Research progress on siRNA delivery with nonviral carriers

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Abstract: RNA interference is a powerful method for the knockdown of pathologically relevant genes. Small interfering RNAs (siRNAs) have been widely demonstrated as effective biomedical genetic-therapy applications for many diseases. Unfortunately, siRNA duplexes are not ideal drug-like molecules. Problems hindering their effective application fundamentally lie in their delivery, stability, and off-target effects. Delivery systems provide solutions to many of the challenges facing siRNA therapeutics. Due to some fatal disadvantages of viral vectors, nonviral carriers have been studied extensively. Aside from liposomes, nanoparticles and cationic polymer carriers have exhibited improved in vivo stability, better biocompatibility, and efficiency for gene silencing with less cellular toxicity. They may represent a promising strategy for siRNA-based therapies, especially as nanomaterials. The present review also summarizes other methods of siRNA delivery and the side effects of the nanoparticles.

Keywords: small interfering RNA, nonviral vector, gene therapy, delivery system, nanoparticles, biocompatibility

Introduction
Since its discovery in 1998 by Fire et al, RNA interference (RNAi) has represented a promising new approach towards the inhibition of gene expression in vitro or in vivo.1–3 In 2001, Elbashir et al4 using synthetic small interfering RNA (siRNA), showed for the first time that RNAi also occurs in mammalian cell lines, making successful development of RNAi possible. Rapid progress in our understanding of RNAi-based mechanisms has led to the application of this powerful mechanism in the study of gene function, as well as therapeutic applications for disease treatment.

RNAi is mediated through approximately 21–23 nt, double-stranded siRNAs that trigger sequence-specific cleavage of mRNA molecules, leading to their subsequent degradation.5 These siRNAs are generated intracellularly through the cleavage of longer double-stranded RNAs,6,7 or are introduced into the cell as chemically synthesized siRNA molecules.4

However, the naked siRNA molecule, with negative charges, is susceptible to serum nucleases, renal clearance, and nontargeted biodistribution, making cellular target sites more difficult to access. Thus, it has many limitations, such as poor stability, short half-life, and low efficiency. The major hurdle faced by current RNAi therapeutic strategies is the efficiency of the delivery system. Recently, viral delivery, such as lentivirus and adenovirus delivery, of DNA-based siRNA constructs for RNAi-mediated vascular endothelial growth factor (VEGF) downregulation showed anti-neovascularization effects in various animal models.8–10 Viral vectors are highly...
efficient delivery systems for nucleic acids; however, the potential for mutagenicity, limited loading capacities, high production costs, and most importantly, safety risks caused by their inflammatory and immunogenic effects severely limit the applicability of viruses. These concerns have led to the pursuit of nonviral alternatives. Thus, the direct, systemic, nonviral administration of siRNA molecules that allows for therapeutic use is most desirable. Nonviral vectors are capable of delivering nucleic acids, including genes, siRNA, or antisense RNA into cells, thus potentially resulting in their functional expression. These vectors are considered an attractive alternative for virus-based delivery systems.

Multiple nonviral siRNA delivery systems include chemical modification of siRNA, liposomes, nanoparticles for siRNA delivery, cell-penetrating peptides, and targeted delivery. Positively charged cationic liposomes and polymers, such as polyethyleneimine, are currently the two major carriers used to complex with negatively charged siRNA for systemic delivery. Some of these novel vehicles can potentially overcome extracellular and intracellular barriers, and facilitate the site-specific delivery, cellular uptake, and intracellular target interactions of siRNA.

The current review discusses the progress in the application of nonviral systems for synthetic RNAi molecule delivery, with focus on the characteristics and advantageous properties of siRNA nanoparticle systems.

