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REVIEW ARTICLE

Production and clinical development of nanoparticles for gene delivery

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Gene therapy is a promising strategy for specific treatment of numerous gene-associated human diseases by intentionally altering the gene expression in pathological cells. A successful clinical application of gene-based therapy depends on an efficient gene delivery system. Many efforts have been attempted to improve the safety and efficiency of gene-based therapies. Nanoparticles have been proved to be the most promising vehicles for clinical gene therapy due to their tunable size, shape, surface, and biological behaviors. In this review, the clinical development of nanoparticles for gene delivery will be particularly highlighted. Several promising candidates, which are closest to clinical applications, will be briefly reviewed. Then, the recent developments of nanoparticles for clinical gene therapy will be identified and summarized. Finally, the development of nanoparticles for clinical gene delivery in future will be prospected.

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INTRODUCTION

Gene therapy has drawn significant attention as a promising strategy for specific treatment of numerous gene-associated human diseases ranging from cancer, hemophilia, hypercholesterolemia, neurodegenerative diseases to autoimmune diseases.[1–4] This strategy is to introduce genes into the target pathological tissues or cells by altering the expression of the endogenous genes to cure or prevent the progression of the related disease.[5,6] Gene therapy has been widely studied in various areas, instead of conventional methods that usually fail to treat many diseases caused by genetic anomalies,[7] and has become one of the most promising biomedical technologies for the clinical application.[8,9] However, naked genetic molecules cannot be internalized efficiently by target cells because of their serum nuclease susceptibility, rapid renal clearance, phagocyte uptake, reduced uptake by target cells and toxic effect arose by immune response stimulation, which severely restricts their clinical application.[10] With the developments of material sciences and the rapid progress of nanotechnology, nanosized materials for gene delivery have attracted worldwide attentions.[11] Recently, some preliminary clinical trials of nanoparticles for gene delivery revealed promising effects.[12,13] However, the development of safe, efficient, and controllable gene delivery nanoparticles for gene delivery is still now a bottleneck to successful clinical applications.[14,15]

Despite having been achieved some initiatory successes, the widespread clinical application of gene therapeutics for disease prevention and treatment meets many unavoidable challenges. The most important points are encapsulation efficiency, stability of nanoparticles, degradation in blood circulation, endocytosis by target cells, endosomal escape, delivery efficiency, and toxicity of pharmacology.[16] To overcome these obstacles, many types of nanoparticles have been evaluated as gene carriers, which include lipid-based nanoparticles,[17] polymer-based nanoparticles,[18] and inorganic nanoparticles.[19]

In this review, we will particularly highlight the clinical development of nanoparticles for gene delivery rather than covering all the aspects of this field. Some promising candidates, which are closest to clinical applications in recent years, will be briefly reviewed. Then, the recent developments of nanoparticles for gene delivery will be identified and summarized in the clinical trials. Finally, the development of nanoparticles for clinical gene delivery in future will be prospected.

PRODUCTION OF NANOPARTICLES

With the great development of bioscience and nanotechnology, gene therapy shows an enormous potentiality in clinical application for many human serious incurable diseases.[20] However, none of gene therapeutics based on nanoparticles has so far been approved by the US Food and Drug Administration (FDA). There are still several problems for the clinical application of nanoparticle-based gene therapy (Figure 1), including biodegradation and biocompatibility, aggregation in physiological fluids, nonspecific adsorption by nondesired tissues, less efficient extravasation to reach target tissues, cellular internalization, and endosomal escape.[12]

Biodegradation is the primary factor for the clinical application of nanoparticle-based gene delivery.[21] Poly(lactic-*co*-glycolic acid) has been an FDA-approved biodegradable polymer since 1969. In recent years, poly(lactic-*co*-glycolic acid) has been explored as a gene vector due to its stable and able to protect DNA from degradation during circulation *in vivo*.[22–24] DNA/RNA can be encapsulated in poly(lactic-*co*-glycolic acid) nanoparticles by double-emulsion

