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Review article

Nanotechnology approaches to eradicating HIV reservoirs

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ABSTRACT

The advent of combination antiretroviral therapy (cART) has transformed HIV-1 infection into a controllable chronic disease, but these therapies are incapable of eradicating the virus to bring about an HIV cure. Multiple strategies have been proposed and investigated to eradicate latent viral reservoirs from various biological sanctuaries. However, due to the complexity of HIV infection and latency maintenance, a single drug is unlikely to eliminate all HIV reservoirs and novel strategies may be needed to achieve better efficacy while limiting systemic toxicity. In this review, we describe HIV latency in cellular and anatomical reservoirs, and present an overview of current strategies for HIV cure with a focus on their challenges for clinical translation. Then we provide a summary of nanotechnology solutions that have been used to address challenges in HIV cure by delivering physicochemically diverse agents for combination therapy or targeting HIV reservoir sites. We also review nanocarrier-based gene delivery and immunotherapy used in cancer treatment but may have potential applications in HIV cure.

1. Introduction

At the end of 2016, the toll of the HIV/AIDS pandemic included 36.7 million infections and 1 million deaths [1]. The combination of antiretroviral drugs (ARVs) for HIV treatment, also referred to as highly active or combination antiretroviral therapy (HAART, cART), can successfully suppress viral replication in plasma to undetectable levels. These treatments have greatly extended the life span and improved the quality of life for people living with HIV-1 infection. However, none of these therapies are capable of eradicating the virus from long-lived cellular reservoirs, which leads to virus rebound once treatment is stopped. Moreover, a lifetime of cART is expensive and causes both short- and long-term side effects such as cardiovascular diseases, neurocognitive disorders, and liver injury [2–5].

Currently, there is no cure for HIV/AIDS, but a singular success has proven that a cure is possible and has galvanized the field [6]. The "Berlin Patient" describes the clinical case study of Timothy Ray Brown who underwent treatment for acute myeloid leukemia (AML), and was cured of both AML and HIV after transplantation with CCR5-deficient hematopoietic stem cells that are inherently resistant to HIV infection [7]. Chemotherapy and radiotherapy was used to eradicate all leukocytes including AML cells. Stem cell transplantation was used to reconstitute the immune system, which also effectively eliminated the need for cART to control plasma viremia to undetectable levels. This remarkable case bolstered support for HIV cure research, but considerable and sustained efforts are needed to develop clinical approaches that can be applied safely and effectively with the lowest barriers to accessing healthcare.

Viral reservoirs are the primary obstacle that must be overcome to realize an HIV cure. These reservoirs can be defined as cellular sites (e.g. long-lived infected memory CD4+ T cells, macrophages) where viral replication in infected cells is arrested, and anatomical sites (e.g., gut-associated lymphoid tissue, lymph node, genital tract, central nervous system) that harbor these latently infected cells [8]. These tissues

Abbreviations: AAV, adeno-associated virus; AML, acute myeloid leukemia; APC, antigen presenting cells; ARVs, antiretroviral drugs; ATI, analytical treatment interruption; BBB, blood-brain barrier; BET, bromodomain extra-terminal; BMVECs, brain microvascular endothelial cells; bnAb, broadly neutralizing HIV antibody; cART, combination antiretroviral therapy; CAR, chimeric antigen receptor; CMV, cytomegalovirus; CNS, central nervous system; CRISPR, clustered regularly interspersed palindromic repeats; DC, dendritic cell; dCA, didehydrocortistatin A; GALT, gut-associated lymphoid tissue; HAART, highly active antiretroviral therapy; HBMECs, human brain microvascular endothelial cells; HDAC, histone deacetylase; HMT, histone methyltransferase; HSPC, hematopoietic stem/progenitor cell; iPSC, induced pluripotent stem cell; LN, lymph node; LRA, latency reversing agent; LTR, long term repeat; MAdCAM-1, mucosal vascular addressin cell adhesion molecular 1; M cell, microfold cell; MMF, mycophenolate mofetil; MPA, mycophenolic acid; NF-κB, nuclear factor κB; NHP, non-human primates; NK cell, natural killer cell; NRTIs, nucleoside reverse transcriptase inhibitors; PAMAM, poly(amidoamine); PD, pharmacodynamic; PD-1, programmed cell death protein-1; PK, pharmacokinetic; PKC, Protein kinase C; P-TEFb, positive transcription elongation factor b; RNAi, RNA interference; RNP, ribonucleoprotein; saCas9, *Staphylococcus aureus* Cas9; SAHA, vorinostat or suberanilohydroxamic acid; sgRNA, single-guide RNA; siRNA, small interfering RNA; SIV, simian immunodeficiency virus; TALEN, transcription activator-like effector nuclease; TAT, trans-acting transcriptor; T_{CM}, central memory CD4+ T cells; T_{EM}, effector memory CD4+ T cells; T_N, naïve CD4+ T cells; T_{SCM}, CD4+ T memory stem cells; T_{TM}, transitional memory CD4+ T cells; ZFN, zinc finger nuclease

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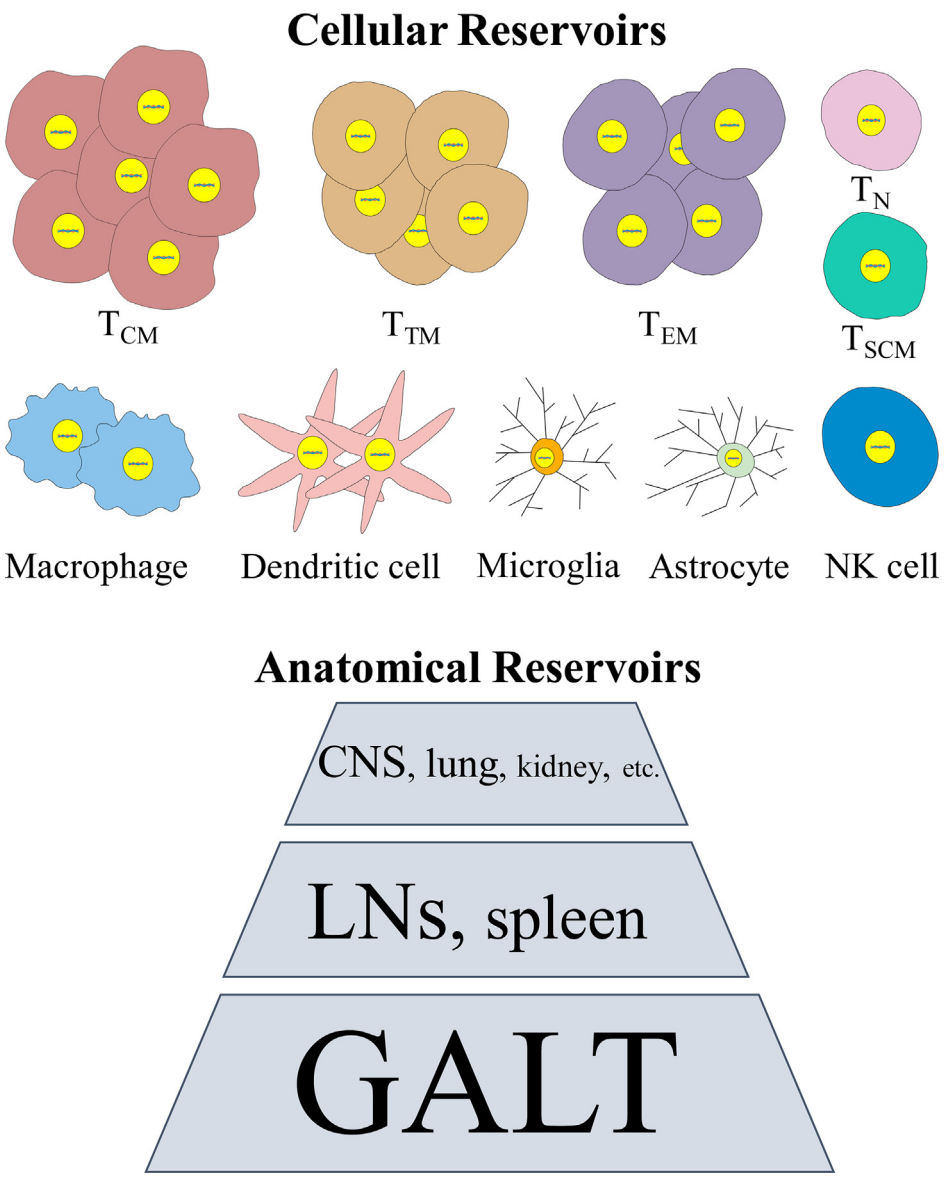

