Gene Therapy (2007) 14, 1057–1064

www.nature.com/gt

***REVIEW***

# *Progress and prospects: RNA-based therapies for treatment of HIV infection*

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*The current treatment regimen for HIV-infected individuals combines two or more drugs targeting different viral proteins such as RT and gag. Resistance to conventional drugs can develop quickly, and typically persists. The prospect of longer, continuous antiretroviral therapy brings with it the need for new antiretroviral drugs and approaches. In this context, gene therapies have the potential to prolong life and quality of life as an additional therapeutic class and may serve as an adjuvant to traditional treatments. This review focuses on RNA-based hematopoietic cell gene therapy for treatment of HIV infection. Recent advances in our understanding of RNA interference (RNAi) make this an especially attractive candidate for anti-HIV gene therapy although ribozyme and RNA decoy/aptamer approaches can be combined with RNAi to make a combinatorial therapy akin to highly active anti-retroviral therapy.*
Gene Therapy (2007) **14,** 1057–1064; doi:10.1038/sj.gt.3302977

**Keywords:** *therapy; RNAi; siRNA; ribozyme; aptamer; HIV-1*

*In brief*

*Progress*

- The repertoire of potential HIV targets is expanding
- Ribozymes and aptamers continue to evolve as therapeutics
- RNAu makes effective use of the spliceosome: external guide sequences (EGS) take advantage of the tRNA processing machinery
- Multi-targeting RNAi approaches to inhibit HIV replication advance, including methods to avoid toxicities associated with saturation of endogenous RNAi pathways
- Immunostimulation by RNAi therapeutics can be avoided
- RNAi-induced transcriptional gene silencing (TGS) presents new possibilities for directed gene silencing
- A lentiviral vector with a triple HIV therapeutic moves toward the clinic

*Prospects*

- New cellular targets are being identified as options for therapeutic intervention
- A better understanding of the role of microRNAs in normal cell biology and disease will provide new options for gene therapies
- RNA-gene therapeutic applications designed to treat cancer and cellular deregulation will expand
- New combinations of RNA-based gene therapeutics against HIV-1 and HCV will be developed.
- Gene therapy proof-of-concept studies in animal models will increase
- Clinical trials will generate information on the efficacy of gene therapy approaches

## Introduction: challenges to conventional therapies

The number of reviews on HIV-1 antiretroviral therapy and the length of *Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents* (compiled by Office of AIDS Research Advisory Council, OARAC, http://AIDSinfo.nih.gov), both attest to the difficulties associated with HIV-1 antiretroviral therapy. Current treatment regimens for HIV are complicated, especially for individuals co-infected by hepatitis C or tuberculosis. Complications of highly active antiretroviral therapy (HAART) include, but are not limited to: hypersensitivity; immune reconstitution syndrome; hyperlipidemia, hyperglycemia, insulin resistance and diabetes mellitus contributing to atherosclerosis and cardiovascular disease; interactions with other medications; renal dysfunction and hepatotoxicity. Although HAART has greatly improved, side effects commonly lead to temporary interruption of HAART. However, in marked contrast to earlier thinking, a recent study shows that compared to

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Received 9 April 2007; revised 12 May 2007; accepted 12 May 2007

continuous antiretroviral therapy, episodic HAART leads to an increased risk of liver cirrhosis, myocardial infarction, renal failure and stroke with no concomitant increase in quality of life.[1] The increase in side effects due to long-term conventional treatment is due in part to the improved lifespan brought on by the very success of antiretroviral therapies. In addition, treatment guidelines traditionally have not recommended initiating therapy in early stages of infection, despite the risks associated with loss of immunological function, increased likelihood of transmission, and the development of a larger pool of viral subspecies that serve as a reservoir for potential resistance. However, there has also been a recent shift towards starting retroviral therapy earlier, before CD4 counts drop below $200 \times 10^6$/l (OARAC, http://AIDSinfo.nih.gov).[2,3] As a possible means of circumventing some of the problems associated with HAART, a number of investigators are focusing their attention on gene therapy either as a stand alone approach or as an adjuvant to pharmacological drug regimens.