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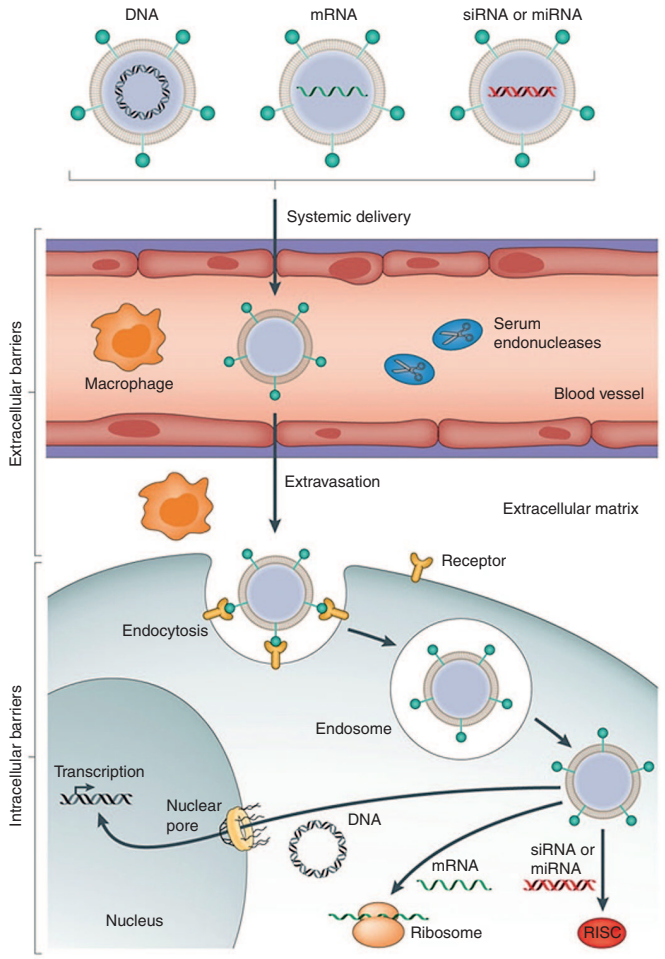

Figure 1 Schematization of nanoparticle-based gene therapy *in vivo* (Copyright 2014 Nature Publishing Group).

solvent evaporation method. For more cellular endocytosis and efficient endosomal escape profile, poly(lactic-*co*-glycolic acid) nanoparticles are modified with biocompatible chitosan or other cationic components so that the nanoparticles possessed positive charges, thus achieving higher gene loading and transfection efficiency.[25] Polypeptide-based cationic polymer is another candidate gene vector for clinical application. Cheng and colleagues[26,27] prepared a series of cationic helical polypeptides and found that these polymers could overcome the efficiency–toxicity poor correlation of normal nanocarriers. Nature polymers, such as cyclodextrin and chitosan, have been intensively investigated due to their preferable biocompatibility.[14,28] Cyclodextrins are generated during the bacterial digestion of cellulose and can form water-soluble complexes with small siRNA molecules. CALLA-01, a targeted nanoparticle system based on cyclodextrins, has been developed for the first in-human phase-1 clinical trial.[8,29] Chitosan is another typical naturally polycation with the advantages of biocompatibility and biodegradability, which will be served as a very promising carrier for gene delivery.[30] Lipid-based nanoparticles are one of the most extensively explored for gene delivery owing to their optimal properties, including high biocompatibility and close resemblance to the lipidic membranes, which facilitate their penetration into the cells.[31,32] Many other biocompatible nanocarriers also attracted great attentions as gene delivery systems, including poly(β-amino ester)s,[33] low-molecular-weight polyethylenimine,[34] polyphosphoesters,[35,36] disulfide cross-linked polymers,[37] and polyamidoamine.[38] Compared with the conventional gene delivery systems, the biodegradable and biocompatible nanoparticle-based systems (Figure 2) show improved formulation stability and safety, which are practically beneficial for the clinical gene therapy in future.

