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Curr Opin Immunol. Author manuscript; available in PMC 2013 October 01.

Published in final edited form as:
Curr Opin Immunol. 2012 October ; 24(5): 625–632. doi:10.1016/j.coi.2012.08.013.

Creating Genetic Resistance to HIV

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Abstract

HIV/AIDS remains a chronic and incurable disease, in spite of the notable successes of highly active antiretroviral therapy. Gene therapy offers the prospect of creating genetic resistance to HIV that supplants the need for antiviral drugs. In sight of this goal, a variety of anti-HIV genes have reached clinical testing, including gene-editing enzymes, protein-based inhibitors, and RNA-based therapeutics. Combinations of therapeutic genes against viral and host targets are designed to improve the overall antiviral potency and reduce the likelihood of viral resistance. In cell-based therapies, therapeutic genes are expressed in gene modified T lymphocytes or in hematopoietic stem cells that generate an HIV-resistant immune system. Such strategies must promote the selective proliferation of the transplanted cells and the prolonged expression of therapeutic genes. This review focuses on the current advances and limitations in genetic therapies against HIV, including the status of several recent and ongoing clinical studies.

Keywords

HIV; gene therapy; HSCs

Introduction

The antiretroviral drug cocktail known as combination antiretroviral therapy (cART) potently inhibits HIV replication, reduces the rates of transmission, and enhances the quality and duration of life of patients. However, cART does not fully restore health and patients still experience chronic inflammation, immunosenescence, and increased risks of non-AIDS morbidity and mortality [1, 2]. It requires daily compliance and lifelong adherence, indefinitely extending the issues of side effects, cost, and drug resistance. Finally, cART does not eliminate latent reservoirs of virus in patients [3] and may fail to suppress completely viral replication despite drug intensification strategies [4, 5].

As an alternative to cART, gene therapy aims to confer "intracellular immunization" by genetically engineering cells for HIV resistance [6]. Such approaches typically utilize one or more therapeutic genes that inhibit steps within the viral infection and replication cycle including RNA-based therapeutics, gene editing enzymes, or protein inhibitors to target viral or host factors. Genetic resistance to HIV in protected cells may be accomplished by encoding therapeutic genes within an integrating vector, such as a retroviral, lentiviral,

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foamy viral, or transposon vector [7]. Alternatively, endogenous genes that are required for HIV infection and not normal cell function can be mutated or deleted using site-specific enzymes. Many anti-HIV gene therapy strategies rely on cell-based delivery, including the *ex vivo* transduction and subsequent transplantation of cells that are natural targets for HIV infection, including CD4+ T lymphocytes and macrophages. Similarly, the transplantation of genetically modified CD34+ hematopoietic stem cells (HSCs) offers further advantages, such as the ability for self-renewal and differentiation into all hematopoietic lineages, including CD4+ T cells, macrophages, and dendritic cells. Likewise, induced pluripotent stem cells (iPSCs) can be reprogrammed into HSCs and further engineered for HIV resistance [8, 9].

Gene therapy targets against HIV

HIV encodes nine viral genes (*gag, pol, vif, vpr, tat, rev, vpu, env,* and *nef*) that control all steps of the viral replicative cycle including entry into the targeted cells, reverse transcription, integration into the host genome, transcription of viral mRNA, assembly of viral proteins, and budding of the newly formed virions. Each of these genes and steps within the viral replication cycle serves as a potential target for novel gene therapeutics. Moreover, gene-based strategies offer the possibility of inhibiting viral genes at the level of DNA, RNA, or protein, thereby expanding the scope of druggable targets for HIV. Such strategies typically require the insertion of therapeutic genes into uninfected cells, though many antiviral genes also inhibit viral replication in infected cells. However, like cART, antiviral gene therapies are susceptible to mutational escape, as the virus can mutate its genome at positions targeted by the therapeutics.