In this review, we will focus on the progress in developing RNA-based anti-HIV gene therapeutics for long-term applications, with particular attention to molecular targets and their mechanisms of action within the context of the special challenges posed by HIV. Gene-based approaches present conundrums and trade-offs analogous to those of conventional drugs. One consideration is the issue of viral versus cellular targets. RNA anti-virals can be designed with high specificity and HIV-1 products are the preferred target; however, many viral RNAs are highly abundant and viral escape is a major problem that can only be partially ameliorated by targeting highly conserved sequences. Cellular targets are far less prone to mutational escape, and are often in lower abundance, but the side effects of downregulating cellular targets for the long term are unknown.

For the sake of discussion, we divide RNA inhibitors into two classes. The first is comprised of autonomous RNA inhibitors such as ribozymes and aptamers that interact with their respective targets in a highly selective fashion. However, colocalization of these RNA molecules with their targets is achieved largely via diffusion, making them behave unpredictably in the cellular milieu. The second class is comprised of RNA triggers that include small interfering RNAs (siRNAs). This class of inhibitors utilizes endogenous cellular proteins to find the target sequences and because of this exhibit highly efficient target inhibition via mRNA degradation. Their efficiency however, can exacerbate related sequence off-target effects and saturation of the cellular pathway can adversely compromise cellular metabolism and development. Finally, we evaluate RNA gene therapies in the light of the emerging consensus that combinatorial gene therapeutics have the greatest likelihood of success, analogous to HAART therapy.

## The repertoire of potential HIV targets is expanding

HIV-1 begins a new cycle of replication (Figure 1) by binding via the viral envelope to the cellular receptor CD4 in conjunction with a coreceptor; either CXCR4, in the case of T-cell-tropic, or CCR5 for macrophage-tropic virus. After entry into the cell, the viral core uncoats, exposing the dimeric genomic RNA, which is converted into double-stranded DNA by HIV-1 reverse transcriptase (RT). The viral DNA forms a pre-integration complex with viral and host proteins, which is imported into the nucleus whereupon the DNA becomes integrated into the host cell DNA as a provirus. Active viral replication can be divided into early and late phases. The early phase begins with the transcription of multiply spliced mRNAs encoding the regulatory proteins Tat and Rev, which re-enter the nucleus. Tat further activates viral transcription, whereas Rev binds to the Rev-response element (RRE) and escorts singly spliced and unspliced transcripts to the nuclear pore for export to the cytoplasm. During the late phase of replication, these mRNAs produce the remaining viral proteins. Viral assembly occurs at the cellular membrane, and the new virion is released by budding. Viral protease continues to process the viral polyproteins into their mature form, condensing the viral genomic RNA core and yielding infectious particles. HAART therapy typically involves a drug cocktail of viral RT and protease inhibitors.

Viral proteins and cellular partners involved in the early events in the emergence of HIV from latency to active replication have been a focus of drug development, because the problems associated with both emergence of viral resistance and perturbation of cellular metabolism as well as viral knockdown vastly increase after the onset of active HIV replication. The Tat–TAR interaction has received special attention due to its central role in the HIV transcriptional transactivation, but cellular cofactors such as NF-$\kappa$B are also targets of drug development.[4] Not surprisingly, drugs against cellular cofactors have high toxicity, but are still being pursued as options in the event of failure of HAART chemotherapy. Other recently identified cofactors in activation of HIV transcription include the Werner's syndrome helicase[5] and p90 ribosomal S6 kinase 2 (RSK2).[6]

There has also been a great deal of interest regarding the TAR-binding protein (TRBP) and its effects on HIV replication. TRBP was initially identified as an activator of HIV transcription, its subsequent recognition as a partner of Dicer[7] led to the suggestion that TAR subverts the RNA interference (RNAi) pathway during HIV infection by sequestering TRBP,[8] which would be expected to lead to a global decrease in endogenous microRNA levels. However, HIV infection results in both up- and downregulation of specific cellular microRNAs.[9] siRNA-mediated knockdown of TRBP decreases expression of RT and HIV total protein levels in HeLa cells transfected with pNL4-3 proviral DNA while actin levels are unaffected, suggesting that TRBP acts predominantly to activate HIV replication.[10] This is consistent with observations that low endogenous expression of TRBP is directly linked to low HIV replication in astrocytes, and increasing expression of TRBP in astrocytoma cells increases viral replication to the same level as observed in HeLa cells.[11,12] TRBP is thought to increase HIV replication through its known effect as an antagonist to PKR (RNA-binding protein kinase), inhibiting PKR activation of the cellular immune response.

