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Advanced non-viral cationic lipids for siRNA-based cancer therapy

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Abstract
siRNA based cancer therapy is a powerful modality for cancer treatment. With the recent FDA approval of Patisiran and Givosiran, siRNA mediated nucleic acid therapy is undergoing a transition from research to clinical space. The primary obstacle for siRNA-based cancer therapy is the delivery of oligonucleotide payloads into the cytoplasm of tumor cells. The success of siRNA-based therapy mainly depends upon the design of carrier systems. Currently, cationic lipids-based vehicles have been widely used to maximize the delivery efficiency of siRNA into the various tumor cells. The objective of this review is to provide an overview of cationic lipid-based carriers in recently published researches focusing on their *in vitro* and *in vivo* studies to provide future research direction to siRNA-based cancer therapy.

Keywords: cationic lipids, siRNA delivery, lipoplex, cancer therapy

1. Introduction
Cancer is one of the major healthcare problems worldwide. Currently, treatment and cure of cancers by nucleic acid therapeutics have gained more attention. Among various nucleic acid-based therapeutics including small interfering RNA (siRNA), messenger RNA (mRNA), micro RNA (miRNA), and plasmid deoxyribonucleic acid (pDNA), siRNA-based therapeutics have been a promising treatment for various cancers by suppressing the target cancer gene [1, 4]. The suppression of genes also helps to eradicate the problems encountered with MDR by sensitizing cells resistant to anticancer drugs [5].
Double stranded siRNA is a polyanionic macromolecule composed of antisense stand (also known as guide strand) and sense strand (also known as passenger strand) which are oriented to each other according to Watson-Crick base pairing. Each strand contains two nucleotides (5′ phosphate end group and 3′ hydroxyl end group) overhangs at the 3′-ends. Generally, siRNA is 21 to 23 base pairs/nucleotides in length with the size less than 10 nm and molecular weight ~13 kDa [6, 8]. RNAi is a natural mechanism of post transcriptional gene silencing and occurs in the cytoplasm of the cells [9]. It is a multistep process [10] and initiates when a long double stranded RNAs (dsRNA) or hairpin RNA are exogenously delivered into the target cells. The cytosolic RNase III-type enzyme Dicer cuts dsRNA or hairpin RNA into smaller fragments called siRNA. Alternatively, synthetic siRNA can be manufactured and exogenously introduced into the target cells without involvement/bypassing of Dicer enzyme [11, 12].
siRNA incorporates into the RNA–Induced Silencing Complex (RISC) with the Argonaute (AGO) protein as the main component. The AGO protein cuts and removes the sense strand of the siRNA molecule and activates RISC. Finally the activated RISC containing only antisense strand finds a homologous target mRNA and initiates the sequence specific gene silencing by cleaving the target mRNA [13, 14]. United States Food and Drug Administration (FDA) approved the first siRNA drug in 2018, Patisiran (ONPATTRO™) and second one in 2019 called Givosiran (GIVLAARI) for treatment of hereditary transthyretin amyloidosis and acute hepatic porphyria respectively. However currently there is no approved cancer therapeutic based on siRNA delivery [15, 17].

2. Biological barriers for systemic use of siRNA delivery
Both extracellular (systemic) and intracellular biological barriers are encountered for the transport of siRNA to the cytoplasm of the target cells which have hindered clinical application via systemic administration. Upon intravenous administration, naked siRNA molecules with a relatively small size, are prone to premature renal clearance by the reticuloendothelial system (RES) and are also rapidly degraded by endonucleases in blood and extracellular compartments [18]. These lead to short half-life of siRNAs (15 min) in plasma, potentially limiting their use [19]. Particularly, ribonucleases bind specifically to siRNAs in systemic circulation and produce fragments for degradation [20]. Several studies displayed that siRNAs themselves can activate innate immunity by sensitizing interferon expression, even at low concentrations. Protein Kinase R (PKR) and toll-like receptor (TLR) 3 signaling pathways are involved in sequence-independent immune activation by siRNAs. Also, certain siRNAs can induce production of proinflammatory cytokines via TLR7 on dendritic cells and TLR8 on monocytes in a sequence-dependent manner [21].
Unfavourable physicochemical properties like the hydrophilic nature, larger molecular weight and intrinsic negatively charged backbone of the siRNA molecule doesn't allow its passive diffusion through the negatively charged hydrophobic phospholipid bilayer of cell membranes leading to poor cellular uptake [22]. Instead of only silencing the specific target