Chemical modification of siRNA

The half-life of unmodified naked siRNAs in vivo ranges in minutes, but this can be significantly improved to hours by chemical modifications. In addition, chemical modification of siRNAs may enhance biological stability without adverse effects on the gene-silencing activity. Furthermore, modified siRNAs with superior potency reduces the dose required for gene silencing, and specific chemical modifications can minimize siRNA side effects, such as the induction of recipient immune responses and inherent off-targeting effects. Various chemical modifications to the backbone, nucleobases, terminals, and sugars of siRNAs have been reported so far, which are mainly focused on increasing its stability and enhancing its cellular uptake.

The most widely used siRNA modifications are on sugar moieties, which commonly include replacement of the 2′-fluoro (2′-F), 2′-O-methyl, 2′-halogen, 2′-amine, and locked nucleic acid (LNA), all of which have shown significant increase in siRNA serum stability. Layzer et al have demonstrated that siRNA modified with 2′-fluoro (2′-F) pyrimidines are more functional in cell culture and have greatly increased stability and prolonged half-life (24 hours) in human plasma, compared with 2′-OH (1 minute) containing siRNAs. Jackson et al showed that 2′-O-methyl modifications to specific positions within the siRNA seed region reduces the number of off-target transcripts and the magnitude of their regulation, without significantly affecting the silencing of the intended targets.

The simplest approach to increase nuclease stability has been to modify the internucleotide phosphate linkage directly. Phosphorothioate (P = S) modifications can be placed in the RNA duplex easily at any desired position and will enhance the stability of siRNA in nuclease environments. Overhoff and Sczakiel stated that phosphorothioate (PTO)-derived oligonucleotides stimulate the physical cellular uptake of siRNA in human cells. On the other hand, siRNAs with boranophosphate (P = B) backbone modifications have much higher nuclease resistance than unmodified ones, with less cytotoxicity. In addition, Hall et al demonstrated that boranophosphate siRNAs are consistently more effective than siRNAs with the widely used phosphorothiate modification. Furthermore, boranophosphate siRNAs are at least 10 times more nuclease resistant than unmodified siRNAs. Therefore, the biochemical properties of boranophosphate siRNAs make them promising candidates for RNAi-based therapeutic applications. Recently, some groups used the same modified siRNA to treat patients with age-related macular degeneration (AMD). The process has reached Phase II clinical trials, and it was found to have no observable side effects.

To date, much of the focus has been on modifying the RNA backbone, and some laboratories have modified the siRNA bases that are centrally involved in target recognition. Terrazas and Kool explored the effects of methyl and propynyl substitution on siRNA duplex stability and cellular RNAi activity. The results suggested that smaller 5-methyl substitutions do not adversely affect gene-silencing activity; furthermore, this modification contributes positively to siRNA stability in human serum.

Terminal nucleotide modifications of the siRNA (also called siRNA conjugates) have also been reported as an efficient delivery strategy. These include peptide modification, such as transactivating transcriptional activator (TAT) peptide, cholesterol conjugation, folate, and aptamer conjugation, which offer opportunities to enhance pharmacological characteristics of or introduce special features to siRNA. Soutschek et al reported that if both 5′- and 3′-terminal modifications of the sense strand are well modified siRNAs, they could silence the endogenous gene.
that encodes apolipoprotein B after intravenous injection in mice.

Chemical modification of siRNA can increase the stability of the RNA duplex to nucleases, minimize the possibility of immunostimulatory responses, and decrease the possibility of off-target effects, as well as improve its pharmacodynamic properties and delivery to target cells. Chemical modification provides solutions to many of the challenges facing siRNA therapeutics, but the remaining challenge is to find universal chemical modification strategies, or to predict reliably which modifications will be effective for a given sequence.32

**Liposome-formulated delivery system**

Liposomes are probably the most extensively used materials for the delivery of gene molecules ever since their ability to transport the preproinsulin gene to the liver33 was demonstrated 25 years ago. Hence, their characteristics are outlined in detail. Liposomes, vesicles with an aqueous compartment enclosed in a phospholipid bilayer, can fuse with cell membranes and enhance drug delivery into cells. Polar drugs can be entrapped in their aqueous center. When lipids combine with nucleic acids to form amorphous particles, they are known as lipoplexes.34 siRNAs can either be entrapped within a lipid core35 or attached to the surface of the lipid materials36 for delivery. The liposome can protect nucleic acids from enzymatic degradation and deliver nucleic acids into cells by interacting with the negatively charged cell membrane. There are two kinds of liposome: neutral and cationic lipid material.