Endogenous genes that are required for HIV infection and/or replication offer several advantages over viral targets. In contrast to viral targets, host genes are not as prone to escape from mutation or saturating levels of virus. Second, host factors may be genetically modified in cells prior to HIV infection, thereby “immunizing” them from the virus. Nevertheless, the virus may evolve to bypass the need for certain host factors, such as the CCR5 co-receptor, or it may produce viral proteins that directly combat host restriction factors (Vif and Vpu for APOBEC 3G and Tetherin).

Design principles for anti-HIV gene therapy

Theoretical models predict that therapeutic genes that inhibit HIV steps prior to integration (Class I) are more effective in promoting the survival and expansion of protected cells than agents that inhibit latter stages of the HIV replication cycle such as viral gene expression (Class II) or viral assembly and budding (Class III) [10, 11]. Importantly, these predictions are consistent with a recent *in vivo* study that concluded that the maC46 fusion inhibitor resulted in higher positive selection of transduced cells than an antisense transcript against the HIV-1 *env* mRNA or a short-hairpin RNA (shRNA) against the HIV *tat/rev* mRNA [12]. Similarly, another computational study investigated several key design principles for effective gene therapy strategies including the potency of viral inhibition in protected cells, the proliferation enhancement of protected cells, the potential value of splitting multiple therapies across cells, and the benefits of small but significant fitness costs associated with viral escape mutations [13].

Hope for a cure (Berlin patient)

In addition to the presence of the CD4 surface receptor on T lymphocytes, HIV requires either co-receptor chemokine receptor type 5 (CCR5) or chemokine receptor type 4 (CXCR4) for viral entry. Inhibition of either CCR5 or CXCR4 co-receptors by gene therapy has been extensively studied in laboratory and animal models of HIV infection, and several therapeutic applications for CCR5 knockdown have reached clinical trials [7]. In fact, CCR5

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offers a promising target for anti-HIV therapeutics, as it is not essential for normal T cell function and homozygous carriers of a CCR5 partial gene deletion (*CCR5Δ32)* are naturally resistant to HIV infection. The concept of targeting CCR5 by gene therapy is bolstered by the famous "Berlin patient" study, in which an HIV patient with acute myeloid leukemia (AML) received CD34+ hematopoietic stem cells (HSCs) from a donor with a naturally occurring homozygous mutation in the CCR5 gene (*CCR5Δ32*), which led to the elimination of all detectable HIV from the patient after 4 years and an apparent functional "cure" of HIV infection [14, 15]. The transplanted CD34+ HSCs differentiated into all hematopoietic lineages, including HIV-resistant CD4+ lymphocytes and macrophages. Due to the preexisting AML, this patient required an allogeneic bone marrow transplant with complete bone marrow ablation and immune suppression, although these invasive procedures are not justifiable options for the treatment of HIV. Furthermore, due to the extremely low frequency of individuals who carry the *CCR5Δ32* allele (5%–14% in individuals of European descent and much less common in individuals of African and Asian descent), finding an HLA-matched donor with HIV-resistant allele is an extraordinarily rare achievement [16]. It is likely that the graft versus host allogeneic effect was critical for elimination of detectible HIV in this patient, and this raises the question of whether gene modified autologous blood stem cells could be as successful. It might be necessary to engineer a selectable marker into such modified cells to ensure adequate expansion of protected progeny, as has been shown in a non-human primate model [17]. Thus, though the case of the "Berlin patient" is not directly applicable for other HIV patients, it strongly supports the notion for an HIV cure and underscores the potential of creating HIV resistance using many of the gene therapy strategies discussed forthwith.