The aggregation and nonspecific adsorption by nondesired tissues are two serious problems which prohibit the clinical application of nanoparticle-based gene delivery, and those are mainly caused by the massive positive charges on the surface of cationic nanocarriers. PEGylation of these carriers is an essential strategy for reducing nonspecific interactions with serum proteins in the bloodstream and avoiding recognition by immune system components.[39–41] Even for the neutral nanoparticles, they will quickly form large aggregates and are adsorbed by serum albumin in physiological salt concentrations without PEG, thus leading to rapid clearance by phagocytic cells and the reticuloendothelial systems.[42,43] Therefore, PEGylated nanoparticles can extend blood circulation time and facilitate accumulation in targeted tissues. Shielding of the positive charges of cationic nanoparticles with polyanions is another strategy for stabilizing the nanoparticles, minimizing nonspecific interactions, and prolonging circulation time *in vivo*.[44] There were numerous reports about the hydrophilic polymers modifying nanoparticles, which exhibited more steric stability with reduction in aggregation and breakdown during circulation.[45,46]

Clinical gene therapy using nanoparticles is also hampered by the lack of targeting ability when delivered into the desired diseased tissues.[47,48] To overcome this drawback, the development of specific ligand receptor-mediated active targeting strategy for gene delivery system is critically required. When the ligands, such as antibody, protein, peptide and aptamer, interact to the receptors of targeted cells, the cellular uptake of nanoparticles will be further enhanced.[41,49,50] To deliver nanoparticles effectively, the specific response of ligand-receptor pairs should be particularly strong and the target receptors should be overexpressed on target cells rather than normal cells.[14,44] Passive targeting is exploited specially for the defective vascular architecture and the inefficient lymphatic drainage of tumors, which leads to the extravasation of nanoparticles into tumor tissues and enhances their retention in the interstitial space. Combined with the strategy mentioned above, PEGylation of nanoparticles is one of the most famous methods to improve the passive targeting by increasing the circulation time and avoiding possible serum aggregation *in vivo*.[51] Stimulus-responsive nanoparticles can produce physical or chemical changes after exposing to diseased tissues or external signals including pH, temperature, light or magnetic field.[52–54] Responsiveness towards internal or external stimuli makes it possible to tailor the time and site of gene therapy precisely, and greatly increase the possibility of clinical application for these nanoparticles.[41]

Effective cellular internalization of therapeutic genes is a critical process for the successful clinical application of nanoparticles for gene delivery. Although, several nanocarriers have been widely used to deliver therapeutic genes to the cells, it is still also urgent to improve the endocytosis of DNA/RNA to meet the ultimate goal of clinical application.[55,56] Cell-penetrating peptides, with membrane translocation sequences or protein transduction domains, have been introduced on the surface of nanocarriers for gene therapy, which evolves quickly cellular uptake of the gene delivery system via direct translocation in addition to the endocytic way.[57,58] In addition, to increase the endosomal escape activity of nanoparticles, endosomolytic agents with the ability to destabilize the endosomal membrane have been introduced.[59] In order to further optimize gene

delivery, novel nanocarriers were developed by combining both endosomolytic agents and cell-penetrating peptides.[60] Harashima and colleagues[61] reported a stearylated derivative INF-modified R8-MEND dual functional gene delivery system and achieved delectable results both *in vitro* and *in vivo*.

In addition, the production of nanoparticles for clinical gene therapy should be designed according to the practical needs in clinic (Table 1). Firstly, the functions of nanocarriers should be tailored by the types and mechanisms of nucleic acid determinants, such as DNA, mRNA, siRNA, and miRNA.[12,62,63] Secondly, the construction of