genes (mRNA), siRNAs may also silence an unknown number of unintended genes that can occur from unintended seed region matches between RNAi guide strands and non-targeted mRNAs causing off-target effects. It can be explained by two mechanisms. First, siRNAs can tolerate many mismatches at the mRNA target and retain their potency to suppress those targets with imperfect complementarity. The second one involves promiscuous entry of siRNAs into endogenous miRNA machinery. As siRNAs are closely identical to the related class of miRNAs, they may recognize mRNAs with their seed region and degrade an unpredictable number of mRNAs [21, 23].

Chemical modifications to the siRNA phosphate backbone and/ or ribose sugar have enhanced RNA stability and can limit off-target effects. RNAi triggers with extensive 2'-O-methyl base modifications have largely avoided the immunogenic reactions to foreign double-stranded RNA. The potency of siRNAs can be also increased by its hydrophobic modifications like siRNAs conjugated with cholesterol [24] avidly binds with serum proteins, greatly enhanced circulation time and promote systemic delivery efficacy [25]. However, chemical modifications can significantly increase cost, reduce siRNA binding affinity for the target sequence [26] and may lead to liver and cardiovascular toxicity, prolonged blood coagulation times and thrombocytopenia [27]. In order to solve these siRNA deliveries associated problem various types delivery vehicles including viral vectors and non-viral vectors have been employed. Viral vectors have high gene silencing efficiency, but they have inherent safety concerns and higher production cost and lower loading capacity compared to other non-viral vectors including cationic lipids, cationic polymers and inorganic nanoparticles [28, 30]. Among these, cationic lipids are particularly attractive, since they have many advantages, for example simple chemistry and easy formulation of nanoparticles with siRNA. These cationic lipids consist of secondary and tertiary amine groups to complex the anionic siRNA molecules through electrostatic interaction to form complex also called as lipoplex. These systems developed the positive charge at low pH of endosomes which facilitate its endosomal escape via proton sponge effect [31].

Some widely exploited commercially available amine-based cationic lipids in siRNA delivery include 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP); N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA, Lipofectin); 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA, Lipofectamine); N-(2-hydroxyethyl)- N,N-dimethyl-2,3-bis(tetradecyloxy-1-propanaminiumbromide) (DMRIE); dioctadecyl amidoglyceryl spermine (DOGS, Transfectam); dimethyldioctadecylammonium bromide (DDAB); and 3b-[N-(NO,N)-dimethylaminoethane) carbamoyl] cholesterol (DC-Chol) [32].

Cationic lipids and siRNA complexes can be formulated in two major groups, i.e., lipid nanoparticles (LNPs) and liposomes. LNPs are comparatively more versatile than liposomes and involve siRNA complexation without formation of lipid bilayers. The ideal cationic lipids for siRNA delivery should be biodegradable and biocompatible, enclose and condense siRNA easily into a nanoparticle complex, neutralize excessive charge, protect siRNA degradation by nucleases, prevent rapid renal clearance and should escape the mononuclear phagocytic system (MPS) and non-toxic [33, 34]. Moreover, the diameters of ideal cationic lipid vectors should be less than 200 nm and should extend the circulation time of siRNA in the blood to allow passive tumor accumulation by the enhanced permeability and retention (EPR) effect which is ascribed to abnormal architecture of tumor blood vessels and gaps between endothelial cells and much larger fenestrations compared with normal tissue vessels and it also should prevent non-specific interactions with blood components like plasma proteins and erythrocytes. Finally, it should allow easy passage of siRNA through the vascular endothelium and the extracellular matrix and deliver siRNA effectively into the target cancerous cells followed by endosomal escape to release siRNA into the cytoplasm [19, 35].