Intracellular Immunization

Gene editing enzymes

The recent development of site-specific DNA editing proteins, including zinc-finger nucleases (ZFNs), has fueled the interest of permanently altering and inactivating a range of host or viral genes necessary for HIV infection and replication [1]. One of the earliest and most promising gene editing therapeutics is a ZFN from Sangamo BioSciences (Richmond, CA, USA) that disables the endogenous *CCR5* gene by creating double-strand breaks and imperfect repair by nonhomologous end joining (NHEJ) at a location upstream of the natural *CCR5Δ32* mutation [18]. This ZFN may be delivered *ex vivo* to human CD34+ HSCs or primary CD4+ T cells by adenoviral vectors [18], integrase-deficient lentiviral vectors [19], or nucleofection [20]. The adenoviral-delivered CCR5 ZFN in autologous CD4+ T cells, known as SB-728-T, is currently in clinical testing in two Phase 1 trials (NCT00842634 and NCT01044654) and two Phase 1/2 studies (NCT01543152 and NCT01252641). While all of these trials use the same SB-728-T therapy, they examine different patient cohorts, including viremic patients who have never received cART, viremic patients who have multidrug cART resistance, aviremic patients who remain on cART, and aviremic patients who have volunteered for scheduled treatment interruptions (STI). These multi-cohort studies aim to evaluate the efficacy and safety of SB-728-T, as well as to explore different conditions that might improve the engraftment and expansion of protected cells.

Although both *CCR5* and *CXCR4* ZFNs function independently, it might be necessary to eliminate both to immunize CD4+ T cells from HIV infection or to combine the *CXCR4* ZFN therapy with the CCR5-specific fusion inhibitor maraviroc. Indeed, elimination of *CXCR4* without the removal of *CCR5* led to the eventual emergence of CCR5 (R5)-tropic virus in NSG mice transplanted with human CD4+ T cells [21]. Despite the risks associated with removing *CXCR4* without also disrupting *CCR5*, adenoviral delivery of the *CXCR4* ZFN provided superior protection from HIV infection over an integrated lentiviral vector expressing CXCR4 shRNAs in NSG mice transplanted with human CD4+ T cells [22].

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While ZFN approaches have aimed at genetically modifying the host *CCR5* and *CXCR4* genes to prevent HIV infection, other gene editing approaches target the viral gene locus in HIV-infected cells. These include an engineered HIV-specific homing endonuclease [23] and an LTR-specific recombinase (Tre-recombinase) that recognizes asymmetric sequences within the 5’ and 3’ LTRs flanking the viral genes [24, 25]. Although such potential therapeutics exist in earlier development stages than their ZFN counterparts, they offer the ability to eliminate HIV from infected cells, including latently infected cells which are currently untreatable by any clinical therapy.

Protein-based inhibitors

Another strategy to block HIV entry utilizes a fusion inhibitor known as C46, a 46-amino acid cell membrane-anchored peptide derived from the second heptad repeat of the HIV envelope glycoprotein gp41 [26]. In a Phase I clinical trial of ten HIV-infected patients, expression of C46 from a gammaretroviral vector (M87o) in autologous CD4+ T cells persisted for up to a year post-transplantation with no observed adverse events [27]. A Phase I-II clinical study at the University Medical Center Hamburg-Eppendorf continues to evaluate the M87o vector in autologous CD34+ HSCs for patients with high-risk AIDS-related lymphoma (NCT00858793). Preclinical studies support the efficacy of the C46 peptide, as its expression from a lentiviral vector protected infection in HSCs in a macaque model of HIV [28] and in primary CD4+ T cells in a humanized mouse model [12]. Furthermore, the C46 peptide can also be expressed as a secreted antiviral entry inhibitor (SAVE), which provides a bystander effect for neighboring unmodified cells [29]. However, C46 is subject to resistance induction by selection of envelope mutations [30], and it will likely be necessary to use C46 as part of combinatorial gene therapy strategies.

In addition to the surface receptors required for viral entry, knockdown screens have identified hundreds of unique host genes that are necessary for HIV infection and replication and may offer targets for novel antiretroviral therapies [31]. One such factor required for HIV integration is the lens epithelium-derived growth factor (LEDGF/p75), and overexpression of the C-terminal portion that contains the HIV-1 integrase (IN)-binding domain (IDB) can inhibit viral replication in both human CD4+ T cells and in the humanized NSG mouse model [32

Alternatively, many types of nonhuman cells, such as murine and simian, are naturally resistant to HIV infection due to species-specific “restriction factors” that could be therapeutically exploited in gene therapy applications, such as APOBEC3G, tripartite motif 5α(TRIM5α), and BST-2/tetherin (reviewed in [33]). Among such restriction factors, engineered versions of APOBEC3G [34–36] and TRIM5 α[37, 38] inhibit HIV infection and replication in preclinical gene therapy applications.