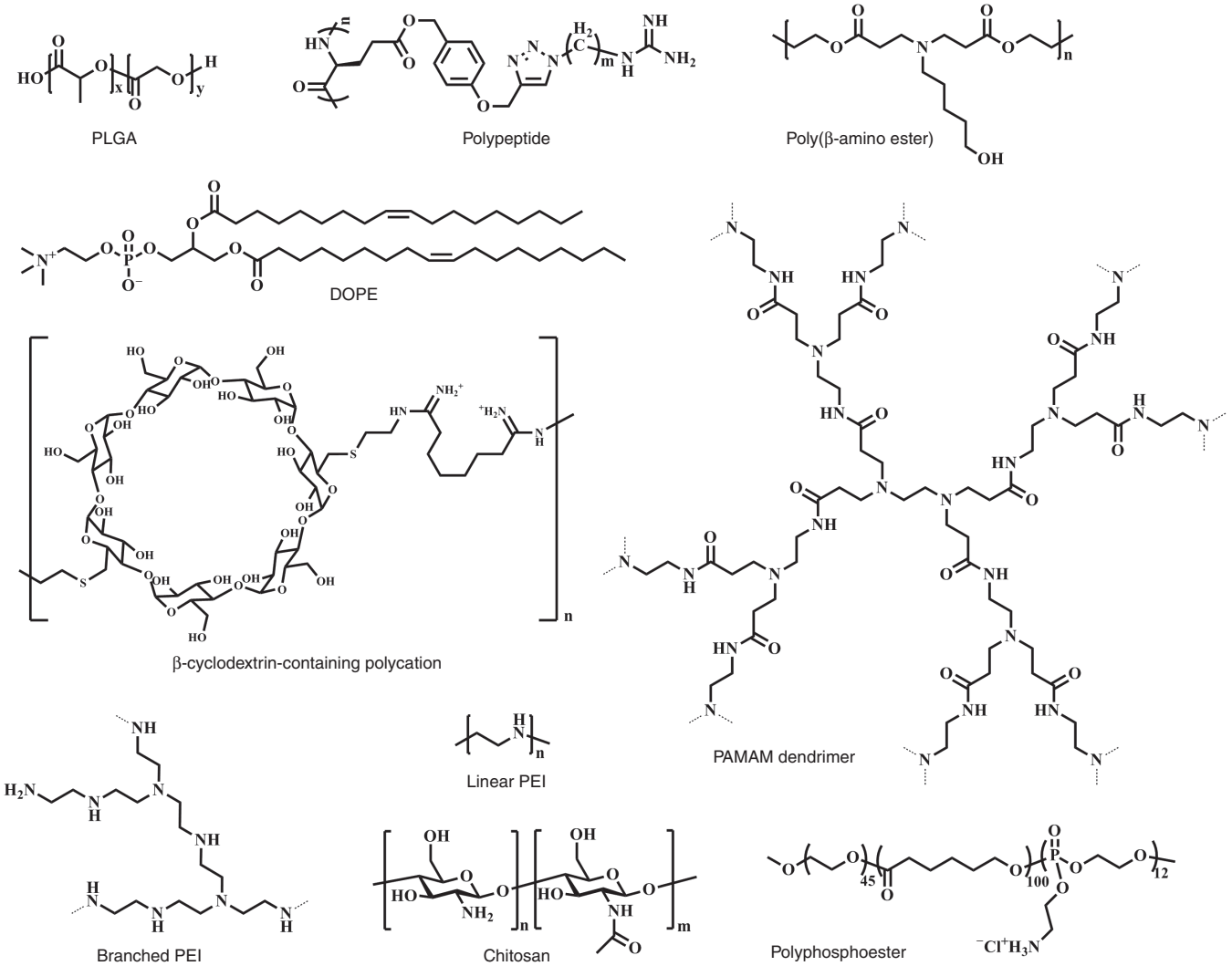

Figure 2 The varieties of nanoparticles for the potential clinical gene therapy.

Table 1 Multifunctional nanoparticles for preclinical gene delivery studies

Functionalization	*Delivery systems*	*Advantages*
PEGylation	PEG-βCD; PEG-PEI	Enhanced of stabilization; prevention of protein absorption; improved circulation time[39,40]
Targeting	RGD-HA-PEI-PBLG; R-PEG20C; transferrin-lipid	Enhanced gene target efficacy *in vivo*[44,48,51]
Stimulus response	pH sensitive; light sensitive; redox sensitive	Enhanced gene target efficacy *in vivo*[52–54]
Cell penetrating	$p(DAH_a\text{-}E/API_b)$	Cross cell membrane; enhanced cellular uptake[58]
Endosome escaping	$(Arg)_7$-FI-PNA	Cross cell membrane; improved endosomal escaping[59]
Nuclear localization	PC/*p*DNA/NLS; VKRKKKP-R_8	Nuclear localization[62,63]

βCD, β-cyclodextrin; NLS, nuclear localization sequence; PC, β-cyclodextrin and polyethylenimine; PEI, polyethylenimine; R-PEG20C, cRGD-PEG-PAsp(DET)-cholesteryl.