The therapeutic applications of siRNA-based gene delivery in cancer treatment mostly rely on the development of rationally designed systemic delivery vehicles. Despite the fact that cationic lipids can condense siRNA, there are still many issues to be addressed such as pharmacodynamics-related challenges in targeting specificity, off-target RNAi activity, immune-sensor-mediated cytotoxicity and pharmacokinetics-related challenges in systemic circulation, cellular uptake and endosomal escape, which should be overcome to allow clinical application [36, 37]. The toxicity of cationic lipids results due to the destabilization of lipoplex in presence of serum and/or nonspecifically binding with blood plasma proteins and erythrocytes, forming aggregates that may occlude the pulmonary vasculature [38]. In addition, premature destabilization of lipoplexes in the systemic circulation by anionic heparin sulfate in the glomerular basement membrane (GBM), can accelerate rapid renal clearance and urinary excretion, minimizing the potential for biodistribution and bioactivity within targeted tumor tissue and organs. While the strong interaction and colloidal stability of siRNA lipoplexes may be ideal in the circulation and for initial cellular penetration, overly-stable packaging can also hinder bioavailability and gene suppression within target cells due to inefficient siRNA release [18]. They may also cause potential toxicity which includes cell shrinking, reduced cell division and cytoplasmic vacuolization [39].

In this review, we summarize the most recent strategies in the design and synthesis of non-viral cationic lipids vectors for siRNA delivery in cancer therapy. Various techniques employing surface modification by coating with polyethylene glycol (PEG), receptor mediated delivery, tumor micro environment responsive systems and other cationic lipids vehicles for concomitant delivery of siRNA and anticancer drugs have been discussed. The cationic lipids mentioned are designed to assess the challenges in systemic siRNA delivery and promising characteristics of these carriers required to bypass the delivery obstacles are also highlighted.

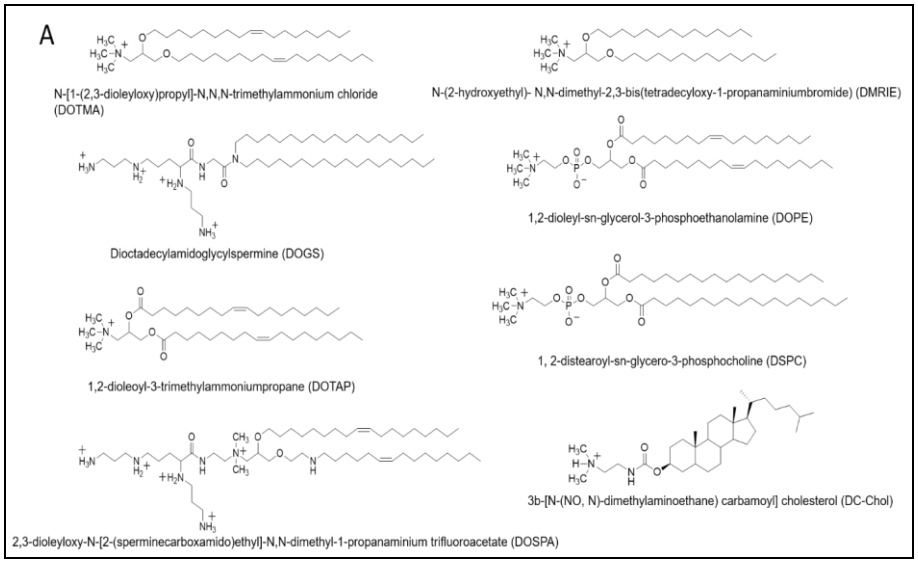

Fig 1: Chemical structures of non-viral Cationic and neutral lipids.

