Clinical Evaluations of Toxicity and Efficacy of Nanoparticle-Mediated Gene Therapy

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Considerable efforts have been devoted to develop safe and efficient gene therapies for life-threatening or inherited diseases. The choice of gene delivery vehicle plays key roles in enhancing the therapeutic effect of nucleic acid cargo. To date, gene therapy approaches involving both viral vectors and nonviral vectors have been evaluated in clinical trials. With improvements in material science and nanotechnologies, positively charged nanoparticles have emerged as potential gene delivery vehicles. In this review, we highlight clinical trials that examined cationic nanocarrier-mediated gene therapy as well as discuss both the toxicity and efficacy of nanocarrier-based therapeutics.

Keywords: gene therapy, nanocarrier, toxicity, clinical trials, nonviral vector

INTRODUCTION

GENE THERAPY PROVIDES METHODS for treating life-threatening disease and is rapidly developing.¹ The efficient delivery of genetic cargo into the targeted tissues and cells plays a key role in advancing therapeutic efficiency. To date, gene delivery approaches involving viral and nonviral carriers have been intensively studied.^{2,3} In general, viral carriers have high efficiency, but can also have issues with immunogenicity and toxicity.⁴ With the development of material science and nanotechnology, numerous nanoscaled drug delivery systems have emerged, including liposomes, dendrimers, and micelles.⁵ Such delivery systems are also being investigated and optimized as potential carriers in gene therapy.⁶

Nonviral nanocarriers are advantageous compared with viral carriers, due to their ease of large-scale production, lower immunogenicity, and ability to deliver large gene payloads.⁷ Such nonviral nanocarriers can be tailored and synthesized to obtain various physical–chemical properties that suit the physical circumstances and pathological features of a given disease.⁸ Moreover, novel therapeutic target discovery in basic research highlighted the im-

portance of various surface modifications that can be performed to yield ideal targeting properties.⁹ For example, to carry anionic gene cargoes, nonviral vectors typically have a cationic surface charge that allows simple encapsulation of anionic gene cargo via electrostatic interactions.⁷ Cationic liposomes and polymers such as polyethylenimine (PEI) and polylysine thus are of significant interest for their potential use as delivery systems in gene therapy.¹⁰ Over the past two decades, a number of clinical trials that examined nonviral vector-mediated delivery have been conducted. In this review, we highlight clinical trials that investigated nanocarrier-based gene therapies, focusing on both the toxicity and the therapeutic efficiencies of these nanocarriers.

NANOPARTICLE CATEGORIES

An appropriate alternative delivery system is critical to deposit nucleic cargoes in target cells or organisms *in vivo*. To date, considerable effort has been devoted to the search for safer and better gene carriers for human gene therapy.¹¹ Nanocarriers can be modified to present a positively charged

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surface that facilitates encapsulation of anionic nucleic cargo. Nanocarriers such as cationic liposomes, polymers, micelles, nanospheres, and hydrogels have been evaluated for their potential to act as gene delivery systems. Although numerous nanoparticles have emerged as potential delivery alternatives in basic research or preclinical studies, few have been evaluated in clinical trials. Lipid-mediated gene transfer was one of the earliest strategies for gene therapy.¹² Therefore, the majority of the clinical trials involved cationic liposomes, or cationic lipid-based nanocomplexes. The cationic liposome cargoes that have been evaluated for safety and efficacy in clinical trials include both plasmid and small interfering RNA (siRNA). Cationic polymers also play roles in human gene therapy because of their diversity and flexibility in preparation and modification. For instance, a linear, cationic cyclodextrin-based polymer has been studied for tumor treatment, and polylysine-based polymers have been investigated for use in cystic fibrosis (CF) gene therapy. Other types of virus-like nanoparticles or particles encapsulated in liposomes were also evaluated in clinical studies.^{13,14}

