptability, which is advantageous because HIV mutates excessively [113]. Didehydro-cortistatin A (dCA), a Tat inhibitor, can be another tool to permanently freeze proviruses. dCA has been shown to induce a long-lived reservoirs by breaking Tat-mediated transcriptional feedback loop and this remains effective even after the drug consumption has been stopped [97]. In another study, dCA suppressed HIV expression *In vivo*, inducing epigenetic silencing by restricting RNAPII recruitment to the promoter in the bone marrow-liver-thymus (BLT) mouse model of HIV latency [23]. The binding of dCA to Tat alters the protein environment, making Tat more

resistant to proteolytic degradation, and also interferes with the Tat-TAR interaction upon which the functions of Tat are dependent on, thereby preventing provirus reactivation [114]. Tat-mediated process can also be prevented by inhibiting splicing factor 3B subunit 1 (SF3B1), a critical HIV dependency factor. This was seen after exposure to various reversal agents and there was no viral activation [98]. Another way of blocking the reactivation of proviruses is to target mTOR, a conserved serine/threonine kinase complex that serves as a regulator of HIV latency [93]. Torin1 and pp242 have been shown to interrupt the activity of mTOR and suppress HIV transcription through interference with the viral Tat mechanism *in vivo* [115]. Post-Transcriptional Gene Silencing is another way of achieving block and lock of the latent reservoir. Small molecule modulators involved in post-transcriptional modification in HIV-1 can be inhibited to achieve post-transcriptional silencing. RNA surveillance proteins UPF2 and SMG6 have been shown to be involved in post-transcriptional silencing by their interaction with UPF1, subsequently reducing viral gene expression in HIV-1-infected primary CD4+ T cells [80]. Synthetic siRNAs targeted to the central region of the V3 loop and CD4 binding site of conserved regions on gp120 has also resulted in gene silencing of HIV gene expression [113].

7. CHALLENGES OF CURRENT CURE STRATEGIES

The Berlin Patient was declared cured of HIV infection after receiving a bone marrow transplantation from a donor with stem cells harboring a CCR5 deletion mutation. Due to this hope, several clinical attempts followed to duplicate this strategy. Unfortunately, these attempts were unsuccessful. The London Patient showed a successful outcome by bone marrow transplantation. Despite that, CCR5 Δ32 mutation

appears to reduce protection against some other viral infections. These studies demonstrated that, while effective for the cure of HIV, bone marrow transplantation is a risky procedure and is not tolerated by most patients; hence it is not a scalable treatment. Gene editing disrupts HIV proviruses with DNA editing. Though it can make gene editing of the HIV genome achievable, it is not known what the optimal targeted HIV genome sequences are. Challenges facing this strategy range from the questions on how to deal with a gene editing escape during the residual replication of HIV in deep tissues to how to effectively deliver the editing system *in vivo* [116]. As pointed out by O'Geen et. al., the main significant challenge of genome editing is the sequence diversity of HIV-1 quasispecies present in patients [117]. Because of this diversity, there is a requirement to target multiple sites, making it a task to edit every provirus as well as an error-prone process. Majorly, gene editing technologies can be confounded by problems such as off-target editing, inefficient or off-target delivery, and stimulation of counterproductive immune responses. These questions still remain and need to be resolved before the technology is tested in patients. Shock and Kill strategy has been proposed as a therapy to reduce the frequency of infected cells by targeting molecular mechanisms of HIV latency. However, whether or not this strategy can achieve a virological control in the absence of ART is still under active investigation. A significant reduction of reservoir size has not been observed in patients yet, indicating that the reactivation of latent HIV reservoirs alone does not necessarily cause a reservoir clearance.

CONCLUSION

There are challenges in developing an HIV cure. However, given the interest in the field over the last several years, a cure for HIV seems possible. It is hoped that advances in therapeutic approaches for eliminating latent HIV described in this review will provide a pathway for the development of scalable and safe methods for eliminating persistent reservoirs of HIV in the future. Importantly, Block-and-lock approach seems a viable alternative for a functional HIV cure because agents under this strategy have the potential to reduce the level of viremia and prevent viral reactivation from latent reservoirs.

CONSENT FOR PUBLICATION

Not applicable.

STANDARD OF REPORTING

The systematic review was designed and developed in accordance with the PRISMA statement.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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