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REVIEW ARTICLE

Small Interfering RNAs and their Delivery Systems: A Novel Powerful Tool for the Potential Treatment of HIV Infections

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Abstract: ***Background:*** Small interfering RNAs (siRNAs) have rapidly developed into biomedical research as a novel tool for the potential treatment of various human diseases. They are based on altered gene expression. In spite of the availability of highly active antiretroviral therapy (HAART), there is a specific interest in developing siRNAs as a therapeutic agent for human immunodeficiency virus (HIV) due to several problems including toxicity and drug resistance along with long term treatment. The successful use of siRNAs for therapeutic goals needs safe and effective delivery to specific cells and tissues. Indeed, the efficiency of gene silencing depends on the potency of the carrier used for siRNA delivery. The combination of siRNA and nano-carriers is a potent method to prevent the limitations of siRNA formulation. Three steps were involved in non-viral siRNA carriers such as the complex formation of siRNA with a cationic carrier, conjugation of siRNA with small molecules, and encapsulation of siRNA within nanoparticles.

Conclusion: In this mini-review, the designed siRNAs and their carriers are described against HIV-1 infections both *in vitro* and *in vivo*.

Keywords: HIV infection, siRNA, delivery system, *in vitro* and *in vivo* studies.

1. INTRODUCTION

The global effects of sexually transmitted infections (STIs) are important. It is clear that the control of viruses involved in these infections such as the human immunodeficiency virus type-1 (HIV-1) is very difficult [1, 2]. There are several highly active antiretroviral therapies (HAART) for the treatment of HIV infections, but some problems including toxicity and drug resistance along with long-term treatment hinder complete success. At present, there is a specific interest in developing siRNAs as a therapeutic agent for HIV infections. As it is known, gene silencing can be stimulated by chemical synthesized short double-stranded RNA including small interfering RNAs (siRNA) and microRNA (miRNA), and double-stranded hairpin RNA (shRNA) transcribed *in vitro* or from viral vectors and plasmids [3]. Among them, siRNAs are used as synthetic tools to silence viral infection in human cells. It was reported that the HIV-1 genome encodes viral siRNA precursors, and natural HIV-1 infection causes nucleic acid-based immunity in human cells. To prevent this cellular defense, there is a suppressor of RNA silencing (SRS) function in HIV-1 Tat protein. The Tat protein reduced the cell's RNA-silencing defense by impairing the ability of Dicer to process precursor double-stranded RNAs into siRNAs [4]. The studies showed that synthetic siRNAs could be utilized to trigger the down-regulation of special genes through transfection into mammalian cells. Regarding the problems related to viral vectors (*e.g.,* safety), there is a special interest in the use of non-viral lipidic and polymeric delivery systems and antibodies for suitable siRNA delivery [5]. However, there are some advantages and disadvantages in using these carriers as shown in Table **1**. In this mini-review, the designed siRNAs against HIV-1 and their delivery into the cells are described. These siRNAs were directed toward the HIV-1 genome or its receptors on the cell surface [6].

2. DESIGN OF siRNAs IN TREATMENT OF HIV INFECTIONS

HIVsirDB is a freely accessible database of siRNAs which can silence HIV genes. This database contains about 750 siRNAs such as 75 partially complementary siRNAs differing by one or more bases with the target sites and over 100 escape mutant sequences. HIVsirDB structure contains sixteen fields such as siRNA sequence, HIV strain (~ 26 types), targeted genome region, efficacy and conservation of target sequences. There are some tools in this database such as siRNAmap for mapping siRNAs on the target sequence, HIVsirblast for BLAST search against the database, and siRNAalign for aligning siRNAs [7]. Targeting multiple HIV

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Table 1. Advantages and disadvantages of major carriers for delivery of bioactive agents [6, 12].