Additional cellular factors involved in HIV replication continue to be identified. These include 11 cellular microRNAs that are upregulated and downregulated[9]

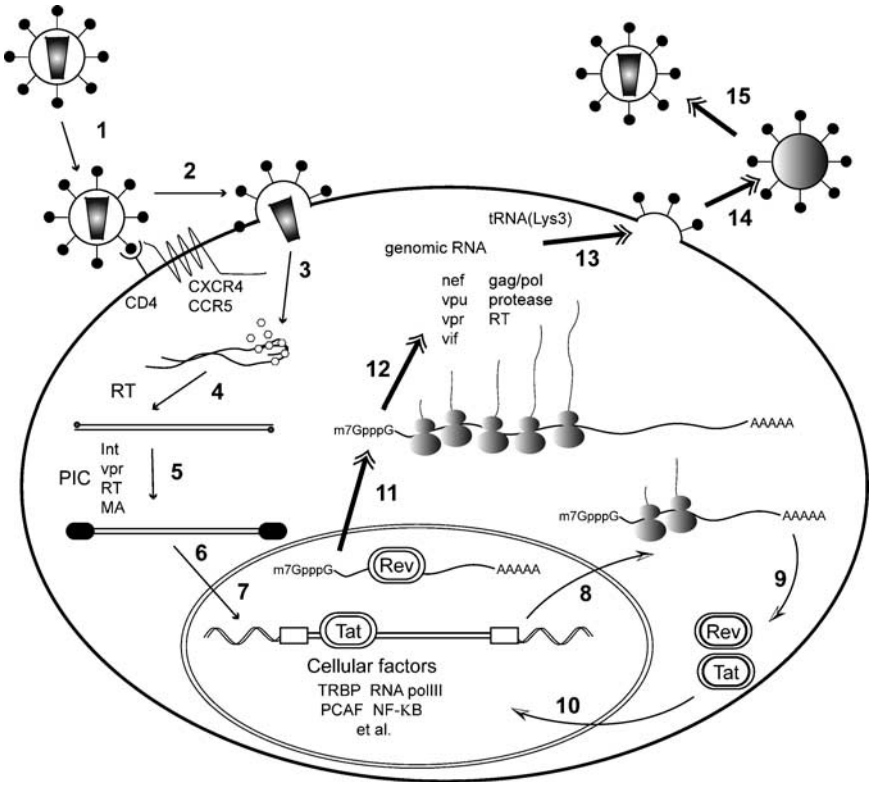


**Figure 1** HIV life cycle. Small arrowheads, viral entry to integration. Curved arrows, early replication. Double-headed arrows, late replication. (1) Adsorption to CD4 receptor and either CXCR4 or CCR5 co-receptor. (2) Fusion. (3) Uncoating of viral genomic RNA dimer. (4) Reverse transcription (RT, reverse transcriptase). (5) Formation of pre-integration complex (PIC). (6) Nuclear import of PIC. (7) Integration of proviral DNA into host genome. (8) Transcription of early multiply spliced mRNAs. (9) Translation of early regulatory proteins, Tat and Rev. (10) Nuclear import of Tat and Rev. Tat increases transcription of viral mRNAs. (11) Rev mediates export of singly spliced and unspliced viral mRNAs. (12) Translation of viral structural proteins. (13) Assembly at the plasma membrane of viral genomic RNA, proteins, and cellular factors including tRNA(Lys3), the obligate primer for reverse transcription. (14) Viral budding. (15) Viral maturation. Cellular factors involved in viral transcription: RNA polIII, TRBP, NF-$\kappa$B and PCAF (see text).

and the polycistronic miR-17/92 cluster encoding multiple microRNAs implicated in B-cell lymphoma and lung cancer development.[13–18] PCAF (p300/CBP-associated factor) is a likely target of two microRNAs derived from the polycistronic pri-miR-17/92, miR-17-5p and miR-17-20a. PCAF is a cofactor for recruiting Tat to TAR and relieving the block in transcriptional elongation upon integration of HIV-1. HIV-induced downregulation of miR-17-5p and miR-17-20a is therefore expected to relieve inhibition of PCAF translation, leading to an increase in PCAF levels in HIV-infected cells, with subsequent increase in HIV proviral transcription and higher rates of HIV replication. Consistent with this model, nuclear transfection of miR-17-5p or miR-17-20a reduces HIV replication, whereas locked nucleic acid inhibitors of miR-17-5p and miR-17-20a as well as nuclear-transfected siRNAs targeting the primary and pre-microRNAs for this cluster increase HIV replication in Jurkat cells.[9]

Recently, another potential class of viral targets has emerged: HIV-derived microRNAs. MicroRNAs have been reported to be derived from nef[19] and possibly other transcripts.[20] Although microRNAs can be successfully targeted for intracellular degradation by synthetic antagomirs (antagonists of microRNAs),[21] modified oligonucleotides complementary to microRNAs that inhibit microRNA, methods for long-term inhibition of microRNAs await development.