Disease conditions and administration route

Gene therapy provides more alternatives to treat many major diseases, particularly those that are life-threatening.¹ Human gene therapy for cancer treatment has been studied intensively in clinical trials.¹⁵ Various types of tumors including melanoma, sarcoma, osteosarcoma, ovarian cancer, glioma, and head and neck cancer have been treated with therapeutic genes delivered by nanoparticles. In particular, previous studies that examined the use of nanoparticles to treat advanced tumors and metastatic tumors have drawn significant attention. One study described a clinical trial involving 41 patients with gastrointestinal, gynecological, or genitourinary tumors and sarcoma to examine RNA interference (RNAi) to target vascular endothelial growth factor (VEGF) and kinesin spindle protein (KSP). Most patients in this trial had hepatic and extrahepatic tumors.¹⁶ Another phase I clinical trial examining delivery of the human tumor suppressor gene p53 as part of the nanocomplex SGT-53 evaluated the treatment of advanced solid tumors including thyroid, rectum, colon, vaginal, and cervical tumors.¹⁷ An interleukin (IL)-12 plasmid formulated with polyethylene glycol (PEG)-PEI-cholesterol lipopolymer was used to treat persistent or recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer in a phase II trial.¹⁸

In addition to cancer therapy, other major diseases have also been treated by nanocarrier-

mediated gene therapy. Transthyretin amyloidosis is a life-threatening disorder caused by deposition of hepatocyte-derived transthyretin amyloid in peripheral nerves and the heart. One study described the effectiveness of two distinct lipid nanoparticles carrying the anti-transthyretin siRNA to reduce levels of transthyretin.¹⁹ CF, which involves mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) that lead to irreversible lung damage, has also been treated by nanocarrier-based gene therapy in several clinical trials.^{20–22} In a double-blind, placebo-controlled trial, therapy with a lipid-DNA complex containing a CFTR gene delivered through the nasal epithelium of patients with CF was evaluated to determine appropriate doses as well as safety and efficacy.²⁰ Another study monitoring a small group of patients with CF treated by cationic lipid-mediated gene transfer to the lungs and nasal tissue showed some success in reducing chloride transport abnormalities.²² Nanoparticles consisting of polyethylene glycol-substituted lysine peptides have also been used to deliver the CFTR gene to the nasal mucosa and were partially successful in reconstituting CFTR function.²¹ On the basis of the above studies, the most efficient lipid-based formulation was selected and in 2013 the UK Cystic Fibrosis Gene Therapy Consortium launched a randomized, double-blind, placebo-controlled phase IIB clinical trial of gene therapy in patients with CF to assess the clinical benefit of these treatments.²³

Nanoparticle-based gene therapy has also been used to prevent disease. Gene therapy to prevent restenosis in patients with *de novo* or restenotic coronary artery lesion (REGENT I extension) was done with an intracoronary iNOS (inducible nitric oxide synthase) lipoplex (CAR-MP583), which was found to be safe.²⁴ Another phase I trial evaluated the safety and efficacy of manganese superoxide dismutase plasmid liposomes delivered orally to protect patients with lung cancer undergoing concurrent chemotherapy and thoracic radiotherapy.²⁵ A novel Toll-like receptor 9 agonist packaged in virus-like nanoparticles demonstrated its clinical efficacy in patients with allergic rhinoconjunctivitis.¹³

The administration route for nanoparticles can vary according to disease characteristics. For cancer therapy, intravenous injection and intratumoral injection are the most frequently used methods for administration. Intraperitoneal administration has been used for ovarian cancer treatment, and nanoparticles were injected into the brain after tumor removal.²⁶ Treatment for transthyretin amyloidosis generally involves systemic injection,¹⁹ whereas CF

treatments involve nasal or airway administration. Moreover, oral administration and intracoronary administration were also used in other cases.²¹ The clinical trials involving nanoparticle-mediated gene therapy referenced in this review are summarized in Table 1.

Toxicity of nanoparticle-mediated gene therapy

Safety profiles of nonviral vector delivery systems have always been a major concern in pre-clinical studies and in clinical evaluation.²⁷ The occurrence of adverse events (AEs) can be drug- and/or procedure-related and is largely associated with the administration route. For example, in cancer therapies intravenous infusion often results in drug-related AEs, whereas intratumoral injection is generally associated with procedure-related discomfort. In contrast to systemic administration, local administration, such as through the airway, has a tendency to elicit inflammatory responses.²⁸ Here we highlight the reported safety

issues and toxicities of nanoparticle therapies seen in clinical trials.