RNA-based therapeutics

RNA-based therapeutics offer several advantages as anti-HIV genes including high levels of expression, low immunogenicity, and design flexibility against a range of viral and cellular targets. Hence, several RNA-based strategies have reached early stages of clinical testing. RNA-based drugs may be classified by the mechanism of activity such as catalytically active RNA molecules (ribozymes), transcripts that inhibit RNA translation by Watson-Crick base-pairing (antisense), RNAs that bind to proteins and other molecular ligands (decoys/aptamers), and small interfering RNAs (siRNAs) that induce RNA interference (RNAi) [39].

Ribozymes

Ribozymes are catalytically active RNAs that can be engineered to induce site-specific cleavage of target RNA molecules in *cis* (the same nucleic acid strand) or in *trans* (a

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noncovalently linked nucleic acid). The first clinical trials using ribozymes consisted of anti-HIV hairpin or hammerhead ribozymes encoded in the attenuated Moloney murine leukemia virus (MMLV) gammaretroviral vector that targeted well-conserved positions in HIV mRNA transcripts [40, 41]. The primary objectives of these trials were to assess the safety of the ribozyme and to determine whether the ribozyme imparted a survival advantaged for the protected cells. Two Phase I clinical trials have examined different anti- HIV ribozymes in autologous [40, 42] and syngeneic CD4+ T lymphocytes [41, 43], while a third anti- HIV ribozyme in autologous CD34+ hematopoietic progenitor cells (HPCs) has reached Phase II trials (NCT00074997 and NCT01177059) [44, 45]. While these trials demonstrated the safety and feasibility of gene-derived ribozymes, none demonstrated a clear survival advantage for the protected cells vs. the empty vector (control) transduced cells.

Antisense

RNA antisense transcripts recognize and bind their cognate mRNA targets by Watson-Crick base pairing to inhibit the function of the sense viral mRNAs. By generating long dsRNA complexes with the target (sense) mRNA, antisense transcripts block the processing of viral mRNA transcripts, induce adenosine to inosine RNA editing by ADAR (adenosine deaminase that acts on RNA), and trigger the degradation of hyper-edited mRNA transcripts by inosine-specific nucleases [46]. VIRxSYS Corporation has developed a gene therapy approach against HIV-1 using a “conditionally replicating” lentiviral vector (VRX496) that encodes a 937 bp antisense sequence against the HIV *env* region of viral transcripts. While VRX496 does not encode any viral genes, it is engineered with full-length 5’ and 3’ long terminal repeats (LTR) and other essential structural nucleic acid elements that enable its replication only in cells also infected with wildtype HIV [47]. While the “conditionally replicating” feature of this gene therapy strategy is designed to provide additional therapeutic protection, highly efficient inhibition of HIV by the therapeutic vector may severely limit the mobilization and persistence of the vector [48, 49].

In a Phase I clinical trial for five HIV patients having resistance to multiple CART regimens, autologous CD4+ T cells were harvested from the patients, transduced *ex vivo* with the VRX496 antisense vector, and transplanted back into the patients. In this trial the T-cell-based infusion of VRX496, referred to as Lexgenleucel-T, was well tolerated for all patients with no detectable insertional mutagenesis, clonal outgrowth, or immunogenicity from the lentiviral vector [47]. The integrated VRX496 DNA was detected for at least one year in PBMCs from two subjects and VRX496 genomic RNA mobilization in plasma was observed for up to two months. Two follow-up Phase II trials (NCT00622232 and NCT00131560) in cART-failed subjects compared single or multiple doses of Lexgenleucel-T in modified CD4+ T cells, though multiple infusions did not confer significant immunological benefits.