Neutral liposomes confer low toxicity to mammalians with low transfection efficiency due to their surface charge. But cationic liposomes can cross the cell membrane and reach the target genes with good biocompatibility.37,38

There are two kinds of liposome, neutral and cationic, based on their lipid composition. Initially, neutral liposomes were used for in vitro and in vivo delivery of nucleic acids because of their low toxicity, low immunogenicity, and easy production. Halder et al19 examined the therapeutic potential of focal adhesion kinase (FAK)-siRNA in the neutral liposome 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC). The results show that treatment with FAK siRNA-DOPC results in decreased microvessel density, decreased expression of VEGF and matrix metalloproteinase-9, and increased apoptosis of tumor-associated endothelial cells and tumor cells.

Given that neutral liposomes confer low transfection efficiency due to their lack of surface charges, this methodology was soon supplanted when cationic liposomes were developed in 1989.40 The cationic charge can electrostatically combine with siRNA to achieve a more robust construct to improve cell entry and protect siRNA against serum degradation, whereas neutral lipids facilitate fusion with the host cell membrane.41 Spagnou et al37 selected a number of cationic lipid/lipid-based systems to investigate the optimum lipid to nucleic acid ratio, mode of delivery, biocompatibility, and dose-response effects. The results demonstrate that the systems mediate a maximal specific gene silencing and knockdown with significantly low toxicity to mammalians. Leal et al42 reported the development of cationic liposome (CL)-siRNA complexes with novel cubic phase nanostructures that exhibit efficient silencing at low toxicity. This finding underscored the importance of understanding the membrane-mediated interactions between the CL-siRNA complex nanostructure and cell components in developing CL-based gene-silencing vectors.

Some cationic liposomes, such as Lipofectamine™ 2000, Oligofectamine™, and Lipofectamine (Invitrogen), are commercially available.41 Huang44 transfected two keratoepithelin (KE)-specific siRNAs into HEK293 cells via Lipofectamine to treat certain types of inherited corneal stromal dystrophy, and observed that KE expression is reduced by approximately 50%–80%. Kim et al45 applied water-soluble lipopolymer (WSLP) for delivering siRNA targeting VEGF in vitro and in vivo, which significantly increased the efficiency of inhibition. WSLP/siRNA complexes can efficiently protect siRNAs from enzymatic degradation in serum-conditioned media.

Many researchers studied immunoliposomes to develop a vehicle that can be systemically administered safely and repeatedly, and will deliver the siRNA specifically and efficiently to the targeted tissues. Pirolo et al46 engineered a nanosized immunoliposome-based delivery complex that, when systemically administered, will preferentially target and deliver siRNA to tumor cells wherever they occur in the body. They enhanced the efficiency of this complex with the inclusion of a pH-sensitive histidine–lysine peptide in the complex and by delivery of a modified anti-HER-2 siRNA molecule. The complex can sensitize human tumor cells to chemotherapeutics, silence the target gene, and inhibit tumor growth in a cancer model.

Successful lipoplex deliveries of siRNA has been reported in several studies, and mechanisms for the release of payload from lipoplexes have been suggested. Continued research into the mechanism of release may lead to the development
of more bioresponsive vectors that can achieve higher levels of silencing for a given dose.\(^{47}\)

**Nanotechnology-based siRNA delivery system**

The birth of nanotechnology has allowed people to transform nature at the molecular and atomic level, enabling the manipulation of single atoms. With the progress of material synthesis and the rise of nanotechnology, the synthesis of nanomaterials with specific functions has become possible. The development of nanotechnology in the biological area has made nanodelivery systems popular. Currently, nanospheres can smoothly pass the blood–brain barrier, testicle–blood barrier, and placenta.\(^{48,49}\) Thus, nanospheres will be suitable transfection carriers for improving the effect of gene therapy.