Cationic lipids and/or liposomes are members of the large family of synthetic non-viral gene delivery systems [40]. Some of the advantages of cationic lipids include acceptable physicochemical properties and biocompatibility (with biomimetic cell membranes) [41], remarkable simplicity and easy to synthesize, good repeatability, high commercial value and broad clinical application and safety [42]. They are composed of three structural domains [43]: i) a positively charged hydrophilic head group such as a primary, secondary or tertiary amine, guanidinium and amidine groups, quaternary ammonium salts, imidazolium, pyridinium etc.; ii) a hydrophobic portion consisting of a steroid group or alkyl chains [44] iii) and a linker which joins the hydrophilic and hydrophobic domains. The linker plays a significant role in determining the chemical stability and biodegradability of cationic lipids and hence directly influences its transfection efficiency and cytotoxicity. Cationic lipids with carbamate and amide linker groups are chemically stable and biodegradable and can therefore efficiently be used for gene delivery carriers [45]. Cationic lipids are generally mixed and accompanied with neutral helper lipids such as DOPE and cholesterol to assist the condensation of siRNA into a stable hexagonal-phase structure, which is advantageous for transfection efficiency. DOPE also facilitates the escape of siRNA from endosomes and also its release from the nano-complex. Similarly, cholesterol increases the structural rigidity and stability of the nano-complex and prolongs its systemic circulation [29].

Cationic liposomes are lipid-based nanoparticles which are commonly studied for siRNA delivery [46]. They consist of a spherical vesicle with at least one lipid bilayer. The aqueous core of liposomes is surrounded by a hydrophobic membrane, which prevents the transport of hydrophilic solutes from the inside to the external phase and allows the incorporation of hydrophobic molecules in the lipid bilayer. Therefore, liposomes can transport both hydrophilic and hydrophobic molecules [47]. Novel methods for liposomal preparations include microfluidics, supercritical reverse phase evaporation, spray drying, membrane contactor technology and crossflow injection [48]. Cationic liposomes, however, are usually associated with toxic effects in the systemic circulation rendering them unsuitable candidates for siRNA delivery. These liposomes non-specifically interact with negatively charged blood components before entering the target cells and form aggregates [49], which activate the complement system and will be rapidly cleared from the blood circulation by macrophages of the RES. Higher concentrations of cationic lipids may also have various undesirable side effects like the generation of reactive oxygen species and increase of intracellular calcium levels [50], and initiation of pro-inflammatory cytokine production and apoptosis [51]. A summary of the recent research on the use of lipids/liposomes for siRNA delivery is shown in Table 1.

Reduction of the cationic lipid/siRNA charge ratio can be a straight forward strategy for decreasing the cationic lipid mediated cytotoxicity. However most cationic lipids are utilized in binding with negatively charged siRNAs for its encapsulation by electrostatic interactions and are not suitable for interacting with endosomal membranes. Therefore, higher molar ratios of cationic lipids versus siRNA are generally employed in most studies. Sato *et al*. successfully developed lipid nanoparticles referred to as low lipid core-nanoparticles (LLC-NPs) with increased gene knockdown activity and a low cationic lipid/siRNA charge ratio. The cationic protein protamine was used to neutralize the negative charges of siRNAs in order to lower the net dose of cationic lipid, YSK05, for endosomal escape, restoring the fusogenic activity and gene silencing efficiency, both *in vitro* and *in vivo*. Additionally, LLC-NPs demonstrated a reduced hepatotoxicity as compared to conventional lipid nanoparticles having relatively higher cationic lipid/siRNA charge ratios [51].

PEGylation of cationic liposomes is one of the strategies to increase the structural stability of siRNA-lipoplexes and to prolong their blood circulation time. PEGylated lipoplexes can be prepared both by pre-insertion and post-insertion techniques. In the pre-insertion technique, liposomes are PEGylated by integrating PEG-conjugated lipids during the formation of liposomes while in the post-insertion technique,

aqueous dispersions of PEG-conjugated lipids are blended with previously synthesized liposomes [35]. Despite the fact that, PEGylated lipoplexes have increased blood circulation times, the cellular uptake and endosomal escape are often decreased, which also leads to an overall decrease of the ultimate gene transfection efficiency which is known as the "PEG dilemma" [52, 53]. Various strategies have been developed to overcome the PEG dilemma with cationic liposomes like- the development of a multifunctional envelope type nano device (MEND) [54] including the conjugation with fusogenic peptides for increasing endosomal or lysosomal escape [55], the use of specific ligands for cellular uptake [56, 57], the application of PEG-peptide-DOPE ternary conjugate (PPD) cleavable PEG systems [58], as well as coating with anionic glycosaminoglycans (GAGs), such as chondroitin, heparin sulfate, and hyaluronic acid [59].