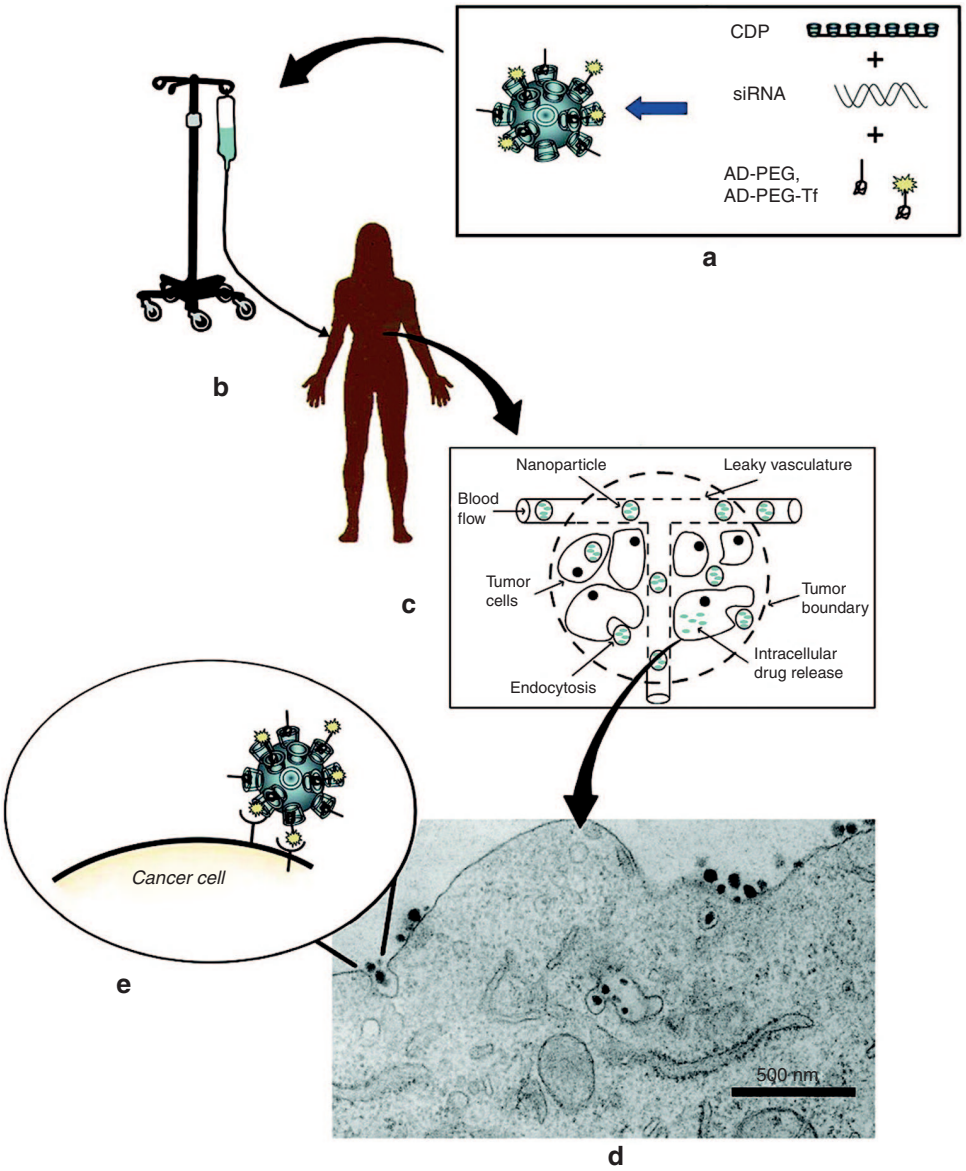

Figure 3 Schematic of mechanism proposal for CALAA-01. (**a**) Nanoparticles are assembled from a linear cyclodextrin-containing polymer (CDP), an adamantane-PEG conjugate (AD-PEG), a targeting component (transferrin, Tf) and the therapeutic gene (siRNA). (**b**) Nanoparticles are infused into patients. (**c**) The circulation of nanoparticles and their escape into tumors. (**d**) Receptor-mediated endocytosis. (**e**) Interactions between targeted nanoparticles and receptors on the surface of the cancer cell.[29] (Copyright 2009 American Chemical Society).

the nanocarriers should also be changed by the variety of genetic diseases including cancers, optic atrophy, hypercholesterolemia, and dry eye, etc.[13] Finally, plentiful of administration routes including local and systemic approaches also provide the opportunities and challenges to the application of nanoparticle-based gene therapy.[64,65]

Clinical development of nanoparticles for gene delivery

Many clinical trials of gene therapy for preventing or treating genetic diseases are rapidly ongoing worldwide although none of the gene therapeutics based on nanoparticles have so far been approved by FDA.[12]

Anderson *et al.*[66] conducted the first in-human clinical trial, which involved systemic administration of adenosine deaminase gene to a 4-year-old girl with severe combined immunodeficiency disease. An initial success of the trail was achieved, and then a research boom of gene therapy was set off worldwide. In 2000, Fischer and colleagues[67] initiated a gene therapy trail for severe combined immunodeficiency-X1, an X-linked inherited disorder characterized by an early block in T and natural killer lymphocyte differentiation, based on the use of complementary DNA containing a retrovirus-derived vector and *ex vivo* infection of $CD34^+$ cells. After a 10-month