Fig. 1. Schematic illustration on major HIV cellular and anatomical reservoirs. The relative size of the reservoir is represented by the size of the compartment as shown. T_{CM}, central memory CD4+ T cells; T_{TM}, transitional memory CD4+ T cells; T_{EM}, effector memory CD4+ T cells; T_N, naïve CD4+ T cells; T_{SCM}, CD4+ T memory stem cells; NK cell, natural killer cell; CNS, central neural system; LNs, lymph nodes; GALT, gut-associated lymphoid tissue.

exhibit limited access to ARVs which may contribute to viral persistence [9,10]. Numerous approaches are aimed at finding and diminishing these HIV reservoirs. For example, a number of studies have suggested that early initiation of cART during acute infection could be a realistic approach to cure HIV at the population level as it may block the initial establishment of latent reservoirs [11,12]. Another approach focuses on reactivation of HIV from latently infected cells by use of latency reversing agents (LRAs). Once these latent cells express viral antigen, they may become vulnerable to the immune system or other therapeutics that result in their elimination [13]. However, clinical studies of LRAs have failed to reduce the reservoir size, which might be due to insufficient potency of LRAs, off-target effects in uninfected cells leading to dose-limiting toxicities, or lack of effective "kill" strategies [14]. Cell and gene therapies have also been investigated for HIV cure, where immune cells have been engineered to be resistant to HIV, or HIV proviral DNA has been targeted for excision from latently-infected cells [15]. Other strategies such as broadly neutralizing HIV antibody (bnAb) [16–18], permanently silencing the HIV provirus [19], and anti-proliferative therapy have also been investigated [20].

Due to the complexity of HIV pathogenesis, more efficient therapeutic combinations and novel reservoir-targeted drug delivery approaches may be needed to optimize current approaches for eradicating latently infected cells. Nanocarrier drug delivery systems have unique attributes that are ideally suited to address challenges with targeting the HIV reservoir and eradicating the latent virus to realize an HIV cure. This technology has already shown enormous potential for use in the diagnosis and treatment of several diseases, with a major emphasis in cancer. Nanocarrier attributes that may be ideally suited for HIV cure strategies include: (1) improvement of bioavailability and pharmacokinetic (PK) profiles of cure agents [21]; (2) reduction of drug toxicity [22], and avoidance of surface efflux pump mediated drug resistance [23]; (3) enhanced or synergistic efficacy through combination of multiple drugs in a single particle [24]; (4) ability to penetrate into HIV reservoir sites such as lymphatic tissues or to target specific vulnerable cells such as CD4+ T cells [25–29]; (5) ability to release therapeutics in controlled rates for sustained drug delivery [30,31].

In this review, we will describe HIV sanctuaries and the current strategies toward diminishing HIV reservoirs and provide an overview

of current and future nanotechnology approaches to address HIV cure.

2. HIV reservoirs are the obstacle to a cure

The main obstacle to HIV eradication is the existence of reservoirs of latently infected cells. Two models have been proposed to explain the establishment of latency in long-lived memory cells. The pre-activation latency model hypothesizes that HIV can directly infect resting CD4+ T cells [32,33], and the post-activation latency model relies on an idea that the activated CD4+ memory T cells revert to a resting state instead of cell death after being infected by HIV [34]. HIV viral DNA integrates at various locations into the genome of these long-lived host cells [35], and the integration at specific sites (e.g. MKL2 and BACH2) has been attributed to cell clonal expansion and therefore persistent infection [36,37]. HIV latency is maintained under several molecular mechanisms including: (1) the recruitment of histone deacetylases (HDACs) and histone methyltransferases (HMTs) to HIV-1 long term repeat (LTR), leading to histone deacetylation and methylation on Nuc-0 and Nuc-1, which can restrict the initiation of transcription [38,39]; (2) sequestration or inactivation of transcription and elongation factors such as nuclear factor κB (NF-κB) and NFAT, or positive transcription elongation factor b (P-TEFb) that are important for initiating or elongating transcription [40,41]; and (3) other mechanisms such as HIV-1 DNA methylation [42], post-transcriptional blocks [43], and cellular microRNAs that inhibit HIV-1 production [44]. Compared to virus-producing CD4+ T cells that have a half-life of only 0.7–1.1 days [45], latently-infected CD4+ T cells have a half-life ranging from 4.6 to 44.2 months based on different studies in patients receiving ART [46–48]. These long-lived infected CD4+ T cells with the ability of clonal expansion keep proliferate without releasing virus, leads to their high stability and persistence in sanctuary sites [49]. The variability of integration sites, complexity of latency maintenance, and clonal expansion of long-lived reservoirs are all challenges for the HIV cure field.

HIV latency can be found in different cell types and tissue compartments, which presents additional barriers to curative strategies (Fig. 1) [8]. Cellular reservoirs include a wide-range of cell types that are susceptible to HIV infections, such as memory CD4+ T lymphocytes and macrophages. These cells are found in the peripheral blood, but also in anatomical reservoirs that include lymph nodes (LNs), spleen, gut-associated lymphoid tissue (GALT) and brain or central nervous system (CNS).

HIV proviral DNA is detected primarily in central memory (T_{CM}), transitional memory T cells (T_{TM}), and effector memory T cells (T_{EM}) which maintain latency and persistence through clonal expansion [49–51]. In particular, a subset of T_{CM} called peripheral T follicular helper cells (pTfh cells) are highly susceptible to HIV infection and contribute to HIV persistence [52]. CD4+ T memory stem cells (T_{SCM}) also significantly contribute to reservoir expansion and viral persistence because these cells are long-lived and differentiate into mature memory T cells, although their proportion in circulating lymphocytes is only 2–4% [53,54]. Macrophages are also a chronic and latent HIV reservoir in infected patients that contributes to disease progression [55]. Macrophages are antigen presenting cells (APCs) that have been shown to spread virus particle to bystander CD4+ T cells [56], as well as recruit and activate latently-infected CD4+ T cells through chemokine and virus antigen production [57]. Macrophages are present in almost all organ systems, and can transmit virus to different anatomical sites including the brain (e.g., microglia as the resident macrophage in the brain and spinal cord) [58]. Infected macrophages have a much longer half-life (> 30 days) and are less prone to cytopathic effects of the virus compared to CD4+ T cells [59,60]. In particular, HIV-infected microglia show a strong correlation with HIV-associated neurological symptoms due to neurotoxin secretion and attraction of adaptive immune responses to the brain that cause neural damage [58,61]., Dendritic cells (DCs) are another type of APC that can be infected directly by HIV-1, although less efficiently than CD4+ T cells, but can transmit virus to T cells [62,63]. It has also been suggested that DC subtypes in the LNs, but not the peripheral blood, act as long-lived HIV reservoirs [64,65].