siRNA Carriers	Advantage	Disadvantage
Liposome	* Biodegradable, biocompatible, flexible * Delivering aqueous and lipidic drugs * Protection of drugs from degradation * Site-specific drug delivery	* Low stability, Short half-life * Oxidation and hydrolysis of lipids * Stimulation of allergic reactions * Accumulation in non-target cells
Polymer	* Higher stability compared to liposomes * Site-specific drug delivery * Controlled drug release * Multifunctional ability * Injection via various routes	* Nanotoxicity * Rapid clearance of unmodified nanoparticles * Physical handling is difficult
Dendrimer	* Water-soluble, biocompatible * Possessing multiple surface groups functionalized for drug delivery * Forming dendriplexes and protecting degradation of nucleic acids * Ability for encapsulation of drugs * Facilitating passive targeting	* Non-specific cytotoxicity * Rapid clearance * Poor control against release of the drugs
Quantum Dots	*High photochemical stability * Resistant to photobleaching	*Toxicity * Non-specific organ uptake
Gold nanoparticles	*Simple synthesis * Biocompatible *Easy conjugation of bioactive agents * Modulation of cytotoxicity	*Not-biodegradable *Nanoparticle aggregation * High cost
Carbon nanotubes	* Large modified surface *Industrial production *Delivery of bioactive agents such as nucleic acids, proteins, drugs *Protecting nucleic acids from degradation	* Toxicity * Poor solubility in water * Not-biodegradable

sites using a combinatorial RNAi-based approach could effectively suppress viral propagation at an early stage [8]. The studies indicated that HIV-1 was susceptible to RNAi. In addition to targeting the virus itself, RNAi-mediated down-regulation of cellular targets encoding receptors required for viral entry also proved to be effective. However, extensive *in vitro* experiments showed potential problems of viral escape mutants and other toxicities caused by the siRNAs that should be considered in their design [9]. The siRNA-mediated knockdown of gene expression offers a new treatment strategy for HIV infections. However, the main problem for clinical use is a practical strategy for the delivery of siRNA to the immune cell types that are important in viral pathogenesis [10]. Recently, the reagents were developed to deliver selectively exogenous siRNAs to immune cells that were targeted by HIV-1 or involved in viral pathogenesis including T cells, macrophages and dendritic cells (DCs). Two antibody-based strategies were used for systemic delivery of siRNA either specifically to T cells through the CD7 receptor or to multiple immune cell types through the lymphocyte function-associated antigen-1 (LFA-1) present on all leukocytes [11].

3. siRNA AND GENERAL METHODS OF ITS DELIVERY

The high negative charge on siRNA inhibits its easy entry into the cells and thus, it is required to design potent and safe siRNA delivery systems. There are two siRNA carriers including exogenous carriers (*e.g.,* different polymeric or lipidic delivery systems suitable for generating immediate and/or short-term effects of siRNAs) or endogenous carriers (*e.g.,* adenoviral and lentiviral-based vectors harboring siRNAs). In endogenous expression, the sense and antisense strands are expressed as two independent transcripts that hybridize within the cells to form functional siRNA duplexes and/ or these strands are expressed as a single transcript separated by a short loop sequence. The transcript forms a

hairpin structure that can be processed by Dicer into functional siRNA [6]. Limitations to the successful use of siRNAs were divided into two groups: a) the inability of unprotected naked siRNA to cross the cell membrane by passive diffusion due to its high anionic charge and large molecular weight, and b) lack of methods to safely and effectively deliver siRNA molecules into target cells [12]. Other problems include rapid degradation of siRNA by endogenous nucleases, non-specific distribution, low endosomal escape, removal by glomerular filtration, and development of siRNA viral mutants due to the high mutation rate of HIV-1 (Table **2**) [12]. The potential of siRNAs to overcome limitations such as the high rate of HIV mutation was investigated by targeting highly conserved regions on viral genes that are important for viral replication. The siRNAs (~ 21-23 nucleotides long) were able to induce selective degradation of complementary mRNA. The RNAi technique was used to suppress HIV replication by its effect on HIV-1 genes or cellular factors *in vitro* and *in vivo* [12]. The HIV-1 genome contains nine genes encoding three main proteins (Gag, Pol, Env), two regulatory proteins (Tat, Rev), and four accessory proteins (Nef, Vpr, Vpu, Vif). The HIV-1 envelope glycoprotein (Env) is important for the entry of the virus into the cell [13-15]. Furthermore, all HIV-1 genes such as *tat*, *rev*, *gag*, *pol*, *nef*, *vif*, *env*, *vpr* and the long terminal repeat (LTR) were susceptible to the RNAi mechanism [12]. However, the expression of HIV-1 structural genes (*env, gag, pol*), and the construction of full-length viral genomic RNA require Tat and Rev proteins, thus, early transcripts such as HIV-1 *tat* and *rev* are suitable targets for siRNA. In 2002, the synthetic and expressed siRNAs that have targeted several early and late HIV-encoded RNAs including *tat*, *rev*, *gag*, *env*, *vif*, *nef* and reverse transcriptase were studied by Capodici *et al*., Novina *et al*., Hu *et al*. and Park *et al*. (for the Gag and Env proteins), Hu *et al*. (for the reverse transcriptase), Elbashir *et al*., Surabhi and Gaynor, and Lee *et al*. (for the regulatory Tat and Rev proteins), and Jacque *et al*. and Das *et al*. (for the two accessory proteins Nef and Vif) [16-25]. The studies showed that targeting genes encoding two proteins which act synergistically are more effective than each one as shown by the targeting of Tat and Rev transcripts by siRNA. The synthetic siRNA targeting the HIV-1 *rev* and *tat* mRNAs inhibited HIV-1 gene expression and replication in primary lymphocytes and human T cell lines [6]. Host cellular genes such as the chemokine receptors CCR5 and CXCR4 that function as HIV-1 co-receptors for M-tropic HIV-1 were also targeted by siRNAs [12]. Martinez *et al*. demonstrated that *in vitro* silencing of CCR5 by siRNAs offered clear shielding from HIV-1 [26]. Although the silencing of $CD4^+$ expression suppressed viral entry and reduced the free viral titers, $CD4^+$ might not be a suitable therapeutic target due to its involvement in the function of T cells [6].