## Ribozymes and aptamers continue to evolve as therapeutics

Ribozymes are antisense RNAs that are also capable of enzymatic cleavage of targeted mRNAs. Although the majority of attention in RNA-based therapeutics has turned toward triggers of the RNAi mechanism, ribozymes targeting both viral and cellular mRNAs continue to be tested. A pair of ribozymes derived from the minus and plus strands of Tobacco Ringspot Virus sTRSV(−) and sTRSV(+), respectively were designed to target separate sequences in the HIV-LTR. These combined ribozymes inhibited HIV replication (as reflected in HIV p24 antigen production) when tethered downstream of a sequence which was antisense to TAR, in transient co-transfection assays of the expressed ribozymes and pNL4-3 proviral DNAs in U87-CD4-CXCR4 glioma cells.[22] A different approach combined the HIV packaging signal with a ribozyme or a short HIV antisense strand in a Tat-inducible lentiviral vector. Both the ribozyme and antisense constructs resulted in the production of defective HIV virions when cells expressing these constructs were challenged with HIV.[23] A novel chimeric anti-Tat ribozyme-TAR decoy chimera in which the TAR decoy sequence was an extension of the ribozyme catalytic core gave greater inhibition than

**Table 1** Characteristics of HIV RNA-based inhibitor cassettes in combinatorial lentiviral construct

| *Description* | *Target* | *Promoter* | *RNA inhibitor type* | *Comments* |
|---|---|---|---|---|
| VA1/CCR5-ribozyme | Cellular mRNA | pol III (viral) | Autonomous | Homozygote deletion (*Δ*32) individuals resistant to infection; Heterozygote individuals are slow progressors. |
| U6/U16-TAR decoy | Viral protein | pol III (cellular) | Autonomous | U16 sequences in chimera concentrate decoy in nucleolus to colocalize with target. |
| U6/tat-rev shRNA | Viral RNAs | pol III (cellular) | Cellular trigger | Potent inhibitor of HIV replication; escape variants are less fit. |

Abbreviation: shRNA, short-hairpin RNA.

either separate component.[24] A combination of a ribozyme and a DNAzyme, each targeting different sequences in HIV showed good antiviral efficacy when transfected into macrophages.[25] Dual expression of ribozymes targeting the CXCR4 and CCR5 HIV coreceptors (Figure 1) reduced HIV replication of both T- and M-tropic strains in peripheral blood mononuclear cells.[26] Although ribozymes typically mediate less knockdown than RNAi, they may still be valuable in multiplexing strategies against HIV where a multiple mechanisms of knockdown may be advantageous (see below and Table 1).

## RNAu and effective use of the spliceosome: external guide sequences take advantage of the tRNA processing machinery

Modified U1 snRNA (mU1), also known as RNAu, in which the first 10 nucleotides of the U1 snRNA are replaced with an antisense sequence complementary to sequences in the 3′-untranslated region (UTR) of HIV has been used to disrupt polyadenylation of HIV mRNAs, resulting in inhibition of HIV-1 replication.[27] Although off-target effects using this approach are a concern due to the short length of the targeting sequence, this concern is partially offset by the positional and accessibility requirements needed to get the modified U1snRNP to interact with a target sequence. tRNAs represent 2% of the total cellular RNA, and undergo several processing steps during maturation including removal of the 5′ leader sequence by the RNAse P complex and trimming of the 3′ trailer sequence by tRNAse $Z^L$ in human cells.[28] Although there are some sequence constraints, specificity is largely conferred by structure, so external guide sequences (EGS), which form sufficiently tRNA-like structures upon annealing with their target sequence can induce target cleavage. Expression can be regulated[29] and has been used to inhibit human cytomegalovirus (HCMV). U373MG cells stably transduced with a retroviral vector expressing a U6-driven RNAse P EGS targeting a common site in the mRNAs for the viral structural genes, capsid scaffolding protein and assemblin, suppressed HCMV growth 800-fold.[30] In the same experimental system, an *in vitro* selected variant EGS targeting a site in both essential immediate early genes 1 and 2, mRNAs reduced HCMV viral growth 3000-fold.[31] COS cells co-transfected with an expressed RNAse P EGS either against a site in HIV-1 *tat* or the 5′-UTR along with a pNL4-3 luc construct reduced viral output in a single-round co-transfection assay.[32] In a follow-up study, a combination of RNAse P and RNAse $Z^L$ EGS were more efficient at reducing HIV-1 replication than EGS alone.[33] The efficacy of these anti-HIV EGS in long-term infectious assays awaits assessment.