The major anatomical sites that harbor infected cells include lymphatic tissues (LNs, spleen, and GALT), CNS, and lung (Fig. 1) [66]. HIV-1 or cells producing viral RNA (vRNA+ cells) can still be detected in many of these tissues of patients whose viral loads in the peripheral blood are undetectable [10]. Estes et al. have reported that in an NHP model, ~98.6% vRNA+ cells reside in lymphatic tissues (LNs, spleen, and GALT) [66]. Ineffective viral suppression in these tissues may be due to poor immune surveillance, less efficient drug penetration, high CD4+ T cell density that favors cell-to-cell HIV transmission, and interactions with autologous B cells or dendritic cells [67–69]. A clear majority of cells that contain HIV proviral DNA (vDNA+ cells) are found in lymphoid tissues harboring significant numbers of memory T cells. A frequency on the order of ~10^5 vDNA+ cells/g was detected in both LN and GALT from patients under ART [66]. The GALT is one of the largest lymphoid organs infected by HIV-1 [70,71] estimated to contain ~62.3% of vRNA+ cells before ART and ~98.0% after the therapy in an NHP model [66]. Distinct from other lymphatic tissues, the frequency of vDNA+ cells in GALT does not decline after ART, leaving it as the largest sanctuary for HIV latency. The gut mucosal CD4+ lymphocytes are uniquely susceptible to HIV infections due to higher expression of chemokine receptor CCR5 and high levels of activation [72]. In addition, HIV-1 infected CD4+ T cells from other parts of the body regardless of the primary infection route can traffic to the GALT mediated by α4β7 and CCR9 [73,74]. It has also been reported that resting central memory α4β7+CD4+ cells are preferential targets of simian immunodeficiency virus (SIV) and contribute to reservoir establishment and expansion in mucosal sites [75]. Aside from these lymphatic tissues, the CNS is another key anatomical reservoir for HIV-1. The virus enters the brain at the early stage of infection and is difficult to eradicate, making the brain a lifelong virus pool [76]. Clinically, most ARVs have limited penetration into the CNS due to tight junctions of the blood-brain barrier (BBB) and transmembrane proteins that exist on the BBB to pump the drug out of the brain [77]. Other tissues such as lung and kidney have also been regarded as HIV reservoirs although they harbor much less proportions of latently infected cells [78,79].

3. Current strategies toward diminishing HIV reservoirs

With the recognition that viral reservoirs are major barriers to an HIV cure, several therapeutic strategies toward finding and diminishing latently infected cells have become the focus of recent research efforts. These cure strategies include early cART, reactivation of latently infected HIV reservoirs, gene therapy, and immunotherapy.

3.1. Minimizing the size of HIV reservoirs by early cART

Many studies suggest that initiation of ART during the acute phase of infection could be an effective first step toward achieving sustained virologic remission. HIV latency is likely established early within days of acquiring infection although the specific timing in human is still uncertain [80,81]. Studies in non-human primates (NHPs) have observed high levels of integrated SIV DNA in resting CD4+ T cells within 10 days post-infection, but the seeding of the reservoir is thought to occur as early as 3 days [82–84]. Patient initiated early ART has resulted in observed faster decay of HIV reservoirs and better preserved immunity against HIV compared to those who delayed ART [11,46,48,85–87]. For example, the VISCONTI study investigating 14 patients who received ART during the acute-early phase of infection found that plasma viremia remained controlled for prolonged periods of time after cessation of therapy [12]. In a clinical case study known as the “Mississippi baby”, an infant born to an HIV-positive mother had ART initiated at 30 h from birth but discontinued therapy at 18 months of age and remained aviremic for more than 2 years [88]. Unfortunately, the child’s plasma viremia ultimately rebounded and ART

had to be reinitiated [89]. Aside from impeding the initial seeding and development of reservoirs, it has been suggested that early ART could also reverse chronic mucosal and systemic immune activation, which is the hallmark of HIV infection, contributes to reservoir size, and viral distribution through preservation of mucosal Th17 cells [90]. While multiple studies have shown that early ART can successfully suppress HIV in peripheral blood to undetectable levels, it has shown limited effect in inhibiting viral replication in lymphatic tissues such as intestinal tissues and lymph nodes which may contribute to viral rebound [91,92]. Collectively, the data suggests that early ART alone may not be sufficient to achieve virologic remission. The practicality of initiating ART at the population level during the early phase of HIV infection also remains a challenge. As such, future direction may need to focus on combination therapy with other cure strategies, such as latency-reversing agents, therapeutic vaccines, or immune-modulating agents, as well as increase drug concentration in anatomical reservoir sites [81].

3.2. Reactivating HIV reservoirs by LRAs

Viral rebound normally occurs within 2–3 weeks after the interruption of ART, mainly due to viral reactivation in latently infected resting CD4+ T cells as well as other cellular reservoirs [93]. Several strategies for reducing the reservoir size have focused on targeting these cells. A widely investigated approach that has reached the clinic known as “shock and kill” reactivates HIV-1 proviruses while maintaining ART in order to prevent the spread of new infections and the establishment of new cellular reservoirs. These reactivated cells are then eliminated by viral cytopathic effects, the host immune response, or by a combination of therapies involving therapeutic vaccines, neutralizing antibodies or immune checkpoint blockade agents [94]. Several classes of LRAs have been identified and developed to overcome obstacles to transcription of the HIV-1 provirus that leads to maintenance of latency (Table 1). HDAC inhibitors (HDACi) unleashes the repression of viral LTR that is maintained by histone deacetylase to allow transcription of provirus [95]. While these drugs are effective and do not lead to global T cell activation, they may affect expression of a large numbers of genes [96]. HMT inhibitors (HMTi) similarly activate viral LTR through inhibition of histone H3 methylation [39]. HMTi can enhance the potency of HDACi but most agents are still under preliminary studies [97]. Protein kinase C (PKC) agonist activates NF-κB which binds to LTR to increase proviral transcription [98,99]. Many agents from this class are highly potent, but have major concerns such as nonspecific induction of many genes, toxicity, and tumor-promoting potential. P-TEFb activators, such as bromodomain extra-terminal (BET) inhibitor JQ1, enhance interaction of P-TEFb with LTR to activate transcription [100]. JQ1 is also advantageous as it suppresses T cell proliferation and activation, which may be beneficial for cure therapy. DNA methyltransferase inhibitors function based on the role of DNA methylation on HIV latency [101]. This role is still controversial, and latency reversal from such inhibitors is relatively weak but could be strengthened when combined with other agents. Some agents may act based on multiple mechanisms. For instance, SAHA as an HDACi also activate latency through the activation of PI3K/Akt pathways [102]. Also, some short chain fatty acids induce latency by activating P-TEFb as well as inducing multiple histone modifications [103].

Several HDACi, (e.g., vorinostat [104–107], panobinostat [108], romidepsin [111], valproic acid [109] and chidamide [114]), disulfiram [126,127], and bryostatin-1 [119] have already been tested in clinical trials (Table 1), and other agents are under ongoing clinical trials (reviewed in [14,128]). Many clinical studies of LRAs have shown an increase in cell-associated or plasma HIV RNA in CD4+ T cells. This is with the exception of bryostatin-1, where no effect on PKC activity or HIV latency reversal was observed, which may due to low plasma concentrations after a single dose [119]. However, while all these LRAs have shown ability to reactivate HIV in patients, none have shown a reduction on the HIV reservoir size [14,129]. This might be due to a lack of effective “kill” approaches used in these clinical trials, as only ART has been incorporated so far to suppress viral spreading. Only few “kill” strategies such as broadly neutralizing antibodies have been tested in combination with LRA in humanized mice [130]. It has been shown that some of these LRAs act synergistically to enhance potency when used in combination. The combination may also reduce the dose of single LRAs to minimize their toxicity. For example, HMTi can enhance proviral reactivation by HDAC inhibitors such as SAHA [97]. Recent studies from several groups have shown that protein kinase C activators such as prostratin, bryostatin 1, or ingenol-B synergistically and robustly induced HIV-1 transcription and virus production from *in vitro* or *ex vivo* models when combined with bromodomain inhibitor (also known as pTEFb-releasing agents as described above) or HDACi, [121,131–134].