Hashiba and coworkers developed siRNA lipid nanoparticles (LNPs) modified with PEG via maleic anhydride, a pH labile linker, and also conjugated N-acetyl-D-galactosamine (GalNAc) ligand for active targeting towards hepatocytes. The *in vitro* results demonstrated that the modified lipid nanoparticles had a suppressed cationic charge and stealth properties up to 1 hour at physiological pH and under slightly acidic conditions rapid detachment of PEG and re-activation of fusogenicity took place within only 2 minutes. The *in vivo* results showed a dramatic increase in the efficiency of hepatocyte-targeting with enhanced gene knockdown activity of a factor 7 in mouse model [60]. In another study, Li *et al*. constructed promising multifunctional lipid nanoparticles (sTOLP) for systemic delivery of FAM-siRNA to human hepatocarcinoma cells (HepG2)-bearing nude mice (Fig. 2.). sTOLP nanoparticles were composed of three regions viz., a protamine complexed siRNA core, supported bilayer incorporating oleoyl-octaarginine (OA-R8) cell penetrating peptide, egg PC, cholesterol and three different cationic lipids (DODMA or DOTMA or DOTAP) and the outermost layer containing a PEG layer and transferrin (Tf) targeting ligand. It was observed that the incorporated OA-R8 in the supported bilayer promoted the cellular penetration and that the conjugated Tf in the outermost layer enhanced the hepatocellular uptake via active targeting of Tf receptors. The *in vivo* results demonstrated potent tumor inhibition (61.7%) without inducing immunogenicity or hepatic or renal toxicity. Furthermore, sTOLP-loaded siRNA had a greater stability in the circulation than free siRNA [61].

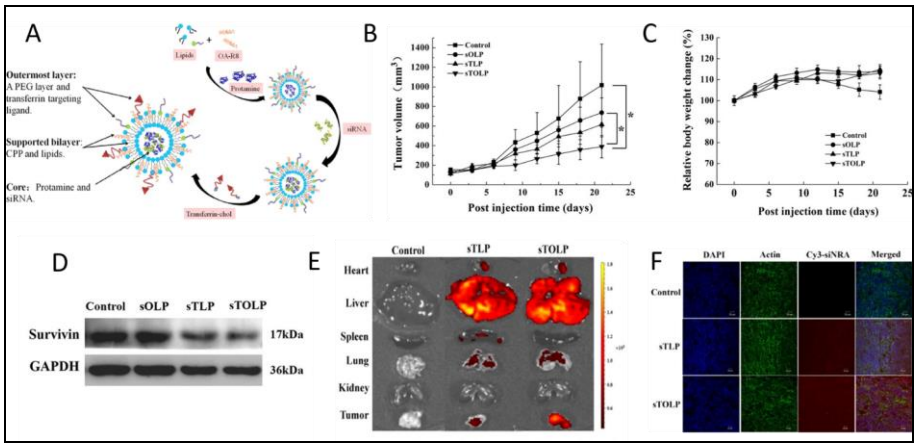

Fig 2: Cell penetrating peptide (CPPs) modified lipid nanoparticle (LNPs) as a vector for siRNA delivery: A: Illustration of steps involved in the synthesis of the multifunctional LNPs. B: Tumor growth curves after treatment. C: Relative body weight of nude mice during treatment. D: Protein levels of survivin after treatment. E: Biodistribution of fluorescence-labeled complexes in all tissues. F: Tumor tissues distribution of complexes after i.v. injection with imaging by confocal microscopy. Adapted with permission from [61].