Each of the above-described methods has some limitations. An accessible target site is required, limiting target selection; consequently, mutations outside the target can cause structural occlusion of the target sequence and viral escape. Specificity is also a concern due to the short lengths of the targeting sequences; 10 nucleotides for mU1 and 12–14 nt for EGS elements. Mutations within the target sequence, especially for mU1, are likely to abrogate the efficacy. In addition, the mU1 expression cassette is rather large. For both approaches, their dependence on the cellular RNA processing machineries adds to their robustness. These methods may be a viable option, particularly in combination with other methods, when the only available highly conserved target sequences are 10–14 nucleotides long, because siRNAs require at least a 19 nucleotide target and are also highly susceptible to mutational escape (see below) and intracellular ribozyme activity is highly variable.

## Multi-targeting RNAi approaches to inhibit HIV replication

HIV-inhibitory RNAi therapeutics continues to be investigated because effective siRNAs mediate very potent target knockdown; both the limitations and strengths of using this approach are becoming clearer. Although RNAi can inhibit HIV-1 replication effectively in the initial stages of infection, a single base change in nearly any position within the target sequence may be sufficient to create siRNA/short-hairpin RNA (shRNA)-resistant viruses after only a few days in culture, even in highly conserved target sequences.[34–36] In addition, mutations outside the RNAi target sequence can allow viral escape to occur by evolving alternative RNA secondary structures.[37] Simultaneous targeting of both wild-type virus and RNAi-escape mutant variants by two or more shRNAs was ineffective in one study, presumably due to competition between shRNAs directed against the wild-type and major escape variant targets.[38]

However, using multiple shRNAs to target separate conserved sites in HIV, akin to the HAART approach, is

more promising, as it prevents cross-resistance between different RNAi effectors or between RNAi effectors and conventional pharmaceuticals. Multiple RNAi effectors would thus have the advantage of limiting escape and targeting a range of sequences as is found in different viral genotypes or quasispecies.[39,40] Viruses which escape the antiviral effects of RNAi can be re-inhibited by targeting different sequences[41] and thus a multiple inhibitory approach should aim to target distinct genomic regions of HIV-1 or, alternatively, target host-derived factors which contribute to viral replication.

A variety of approaches are being explored to express multiple siRNAs.[42] The target sequence of a typical siRNA is 21 nucleotides. Long-hairpin RNAs (lhRNAs) greater than 50 bp in length can be expressed in cells and create multiple siRNAs via Dicer-mediated processing.[43–45] Expressed lhRNAs have been shown to be effective in cell culture against targets for HCV[46] and HIV[38,47,48] and in an *in vivo* murine hydrodynamic injection model of HBV replication for targets against HBV.[44] However, processing and knockdown efficacy of these substrates is asymmetric, being greatest at the base of the hairpin and tapering-off across the full length of the duplex.[44] A 50 bp U6 lhRNA against a conserved HIV-1 *int* region suppressed HIV replication in a variant resistant to a shorter shRNA in the same target.[38] A replication-competent *nef*-deleted HIV-1 variant with a 300 bp lhRNA targeted to *nef* showed significant inhibition of HIV-1 in *trans* although, intriguingly through a non-RNAi mediated mechanism.[47]

Another approach is the use of polycistronic shRNAs, where several hairpins are expressed as a single transcript. Unlike the lhRNAs that typically target adjacent sequences, different short hairpins (usually 19–29 nt in length) in the primary transcript can be directed against widely separated targets. The individual units can be either simple hairpins or modeled on microRNAs, and potentially processed individually for better control of their individual activity.