Systemically delivered nanoparticle therapeutics. Systemic administration of nanoparticle-based therapies has frequently been used to treat cancer, although several reports noted the occurrence of AEs after treatments. Clinical studies based on cationic liposomal delivery system have reported AEs and described premedication strategies to reduce the likelihood of drug-related AEs. In a phase I study using a liposome-entrapped *c-raf-1* antisense oligonucleotide (LErafAON) to treat advanced solid tumors, four dose cohorts were treated via intravenous infusion.²⁹ Two of four patients experienced grade 3 or 4 hypersensitivity reactions, manifested as flushing, diaphoresis, low-grade fever, back pain, and dyspnea even at the lowest dose of 1 mg/kg/week. These reactions occurred within minutes of initiating the therapy and were described as drug-related but not dose-related. Similar hypersensitivity reactions were also seen in patients

Table 1. Clinical trials involving nanoparticle-mediated gene therapy

Carrier	Gene	Administration route	Disease	Phase	Reference
Cationic liposome	siRNAs targeting VEGF and KSP	Intravenous	Cancers	Phase I	16
Cationic liposome	p53 gene	Intravenous	Advanced solid tumors	Phase I	17
Cationic liposome	<i>c-raf-1</i> antisense oligonucleotide	Intravenous	Advanced solid tumors	Phase I	29
Cationic liposome	<i>c-raf-1</i> antisense oligonucleotide	Intravenous	Advanced solid tumors	Phase I	30
Cationic liposome	TUSC2/FUS1 gene	Intravenous	Lung cancer	Phase I	31
Cationic liposome	HLA-B7 gene	Intratumoral	Renal cancer	Phase I	33
Cationic liposome	HLA-B7 and β_2 -microglobulin	Intralesional	Melanoma	Phase II	34
Cationic liposome	HLA-B7 and β_2 -microglobulin	Intralesional	Melanoma	Phase II	42
Cationic liposome	Interleukin-2 gene	Intralesional	Head and neck cancer	Preclinical, phase I	39
Cationic liposome	Interleukin-2 gene	Intratumoral	Renal cancer	Not mentioned	35
Cationic liposome	Interferon- β gene	Intratumoral	Melanoma	Pilot study	41
Cationic liposome	E1A gene	Intratumoral	Head and neck cancer	Phase II	36
Cationic liposome	E1A gene	Intratumoral	Breast and head and neck cancer	Phase I	37
Cationic liposome	E1A gene	Intracavitary	Breast and ovarian cancer	Phase I	38
Cationic liposome	CFTR gene	Nasal	Cystic fibrosis	Not mentioned	20
Cationic liposome	CFTR gene	Nasal	Cystic fibrosis	Not mentioned	21
Cationic liposome	CFTR gene	Nasal	Cystic fibrosis	Phase IIB	23
Cationic liposome	CFTR gene	Nasal	Cystic fibrosis	Not mentioned	22
Cationic liposome	CFTR gene	Airway	Cystic fibrosis	Safety trial	28
Cationic liposome	Interferon- β gene	Into brain after tumor removal	Glioma	Phase I	26
Cationic liposome	Inducible NO synthase gene	Intracoronary	Prevention of restenosis	Safety and tolerability	24
Cationic liposome	Manganese superoxide dismutase gene	Oral	Damage induced by chemoradiotherapy in cancer	Phase I	25
Liposomal formulations	siRNA to transthyretin	Intravenous	Transthyretin amyloidosis	Phase I	19
Cationic polymer	siRNA to the M2 subunit of ribonucleotide reductase	Intravenous	Cancers	Phase Ia/Ib	32
PEG-PEI-cholesterol lipopolymer	IL-12 gene	Intraperitoneal	Ovarian cancer	Phase II trial	18
PEG-substituted lysine peptides	CFTR gene	Nasal	Cystic fibrosis	Safety and tolerability	43
Virus-like particles	TLR9 agonist oligonucleotide	Subcutaneous	Allergic rhinoconjunctivitis	Phase IIb	13
Liposomally encapsulated virus	IL-12 gene	Intratumoral	Glioblastoma multiforme	Phase I/II	14

CFTR, cystic fibrosis transmembrane conductance regulator; IL, interleukin; KSP, kinesin spindle protein; NO, nitric oxide; PEG, polyethylene glycol; PEI, polyethylenimine; siRNA, small interfering RNA; TLR9, Toll-like receptor 9; TUSC2/FUS1, tumor suppressor candidate 2; VEGF, vascular endothelial cell growth factor.