“Shock and Kill” is still a controversial strategy especially based on recent safety issues and unenthusiastic outcomes from clinical studies. As such, whether or not a single LRAs is able to significantly reduce the size of HIV reservoirs is still questionable. A stronger focus is now on the use of LRAs combinations and combination with other therapeutics, as well as novel drug delivery systems to enhance therapeutic effects, avoid global immune cell activation, and reduce off-target side effects.

Table 1
Major latency reversing agent (LRA) categories and their representatives.

Categories	Representative agents	Clinical trial number[a]	Reference
HDAC inhibitor	vorinostat (SAHA)	NCT01365065; NCT03198559; NCT02707900; NCT01319383; NCT03212989; NCT02475915; NCT02336074	[102,104–107]
	panobinostat	NCT01680094; NCT02471430	[108]
	valproic acid (VPA)	NCT00312546; NCT00614458; NCT00000629; NCT00289952	[109,110]
	romidepsin	NCT02616874; NCT02092116; NCT01933594; NCT03041012; NCT02850016	[111–113]
	chidamide	NCT02513901; NCT02902185	[114]
	sodium butyrate	Not tested	[115]
HMT inhibitor	BIX01294	Not tested	[116]
	Chaetocin	Not tested	[39]
PKC agonist	prostratin	Not tested	[117,118]
	bryostatin-1	NCT02269605	[119,120]
	ingenol derivatives	Not tested	[99,121,122]
P-TEFb activator	hexamethylene bisacetamide	Not tested	[123]
	JQ1	Not tested	[100]
DNA methyltransferase inhibitors	decitabine and its analog azacytidine	Not tested	[124]
unclassified	disulfiram	NCT00878306; NCT01944371; NCT03198559; NCT01286259; NCT00002065	[125–127]

HDAC, histone deacetylase; HMT, histone methyltransferase; PKC, protein kinase C; P-TEFb, positive transcription elongation factor b.
[a] Source: clinicaltrials.gov.

3.3. Gene-editing approaches

Gene-editing technologies are also being investigated for HIV cure strategies [15]. Gene-editing enzymes such as homing endonucleases, evolved recombinases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9 have been used to directly excise HIV provirus from the host genome and have proven effective in culture with cell lines and primary cells [135–138]. Recent studies in humanized mouse models have shown successful proviral excision in most major organs after single intravenous administration of *Staphylococcus aureus* Cas9 (saCas9) and multiplex single-guide RNAs (sgRNAs) using adeno-associated virus (AAV) [139,140]. However, due to various integration sites of latent virus and low percentage of latently infected cells, off-target effects and poor gene delivery efficiency remain big challenges towards clinical trials.

Gene-editing tools have also been used to knock out CCR5 in CD4+ T cells or hematopoietic stem/progenitor cells (HSPCs) in order to block virus entry [141], one of the mechanisms underlying the "Berlin Patient" case. ZFNs have been used to modify autologous CD4+ T cells and tested in HIV-infected patients [142]. However, patients that completed analytical treatment interruption (ATI) all had viral rebound after the therapy, even though the therapy resulted in a slower decay rate for the CCR5-modified CD4+ T cells compared to unmodified cells. A challenge for this approach is the low frequency of modified CD4+ T cells that are adoptively transferred, which likely limits its effect. CRISPP/Cas9 has been used successfully to disrupt CCR5 as well as CXCR4 on primary CD4+ T cells *in vitro* [143–145]. Gene-editing of primary CD4+ T cells *in vivo* is also likely to result in a low frequency of modified cells and would be associated with relatively high off-target effects. Disrupting CCR5 on HSPCs, which gives rise to all cell lineages that HIV-1 infects, has the advantages of producing longer-term effects than targeting CD4+ T cells. ZFNs or CRISPR/Cas9 have been used to modify HSPCs, showing successful CCR5 disruption and anti-HIV effect in reconstituted or transplanted mice [146–148]. Similarly, induced pluripotent stem cells (iPSCs) have been modified and can be differentiated into HIV-resistant monocytes/macrophages *in vitro* [149–151]. Aside from generating HIV-resistant immune cells, human hematopoietic stem cells (HSCs) have also been induced to HIV-specific cytotoxic T lymphocytes to inhibit viral replication *in vivo* [152].

Permanently silencing the non-expressing provirus in latently infected cells has been investigated using different strategies. RNA interference (RNAi) with small interfering RNA (siRNA) silences the expression of viral RNA or cellular mRNAs that are necessary for viral production, and has been shown to control viral replication (reviewed in [19]). HIV-1 encoded genes such as *tat, rev, vif, gag, nef, pol, env, vpr* that are important for viral replication and are targets for siRNA silencing.

siRNAs have potential to overcome the high rate of HIV mutation through targeting highly conserved sequences [153]. These agents may offer an effective and safe approach towards an HIV cure, but still have challenges for reaching the clinic such as low delivery efficiency and instability. Challenges with gene editing strategies have included delivering genes or editing enzymes specifically to latently infected resting CD4+ T cells, off-target effects, and insufficient activity of enzymes that reach target cells *in vivo* [154]. These issues will collectively impact the efficacy and safety of gene editing therapies for HIV cure.

3.4. Immunotherapy

HIV therapeutic vaccines have focused on inducing HIV-1 specific CD8+ T cells responses to selectively kill virus-producing cells [155,156], and ultimately control the disease. Several therapeutic vaccines have been tested on HIV-1 infected individuals including vector-based vaccines that express HIV-1 antigens from harmless or attenuated viruses and plasmid DNA vaccines [157–160], but most have failed to show sufficient efficacy and some have raised safety concerns. Nonetheless, several promising vaccines have been tested in preclinical studies and will go into clinical trials in the near future. For example, a vaccine based on cytomegalovirus (CMV) has been investigated and showed induction of broad cellular immune responses against novel epitopes resulting in efficient control of pathogenic SIV infection [161,162].

The use of broadly neutralizing antibodies (bnAbs) has also attracted attention as a therapeutic to prevent and treat HIV infection based on promising animal and human data. These bnAbs are also regarded as a potential cure strategy based on their antiviral activity as well as their ability to reduce reservoir size [163]. For the treatment of HIV, bnAbs have demonstrated remarkable efficacy in reducing viral load through clearance of free virus, elimination of infected T cells, as well as reduction of proviral DNA in the LN and GALT [16–18,163,164]. bnAbs can also act as vaccines by enhancing autologous neutralizing antibody responses [165], or boosting and broadening humoral immunity [166]. Another strategy combines bnAbs and LRAs showed reduced viral rebound from the reservoir in established infections in humanized HIV- infected mice [130]. Currently, bnAbs still have challenges such as limited accessibility to certain anatomical reservoirs, as well as possible hurdles for clinical application related to their bioavailability, PK profile and high cost.

Many regulatory pathways designed to blunt cell activation are turned on during chronic infection, and one of the well-characterized immune regulators is the programmed cell death protein-1 (PD-1). For example, HIV-specific CD8 T cells that express high levels of PD-1 show functional exhaustion with low proliferation and cytotoxic effects [167]. The ligands for PD-1 (e.g., PD-L1 and PD-L2) are widely expressed in tissues, and inhibitors or blockades of the PD-1 pathway result in restoration of T cell function [168]. Thus, the blockades may enhance immune responses to clear chronic viral infections [169]. It has been demonstrated that the size of the reservoirs is positively correlated with the frequency of PD-1 expressing cells [49,170]. PD-1 inhibitors have been administered into SIV-infected macaques, and induce a significant viral load reduction and prolong survival [171]. It has been further shown that PD-1 blockade reduces hyperimmune activation in the blood and colorectal tissue and decreases microbial translocation [172].