4. *IN VITRO* AND *IN VIVO* DELIVERY OF siRNAs

Non-viral approaches for exogenous siRNA delivery include the use of liposomes, polymeric nanoparticles, dendrimers, quantum rods, carbon nanotubes, and inorganic nanoparticles [27]. Cell penetrating peptides (CPPs) were successful in delivering a variety of cargoes including peptides, DNA, siRNA, oligonucleotides and proteins into different cell types and do not damage the cell membrane as observed by physical and chemical transfection [28]. The ideal delivery system must bind or encapsulate the siRNA in a reversible pathway to facilitate siRNA delivery, protect the siRNA from degradation in endosomes, and also suppress clearance by the liver and kidney. Moreover, carriers should be biocompatible and biodegradable. Targeted delivery of siRNA using siRNA conjugation with aptamers without the use of a carrier was also evaluated in some studies [27]. Cationic liposomes were used as a non-viral vector for *in vitro*/ *in vivo* siRNA delivery. The liposome size and the number of bilayers affect the circulation half-life and drug loading, respectively [6, 12]. A liposome-siRNA complex (lipoplex) showed efficient protection of siRNA against RNase degradation and improved the endocytosis-mediated cell uptake of siRNA. The cationic formulation used for siRNA delivery led to *in vivo* limited use because of the cytotoxicity and instability in the presence of serum. Thus, to overcome these restrictions, Lavigne *et al.* developed an anionic lipid-based carrier, Nutraplex (Nx). The efficiency of three Nx formulations (cationic, neutral, and anionic) was compared for the delivery of anti-CXCR4 siRNAs *in vitro*. The data indicated that although cationic formulations were the most effective carriers for actively silencing siRNA delivery, anionic carriers were taken up by the cells, delivered active siRNAs, and had low cytotoxicity. Thus, anionic delivery systems have potential for *in vivo* siRNA therapeutics [6, 29]. On the other hand, enhanced stability, efficient delivery and regulated release are the advantages of biodegradable polymeric nanocarriers. Non-viral polymeric materials involved in siRNA delivery can be divided into natural (*e.g.,* chitosan, albumin and gelatin) and synthetic (*e.g.,* cyclodextrin polyethylene glycol, polyethyleneimine and poly (D, L-lactideco-glycolic acid: PLGA) polymeric carriers [30]. Natural biopolymers were divided into polysaccharides (*e.g.,* chitosan, alginate), and proteins (*e.g.,* collagen) for delivery of genes and nucleic acids. The polyesters such as poly (lactic acid) (PLA), poly (glycolic acid) (PGA), polycaprolactone (PCL), and their copolymers such as poly (lactide)-co-(glycolide) (PLGA) are the most widely used polymers due to their biocompatibility, non-immunogenic and non-toxic properties [12]. In addition, dendrimers are monodisperse, polybranched, highly symmetrical synthetic three-dimensional polymers in the nano-range. The most broadly used dendrimers in biomedicine and drug delivery are polyamidoamine (PAMAM) and polypropylenimine (PPI) dendrimers. Indeed, the drugs can be encapsulated in the dendrimer through non-covalent mechanisms and/ or complexation to charged functional groups for the delivery of nucleic acids. Weber *et al.* reported the synthesis of second-generation ammonium-terminated carbosilane dendrimers containing 8 or 16 positive charges (named as 2G-NN8 and 2G-NN16, respectively), which bind different siRNAs by forming dendriplexes including siP24 (siRNA targeted to HIV *p24* gene), siGAG1 (siRNA targeted to HIV *gag* gene), and siNEF (siRNA targeted to HIV *nef* gene) and suppress HIV in HIV-infected peripheral blood mononuclear cells (PBMC) [31]. Furthermore, Wrobel *et al*. showed that the 2G-NN16 dendrimers may be suitable for siRNA delivery due to lower toxicity and less tendency to form aggregates [32]. Jimenez *et al*. demonstrated the ability of the 2G-NN16 amino-terminated carbosilane dendrimer to deliver siRNA to HIV-infected human astrocytes and to cross the blood-brain