follow-up period, T, B, and NK cell counts and function of the two patients were comparable to those of age-matched controls. However, the two youngest boys revealed the symptoms of leukemia 3-years after gene therapy, which was mainly due to the retrovirus vector integration in proximity to the proto-oncogene and triggered deregulated premalignant cell proliferation with unexpected frequency.[68] Thus, the broad application of gene therapy was restricted severely by the safety concerns aroused by viral vectors.[69]

Recently, nanoparticles are being investigated to utilize as gene delivery systems to overcome the delivery barriers due to their properties associated with safety, nonimmunogenicity,

Table 2 Nanoparticle-based gene therapy under clinical evaluation

Delivery system	*Product*	*Sponsor*	*Disease*	*Administration*	*Phase*	*Status*	*Gov identifier*
PEI-based nanoparticles	BC-819/PEI	BioCancell	BC	Local	2	Active	NCT00595088
	BC-819	BioCancell	OC	IP	1/2	Completed	NCT00826150
	DTA-H19	BioCancell	PN	Local	1/2	Completed	NCT00711997
	EGEN-001	Gynecologic Oncology Group	Cancer	IP	2	Active	NCT01118052
Lipid-based nanoparticles	TKM-080301	National Cancer Institute	HM	IA	1	Completed	NCT01437007
	TKM-080301	Tekmira Pharmaceuticals Corporation	HC	IV	1/2	Recruiting	NCT02191878
	TKM-080301	Tekmira Pharmaceuticals Corporation	NET; ACC	IV	1/2	Completed	NCT01262235
	Atu027	Silence Therapeutics GmbH	ASC	IV	1	Completed	NCT00938574
	ALN-TTR02	Alnylam Pharmaceuticals	TTR-A	IV	2	Completed	NCT01617967
	DOTAP-Chol-fus1	MD Anderson Cancer Center	LC	IV	1	Completed	NCT00059605
	DCR-MYC	Dicerna Pharmaceuticals	ST; MM; NHL	IV	1	Recruiting	NCT02110563
	DCR-MYC	Dicerna Pharmaceuticals	HC	IV	1/2	Recruiting	NCT02314052
	ND-L02-s0201 Injection	Nitto Denko Corporation	EHF	IV	1	Recruiting	NCT02227459
PLGA-based nanoparticles	siG12D LODER	Silenseed	PC	Local	2	Active	NCT01676259