Chimeric antigen receptor (CAR) T cell therapy has shown great promise in treating multiple cancers such as leukemia [173], and is also being investigated for HIV cure. The immunotherapy uses gene-editing tools to engineer T cells to express receptor on their surface in order to recognize and bind to specific target cells and then mediate cell lysis [174]. Previously, this technology has been applied to engineer CD8+ cytotoxic T cells to bind to and lyse HIV-infected CD4+ T cells [175], but has shown limited efficacy in clinical studies [176]. A recent study induced CAR onto HSPCs to consistently generate CAR T cells against SHIV infection in an NHP model of suppressive SHIV that are relevant to HIV cure research. They observed long-term (> 2 years) and stable production of CAR T cells in multiple lymphatic tissues, as well as a lower viral rebound after cessation of cART [177].

3.5. Other strategies

While early cART, reactivation of latently infected HIV reservoirs, gene therapy, and immunotherapy represent the most active areas of HIV cure research, several other approaches are also being actively pursued. One strategy known as "block and lock" is proposed to permanently inhibit transcription to prevent viral reactivation from latency, which may provide a functional cure for HIV infection. For example, ruxolitinib and tofacitinib are FDA-approved for myelofibrosis and rheumatoid arthritis (RA), respectively, and inhibit the JAK-STAT pathway [178,179]. Another agent, didehydro-cortistatin A (dCA) binds to the HIV regulatory protein Tat and suppresses transcription [180,181]. While most of these agents are still under preclinical studies, the JAK inhibitor ruxolitinib (clinicaltrials.gov # NCT02475655) is

currently being tested clinically in a phase 2 study. There is still concern about whether or not these agents alone are sufficient enough to permanently block viral reactivation, and they may need to be integrated with other strategies to realize an HIV cure in the future.

An innovative idea in cure research hypothesizes that reducing the proliferation of long-lived latently infected T cells could deplete the reservoir. Reeves et al. predicted that substantial reductions in the reservoir size may result from modest but continuous decreases in the proliferation rate of latently infected CD4 T cells with the use of mathematical modeling of the HIV reservoir under suppressive cART [20]. They identified that sustained anti-proliferation and ART treatment could potentially induce a functional cure within 2–10 years, which is much shorter than is predicted to occur with LRA and ART treatment. Mycophenolate mofetil (MMF) or its active metabolite, mycophenolic acid (MPA) have been tested in patients, and clinical trials for evaluating its effect on reducing reservoir size are currently enrolling patients [20] (clinicaltrials.gov # NCT03262441).

A recent study treated SIV+ macaques with a monoclonal antibody against the α4β7 integrin in combination with ART, and observed normal CD4+ T cell counts as well as undetectable viral loads in both plasma and GALT, even after cessation of ART [182,183]. α4β7 integrin is involved in trafficking of CD4+ T cells to GITs, where there are high levels of viral replication and viral reservoirs can be rapidly established [70]. CD4+ T cells that express high levels of α4β7 are also preferential targets of HIV during acute infection [74,75,184]. While the mechanisms by which α4β7 mAb therapy promoted virologic control remains to be defined, it is hypothesized that the antibody can block α4β7 binding to mucosal vascular addressin cell adhesion molecular 1 (MAdCAM-1) leading to interrupted cell migration to the GALT. It is noteworthy that α4β7 integrin is also a target for the HIV-1 envelop protein gp120, and binding leads to rapid activation of LFA that contributes to forming of virological synapses and facilitating cell-to-cell virus spreading [185]. More research efforts are needed to investigate this promising therapy for use in HIV treatment towards eradicating viral reservoirs.

4. Nanocarriers for eradicating HIV reservoirs

Many HIV cure strategies still face hurdles that limit their clinical translation. For example, clinical studies of LRAs have failed to meaningfully reduce reservoir size [42]. Sufficient reduction of the latent pool to curative levels may require mechanistically distinct LRAs used in combination. Many LRAs differ in solubility, bioavailability, and toxicity which make dosing and formulation challenging [131,186]. Gene-based therapies must overcome challenges associated with off-target effects, low delivery efficiency, as well as safety issues especially when using viral-based vectors *in vivo* [187]. The complexity of HIV infection and latency may also require complimentary strategies for curing HIV, making it important but challenging to deliver physicochemically diverse cure agents simultaneously. HIV persistence in some anatomical sites has been attributed to lower concentrations of ARV drugs [10,188], which could be addressed by novel drug delivery system to improve the bioavailability of cure therapeutics. New approaches are required to address these multiple complex molecular mechanisms associated with latent reservoir dynamics in cells and tissue compartments that present physiological barriers to therapeutic delivery *in vivo*. Nanotechnology has emerged as a promising approach for HIV cure due to several key attributes including ability to encapsulate diverse agents, increase circulation or tissue retention time, sustain drug release, enhance solubility and bioavailability, reduce toxicity or side effects, and enhance drug potency [22,189]. Some nanocarriers can also be modified to target specific cells or tissues, which have shown broad applications in cancer therapy [190]. Various types of nanocarriers including liposomes, polymeric nanoparticles, solid lipid nanoparticles, micelles, dendrimers, and inorganic nanoparticles have been reported for ARV drug delivery (reviewed in [191]). Some prodrugs or analogs of ARV drugs have also been developed and used to enhance drug loading or extend half-life when encapsulated in nanocarriers [192,193]. LRAs have been incorporated into nanocarriers for HIV cure applications and demonstrate CD4+ T cell specific reactivation both *in vitro* and *in vivo* [29,194]. Here, we summarize current nanocarrier-based anti-HIV therapeutics and focus on strategies using drug combination or targeted nanocarriers to enhance drug potency and reduce toxicity. We also review nanocarrier-based gene delivery and immunotherapy approaches that have attracted more attention in cancer therapy and may have potential applications for curing HIV.

4.1. Combination therapy using nanocarriers

Based on multiple cellular mechanisms that suppress viral reactivation in latent cells and from results of preclinical studies, LRA combinations have been proposed to achieve a clinical cure by maximizing potency and minimizing toxicity [131]. However, there are several challenges that may limit such combinations using conventional drug formulations [195]. Co-dissolving of multiple hydrophobic drugs for injection may result in precipitation or aggregation, inaccuracy of dosing and risk of embolisms. Also, different drugs have their own bioavailability and PK profiles that make it more difficult to reach sufficient and safe concentrations in target tissues when delivered simultaneously.

Several nanotechnology-based systems have been developed to deliver incompatible drug combinations simultaneously, which is especially important and beneficial for both ARV and LRA therapies being considered for HIV cure. Table 2 summarizes recent studies that use nanocarriers to co-deliver multiple anti-HIV drugs to enhance their therapeutic effects. ARV drugs are physicochemically diverse and are used clinically in combination, but have been incorporated in nanocarriers for the dominant purpose of enhancing potency and prolonging drug residence time to reduce dosing frequency [24,196–202]. For example, the Ho group has developed a long-acting triple-ARV drug combination using lipid nanoparticles that have shown enhanced drug exposure in primate blood and lymph nodes, as well as persistent drug levels in peripheral blood mononuclear cells (PBMCs) and lymph node mononuclear cells (LNMCs) [196,197,203]. The synergistic enhancement of antiviral activity may be attributed to higher intracellular ARV drug concentrations, which has been observed by several studies (Table 2). Similar to ARV drugs, LRAs are mostly small molecule compounds and are physicochemically and mechanistically diverse. Several LRAs have been incorporated into nanocarriers individually. For example, Buehler et al. have reported a self-assembling vault nanoparticle incorporating a PKC agonist bryostatin-1. They demonstrated its ability to reactivate latent virus in a human cell line and induce CD69 expression in primary human PBMCs and mice after intravenous administration. However, whether or not the nanoparticle had any effect on the potency of free drug was not investigated. From studies investigating co-delivery of ARV drug combinations, nanocarriers also have the potential to deliver LRAs in combination. To date, single LRAs have been co-delivered with ARVs using nanocarriers [29,30,204]. For example, Kovochich et al. loaded bryostatin-2 and a protease inhibitor nelfinavir into lipid nanoparticles. They observed that the nanoparticle formulation enhanced latency reactivation both in human T cell lines and PBMCs from humanized mice with the ability to control viral spreading. In another study, Jayant et al. established ultrasmall magnetic nanoparticles (MNPs) used a layer-by-layer process to incorporate tenofovir (ARV) and vorinostat (LRA) for reactivating and suppressing HIV in a sustained manner for 5 days *in vitro* [30]. These promising preclinical studies will need to demonstrate PK, safety and efficacy *in vivo* before advancing into human clinical studies. Prioritizing drug combinations that are safe and effective for HIV cure is also challenging since there is a large pool of multiple combinations that are difficult to test in complex, expensive models such as patient blood or HIV- or SIV-infected animal models. These examples

Table 2
Nanocarriers incorporating combination therapeutics.