Table 2. Limitations of siRNA application in clinical trials [12].

1. Inability of the naked siRNA to cross the cell membrane by passive diffusion
2. Lack of safe and effective approaches to deliver siRNA molecules into target cells
3. Non-specific distribution
4. Low endosomal escape
5. Removal by glomerular filtration
6. Development of siRNA viral mutants

barrier (BBB) [33]. Moreover, the nano-conjugate formed by 2G-03NN24/siRNA-Nef indicated the highest inhibition of HIV-1 replication. In general, dendrimers showed safety properties because they did not induce proliferation on CD4 T lymphocytes and decreased the release of TNF-α and IL-12p40 by macrophages. Both dendrimers (2G-NN16 and 2G-03NN24) also reduced the phagocytosis activity. The 2G-03NN24 dendrimer also decreased the CCL2 and CCR2 expression in macrophages. Carbosilane dendrimers 2G-NN16 and 2G-03NN24 could be used as potent non-viral vectors for gene therapy especially in the treatment of HIV infection [34]. It was reported that the dendrimer complexed with anti-*tat*/ *rev* siRNA or a combination of anti-*tat*/ *rev* siRNA, anti-CD4, and anti-Transportin 3 (TNPO3) siRNAs administered by intravenous (IV) injection to HIV-infected humanized mice (RAG-hu) suppressed HIV-1 infection and protected them against virus-induced $CD4^+$ T cell depletion [12]. On the other hand, polyethyleneimine (PEI) polymer has various functions in nucleic acid delivery due to its large cationic amine groups. Recently, Weber *et al*. synthesized polyplexes by PEI-grafted-polyethylene glycol (PEG) block copolymers, and reported the high transfection efficiency of polyplex within 24 hours of treatment in hard-to-transfect T cells [35]. Generally, biodegradable polymers could deliver siRNA and achieve effective silencing of the target gene in different types of cells [36]. The cytotoxicity results showed that dendriplex had lower toxicity compared to the dendrimer alone [6, 35]. Also, the linkage of polyplexes to the biocompatible molecule (*e.g.,* PEG) could reduce the cytotoxicity of polyplexes [35]. Furthermore, inorganic nanoparticles such as carbon nanotubes, quantum dots, gold and silica were successfully used for the delivery of nucleic acids. The use of quantum dots (QRs) for siRNA delivery was reported in HIV therapy. For example, a quantum rod-siRNA complex (QR-si510 HIV-1 siRNA complex) targeted the transacting responsive (TAR)/ poly A region of the HIV-1 LTR and thus inhibited HIV-1 viral replication. Gold nanoparticles such as nanospheres, nanocages and nanorods were also used for the delivery of bioactive agents to target organelles through covalent conjugation (*e.g.,* thiols) or other non-covalent mechanisms [30]. For the delivery of nucleic acids, gold nanoparticles were functionalized with positively charged quaternary ammonium, or branched PEI 110 and/ or coated with a cationic lipid bilayer [37]. Moreover, the attachment of oligonucleotides to the surface of gold nanoparticles was also reported [38]. For HIV therapy, single-walled carbon nanotubes (SWCNTs) were shown to deliver siRNA into human T cells and primary cells for the efficient degradation of CXCR4 and CD4 mRNAs. Knocking down these mRNAs led to the depletion of CXCR4 and CD4 receptors on human T cells and peripheral blood mononuclear cells [39].