ACC, adrenocortical carcinoma; ASC, advanced solid cancer; BC, bladder cancer; EHF, extensive hepatic fibrosis; HC, hepatocellular carcinoma; HM, hepatic metastases; IA, intra-arterial; LC, lung cancer; MM, multiple myeloma; NET, neuroendocrine tumors; NHL, non-Hodgkins lymphoma; OC, ovarian cancer; PC,pancreatic cancer; PEI, polyethylenimine; PLGA, poly(lactic-*co*-glycolic acid); PN, pancreatic neoplasms; ST, solid tumors; TTR-A, transthyretin amyloidosis.

Table 3 Combination of gene therapy and chemotherapy under clinical evaluation

Delivery system	*Product*	*Sponsor*	*Disease*	*Administration*	*Phase*	*Status*	*Gov identifier*
PEG-PEI-cholesterol	EGEN-001 + carboplatin + docetaxel	EGEN	ON	IP	1	Completed	NCT00473954
PEG-PEI-cholesterol	EGEN-001 + PLD	Gynecologic Oncology Group	R/POEC; FTC; PPC	IP	1	Recruiting	NCT01489371
PEG-PEI-cholesterol	GEN-1 + SNC	Celsion	EOC; FTC; PPC	IP	1	Recruiting	NCT02480374
Lipid	SGT-53 + nab-paclitaxel/ gemcitabine	SynerGene Therapeutics	MPC	IV	2	Recruiting	NCT02340117

EOC, epithelial ovarian cancer; FTC, fallopian tube cancer; MPC, metastatic pancreatic cancer; ON, ovarian neoplasms; PEI, polyethylenimine; PLD, pegylated liposomal doxorubicin; PPC, primary peritoneal cancer; R/POEC, recurrent or persistent ovarian epithelial cancer; SNC, standard neoadjuvant chemotherapy.

controllability, and low cost.[70] Davis and colleagues[8] reported the first nanoparticle-based gene delivery system named CALAA-01 in a phase-1 clinical trials against cancers. CALAA-01 consists of siRNA targeting the M2 subunit of ribonucleotide reductase (RRM2), cyclodextrin containing polymer, PEG steric stabilization agent, and transferrin targeting ligand for binding to transferrin receptors upregulated on cancer cells.[71] The results showed that this "drug" could deliver siRNA to melanoma cells by systemic administration and demonstrate potent antiproliferative activity across multiple types of cancer cells.[29] (Figure 3)

Since then, many nanoparticle-based gene delivery systems have been developed for clinical trials.[13] PEI has been carried out for local clinical gene therapy of various cancers (Table 2). However, the substantial cytotoxicity severely hampered its further application and a range of modifications to PEI had been investigated. Polyethylene glycol-polyethylenimine-cholesterol (PEG-PEI-cholesterol) was successfully developed as a gene delivery carrier for immunotherapy of epithelial ovarian by enhanced expression of cytokine interleukin-12.[72] Lipid formation of nucleic acid shows the most clinical trials among nanoparticles for gene delivery. A phase-1 clinical trial for ALN-VSP, a lipid nanoparticle formulation encapsulating the siRNAs that can specially target the mRNA of vascular endothelial growth factor (VEGF siRNAs), was initiated by Alnylam Pharmaceuticals and showed that systemically therapy could induce the regression of liver metastases and improve the potentially sensitize of cancer cells to chemotherapy.[73] ALN-TTR02 was an intravenously delivered lipid-based siRNA formulation in patients with TTR amyloidosis. The safety, tolerability, pharmacokinetics, and pharmacodynamics of multiple doses of ALN-TTR02 were evaluated. The data showed that specific knockdown of up to 94% was achieved in serum TTR protein levels and the knockdown effect could sustain for 1 month. Furthermore, no infusion reactions were observed at the high dose of 0.3 mg/kg

in the phase-2 study.[74,75] Allovectin-7, which was consisted of DMRIE-DOPE and a plasmid DNA, had successfully passed phase-2 clinical trials, but failed to meet its endpoints in increasing percent DDR or overall survival in a phase-3 clinical trial for treatment of advanced metastatic melanoma and finally the development of this treatment had been abandoned.[12,76] Nonetheless, various lipid-based formulations continued to be developed in clinic. (Table 2)