Combination agents	Nanosystems	Test model	Observed effects	Ref.
ARVs (loponavir, ritonavir, and tenofovir)	Lipid nanoparticles	Nonhuman primates	Higher intracellular ARV concentrations in lymph nodes (50-fold higher than free drugs), long-acting plasma and lymphatic PK profiles	[197,198,204]
ARVs (loponavir/ritonavir and efavirenz)	Biodegradable nanoparticles	*In vitro* cell lines	Higher intracellular ARV concentration	[199]
ARVs (binary or trinary combination of maraviroc, etravirine and raltegravir)	PLGA nanoparticles	*In vitro* cell lines, and *ex vivo* macaque cervicoviginal tissue	Higher intracellular ARV concentration, enhanced antiviral activities in relevant *in vitro* and *ex vivo* models	[24]
ARVs (tenofovir, alafenamide, and elvitegravir)	PLGA nanoparticles	Mice	Long residence time and exposure for both drugs	[200]
ARVs (atazanavir, ritonavir, and efavirenz)	NanoART	*In vitro* cell lines, *ex vivo* human primary cells, and *in vivo* in humanized mice	Attenuated viral replication and preserved CD4+ T cell numbers	[201,202]
ARVs (abacavir and lamivudine)	Glucose-coated gold nanoparticles	*In vitro* cell lines	Co-deliver and pH-mediated release of the drug and similar antiretroviral activity compared to free drug	[203]
ARVs (NNRTI: DAAN-14f, and HIV-1 fusion inhibitor: T1144)	PEG-PLA nanoparticles	*In vitro* cell lines and *in vivo* pharmacokinetic studies in rats	Enhanced *in vitro* antiviral activity against various HIV-1 strains and sustained controlled release both *in vitro* and *in vivo*	[206]
ARVs (zidovudine, efavirenz, and lamivudine)	Lactoferrin nanoparticles	*In vitro* cell lines and *in vivo* pharmacokinetic and safety studies in rats	Enhanced *in vitro* antiviral activity and improved pharmacokinetic and biodistribution profiles *in vivo*	[207]
LRA (bryostatin-2) + ARV (nelfinavir)	Lipid nanoparticles	*In vitro* cell lines, and *ex vivo* cells from an HIV-infected humanized mouse model	Reactivated latent virus and inhibited viral spreading simultaneously	[29]
LRA (vorinostat) + ARV (tenofovir)	Magnetic nanoparticles	*In vitro* HIV-infected human astrocytes	Reactivated latent virus and suppressed the viral replication	[30]
LRA (vorinostat) + ARV (nelfinavir)	PLGA-PEG nanoparticles	*In vitro* cell lines	Reactivated latent virus and suppressed the viral replication	[205]
ARV (nelfinavir) + sigma receptor antagonist (rimcazole)	Magnetic nanoparticles	*In vitro* cell lines	Mitigated co-effect of drug of abuse and inhibited HIV-1 infection	[208]
siRNAs (TNPO3, CD4, *tat, rev*)	PAMAM dendrimer	*In vitro* cell lines, *ex vivo* human primary cells, and *in vivo* in humanized mice	Suppressed HIV-1 infection and protected mice from CD4+ T cell loss	[209]
siRNAs (*p24*, *gag1*, *nef*)	Carbosilane dendrimers	*In vitro* cell lines, *ex vivo* human primary cells	Reduced HIV-1 replication	[210]
siRNAs (CCR5, *vif*, and *tat*)	Peptide carriers	*In vitro* cell lines and *in vivo* humanized mouse models	Suppressed HIV-1 replication and prevented mice from CD4+ T cell loss	[153]
siRNA (*tat/rev*) + RNA aptamer (gp120)	RNA nanoparticles	*In vitro* cell lines and *in vivo* humanized mouse models	Suppressed HIV-1 replication and prevented mice from CD4+ T cell loss	[211,212]

ARV, antiretroviral drugs; dsiRNA, dicer substrate small interfering RNA; NNRTI, Non-nucleoside reverse-transcriptase inhibitors; PAMAM, poly(amidoamine); PEG-PLA, polyethylene glycol–polylactic acid; PLGA, poly (lactic-co-glycolic acid).

demonstrate that nanocarriers can overcome challenges with co-formulating physicochemically diverse drugs. Due to the higher potency of some combination drug therapies, dose-reduction could reduce toxicity that may be associated with single drugs. The toxicity associated with LRAs arises primarily from nonspecific induction and systemic release of cytokines [132]. Using biodegradable and non-toxic nanoparticles may further protect drug from degradation, increase circulation half-life and exhibit improved PK profiles, and lowering toxicity [22]. Thus, nanotechnology could provide alternative strategies to optimize latency reversal therapies. Future studies are being directed towards using a single nanocarrier to combine delivery of mechanistically distinct LRAs alone or co-formulated with other small molecule cure agents.

Many other HIV cure agents such as antiproliferative agents, bnAbs, and oligonucleotides drugs may also need combinations for achieving improved efficacy in eradicating HIV reservoirs. For example, antiproliferative agents are required to be dosed with ARVs in order to suppress both viral replication and reservoir proliferation, and show potential benefits when combined with LRAs [20]. Preclinical studies in humanized mice have demonstrated improved anti-HIV effects from treatment with a combination of multiple bnAbs [212,213] as well as single bnAbs combined with ARV drugs [214]. Combination gene therapies that target multiple HIV transcriptional genes as well as expression of CCR5 are being investigated to address rapid viral mutations and the complexity of HIV infection and latency maintenance [215].

Such combinations of antibodies, or nucleic acids with small molecules can be even more challenging due to their incompatibility, requirement of different vectors and administration routes, various PK or pharmacodynamic (PD) properties, and site of action. Nanocarriers have demonstrated their utility for co-delivering combinations of antibodies with chemotherapeutics [216], different RNAi targets [217], or siRNA with miRNA [218]. Due to the complexity of HIV integration sites and rapid mutation, combination might also bolster efficacy even for single gene therapies such as delivery of multiple siRNA. For example, Zhou et al. demonstrated the ability of poly(amidoamine) (PAMAM) dendrimer to deliver a combination of siRNAs to suppress HIV infection [208]. These siRNAs target viral as well as cellular transcripts for minimizing viral escape mutants and resulted in prolonged HIV suppression in humanized mice. However, dendrimer-siRNA nanoparticles primarily accumulated in PBMCs and liver, and biodistribution data was not provided for other major lymphatic tissues such as lymph nodes and GALT. Further investigation on biodistribution and PK profiles of these nanoparticles are needed to develop nanocarriers that can deliver multiple agent more efficiently to different target sites relevant for HIV cure.

4.2. Targeting tissue and cellular HIV sanctuaries by nanocarriers

The compartments that harbor HIV are primarily the blood, lymphatic systems (e.g. lymph nodes, GALT, spleen, etc.), and CNS. Inconsistent ARV drug penetration and accumulation in some of these sites is a challenge for effective HIV treatment and can lead to HIV persistence [10]. Targeted nanocarriers have been investigated for delivering ARV drugs or HIV cure agents to specific cells or tissues that harbor latent virus (Table 3).