receiving escalated doses of infusion, and thus a pretreatment regimen including oral diphenhydramine (50 mg), oral famotidine (20 mg), and intravenous methylprednisolone (100 mg) was added before each LErafAON infusion. At the 4-mg/kg/week dose, one patient could not complete the infusion because of severe AEs such as rigors and grade 3 hypoxia. At all doses patients experienced thrombocytopenia, but showed variable platelet recovery 4 weeks after completing the treatment course.²⁹ Another study of liposome-encapsulated *c-ras* siRNA documented serious AEs in intravenously treated patients who exhibited acute infusion-related symptoms.³⁰ Similar results were reported in a phase I clinical trial of systemically administered cationic liposomes complexed with the tumor suppressor gene TUSC2 (FUS1).³¹ The first patient developed grade 2 fever within 3 h of the DC-TUSC2 infusion and, after lowering the dose, three patients nonetheless developed grade 2 or 3 fever and one patient developed grade 3 hypotension. The U.S. Food and Drug Administration allowed the protocol to be amended to require dexamethasone and diphenhydramine premedications to prevent these AEs.³¹ Another phase I study based on systemic delivery of liposomes complexed with p53 to treat advanced solid tumors also reported the occurrence of drug-related grade 1 or 2 AEs such as transient fever lasting 12–16 h (5 of 11; 46%) and transient hypotension over a similar time frame (7 of 11; 64%).¹⁷

In another phase I study, the cationic polymer-siRNA nanoparticle therapeutic, CALAA-01, also exhibited toxicity after systemic administration to patients with cancer.³² Of the 19 patients in this trial, the most common treatment-related AEs of any grade were fatigue ($n = 12$), chills ($n = 12$), and fever ($n = 10$). Importantly, five patients (21%) in the study discontinued treatment because of an AE. CALAA-01 treatment also resulted in a dose-dependent reduction in platelet count, but did not alter serum complement function. Serum cytokine release and apparent treatment-related elevations in IL-6, IL-10, tumor necrosis factor (TNF)- α , and interferon (IFN)- γ were also investigated in this study. IL-6 and TNF- α serum levels were transiently elevated, suggesting that the cationic polymer complex might induce an inflammatory response in some patients. This finding was consistent with another study that also found evidence of serum cytokine elevation on systemic administration of RNAi-mediated therapy to reduce VEGF and KSP levels.¹⁶ In this study, cationic liposomes used to encapsulate RNAi-based therapeutics for patients with cancer produced dose-dependent transient

increases in serum levels of the proinflammatory cytokines IP-10 (IFN- γ -inducible protein of 10 kDa) and IL-1RA (IL-1 receptor antagonist). Levels of other cytokines including IL-6, granulocyte colony-stimulating factor (G-CSF), and TNF- α also increased at higher doses, whereas levels IL-1 β , IFN- α , and IFN- γ were not affected by the treatment.¹⁶

Locally delivered nanoparticle therapeutics. Local delivery of nonviral therapeutics in gene therapy appears to promote a milder toxicological response compared with systemic administration. Intraleisional injection of lipid-formulated plasmid DNA encoding the MHC HLA-B7 gene was evaluated in a phase I clinical study of patients with renal carcinoma.³³ Grade I or II toxicities, including pain and/or bleeding at the biopsy/injection site, fatigue, anemia, and nausea, were recorded. One patient discontinued the therapy because of grade II pain and bleeding complications from tumor injection. However, no serious systemic toxicities (grade III or IV) related to the study drug were observed in any of the trial participants.³³ Another study that examined intralesional administration of a HLA-B7/ β_2 -microglobulin DNA-liposome complex recorded only mild to moderate drug-related toxicities and no grade III toxicity. Moreover, procedure-related toxicities such as pneumothoraces, ecchymoses, and pain at the injection site were minor.³⁴ Other intratumoral studies involving the tgDCC-E1A gene or IL-2 also noted only mild toxicities,^{35–37} whereas a study to evaluate intracavity injection of nanoparticles carrying the E1A gene in patients with breast and ovarian cancer documented treatment-related toxicities including fever, nausea, vomiting, and/or discomfort at the injection sites.³⁸ Intraperitoneal injection of lipopolymer-encapsulated IL-12 plasmid also produced a similar toxicity effect manifested as grade I/II fatigue, fever, chills, abdominal pain, nausea, vomiting, anemia, thrombocytopenia, and leukopenia.¹⁸