Recently, great attentions have been paid to the clinical application of combination approaches of gene with drug, radiotherapy, photodynamic therapy, or immunotherapy.[13,48] A phase-1 study of the safety and biological activity of intraperitoneal GEN-1 (IL-12 plasmid formulated with PEG-PEI-cholesterol lipopolymer) administered in combination with standard neoadjuvant chemotherapy was carried out to diagnose with epithelial ovarian, fallopian tube, and primary peritoneal cancer (Table 3). A phase-2 study of the combination of intravenously administered SGT-53 (P53 gene therapy sponsored by SynerGene Therapeutics) and oral temozolomide was ongoing for the treatment of recurrent glioblastoma. SGT-53 nanodelivery system consisted of a cationic liposome, an antitransferrin receptor single chain antibody fragment and the wtp53 plasmid DNA.[48] This trial will evaluate the nanoparticle delivery to tumor site, the induction of apoptosis in the tumor, antitumor activity, and safety (Table 3).

Conclusion and outlook

In the past two decades, substantial nanoparticle-based gene therapies have been constructed in the treatment of various diseases due to the rapid development of nanotechnology and genomics.[12] The clinical application of gene therapy is hindered by the challenges associated with its delivery system, including rapid degradation and clearance in circulation, insufficient half-life, nonspecific uptake, reduced uptake by target cells, inability to escape endosomes and toxic effect arose by immune response stimulation.[12,77] Although several viral-based vectors have been used for clinical gene therapy due to their high transduction efficiency,[78,79] they still face serious challenges, including adverse effects and high costs.[80] Therefore, it is necessary to develop safe and effective nonviral gene delivery systems. Among them, nanoparticle-based delivery systems have shown their potential application in clinical gene therapy.[13] Furthermore, many strategies have been introduced to improve the intelligences of nanoparticles for gene delivery systems, such as biodegraded, PEGylated, targeted, and modified with cell-penetrating peptides or endosomolytic agents.[3,81,82]

Since the first cyclodextrin-based phase-1 clinical trial for gene therapy was achieved in 2010, a number of nanoparticle-based gene delivery systems have been developed for clinical trials.[13] Most of them consisted of cationic polymer for binding nucleic acids, PEG steric stabilization agent, and targeting ligand for binding to the receptors on target cells. However, some of the clinical trials finally failed to meet their endpoints and none of gene therapeutics based on nanoparticles has so far been approved by FDA. To our knowledge, the primary obstacle comes from the complexity of the disease and the precise interpretation of its pathogenesis by molecular biological approach will be a prerequisite for effective clinical gene therapy. Moreover, successful clinical gene therapy is also seriously restricted by the safety and effectiveness of gene delivery systems. Despite these difficult conditions, gene therapy still owned its great potentiality for preventing or treating genetic diseases.

Further clinical progress of nanoparticle-based gene therapy will be facilitated by additional biological insights and nanotechnology into the key rate-determining steps that limit the effective therapy. Moreover, structure–function relationships, anatomical barriers, nucleic acids stability and availability, immunoreactivity, delivery routes are all major clinical challenges. It is expected that gene therapy based on local administrations can reach its goals and be approved by FDA more easily due to foreseeable features. Immunotherapy based on gene delivery will be a very promising approach for the treatment of genetically related diseases by subcutaneous injection. However, the systemic application of DNA/RNA will be seriously hindered by the complicated *in vivo* microenvironment. Particularly, it is impossible to completely avoid the possible cross-reactivity between nucleic acid drug and all body cells, which will bring about unexpected side effects by systemic therapies. Based on it, a secure targeted delivery strategy will be especially necessary. Finally, combination approaches of nanoparticle-based gene therapy with drug, radiotherapy, photodynamic therapy, immunotherapy or others will be the one of the most emphasis in future clinical studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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