Cell-specific accumulation of nanocarriers is achieved by the use of targeting moieties. Due to their high specificity, antibodies have also been widely used for targeting nanocarriers to T cells in various applications [26–28,219,238]. It has been suggested that anti-CD4 monoclonal antibodies, or its fragments, are good candidates to direct negatively-charged nanoparticles to CD4+ T cells [239]. Kovochich et al. reported lipid nanoparticles decorated with an anti-CD4 antibody for targeting both LRAs and ARVs to primary human CD4+ T cells in order to activate latent virus and inhibit viral spread [29]. The CD4 antibody led to specific targeting of nanoparticles to CD4+ T cells in PBMC culture, as well as preferential activation on CD4+ T cells over CD8+ T cells. This system shows great potential in systemic T cell targeting, however, PK profiles and biodistributions in some key HIV anatomical reservoirs were not investigated but would be important for future applications. Among CD4+ T cells, latent HIV viruses typically reside in resting memory (CD45RO+) T cells. Tang et al. conjugated anti-CD45RO antibody to PLGA-PEG nanoparticles that were loaded with a HDACi and protease inhibitors for targeting and eliminating latent HIV reservoirs *in vitro* [204]. They demonstrated that CD45RO antibody enhanced nanoparticle binding to human memory T cells, but the binding specificity was not measured and it is unclear how targeting improved drug potency. Aside from CD4+ T cells, macrophages are another target for ARV drug delivery and have been found to store and transport ARV drugs to lymph nodes and the CNS. Puligujja et al. developed folic acid (FA)-linked polymer-coated nanoformulated antiretroviral therapy (FA-nanoART), that induced five-fold enhanced plasma and tissue drug levels in mice and enabled 2.5-fold improvement in treating compared to untargeted nanoART [222,223]. These targeted nanocarriers demonstrate the potential for applications in HIV cure research, but most are still in early stages of development and need to be tested in relevant animal models. Also, there is a need of more options to select binding ligands aside from whole antibodies to overcome high costs, nonspecific conjugation to nanocarriers, and effect on reducing nanocarrier circulation half-life for *in vivo* cellular targeting applications [240].

Several studies have focused on developing nanocarriers that preferentially distribute to lymph nodes after subcutaneous administration to address insufficient drug concentrations in these tissue [241,242]. Therapeutics administered subcutaneously are preferentially taken up by blood capillaries or lymphatic vessels depending on their physicochemical properties. Small molecular drugs, peptides, and proteins (< 16 kDa) have been shown to be absorbed by blood capillaries while particulates between 10 and 100 nm diameter are transported through the interstitium and preferentially into the lymphatic system [243]. It has been reported that a lipid-drug or lipid nanoparticles (50–80 in diameter) enhanced delivery of ARV drugs to lymph nodes, increased intracellular drug concentration, acted as a long-acting dosage form, and led to significant virus load reduction after subcutaneous administration to NHP models [196,197,225]. Such nanoparticle systems can be potentially used to enhance accumulation of other HIV cure agents in the lymph nodes, or direct multiple drugs with different PK profiles to lymph nodes for eradicating latent virus.

The GALT is an important site for early HIV replication and reservoir establishment, and has also shown significantly lower ARV concentrations compared with peripheral blood in patients under ART that are considered fully suppressive [10]. Nanoparticles have been used to target the GALT for various applications. For example, a variety of biodegradable antigen delivery systems have been developed for oral vaccines. Some of these were decorated with microfold cells (or M cells)-specific ligands to actively target delivery of nanoparticles to these cells in the GALT that transport nanoparticles across the intestinal epithelium [244]. Some limitations for this M cell targeting strategy may be the low efficiencies for GALT delivery due to a small percentage of M cells in the follicle-associated epithelium [245]. Peers et al. used antibodies against the β7 integrin to target mutilamellar vesicles to specific leukocytes subsets involved in gut inflammation, showing more than 10-fold higher accumulation in the gut compared to an isotype antibody control group administered intravenously in mice [26]. These nanocarriers have been barely studied in the HIV field, but have potential to deliver HIV cure agents to the GALT.

Nanotechnology-based methods are also being developed to deliver ARVs into the brain, which shows limited accessibility of many drugs and is another key anatomical reservoir harboring the virus [76,77,246]. Most investigations focus on crossing the BBB by developing nanocarriers with increased permeability, uptake, or transcytosis by brain microvascular endothelial cells (BMVECs) [247]. Many types of nanocarriers have been applied for the management of HIV infection in the CNS such as liposomes [226], micelles [227], nanoemulsions

Table 3
Nanocarriers targeting HIV sanctuaries.

Target	Targeting ligands	Therapeutics	Nanosystems	Test model	Reference
Cellular Reservoirs					
Leukocytes	LFA-1	anti-CCR5 siRNA	Liposome	*Ex vivo* human lymphocytes and *in vivo* humanized mouse model	[220]
CD7-expressing T cells	Anti-CD7 single-chain antibody	siRNAs (CCR5, *vif* and *tat*)	Peptide carrier	*In vitro* cell lines and *in vivo* humanized mouse model	[153]
CD4 + T cells	CD4-binding peptides	ARV (indinavir)	Lipid nanoparticles	*In vitro* cell lines	[221,222]
	anti-CD4 antibody	LRA (bryostatin-2) + ARV (nelfinavir)	Lipid nanoparticles	*In vitro* cell lines and *ex vivo* latently infected cells from humanized mouse model	[29]
Resting memory T cells	CD45RO	LRA (vorinostat) + ARV (nelfinavir)	PEG-PLGA nanoparticles	*In vitro* cell lines	[205]
Macrophage	Folate	ARV (atazanavir)	Polymer coated ARV nanoformulations (NanoART)	*In vitro* cell lines, *ex vivo* human primary cells, and *in vivo* mice	[223,224]
Astrocytes	bradykinin B2 antibody	siRNA	Chitosan nanoparticles	*In vitro* cell lines	[225]
Target	Administration route	Therapeutics	Nanosystems	Test model	Reference
Anatomical Reservoirs					
Lymph nodes	SC	ARV (Indinavir)	Lipid-drug complexes	HIV-2287-infected macaques	[226]
	SC	ARV (lopinavir, ritonavir, and tenofovir)	Lipid nanoparticles	Pig-tailed macaques	[197,198]
CNS	N/A	ARV (azidothymidine 5′-triphosphate, AZTTP)	Magnetic liposomal nanoformulation	*In vitro* BBB model	[227]
	IN	ARV (efavirenz)	PEO-PPO micelles	Rats	[228]
	IV	ARV (indinavir)	Transferrin coupled submicron lipid emulsions	Mice	[229]
	Oral or IV	ARV (saquinavir)	Nanoemulsion	Mice	[230]
	IP	ARV (azidothymidine)	Nanogel decorated with the peptide binding brain-specific apolipoprotein E receptor	Mice	[231]
	IV	ARV (nevirapine)	Surface modified nanosuspensions	Rats	[232]
	N/A	ARV (stavudine, delavirdine, or saquinavir)	PBCA, MMA-SPM, and SLN	*In vitro* BBB model, human brain-microvascular endothelial cells (HBMEC)	[233,234]
	IV	ARV (ritonavir)	TAT peptide conjugated PLA nanoparticles	Mice	[235]
	SC	ARV (atazanavir and ritonavir)	macrophage-carriage system for nanoformulated crystalline ART (nanoART)	*In vitro* cell lines, *ex vivo* human primary cells, and *in vivo* mice	[236]
	Retro-orbital injection	siRNA (*nef*)	carbosilane dendrimer	*In vitro* BBB model, human brain-microvascular endothelial cells (HBMEC), and *in vivo* mice	[237,238]

ARV: antiretroviral drugs; BBB: blood brain barrier; CNS: central neural system; LRA: latency-reversing agents; MMA-SPM: methylmethacrylate-sulfopropylmethacrylate; GALT: gut-associated lymphoid tissue; IN: intranasal; IP: intraperitoneal; IV: intravenous; PBCA: Polybutylcyanoacrylate; PEG-PLGA: Poly(ethylene glycol) methyl ether-block-poly(lactide-co-glycolide); PEO-PPO: poly(ethylene oxide)-poly(propylene oxide); PLA: Poly(lactic acid); SLN: solid lipid nanoparticle; TAT: trans-activating transcriptor; SC: subcutaneous.