Gene therapy for cystic fibrosis involves local administration, mainly through the airway.^{20–23} Innate immune responses stimulated by the delivery of cationic lipid and plasmid complexes through aerosolized administration has drawn significant attention. During a dose escalation safety trial of an aerosolized CFTR cDNA complex, one-half of the enrolled patients (four of eight) enrolled developed a pronounced clinical syndrome of fever, myalgias, and arthralgia as well as elevated serum IL-6 levels hours after administration.²⁸ This study showed a synergistic effect of the cationic carrier and DNA, suggesting that immunologic responses to

lipid–DNA conjugates are independent of unmethylated CpG sequences.²⁸

Safety evaluations of nonviral vectors delivered by other routes have also been conducted. Intracoronary gene therapy using the iNOS lipoplex CAR-MP583 produced no complications and no signs of inflammatory responses or hepatic or renal toxicity.²⁴ Another study that investigated the ability of oral manganese superoxide dismutase plasmid liposomes to protect patients from ionizing irradiation damage showed no grade III or IV toxicity related to the treatment.²⁵

Efficacy of nanoparticle-mediated gene therapy

Cancer therapy. Gene therapy strategies for cancer vary by cancer type and produce a variety of effects, including elevated levels of inhibitory cytokines (IL-2, IFN, IL-12),³⁹ restoration of normal human tumor suppressor (p53), and interference with the activity of antiapoptotic molecules (e.g., c-Raf). The efficacy of these therapies can be characterized according to the extent of tumor regression after treatment, and is typically described as complete response, partial response, mixed response, stable disease, and tumor progression. However, gene therapy mediated by nonviral vectors showed mild effects in most cases.

Immunocytokine-based therapies have demonstrated potent antitumor effects in many basic studies.⁴⁰ But in a study involving 16 patients with ovarian cancer treated by intraperitoneal delivery of a plasmid encoding IL-12 complexed with lipopolymer, none showed any partial or complete response, and seven and nine patients had stable and progressive disease, respectively, after treatment.¹⁸ An evaluation of IL-2 gene therapy for 31 patients with metastatic renal cell carcinoma resulted in an overall response rate of 10%, with two patients showing a partial response and one patient showing a complete response.³⁵ Tumor samples from these patients after therapy showed elevated IL-2 expression levels and the number of CD8-positive lymphocytes relative to those seen before treatment.³⁵

Use of human IFN- β (HuIFNb) gene (IAB-1) therapy for five patients with glioma in a pilot study produced either no change, a mixed response, or progressive disease.⁴¹

Restoration of normal activity of human tumor suppressor (p53) is also considered a potent therapeutic strategy to treat various cancers. In a phase I study involving delivery of liposomal p53 in patients with advanced solid tumors, exogenous p53 transgene was detectable in metastatic tumor tissues by PCR after treatment, although the therapy pro-

duced no significant disease regression (seven of 10 enrolled patients had stable disease and three of 10 had progressive disease).¹⁷ Restoration of another tumor suppressor gene, TUSC2/FUS1 (TUSC2), in patients with lung cancer produced a similar result in that 18 of 23 had tumor progression and the remaining five had stable disease.³¹

Introduction of expression of allogeneic MHC antigens could promote an immune response in tumor tissues. One study that used direct intralesional gene transfer of HLA-B7 to treat renal carcinoma found no significant reduction in tumor size and either stable or progressive disease.³³ In contrast, a phase II study using intralesionally delivered HLA-B7/ β_2 -microglobulin DNA–liposome complexes to treat metastatic melanoma elicited regression of the injected lesion in 18% of patients, including one complete response and three partial responses.³⁴ This study documented an overall disease response rate of 4%, and nine patients had stable disease after their initial administration cycle. In a phase II dose escalation study of HLA-B7/ β_2 -microglobulin gene therapy, 15 of 127 patients (11.8%) achieved an objective response, with a median duration of response of 13.8 months.⁴²