Due to their low toxicity, biodegradability, and bio-compatibility, many nanomaterials are used as transfection carriers, such as chitosan, cyclodextrin, polyethyleneimine (PEI), poly(lactic-co-glycolic acid) (PLGA), dendrimers, magnetic nanoparticles, carbon nanotubes, and gold nanorods. Because of its high nuclease resistance and mucoadhesive properties, chitosan, natural polymers extracted from crustaceans, has become popular siRNA vectors. Howard et al\(^{50}\) used a chitosan-based siRNA nanoparticle delivery system for RNA interference in vitro and in vivo. They observed, using fluorescence microscopy, that Cy5-labeled nanoparticles were rapidly uptaken by NIH 3T3 cells within 1 hour and are accumulated over a 24-hour period. In vivo, effective RNAi was achieved in bronchiole epithelial cells of transgenic enhanced green fluorescent protein (EGFP) mice after nasal administration of chitosan/siRNA formulations. Ghosn et al\(^{51}\) reported the use of imidazole-modified chitosan-siRNA nanoparticles to mediate gene silencing via either intravenous or intranasal administration. The results showed that intravenous delivery demonstrated significant knockdown of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) enzymes in both the lungs and the liver at as low as 1 mg/kg siRNA dose. For intranasal delivery, significant silencing of GAPDH protein expression was seen in the lungs with only 0.5 mg/kg/day siRNA delivered over three consecutive days.

As a cationic polymer, PEI with its high-density charge, which can protect DNA from degeneration by nuclease, has been widely studied in gene delivery. Höbel et al\(^{11}\) established the therapeutic efficacy and safety of PEI F25-LMW/siRNA-mediated knockdown of VEGF in tumor cells. The results showed that PEI F25-LMW/siRNA complexes, which can be stored frozen as opposed to many other carriers, represent an efficient, safe, and promising modality in antitumor therapy. Modification of PEI with several functional moieties revealed that PEI succinylation resulted in up to a 10-fold reduction of polymer toxicity compared with unmodified PEI. Moreover, succinylated PEI/siRNA complexes were able to induce remarkable knockdown of the target luciferase gene at the lowest tested siRNA concentration.\(^{52}\)

In the study by Wang,\(^{53}\) TAT-conjugated PEGylated magnetic polymeric liposomes (TAT-PEG-MPLs) formed with superparamagnetic nanoparticles and TAT were successfully prepared and evaluated in vitro and in vivo. The results indicated that TAT-PEG-MPLs were spherical and nonaggregated in solution, with significantly small mean diameters (83.2 nm) and high magnetization. In cell penetration tests performed through fluorescein isothiocyanate (FITC) labeling, the uptake of TAT-PEG-MPLs by MCF-7 cells was greater than that of PEG-MPLs. Most importantly, in vivo animal experiment, MRI, histological analysis, and atomic absorption spectrophotometry revealed that TAT-PEG-MPLs nanoparticles significantly accumulated around the target site, and even inside nerve cells.

PLGA is an amorphous polymer, with molecular weights ranging from 5000–300,000. It can be used as medical film and carrier material for sustained-release dosage systems, with lower toxicity, less irritation, and minimal inflammatory responses. Luo et al\(^{54}\) incorporated the siRNA sequence of the methyl-CpG binding domain protein 1 (MBD1) plasmid into a PLGA-poloxamer carrier to test the therapeutic effect of this compound on BxPC-3 human pancreatic cancer cells. They found that the PLGA-poloxamer carriers can successfully transfect the MBD1 siRNA plasmid into tumor cells and that the PLGA-MBD1 nanoparticle compound can inhibit cell growth and induce apoptosis.