Recently, many studies have been carried out by using lipid-polymer hybrid nanoparticles as an emerging technology for delivery of siRNA [62]. Sun *et al*. reported PEI-modified liposomes (SF-PL/siGPC3) prepared by a thin-film hydration method for co-delivery of sorafenib (SF) and GPC3 siRNA to treat hepatocellular carcinoma (HCC). It was observed that co-delivery of the nanomedicines effectively suppressed the overexpression of GPC3 and cyclin D1 proteins in HepG2 cells and greatly increased the sensitivity of tumors to SF treatments. Moreover, a study with a nude mouse model bearing HepG2 xenografts displayed a significant antitumor effect of SF-PL/siGPC3 via multiple mechanisms including the apoptosis of HCC cells induced by SF/siGPC3 and the inhibition of proliferation of these cells upon downregulation of cyclin D1 (Fig. 3.) [63].

The incorporation of polymers like sodium alginate into the cationic lipid carriers can also increase the stability of the delivery system with better encapsulation, prolonged integrity, low cytotoxicity, enhanced transfection and siRNA release kinetics. Arruda *et al*. reported cationic liposome-based siRNA delivery vectors (siLex) containing anionic sodium alginate polymer (Nalg-siLex), which have a higher efficiency and stability than siLex. siRNA lipoplexes were prepared by auto-assembly of siRNA and DMAPAP/DOPE (equimolar mixture) cationic liposomes which were subsequently mixed with sodium alginate. The addition of sodium alginate leads to the formation of smoother and heterogeneous particles. Also, Nalg-siLex did not induce the remarkable hepatotoxicity and inflammatory cytokine secretion as was noticed with siLex [64].

In another example**,** Hong *et al*. reported an acid-degradable polymer-caged lipoplex (PCLi) carrier composed of a cationic

lipoplex core and a biocompatible, pH-responsive polymer shell for delivery of EGFP-siRNA into human breast cancer (MDA-MB-231) and human cervical cancer (HeLa) cell lines. The unmodified siRNA was directly loaded into a lipoplex core consisting of either a pH-responsive cationic lipid DODAP or a non-pH-responsive DOTAP which was further modified around with a biocompatible polymer shell composed of cholesterol-terminated poly (acrylic acid) (Chol-PAA) using acid-cleavable diamine linkers to provide the PCLi system.

The investigators observed that the acid-cleavable diamine linkers were involved in acid-triggered release of encapsulated siRNA from acidic cellular endosomes, whereas the pH-responsive lipid DODAP was not released. The results showed that the acid-degradable PCLi delivery platform presented ~45- and ~2.5-fold enhancement of EGFP knockdown in cancer cells in comparison to either free siRNA or siRNA-loaded in non-acid-degradable lipoplex formulations, respectively [65].