[228,229], nanogel [230], nanosuspensions [231], and polymeric nanoparticles [232–234]. Ligands such as transferrin and trans-acting transcriptional activator (TAT) peptide are widely used for targeting nanocarriers to the brain. For example, transferrin has been linked to indinavir lipid nanoemulsions and resulted in up to 5 times higher bioavailability in brain compared to free drug [228]. This has been attributed to the overexpression of transferrin receptors on brain cells that can mediate endocytosis and transcytosis of transferrin-coupled substances. The TAT-peptide has been shown to penetrate through cell membranes and enhance transport of nanocarriers across the BBB. Rao et al. demonstrated that TAT-conjugated poly (L-lactide) (PLA) nanoparticles efficiently enhance CNS bioavailability and maintain drug level of encapsulated ritonavir in the brain [234]. A peptide that binds to brain-specific apolipoprotein E receptor has also been decorated to cationic nanogels for delivering nucleoside reverse transcriptase inhibitors (NRTIs) against HIV infection in the brain [230]. The macrophage-targeting nanoART described above showed the ability to be transferred from macrophages to human brain microvascular endothelial cells (HBMECs), and led to four-fold higher ARV levels in the mice brain compared to untargeted nanoART [235]. Alternative delivery routes such as intranasal delivery also offer a non-invasive way to transport drugs through olfactory neurons to the brain [227]. These solutions have demonstrated significant increases of ARV drug concentrations in the brain compared to free drug administration, therefore showing unique promise as efficient tools to deliver HIV cure agents into the brain.

4.3. Future directions: nanocarrier-based gene- or immunotherapy

Gene therapy offers promising solutions in treating multiple diseases including HIV, and has greatly advanced since the discovery of CRISPR as an easy and robust gene-editing tool. While current gene therapy for HIV cure has mostly relied on viral vectors, the advances in nanocarrier-based gene delivery technology may enhance its impact on the HIV cure field due to its lower risk, potential targeting abilities, and lower off-target effects. Some big hurdles for applying gene therapy to HIV cure research, particularly for strategies proposing to knock-out HIV provirus in the host genome, are low efficiency and targeting specificity *in vivo*. Currently, viral vectors such as adenoviruses and AAVs are still the most widely used for *in vivo* gene delivery due to their relatively high transfection efficiency but have associated concerns with off-target effects, immunogenicity, and toxicity. Non-viral vectors such as nanocarriers have been investigated as an alternative and safe way to deliver genes (reviewed in [248–250]). Studies have used lipid-based or polymer-based nanocarriers, or dendrimers as non-viral vectors to deliver exogeneous nucleic acids such as DNA, mRNA, siRNA and miRNA [249]. Many reports use nanoparticles for anti-HIV siRNA delivery [153,208–211,219,236,237,251–255]. For example, Weber et al. have used carbosilane dendrimers to deliver siRNAs targeting HIV *p24*, *gag1*, or *nef* genes [209]. The dendrimer-siRNA complex showed highest transfection efficiency in both cell line and HIV-infected PBMCs without causing cytotoxicity, and protected siRNA from rapid degradation in the presence of RNase. A similar dendrimer system was also tested in mice and efficient siRNA transport across BBB was observed [237]. More investigations on siRNA transfection *in vivo,* particularly anti-HIV effects in relevant animal models are needed for future clinical applications. There are relatively few examples of using non-viral vectors to deliver whole genome editing systems such as ZFNs and CRISPR-Cas9 systems because they are large and complex [250]. Recently, Lee at al. developed a polymer-coated gold nanoparticle that can co-deliver CRISPR components, including the Cas9 and gRNA ribonucleoprotein (RNP) complex and donor DNA, resulting in efficient correction on the mutated dystrophin gene with low off-target effects [256].

HIV therapeutic vaccines, bnAbs, PD-1 blockades, and CAR T cell therapy have all shown promise in HIV cure but also have limitations such as low delivery efficiency to specific tissues or cells. Over the past decades, nanocarrier-based delivery platforms such as liposomes, polymeric nanoparticles, lipid-polymer hybrid nanoparticles, and virus-like nanoparticles have been used as carriers for vaccine delivery in the field of cancer immunotherapy (reviewed in [257]). The advantages of nanocarriers for delivering vaccines include simultaneous delivery of multiple antigens and adjuvants, rapid endocytosis by immune cells (especially DCs and macrophages), and accumulation to lymphatic tissues based on their size [257]. These attributes could be employed for nanocarriers to deliver HIV immunotherapeutics to specific cells or tissues that are inaccessible with current dosage forms.

PD-1 blockades, when delivered systematically, have concerns with systemic immune stimulation that is associated with some immune-related adverse effects [258]. The use of a drug delivery system to enhance accumulation of these blockades to specific cells or tissues may reduce these side effects. For example, Kosmides et al. developed nanoparticles decorated with both anti-PD-L1 antibody and anti-4-1BB antibody (a costimulatory molecule), and observed increased targeting of CD8+ T cells, enhanced anti-tumor activities and low off-target toxicity *in vivo* [259]. Alternatively, Schmid et al. have used CD8 and PD-1 dual-targeting nanoparticles to deliver a TGFβR1 inhibitor, another immunostimulatory drug, to restore CD8+PD-1+ T cell functions for killing cancer cells [260]. These delivery systems may also be applied in delivering PD-1 blockade with other stimulators for targeting and activating HIV-specific CD8+ T cells that highly express PD-1. This targeted delivery system could achieve specific restoration of T cell function towards eradication of HIV infection.

Current CAR T cell-based immunotherapy that engineers T cells *ex vivo* may be too elaborate for widespread implementation for HIV and cancer treatment. This therapy involves complex procedures that requires expensive equipment and technical expertise for T cell isolation, modification, and expansion followed by infusion back into patients. Nanocarriers could offer an alternative and inexpensive solution by delivering modifying genes to program host T cells *in vivo*. Recently, Smith et al. have developed nanoparticles decorated with anti-CD3 antibody and loaded with leukemia-targeting CAR genes to target and engineer tumor-specific T cells directly *in situ* [261]. Such technology has potential to be applied to CAR T cell strategy to engineer CD8+ cytotoxic T cells *in vivo* to kill HIV-infected CD4+ T cells.

5. Conclusion

A variety of strategies have been developed to minimize HIV reservoirs, but several obstacles remain, such as insufficient potency and off-target effects in normal cells that may also result in dose-limiting toxicities [14]. Nanocarriers offer a promising treatment for HIV infection by enhancing drug potency using synergistic combinations, or targeting specific and hard-to-reach sites that harbor virus. Nanocarriers also offer advantages compared to traditional delivery systems that include encapsulation of physicochemically diverse agents, sustained drug release, lower drug dosing, better bioavailability, and fewer or less severe side effects. These promising properties of nanocarriers help address problems in current novel strategies toward HIV cure, such as LRA combination for reactivating latently infected cells. There are still many hurdles that remain in the translation of nanocarriers from the lab to the clinic. Interdisciplinary collaborations on nanocarrier development and evaluation, as well as appropriate *in vitro*, *ex vivo* and *in vivo* models are the best way to ensure rapid translation of nanomedicine for curing HIV.

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