RNA aptamer/decoy

RNA aptamers are single stranded molecules that bind target ligands with high affinity and specificity due to their stable three-dimensional structure. Aptamers can be generated and identified *de novo* through a selection process known as systematic evolution of ligands by exponential enrichment (SELEX) for a specific function, such as affinity for a particular protein ligand. Alternatively, RNA aptamers known as RNA decoys are mimics of natural RNA molecules that competitively target the cognate RNA-binding protein or ligand. Two RNA decoys that mimic either the HIV-1 Rev response element (RRE) or the transactivating region (TAR) have reached clinical trials as anti-HIV therapeutics. In the earliest anti-HIV RNA decoy trial, conducted by researchers at Children’s Hospital Los Angeles, autologous CD34+ HPCs were transduced *ex vivo* with a retroviral vector expressing the 41-nt RRE decoy, which is designed to bind and inhibit the HIV Rev protein [50]. Though no adverse

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events among the four HIV infected subjects were associated with the gene therapy, the low frequency of retroviral gene transfer and engraftment precluded conclusions about the anti-viral potency of the RNA decoy. A different clinical study from the City of Hope and Benitec, Inc used a nucleolar-localizing anti-HIV TAR decoy as one component of an all RNA-based gene therapy that also included a shRNA and a ribozyme [51]. All components of the anti-HIV therapy (pHIV7-shI-TAR-CCR5RZ) were expressed from a lentiviral vector in autologous CD34+ HPCs and transplanted in patients with AIDS-related non-Hodgkins lymphoma.

siRNA/shRNA

The cellular process of RNA interference (RNAi) refers to the conserved sequence-specific degradation of messenger RNA (mRNA) mediated by small double-stranded RNAs. Due to the high sequence-specificity of RNAi, synthetic small interfering (siRNAs) or their precursor short hairpin RNAs (shRNAs) can be designed to specifically target any complementary sequence within a particular gene. RNAi knockdown of a targeted mRNA, defined as post transcriptional gene silencing (PTGS), can be induced by delivering siRNA molecules directly to the cells as ~19–23 bp dsRNAs or by the *de novo* transcription shRNA precursors and their subsequent processing into siRNAs. For prolonged intracellular immunity, constitutive expression of anti-HIV shRNAs from integrated lentiviral vectors can induce long-term gene silencing for the duration of the shRNA transcription and biogenesis.

SiRNA/shRNAs against viral target sites may become ineffective with the emergence of nucleotide substitutions or deletions within the viral genome at the target site [52, 53] or mutations of the viral genome at sites outside of the target site that confer some fitness advantage for the virus under the selective pressure of RNAi [54, 55]. To minimize potential mutational escape from any specific siRNA, multiple shRNAs may be expressed by different RNA Pol III promoters or as a multicistronic transcript from the same promoter [56–60].

Combinations of anti-HIV genes

Combinations of multiple anti-HIV genes in a single therapeutic vector or delivery system may increase the overall antiviral potency, reduce the required dosage level for each component, and decrease the potential for mutational escape. In contrast to combining multiple anti-HIV shRNAs into a single therapeutic, as discussed in the previous section, strategies that incorporate multiple types of therapeutics (ribozymes, decoys, etc.) minimize the competition and toxicity concerns associated with multiple shRNAs. Moreover, these strategies aim to inhibit HIV replication at multiple steps and typically include at least one Class I inhibitor, which inhibits early steps in the viral cycle prior to integration [7]. One design for a combinatorial lentiviral vector aimed to inhibit each of the three steps in the HIV replication cycle by expressing a CCR5 shRNA (pre-entry), a human/rhesus TRIM5α chimera (post-entry/pre- integration), and a TAR decoy (post-integration) [61]. This triple-agent vector was transduced into human CD34+ HSC transplanted NRG mice, leading to gene-modified CD4+ T cells and macrophages that were resistant to infection with either R5-tropic or CXCR4 (R4)-tropic HIV [62].