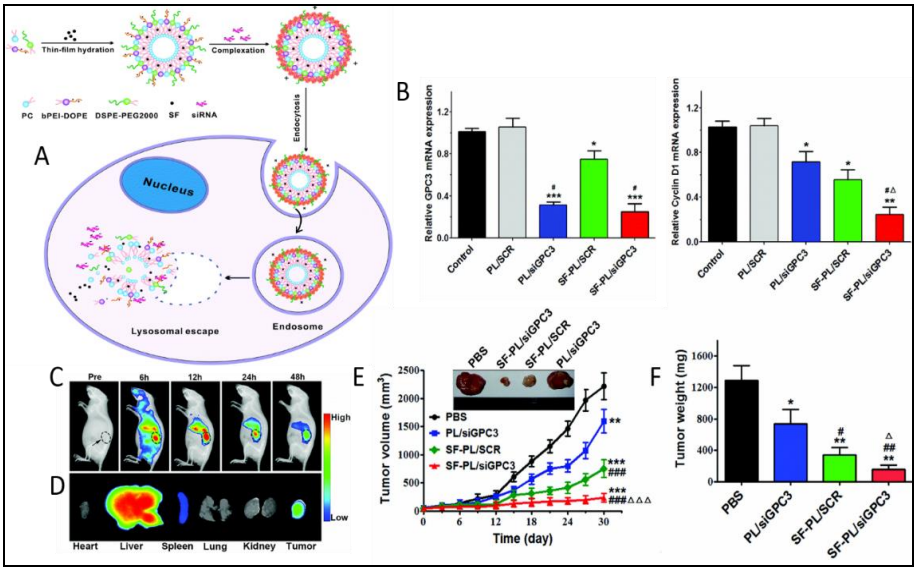

Fig 3: PEI-modified lipid (bPEI-DOPE) and liposome (PL) carrier system. A: Illustration of nanocarrier formation and mechanism for co-delivery of sorafenib (SF) and siRNA targeting GPC3 gene (siGPC3). B: Relative expression of GPC3 and cyclin D1 mRNA level by RT-PCR in HepG2 cells receiving different treatments. C: *In vivo* fluorescence imaging demonstrating tumor accumulation of PL/AF750-CSR in a mouse at different time frames after tail vein injection. D: Ex vivo fluorescence imaging of the major organs and tumors from the same mouse sacrificed at 48 hours. E: The tumor growth curve after treatment. F: Tumor weights. Adapted from Ref. [63]

Cationic liposomes have been widely used in the combination therapy of siRNAs and chemotherapeutics as an alternative strategy for achieving enhanced anticancer effects [66, 67]. Zhang *et al*. constructed a multifunctional drug delivery system (DOX+siRNA/ePL) with pH-sensitive and EphA10 antibody-mediated active targetability for simultaneously delivery of MDR1-siRNA and DOX to overcome the MDR effect. Their results showed that lipoplexes could form stable nanosized particles with incremental cellular uptake, enhanced P-gp downregulating efficacy, as well as better cell cytotoxicity in a human breast cancer cell line/Adriamycin drug-resistant (MCF-7/ADR) cells. The *in vivo* antitumor activities suggest that after intravenous administration into mice, DOX+siRNA/ePL had relatively long circulation times and was concentrated in the tumor cells via receptor-mediated endocytosis. Additionally, the results of the histological study indicated that DOX+siRNA/ePL could inhibit the proliferation of tumor cells, induce apoptosis, and suppress the P-gp expression *in vivo* [68]. In another example, Wang and coworkers formulated a lipid-based delivery system (LPs) to codeliver of Mcl-1 siRNA and gemcitabine (Gem) for pancreatic cancer treatment, named LP-Gem-siMcl-1. The results from the cellular uptake analyses of LP-Gem-siMcl-1 displayed an increased cellular uptake, enhanced Mcl-1 downregulation efficacy, and significant cytotoxicity in the human pancreatic carcinoma cell lines PANC-1 and BxPC-3. Moreover, tumor inhibition *in vivo* proved that LP-Gem-siMcl-1 had more potent anti-tumor efficiency than LP-siMcl-1 plus LP-Gem, indicating the synergistic anti-tumor activities of Gem and siMcl-1. Furthermore, histological analysis exhibited that LP-Gem-siMcl-1 could efficiently co-deliver Gem and Mcl-1 siRNA to cancerous cells overcoming the resistance of Gem [67].

Table 1: Cationic lipids/liposomes mediated siRNA delivery for cancer treatments

Composition of Lipids/liposomal carrier	siRNA type/Anticancer drug	Experimental Level	Cell lines/Disease	Ref.
Carboxymethyl chitosan-modified cationic liposomes (CMCS-SiSf-CL)	Cy3 and Cy5 siRNA/Sorafenib	*In vitro* and *In vivo*	HepG2 and H22 cells	69
Angiopep-2 and tLyP-1 dual peptides-modified	VEGF siRNA/ docetaxel	*In vitro* and *In*	C6 cells (rat glioma cells)/Glioblastoma	70