Recently, a variety of aptamers for HIV-1 proteins as well as host proteins that interact with HIV-1 have been developed and some of them have potent viral neutralization ability and inhibition of HIV-1 infectivity. The aptamer-siRNA approach was found to inhibit HIV-1 replication. Aptamers were also used to deliver anti-HIV siRNAs to $CD4^+$ T cells [40-42]. It is important that siRNA delivered by liposomes or polyplexes could non-specifically activate inflammatory cytokine secretion (TNF-α, IL-6, and IL-12) as well as IFN-responsive genes, and finally trigger cellular toxicity. In contrast, the treatment of HEK-293 cells with anti-gp120 aptamer-siRNA chimeras did not significantly induce the expression of the IFN-β and p56 genes [43]. There are many studies on the use of siRNAs *in vitro* and *in vivo* using a variety of delivery systems. These studies contain the cell type-specific delivery of anti-HIV siRNAs through fusion to an anti-gp120 aptamer or anti-CD4 aptamer (anti-gp120/ anti-CD4 aptamer-siRNA chimera) [41, 44], delivery of plasmids encoding HIV-1-specific siRNAs (psiRNAs) as microbicides through multifunctional chitosan-lipid nanocomplexes [45], dual-antibody-modified chitosan/small interfering RNA (siRNA) nanoparticles [46], delivery of anti-CCR5 siRNA by the lymphocyte function-associated antigen-1 (LFA-1) integrin-targeted immunoliposome [10], aptamer bridge-construct complexed with three different Dicer substrate siRNAs (DsiRNAs) [47], T cell-specific anti-CCR5 siRNA delivery by CD7-specific single-chain antibody conjugated to oligo-9-arginine peptide (scFvCD7-9R) [48], dual function of the gp120 (A-1) aptamer conjugated to 27-mer Dicer-substrate anti-HIV-1 siRNA (dsiRNA), 5' long terminal repeat (LTR)-362 [49], delivery of the PEI-siRNA nano-complex to the central nervous system [50], cationic PAMAM dendrimers as a siRNA delivery system containing a cocktail of dicer substrate siRNAs (dsiRNAs) targeting both viral and cellular transcripts [51], siRNA targeted to the HIV *nef* gene and delivered by the PEG-PEI copolymers [35], delivery of HIV-1 Env gp140 LNP formulations with cationic and ionizable lipids with siRNA [52], antibody-protamine conjugates bearing anti-gag siRNA [53], a gold nanorod (GNR)-galectin-1-siRNA nanocomplex [54] as shown in Table **3**.

Nozari and Berezovski showed that cluster of differentiation (CD) proteins are among the most popular antigens for

Table 3. ***In vitro*** **and** ***in vivo*** **delivery of siRNAs for HIV-1 therapy.**