The use of nanoparticle-mediated gene therapy to deliver RNAi directed against the M2 subunit of ribonucleotide reductase (RRM2) or the antiapoptotic protein c-Raf has also been characterized.^{30,32} A phase I clinical trial that examined the effectiveness of RRM2 siRNA for cancer treatment showed no objective tumor responses, and nearly one-third of enrolled patients discontinued the study because of progressive disease.³² Similarly, in a study of liposomal *c-raf-1* antisense oligonucleotide to treat patients with advanced solid tumors, no objective responses were seen among the 22 patients enrolled. Eight patients discontinued the therapy because of toxicity and evident disease progression, and five and nine of the remaining 14 showed stable and progressive disease, respectively, on initial evaluation.²⁹ On the other hand, in a trial of combined radiation therapy and liposomal *c-raf* antisense oligodeoxyribonucleotide infusion, one-third (four of 12) of patients had a partial response whereas the others had either stable or progressive disease.³⁰ Those patients who had a partial response or stable disease also showed downregulation in levels of *c-raf-1* mRNA and Raf-1 protein.³⁰ In addition, a clinical trial that examined siRNAs targeting VEGF and KSP reported a complete response for one patient after 40 doses of treatment.¹⁶

Other diseases. Nonviral vector-mediated gene therapy to treat diseases other than cancer has

shown some positive results. Treatment of patients with CF by administration of the CFTR gene in complex with cationic lipids had a significant positive effect on chloride abnormalities in the patients who received gene therapy, but not in those who received the placebo, suggesting that cationic lipid-mediated CFTR therapy could attenuate chloride transport defects in the lungs of patients with CF.²² Delivery of the CFTR gene by means of modified lysine peptides also produced partial to complete reconstitution of CFTR activity in a double-blind, placebo-controlled trial.⁴³ Gene therapy for transthyretin amyloidosis, using lipid nanoparticles to deliver anti-transthyretin siRNA, produced a sustained reduction in transthyretin levels in patients treated with higher doses.¹⁹ Treatment of rhinoconjunctivitis symptoms with a Toll-like receptor 9 (TLR9) agonist contained in virus-like particles also yielded positive results in patients receiving a higher dose compared with those who received the placebo.¹³

CONCLUSIONS AND PERSPECTIVES

Considerable effort has been devoted to developing nonviral vector-mediated human gene therapy, although the effective use of these therapies for cancer and other inherited diseases remains challenging. Toxicity represents the main barrier to the application of cationic nonviral vectors, especially for systemic administration. Several studies have had to amend the treatment protocol or add premedication during the clinical trial because of infusion syndrome and also to reduce the incidence of AEs.^{29,30} Additional basic and preclinical research is needed to increase our understanding of the mechanisms associated with toxicity to avoid AEs in human gene therapy. The presence of infusion syndrome, such as fever after treatment, suggests that an inflammatory response is induced.^{29,30} Our group showed that systemic injection of cationic gene carriers (*e.g.*, cationic liposomes, PEI, chitosan) promotes cell necrosis and subsequent release of mitochondrial molecules such as mitochondrial DNA that can trigger an inflammatory response.⁴⁴ These findings suggest that positive surface charges could play a critical role in inducing acute cell necrosis and in turn toxicity. Several studies detected activation of neutrophils within hours of systemic injection of cationic nanocarriers *in vivo*, and this

activation could be due to the release of damage-associated molecular patterns (DAMPs) from necrotic cells.^{44–46} This result correlates with the results of clinical trials that documented transient fever after liposome infusion.

For most cancers, gene therapy efficacy has been restricted to disease control, and an objective response largely remains elusive. The simplest nonviral gene delivery was delivery of “naked” DNA. Although several clinical trials to examine the effects of administration of naked DNA^{47–49} showed that the treatments were well tolerated and thus had potential for effective gene therapy, delivery efficiency was low. In addition to the nanocarriers discussed in this review, other vectors for gene delivery have been intensively studied, such as siG12D-LODER, a miniature biodegradable polymeric matrix encompassing anti-K-ras^{G12D} siRNA cargo that was administered to patients with pancreatic adenocarcinoma in combination with chemotherapy.^{50,51} For nonviral vector-based gene therapies in general, therapeutic efficacy could be improved by developing more efficient targeting carrier and novel molecular targets.⁵² With the development of material science and nanotechnology, new surface modifications for delivery systems, as well as multifunctional materials, which have shown promising results in animal studies, could provide improved delivery efficiency with reduced toxicity.⁵³ Improvements in genome-editing platforms, such as the CRISPR/Cas9 system, could also be important components of future gene therapies,⁵⁴ although preclinical screening and evaluation of the toxicity will be essential for all developing therapeutic approaches. Despite the current challenges in nanoparticle-mediated gene therapy, continued improvements will likely yield promising new treatment options that provide effective targeting of disease with minimal toxicity.

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AUTHOR DISCLOSURE

The authors have no conflicts of interest to declare.

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