Katas et al\(^{55}\) incorporated PEI into PLGA particles to produce spherical, positively charged nanoparticles that are able to protect siRNA against nuclease degradation. Cell culture studies showed that PLGA-PEI nanoparticles with encapsulated siRNA are more efficient in silencing the targeted gene than PEI alone, with certain biocompatibility.

Ladeira et al\(^{56}\) described a novel approach to siRNA delivery by single-walled carbon nanotubes. In the study, the siRNA delivery system showed nonspecific toxicity and transfection efficiency greater than 95%. This approach offers the potential for siRNA delivery into different types of cells, including hard-to-transfect cells such as neuronal cells and cardiomyocytes.
Gold nanoparticles/nanorods (GNPs/GNRs) have the distinct advantage of being easily modified. Hence, with their positively charged surfaces, GNPs/GNRs can easily attach to negatively charged siRNA. Bonoiu et al. applied GNRs as siRNA delivery to explore the dopaminergic brain signaling pathway in vitro. Gene silencing in these cells was evident, with no observed cytotoxicity. Moreover, these nanoplexes can transmigrate across the model of the blood–brain barrier. However, reports about GNPs as gene carriers are rare.

Nevertheless, research regarding siRNA delivery was inspired by the molecular machinery within the phi29 bacteriophage DNA packaging motor, which contains six copies of packaging RNA (pRNA) molecules that form a hexameric ring, which is the crucial part of the motor. Utilizing the novel properties of this pRNA, we constructed pRNA dimers and trimers with potential to serve as parts in nanotechnology. pRNA-derived nanoparticles have small sizes (20–40 nm), making them particularly suited for in vivo systemic delivery; the optimal size range for cell uptake is 10–100 nm. Tarapore et al. explored the potential of pRNA as a vehicle in carrying siRNA to target metallothionein-IIa (MT-IIA) messenger RNA (mRNA) specifically. They found that pRNA chimeras targeting MT-IIA are localized within the GW/P-bodies, and are more potent than siRNA alone in silencing MT-IIA expression. Therefore, the pRNA system, which can assemble into multivalent nanoparticles, has great potential as a highly potent therapeutic agent.

**Targeted siRNA delivery system**

Targeted RNAi therapy is a relatively new approach that can be used to silence genes reversibly in vivo by selective targeting. Targeting the diseased cell, organ, or tissue will increase the silencing potency of a given dose of siRNA. Specific cell targeting will also prevent side effects by avoiding nondiseased cells. Aptamers, antibodies, small peptides, and other ligands that bind to the signature molecules with high specificity and affinity have been studied extensively for their ability to guide siRNA to the target tissues and cells.

Dickerson et al. designed nanoparticles functionalized with peptides that specifically target the erythropoietin-producing hepatocellular (Eph) A2 receptor to deliver siRNAs targeting epidermal growth factor receptor (EGFR). The results showed that the nanoparticles decreased EGFR expression levels and significantly increased the sensitivity of this cell line to docetaxel. Agrawal et al. designed dendrimer-conjugated magnetofluorescent nanoworms called “dendriworms” as a modular platform for siRNA delivery in vivo. Their study showed that dendriworms carrying siRNA against EGFR reduced EGFR protein levels in human glioblastoma cells by 70%–80%; anti-EGFR dendriworms led to specific and significant suppression of EGFR expression.

The folate receptor is also an important target for anticancer drug delivery. Many anticancer drugs, such as prodrug enzymes, toxic proteins, liposome drugs, and nucleic acid molecules, including siRNA, can be combined with folic acid to achieve target specificity. For example, liposome-wrapped siRNA nanoparticles can be modified with folic acid to inhibit the growth of a targeted tumor.