siRNA Target	Delivery System	Results	Year	References
Anti-*tat/rev* siRNA	anti-gp120 aptamer	* The anti-gp120 aptamer-siRNA chimera was specifically taken up by cells expressing HIV-1 gp120 * The attached siRNA was processed by Dicer releasing an anti-*tat/rev* siRNA which inhibited HIV replication (*in vitro* study)	2008	[41]
Fluorine-derivatized siRNA resistant to RNase A	-	* Treatment of HIV-infected activated $CD4^+$ T cells with the derivatized siRNA in the presence of serum led to inhibition of HIV infection (*in vitro* study)	2002	[57]
Plasmids encoding HIV-1-specific siRNAs (psiRNA cocktail)	Chitosan-lipid nanocomplexes (chlipid)	* Significant protection against HIV in both *in vitro* and *in vivo* models * Prophylactic injection of the chlipid to deliver a psiRNA cocktail intravaginally with a cream formulation in a non-human primate model showed substantial reduction of simian/human immunodeficiency virus SF162 titers (*in vitro* and *in vivo* studies)	2015	[45]
Anti-*tat* siRNA	Dual-antibody-modified chitosan	* Transferrin (Tf) antibody and bradykinin (B2) antibody could specifically bind to their receptors, respectively, and deliver siRNA across the BBB into astrocytes as potential targeting ligands * The combination of anti-Tf antibody and anti-B2 antibody significantly increased the knockdown effect of siRNA-loaded nanoparticles (*in vitro* study)	2017	[46]
Anti-CCR5 siRNA	Immunoliposome method targeting the lymphocyte function-associated antigen-1 (LFA-1) integrin expressed on all leukocytes	* *In vivo* injection of the LFA-1 integrin-targeted nanoparticles led to the selective uptake of siRNA by T cells and macrophages * *In vivo* injection of anti-CCR5 siRNA/LFA-1 nanoparticles led to leukocyte-specific gene silencing that was sustained for 10 days * Humanized mice challenged with HIV after anti-CCR5 siRNA treatment indicated an increased resistance to infection (*in vivo* study)	2010	[10]
Dicer substrate siRNAs (DsiRNAs)	Gp120 aptamer with a carbon linker was bound to a 16-nucleotide GC rich bridge sequence	* The aptamer bridge-construct complexed with three different Dicer substrate siRNAs (DsiRNAs) led to knockdown of target mRNAs and potent inhibition of HIV-1 replication *in vivo* (*in vivo* study)	2013	[47]
Anti-CCR5 siRNA	CD7-specific single-chain antibody conjugated to oligo-9-arginine peptide (scFvCD7-9R)	* Treatment with antiviral siRNAs complexed to scFvCD7-9R suppressed endogenous virus and restored CD4 T cell counts in HIV-infected Hu-PBL mice * scFvCD7-9R could deliver antiviral siRNAs to naive T cells in Hu-HSC mice and effectively inhibit viremia in infected mice (*in vivo* study)	2008	[48]
27 mer Dicer-substrate anti-HIV-1 long terminal repeat (5'LTR-362) siRNA (dsiRNA)	Gp120 (A-1) aptamer conjugated to dsiRNA	*Treatment of the aptamer-siRNA conjugates inhibited viral p24 levels and HIV-1 infection *Systemic delivery of the LTR-362 27-mer siRNA conjugates protected $CD4^+$ T cell levels in viremia Hu-NSG mice (*in vivo* study)	2018	[49]
siRNA against the host autophagic protein (Beclin1)	Cationic linear polyethylenimine	* Intranasal delivery of the siRNA targeting Beclin1 (the PEI-siRNA nanocomplex) significantly depleted the target protein expression levels in brain tissues with no toxicity in the HIV-infected brain (*in vivo* study)	2017	[50]
The cocktailed dsiRNAs	Cationic PAMAM dendrimers	*The dendrimer-dsiRNAs treatment suppressed HIV-1 infection and induced protection against $CD4^+$ T-cell depletion * The dendrimer-dsiRNAs accumulated in PBMC and liver, and did not show any significant toxicity *Effective combinatorial delivery of anti-host and -viral siRNAs for HIV-1 treatment was performed in humanized mice (RAG-hu) (*in vivo* study)	2011	[51]

siRNA Target	Delivery System	Results	Year	References
Anti-*nef* siRNA	Polyethylene glycol (PEG) moieties linked to the PEI polymers with the improving stability and reducing cytotoxicity	*siRNA targeted to the HIV *nef* gene and delivered by one of the PEG-PEI copolymers in repetitive treatments could suppress HIV replication (*in vivo* study)	2012	[35]
The particle self-amplifying RNA (saRNA), a large (~ 9500 nt), negatively charged molecule	Lipid nanoparticles (LNPs) formulations with cationic and ionizable lipids	* LNPs formulated with cationic lipids protected saRNA from RNAase degradation * Cationic and ionizable LNPs formulations induced antibodies against HIV-1 env gp140 with lower dosing than mRNA (*in vivo* study)	2019	[52]
Anti-*gag* siRNA	Antibody-protamine conjugates	*The HIV-1-specific antibody-protamine siRNA nanoparticles inhibited viral replication in difficult-to-transfect T lymphocytes (*in vitro* study)	2005	[53]
Galectin-1 siRNA	Gold nanorod	*A gold nanorod (GNR)-galectin-1-siRNA nanocomplex inhibited gene expression of galectin-1 in human monocyte-derived macrophages * Decreased HIV-1 infectivity (*in vitro* study)	2012	[54]
Anti-CXCR4 siRNA	Liposome	*Reduced CXCR4 mRNA expression (~ 70%, *in vitro* study)	2013	[29]
Anti-synaptosomal-associated protein 23 (SNAP23) siRNA	Polymeric nanoparticles	*Inhibition of SNAP23 expression, Vaginal pre-exposure prophylaxis for HIV-1 infection (*in vivo* study)	2015	[58]
Anti-p24, gag, nef siRNAs	Dendrimer	*Inhibition of HIV-1 replication (*in vitro* and *in vivo* studies)	2008	[59-66]
Anti-transportin 3, CD4, *tat*, *rev* siRNAs	Dendrimer	*Down-regulation of targets *Protection against $CD4^+$T cell depletion (*in vivo* study)	2011	[27]
Anti-transactivator (TAR)/poly A of LTR siRNA	Quantum Dot	*Significant reduction in p24 production (*in vitro* study)	2011	[30]
Anti-CXCR4, CD4 siRNAs	Carbon nanotube	*Decreased HIV-1 infectivity (*in vitro* study)	2018	[39]
Anti-CD4, CCR5 siRNAs	INTERFERin	*Reduction in CD4 and CCR5 expression (*in vivo* study)	2011	[34]
Anti-CD4, *vif*, *tat* siRNAs	Peptide carrier	*Reduction in CD4 expression *Inhibition of HIV-1 replication (*in vivo* study)	2008	[67]
Anti-*tat*/*rev* siRNAs	Dual functional RNA nanoparticles containing Phi29 motor pRNA and anti-gp120 aptamer	* Decreased HIV-1 infectivity * Inhibition of HIV-1 replication (*in vitro* and *in vivo* studies)	2011	[68, 69]