A different combinatorial strategy used a foamy virus vector to express the C46 envelope fusion inhibitor and two anti-HIV shRNAs against *tat* and *rev* viral genes and the endogenous *ccr5* mRNA [63]. Additionally, the vector expresses the MGMTP140K gene from a separate promoter to select for gene modified cells *in vivo*. The vector was tested in human CD34+ HSCs transplanted in NSG mice and can also be used to inhibit simian immunodeficiency virus/HIV-1 (SHIV) chimera that is useful in monkey AIDS models.

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To date, the only clinical trial to use a combinatorial gene therapy strategy against HIV was performed as a human pilot feasibility study from the City of Hope (Duarte California, USA) and Benitec (Melbourne, Australia). This study, which consisted of four patients with AIDS-related non-Hodgkin's lymphoma (NHL), examined the safety and tolerability of autologous CD34+ HSCs modified with a replication incompetent lentiviral vector that encoded three anti-HIV small RNAs (pHIV7-shI-TAR-CCR5RZ) [51]. The cells were transduced *ex vivo* and then transplanted to express an shRNA against the overlapping reading frames of viral genes *tat* and *rev*, a nucleolar-localizing mimic of the viral RNA hairpin TAR that serves as a decoy that binds and sequesters the Tat protein, and a ribozyme targeting the mRNA of the endogenous CCR5 co-receptor. For all patients, the therapy was well tolerated and the siRNA was detected by quantitative PCR (qPCR) in primary blood mononuclear cells (PBMCs) and/or primary blood granulocytic cells (PBGCs) for at least 6 months post-treatment and beyond 36 months for one patient. Furthermore, levels of gene marking increased in two patients after increases in viremia, suggesting that the protected cells offered a selective advantage over unprotected cells in the presence of HIV [64].

Conclusions

With more than ten anti-HIV gene therapeutics having reached clinical trials and dozens more in preclinical testing, the future remains promising for HIV-resistant gene therapy. However, many issues remain unresolved for the development of efficient and robust gene therapy that provides comparable *in vivo* viral suppression to cART. Similar to the cocktail strategies of cART, many gene therapy applications for HIV use combinations of antiviral genes, including the usage of one or more Class I inhibitors. Such approaches aim to minimize the likelihood of mutational escape and enhance the proliferative capacity of the HIV-resistant engineered cells. However, careful design must ensure that the expression of antiviral genes does not exhibit cellular toxicity or confer any growth disadvantage to the modified cells, since the prolonged benefits of gene therapy require the engineered cells to replace the existing unmodified cells after transplantation. Myeoablative conditioning increases the engraftment capacity of transplanted HSCs and alternative non-myeoablative conditioning regimens are currently under exploration in various animal models [1]. Brief analytical treatment interruptions of cART may enhance *in vivo* selection of the protected cells and are useful in analyzing the antiviral efficacy of the gene therapy. Animal models are also proving useful to explore additional strategies for *in vivo* selection, such as the inclusion of chemically inducible survival factors [65, 66] or the co-expression of a chemotherapeutic resistance gene under drug selection [17, 63].

While the Berlin patient offers hope that HIV eradication may be achievable by gene therapy, this occurred in an allogeneic setting, and additional hurdles remain in adapting gene therapy for the general population of HIV patients using autologous cells. In fact, it remains to be determined whether, in the absence of an allogeneic effect, the HIV reservoir can be eliminated. Besides the technical issues surrounding the efficacy and duration of such cellular therapy, the significant safety and cost of such procedures must be less than those associated with a lifetime of cART. Moreover, although initial clinical studies support the safety of anti-HIV gene therapy, the general risks associated with cell transplantation and conditioning must improve for it to become a practical and feasible therapy for most HIV patients. Thus, current efforts in preclinical and clinical studies must continue to improve the efficacy, safety, and affordability of gene therapy schemes to realize the goal of creating genetic resistance for HIV.

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Highlights

- Gene therapy aims to create HIV-resistant cells to replace the need for cART.
- Anti-HIV genes include protein or RNA-based agents and gene-editing enzymes.
- Combinations of anti-HIV genes reduce the likelihood of viral resistance.
- Current therapies require autologous transplantation of gene-modified cells.
- Stem cell-based approaches aim to establish an HIV-resistant immune system.