Multiple shRNAs may be expressed from individual promoters, typically the U6 and H1 polymerase III promoters. Recent studies show that it is possible to saturate the RNAi pathway by high levels of expression of shRNAs[49,50] resulting in cellular toxicity, particularly with the U6 promoter.[49] We recently described the use of $tRNA^{Lys3}$-shRNA chimeric cassettes that mediate graded shRNA knockdown which may be valuable in multiplexing strategies,[51] particularly if the principles can be extended to other tRNA isotypes. Use of lower multiplicities of infection when introducing multiplexed shRNAs in lentiviral backbones can alleviate toxicity; in one study three separate shRNAs targeted to regions within *pol* and *gag* were capable of inhibiting HIV-1 with the addition of each shRNA-expressing cassette providing an additive inhibitory effect.[52]

## Immunostimulation by RNAi therapeutics can be avoided

One general concern regarding the use of RNA-based gene therapy approaches remains the potential to activate cellular interferon responses and inflammatory cytokine production. The intracellular presence of duplexed RNA often activates components of the innate immune system, and specifically the cytoplasmic receptors double-stranded RNA (dsRNA)-dependent protein kinase (PKR) and retinoic-acid-inducible gene-I (RIG-I), leading to a type 1 interferon (IFN) response (reviewed by Garcia-Sastre and Biron[53]). Over 300 IFN-stimulated genes are activated by increased circulating levels of cytokines IFN-$\alpha$ and -$\beta$.[54] In particular, the activation of 2′–5′-oligoadenylate synthetases leads to apoptosis via the nonspecific degradation of cellular mRNAs by activated RNase L (reviewed by Garcia-Sastre and Biron[53] and Karpala *et al*.[55]). Exogenously introduced RNAs (single-stranded RNAs and dsRNAs) are also capable of interacting with different endosomal Toll-like receptors (TLRs), leading to a signaling cascade that elicits the IFN pathway. However, we have recently shown that endogenously (nuclear) expressed shRNAs evade detection by TLRs, RIG-1 and PKR when integrated in CD34+ progenitor hematopoietic stem cells.[56] The expression of lhRNAs (>30 bp) also appears to be well tolerated and does not induce IFN gene activation, whether transiently expressed in cells[43,45,48] or *in vivo* when injected in mice by hydrodynamic tail–vein injection.[44] These results suggest that expressed dsRNAs may mimic nuclear-derived natural RNAi precursors and augurs well for future gene therapy clinical trials.

## RNAi-induced Transcriptional Gene Silencing presents new possibilities for directed gene silencing

Transcriptional gene silencing (TGS) is a phenomenon first described in plant and fungal cells where siRNAs and the RNAi machinery mediate repression of gene expression through chromatin changes. Exogenous siRNAs targeting specific promoters in mammalian cells have been shown to affect expression of the corresponding gene.[44,57–64] Although the mechanism is not fully understood, the possibility of using TGS to intervene earlier in the viral replication cycle by repressing transcription is very attractive, especially because it avoids the opportunities for viral escape that active replication provides. Also, strategies targeting HIV cellular cofactors that by themselves mediate only moderate suppression of HIV, but avoid toxicity, may be more effective in combination with TGS.

## A lentiviral vector triple HIV therapeutic moves toward the clinic

We have described previously a triple combination lentiviral construct comprised of a U6-driven TAR RNA decoy appended upon a U16 snoRNA for nucleolar localization, a U6 promoted shRNA targeted to both *tat* and *rev* open reading frames, and a VA1 promoted chimeric anti-CCR5 *trans*-cleaving hammerhead ribozyme.[65] The triple construct efficiently transduced human progenitor CD34+ cells and demonstrated improved suppression of HIV-1 over 42 days when compared to a single anti-*tat*/*rev* shRNA or double combinations of shRNA/ribozyme or decoy.[65] These three expression cassettes utilize a broad spectrum of the alternatives available in RNA therapeutics (Figure 2