aptamers on the cell surface. These anti-CD aptamers could be used in cell phenotyping as well as in the diagnosis and treatment of HIV. The aptamers can act simultaneously as agonists and antagonists of CD receptors depending on the degree of aptamer oligomerization. Moreover, aptamers could deliver siRNA to silence critical genes in CD-positive cells [55]. Although aptamers are capable of binding various molecules with high affinity and specificity, there are some limitations regarding their wide application in clinical trials. These disadvantages include aptamer degradation, aptamer excretion from the bloodstream by renal filtration, control of the duration of action, the interaction of aptamers with intracellular targets, generation of aptamers using impure target proteins, and aptamer cross-reactivity [56]. Thus, it is required to solve these problems for the development of aptamers as a suitable delivery system.

CONCLUSION

The evaluation of the therapeutic potential of RNAi for HIV infection has been hindered by the challenges of siRNA delivery and lack of suitable animal models. Among different delivery systems, the combined use of small interfering RNA (siRNAs) and aptamers could effectively block viral replication and prevent the generation of resistant variants. However, the aptamer-siRNA conjugates must be further optimized for clinical trials. Indeed, it is important to develop novel aptamers against the cellular receptors and improve detection methods for the identification of cell-specific aptamers. However, the effective siRNA-mediated delivery of molecules to HIV-infected target cells including T lymphocytes and monocytes is challenging *in vivo*. Moreover, because of the short plasma half-life of synthetic siRNA ,

repeated injections for years will be required for effective treatment of the infection. The targeting of various critical stages of the HIV-1 life cycle by the combination of different siRNAs is more effective than individual gene silencing.

LIST OF ABBREVIATIONS

HIV	=	Human Immunodeficiency Virus
HAART	=	Highly Active Antiretroviral Therapy
siRNA	=	Small Interfering RNA
STI	=	Sexually Transmitted Infection
miRNA	=	MicroRNA
shRNA	=	Double-Stranded Hairpin RNA
SRS	=	Suppressor of RNA Silencing
DC	=	Dendritic Cells
LFA-1	=	Lymphocyte Function-Associated Antigen-1
Env	=	Envelope Glycoprotein
CPP	=	Cell Penetrating Peptide
PLGA	=	Poly (lactide)-co-(glycolide)
PAMAM	=	Polyamidoamine
PPI	=	Polypropylenimine
PBMC	=	Peripheral Blood Mononuclear Cells
BBB	=	Blood-Brain Barrier
TNPO3	=	Anti-Transportin 3
PEI	=	Polyethyleneimine
IV	=	Intravenous
QR	=	Quantum Rod
TAR	=	Transacting Responsive
SWCNT	=	Single-Walled Carbon Nanotube
DsiRNA	=	Dicer Substrate siRNA
GNR	=	Gold Nanorod
CD	=	Cluster of Differentiation

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

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