liposomes		*vivo*	multiforme	
A new cationic lipid-like compound (G0-C14)	prohibitin 1 (siPHB1) siRNA	*In vitro*	Luc-HeLa cells and A549 lung carcinoma cells	71
Core-Shell type lipid/rPAA-Chol polymer hybrid nanoparticles	Anti-EGFR siRNA	*In vitro* and *In vivo*	Human breast cancer MCF-7 cell line	72
BHEM-Chol/mPEG-PLA	polo-like kinase 1 (Plk1) siRNA	*In vitro* and *In vivo*	Human breast cancer cell BT474	73
Poly (DL-lactic-co-glycolic acid) (PLGA) nanocarriers modified with DOTAP	EGFP siRNA	*In vitro*	Human non-small lung carcinoma cell line H1299	74
Photolabile-caged cell-penetrating peptide (pcCPP) and asparagine-glycine-arginine peptide (NGR) conjugated cationic lipids	c-myc siRNA	*In vitro* and *In vivo*	HT-1080 cells and MCF-7 cells	75
Cationic quaternary ammonium sulfonamide amino lipids	anti-Luciferase siRNA	*In vitro* and *In vivo*	HeLa−Luc cells	76
Cationic amphiphilic macromolecule (CAM)-lipid complexes	anti-Luciferase siRNA	*In vitro*	U87 and U87-LUC cells	77
GE11 peptide conjugated nanoliposomes	ABCG2- siRNA/docetaxel	*In vitro* and *In vivo*	Hep-2 cells	78
PEGylated cationic nanoliposome	JNK-interacting protein 1 (JIP1) siRNA	*In vitro*	MG-63 osteosarcoma cell	79
Antibody-conjugated lipid-based nanoparticles	siRNAs against CD45, luciferase and luciferase-Cy5 (siCy5)	*In vitro* and *In vivo*	Anti-rat IgG2a hybridoma, RAW 264.7 cells, HEK293 cells, Tk1 cells, Granta 519 cells	80
Lipid-polymer hybrid nanoparticles (RSC-HA)	FAM-siRNA, Cy5-siRNA	*In vitro* and *In vivo*	Human lung adenocarcinoma epithelial (A549) cells	81
Lipid-based nanoparticles coated with anti-CD38	siRNAs against cyclin D1	*In vitro* and *In vivo*	MCL-cells	82

3. Conclusion and future perspectives

The use of siRNA-based therapeutics has emerged as a highly promising strategy for the treatment of various types of cancers owing to their high specificity, low dose requirement, relatively simple drug development process and also avoids the possible genomic mutations associated with DNA therapies [83, 84]. With the recent FDA approval of Patisiran and Givosiran, systemic delivery of RNAi-mediated gene therapy is undergoing a transition from research to the clinical space [85]. Despite the impressive advances made in the field of *in vivo* siRNA drug development, the delivery of siRNA still remains challenging due to its instability in the systemic circulation, unexpected adverse effects including off-target effects, innate immune responses, proinflammatory cytokine induction and its inability to cross cellular membrane and endosomal escape and hence advanced carrier systems are required. During the past two decades, immense amounts of time and large capital investments have been made to develop different types of cationic lipid-based nanocarriers for improved *in vivo* siRNA delivery. However, to realize improved siRNA-based therapeutics, further research has to be focused on the design and synthesis of cationic lipids, which can be more efficiently delivered to target cells.

There has been continuous progress in cationic lipid-based carriers to reduce their potential toxicity and also improving their specificity to target cells for translating them to clinical diagnosis and treatment. The higher cytotoxicity of cationic lipids due to increased number and density of amine groups have been reduced by coating these delivery systems mainly with polyethylene glycol (PEG). Similarly, in order to make them tumor-specific, they have been conjugated with various external targeting moieties such as targeting peptides, ligands, antibodies and other small molecules having tumor-targeting abilities. The slow and inefficient siRNA release inside tumor cells generally displayed for most biodegradable cationic lipids-based nanoparticle can result in compromised gene silencing efficacy. By using stimuli-responsive groups like disulfide bonds, cationic lipids based lipoplex can be made responsible to tumor micro environment of cancer cells (e.g., pH, enzyme, redox and hypoxia), which can trigger siRNA release in tumor cells. Combination therapy has also become an important strategy to overcome the multidrug-resistant due to the increasing number of cancer-related gene targets and complexity. A large number of cationic lipids are being used for co-delivery of siRNA and chemotherapeutic drugs in an attempt to increase the anticancer effects by overcoming MDR or initiating different apoptosis pathways. Despite these advances, siRNA-delivery strategies using non-viral cationic lipids have poorly translated into the clinical setting because of preclinical experimental design considerations that poorly predict the therapeutic efficacy. Therefore, further studies are needed to optimize these delivery carriers for efficient and successful systemic siRNA delivery in humans to treat the cancer cells.

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