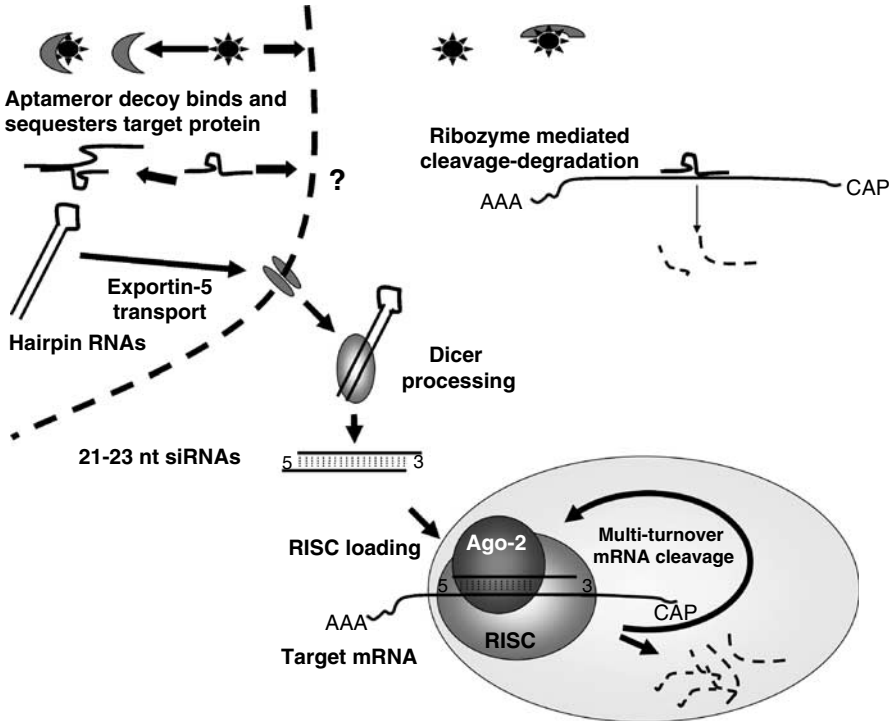


**Figure 2** Expression and function of anti-HIV RNAs. The RNAs are expressed from Pol II or -III promoters from viral vectors. Expressed hairpins are exported to the nucleus via the Exportin 5-Ran GTP carrier. In the cytoplasm, the RNAseIII enzyme Dicer processes the hairpins into siRNAs. One of the strands is chosen as the guide and enters the RNA-induced silencing complex, or RISC, where it serves to guide RISC cleavage and functional destruction of targeted RNAs. The ribozymes are either exported to the cytoplasm via mRNA export pathways or are retained in the nucleus, as the RNAse P guide sequence. The ribozymes either cleave the target autonomously, or use RNAse P to assist in the cleavage mechanism. The aptamers or decoys bind and sequester proteins that they are designed to interact with. This can take place either in the nucleus or cytoplasm.

and Table 1), demonstrating the potential power of this approach. This triple combination is about to enter human clinical trials for AIDS/lymphoma patients using autologous hematopoietic progenitor cells as the targets for vector insertion. A second trial in which the same construct will be inserted in autologous T lymphocytes will most likely initiate in late 2007 or early 2008.

## Prospects

The successful use of RNA mediators of anti-HIV activity in human hematopoietic cells has now been validated by many different investigators. With the development of genetically modified viral vectors that are capable of transducing hematopoietic cells with therapeutic RNA encoding constructs, there will be more proof-of-concept studies in animal models within the next couple of years. Ongoing clinical trials using anti-HIV ribozymes and antisense RNAs have demonstrated the safety of hematopoietic based gene therapies. The next step is to prove efficacy of these RNA-based inhibitors. For HIV-1 gene therapy, introduction of expressed RNA antivirals into CD34+ hematopoietic stem cells theoretically affords protection and possibly immune reconstitution to all derived hematopoietic lineage cells. Lentiviral vectors are the delivery vehicle of choice, because unlike murine retroviral vectors, they can transduce nondividing stem cells at high efficiency and are less subject to some forms of transcriptional silencing. The challenge of producing high-titer lentiviral vectors carrying expressing antivirals is also being overcome.[65,66] Once efficacious use of antiviral RNAs in a gene therapy setting has been demonstrated, this will open the door for expanded clinical applications.

## Conclusions

Although a cure for AIDS does not appear to be imminent, the success of HAART therapy presents the very real possibility of maintaining HIV as a chronic condition. Although many hurdles need to be overcome, the hope is that gene therapy will provide an added option when pharmacological drugs fail and perhaps an adjuvant to current therapies that promotes longer and better quality of life.

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