Aptamers are oligonucleic acid or peptide molecules that are normally selected from a large random sequence pool to bind to a specific target molecule. Chimeric RNA molecules that contain an RNA aptamer directly linked to the passenger strand of siRNA can be transcribed in vitro and readily purified in large quantities. Therefore, aptamers can enhance the ability of siRNAs to target different cells. To date, only a couple of aptamers have been harnessed for targeted siRNA delivery to specific cell populations. Some studies have shown enhancement of target gene silencing activity and specificity using aptamer–siRNA chimeras. Furthermore, anti-tumor activity is further enhanced by appending a polyethylene glycol moiety, which increases the circulating half-life of the chimeras.

**Other delivery systems**

Lee et al. developed pluronic/polyethylenimine shell crosslinked nanocapsules with embedded magnetite nanocrystals (PPMCs) for magnetically triggered delivery of siRNA. PPMC/siRNA–PEG complexes were efficiently taken up by cancer cells upon exposure to a magnet, thereby enhancing intracellular uptake and the silencing effect of siRNA. The study suggests that these novel nanomaterials could be potentially utilized as magnetically triggered delivery of various nucleic acid-based therapeutic agents.

Recently, ultrasound-mediated gene delivery with nano- and microbubbles was developed as a novel nonviral vector system. This system can directly deliver plasmid DNA and siRNA into the cytosol without endocytosis. Therefore, these genes are able to escape lysosomal degradation, resulting in enhanced gene expression efficiency. Zhou et al. transfected plasmid DNA of pigment epithelial derived factor (PEDF) into human retinal pigment epithelium (hRPE) cells and choroidal neovascularization (CNV) animal models by ultrasound-mediated microbubbles. The results showed that
microbubbles with ultrasound irradiation could significantly enhance PEDF delivery compared with microbubbles or ultrasound alone, and that the CNV of rats was inhibited effectively.

**Limitations and other side-effects of siRNA delivery**

siRNA is easily degraded by enzymes in blood, tissues, and cells. Several types of chemically modified siRNA have been produced and investigated to improve stability; however, target site accumulation after administration is still extremely low, even when stability is improved.69 Moreover, the cost and safety of these formulations must also be considered,70 and many groups have found that large numbers of 2′-O-Me modifications (in either strand) decrease siRNA activity.71–73

Even though an increasing number of cationic liposomes are used as nonviral-based gene vectors, studies have demonstrated that these liposomes still cause significant toxicity,74,75 such as cell contraction, mitotic inhibition, formation of aggregates in blood, and the tendency to induce inflammatory response. Some researchers have been trying to design new liposomal structures to reduce their cytotoxicity, and found that modifying the cationic liposome structure can be an effective strategy for controlling its toxicity.76 Hundreds of lipids share the common structure of a positively charged hydrophilic head and hydrophobic tail that are connected via a linker structure. Most of the linkages between the hydrophilic and hydrophobic moieties are ether, ester, carbamate, or amide bonds that can affect the rate of biodegradation.77 Moreover, their transfection efficiency needs further improvement for in vivo application.

Aside from studies of its efficacy, some researchers investigated the biosafety and toxicity issues of chitosan. A recent study by Chellat et al78 found that high chitosan/DNA nanoparticles could lead to certain organ damage.82–84

Watari et al81 revealed the micro/nanosizing effect of materials on living organisms. Various sizes of particles, from 500 nm to 150 µm, were used to co-culture with human neutrophils, which play a central role in the initial stage of inflammation against foreign bodies, as probe cells. Particles were also inserted into the subcutaneous connective tissue in the abdominal region of Wistar rats. The results showed that: 1) the toxicity level, shown as an absolute intensity of superoxide production, as well as the expression of cytokines IL-1β and TNF-α by micro/nanoparticles is very low; 2) the reaction is nonspecific and any particle below cell size are regarded by cells as a foreign object, including neutrophils and macrophages; and 3) for the materials as implants, there is no strong rejection and it is, therefore, generally acceptable to any person.