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Table 1

Anti-HIV gene therapeutics in clinical trials

Mechanism of anti-viral	Name	Target	Delivery of Genes	Cell transplantation	Company	Phase	Status of Clinical	References
ZFN	SB-728-T	Host (CCR5 DNA)	Adenoviral vector	autologous CD4+ T cells	Sangamo Biosciences	I-II	Ongoing	[18]
C46 peptide	M87o	Viral (env protein)	Retroviral (MMLV)	autologous CD4+ T cells	University Medical Center Hamburg-Eppendorf	I	Ongoing	[27]
ribozyme	RRz1 (OZ1)	Viral (tat-vpr mRNA)	Retroviral (MMLV)	autologous CD34+ HPC	Janssen-Cilag Pty Ltd., UCLA	I-II	Ongoing	[44,45]
ribozyme	MY-2	Viral (U5 and pol mRNA)	Retroviral (MMLV)	autologous CD4+ T cells	UCSD	I	Completed	[40,42]
ribozyme	RRz1	Viral (tat-vpr mRNA)	Retroviral (MMLV)	syngeneic CD4+ T cells	Johnson & Johnson, St. Vincent's Hospital	I	Completed	[41,43]
ribozyme	L-TR/Tat-neo	Viral (tat-rev mRNA)	Retroviral (MMLV)	autologous CD34+ HPC	Ribozyme, City of Hope	II	Completed	[67]
antisense	Lexgenleucel-T (VRX496)	Viral (env mRNA)	Lentiviral vector (LTR HIV)	autologous CD4+ T cells	VIRxSYS Corporation	I-II	Ongoing	[47]
antisense	HGTV43	Viral (TAR, tat/rev)	Retroviral (MMLV)	autologous CD34+ HPC	Enzo Biochem	I-II	Ongoing	[68]
RNA decoy	L-RRE-neo	Viral (rev protein)	Retroviral (MMLV)	autologous CD34+ HPC	Children's Hospital Los Angeles	pilot	Completed	[50]
shRNA RNA decoy (TAR) ribozyme	Tat/Rev shRNA TAR decoy CCR5 ribozyme	Viral (tat-rev mRNA) Viral (tat protein) Host (CCR5 mRNA)	Lentiviral vector (SIN HIV)	autologous CD34+ HPC	City of Hope, Benitec	pilot	Ongoing	[51]

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Table 2

Anti-HIV gene therapeutics in pre-clinical animal studies

Type of HIV Inhibitor	Name	Target	Delivery of Genes	Cell transplantation	Experimental System	References
Gene editing	SB-728 ZFN	host CCR5 gene	Nucleofection	CD34+ HSCs	Humanized HIV mouse	[20]
Gene editing	Ad5/F35 X4-ZFN	host CXCR4 gene	Adenoviral vector	CD4+ T cells	Humanized HIV mouse	[21,22]
Protein-based	C46 peptide	viral Envelope	Lentiviral vector	CD34+ HSCs	HIV macaque	[28]
Protein-based	C46 peptide	viral Envelope	Lentiviral vector	CD4+ T cells	Humanized HIV mouse	[12]
Protein-based	LEDGF/p75 KD $LEDGF_{325-530}$	viral Integrase	Lentiviral vector	CD4+ T cells	Humanized HIV mouse	[32]
Protein-based	TRIM5α	viral Capsid protein	Lentiviral vector	CD4+ T cells	humanized HIV mouse	[37,38]
Combinatorial	CCR5 shRNA TRIM5α TAR decoy	CCR5 gene viral Capsid protein viral Tat protein	Lentiviral vector	CD34+ HSCs	Humanized HIV mouse	[62]
Combinatorial	C46 peptide tat/rev shRNA CCR5 shRNA	viral Envelope viral mRNA host CCR5 mRNA	foamy virus vector	CD34+ HSCs	humanized HIV mouse	[63]