However, other researchers reported different results. Nanoscale gene vectors, after being transfected into the body as exogenous materials, are taken up by the reticuloendothelial mononuclear phagocytic system, which is mainly concentrated in the spleen, liver, lungs, bone marrow, and lymph nodes. The nanoparticles could be retained in the tissues for over 30 days; therefore, if not effectively removed, accumulation of the nanosized vectors could lead to certain organ damage.82–84

Low immunogenicity is one of the unique characteristics of nonviral gene vectors. However, studies have shown that the DNA–cationic liposome complex (lipoplex) systemic drugs can activate the innate immune system.85 Sakurai et al86 found that the administration of lipoplex produces a greater amount of inflammatory cytokines, such as IL-6, IL-12, and TNF-α, than adenovirus vector administration. On the other hand, chitosan/DNA nanoparticles and single-walled carbon nanotubes are not available for immune stimulation.87–89

**Clinical trials**

From the first in vivo evidence of RNAi-based therapeutic efficacy in an animal disease model in 2003,90 the pace of its drug development has been rapid. Most of the clinical trials approved by the FDA are nontargeted, designed for intravitreal injection or inhalation to cure age-related macular degeneration (AMD), a leading cause of blindness, or respiratory syncytial virus (RSV), the leading cause of pediatric hospitalizations in the United States today. These trials with naked siRNA have shown certain results without untoward toxicity.14,91

Multiple nanoparticle formulations of siRNA for oncology are currently under clinical development.92 The first targeted-delivery siRNA therapeutic agent approved by the US Food and Drug Administration (FDA) is CALAA-01, a PEGylated, transferrin-targeted nanoparticle that can combine siRNA molecule with the transferrin receptor on
the tumor cell surface. This is currently being investigated clinically in patients with solid cancers in the United States.\(^93\) The results demonstrate that the siRNA nanoparticles are able to provide transient inhibition of tumor growth.

Atu027 is a liposomal formulation of siRNA against protein kinase N3, developed by Silence Therapeutics AG, and is currently being investigated in Phase I clinical trials in Germany.\(^94\) Atu027 comprises neutral fusogenic and PEG-modified lipid components with improved pharmacokinetic properties, cellular uptake, and efficient siRNA release from endosomes after endocytosis.\(^95\)

ALN-VSP, a nontargeted liposomal formulation of two siRNAs targeting kinesin spindle protein and VEGF, is in clinical development in the United States for the treatment of liver cancers. Due to interests in the potent, promising, and novel siRNAs and the need to protect them from degradation within the circulation, interest and research into nanoparticle formulations of nucleic acids is likely to grow continually.\(^92\)

**Perspective of nonviral vector**

siRNA technology, which is attracting much interest in the research community, holds great promise as a therapeutic intervention for targeted gene silencing in cancers and other diseases. Several siRNA-based therapeutic agents are already in clinical trials. Further development of siRNA therapy depends on the development of safe and effective carriers for systemic administration. As described in the present study, cationic nanoparticles have emerged as one of the most attractive carriers because of their ability to form complexes with negatively charged siRNA and their high transfection efficiency. As a whole, the transfection efficiency reported thus far for nonviral approaches is still below that of the highly efficient viral vectors. Further improvements to increase the efficiency and reduce the toxicity of nonviral vectors are needed before their clinical significance can be maximized. Therefore, to achieve more efficient, long-lasting, and nontoxic gene delivery vectors, optimized delivery systems still have many challenges to overcome.

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The authors report no conflicts of interest in this work.

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11. Höbel S, Koburger I, John M, et al. Polyethylenimine/small interfering RNA nanoparticles for systemic delivery in vivo: novel siRNAs and the need to protect them from degradation within the circulation, interest and research into nanoparticle formulations of nucleic acids is likely to grow continually.\(^92\)