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Liposomal siRNA nanocarriers for cancer therapy ☆

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ABSTRACT

Small interfering RNAs (siRNA) have recently emerged as a new class of therapeutics with a great potential to revolutionize the treatment of cancer and other diseases. A specifically designed siRNA binds and induces post-transcriptional silencing of target genes (mRNA). Clinical applications of siRNA-based therapeutics have been limited by their rapid degradation, poor cellular uptake, and rapid renal clearance following systemic administration. A variety of synthetic and natural nanoparticles composed of lipids, polymers, and metals have been developed for siRNA delivery, with different efficacy and safety profiles. Liposomal nanoparticles have proven effective in delivering siRNA into tumor tissues by improving stability and bioavailability. While providing high transfection efficiency and a capacity to form complexes with negatively charged siRNA, cationic lipids/liposomes are highly toxic. Negatively charged liposomes, on the other hand, are rapidly cleared from circulation. To overcome these problems we developed highly safe and effective neutral lipid-based nanoliposomes that provide robust gene silencing in tumors following systemic (intravenous) administration. This delivery system demonstrated remarkable antitumor efficacy in various orthotopic human cancer models in animals. Here, we briefly overview this and other lipid-based approaches with preclinical applications in different tumor models for cancer therapy and potential applications as siRNA-nanotherapeutics in human cancers.

Contents

1. Introduction

1.1. Gene silencing by small-interfering RNA

The discovery of RNA interference (RNAi), including micro RNA (miRNA) and small-interfering RNA (siRNA) mediated gene silencing, is considered one of the most important advancements in biology in the last decade [1–3]. siRNA is now commonly used as a powerful tool for silencing post-transcriptional gene expression and investigating gene. More importantly, potential applications of siRNA have led to a great interest in harnessing this technology for therapeutic use in cancer and other diseases. A specifically designed siRNA can bind the target gene (mRNA) in a sequence specific manner and induce degradation of mRNA translation [3]. These short double-stranded (ds) RNAs are cleaved into fragments called siRNA (21-base pairs) by DICER protein.

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The target mRNA is bound by the antisense strand after forming a complex with proteins, designated as the RNA-Induced Silencing Complex (RISC). An RNA endonuclease (Argonaute 2) within the complex cleaves the target mRNA and leads to its degradation, shutting down protein expression (Fig. 1). For therapeutic applications, synthetic siRNA is used for targeting oncogenes and genes that are involved in cancer cell proliferation, survival, invasion, angiogenesis, metastasis, and resistance to chemotherapy or radiotherapy in cancer and for targeting disease-causing genes in other pathologies [4,5].

1.2. Obstacles for systemic use of siRNA-based therapeutics

The broad therapeutic applications of siRNA-based therapeutics in cancer are largely dependent on the development of rationally designed systemic delivery systems that can efficiently deliver the siRNA molecules into tumors and target cells [6,7]. The major limitations of the systemic use of siRNA-based therapies include rapid degradation by nucleases (half-life ~15 min in serum) and renal clearance following systemic administration [8]. Thus earlier studies with siRNA-based therapies entered into clinical trials relied on the local administration, including the intravitreal or intranasal routes [7,8]. To enhance the stability of various siRNA chemical modifications, such as backbone (phosphorothioate, boranophosphate) and sugar modifications (2′ modifications to the sugar ring, namely 2′-OMe, 2′-fluoro, and 2′-*O*-methoxyethyl (2′-MOE)), have been used [7]. However, poor cellular uptake remains an important issue due to negatively charged cell membranes preventing efficient intracellular uptake of siRNA molecules, which also have a negatively charged backbone, leading to electrostatic repulsion, requiring a carrier to increase the uptake into cancer cells. Rationally designed specific siRNA for the exclusion of partially complementary sequences and certain motifs that induce immune response and the use of the minimum effective dose of siRNA may also enhance unwanted side effects [4]. Overall, developments of safe, stable, effective and tumor-specific delivery systems for systemic administration are important goals for translation of siRNA-based therapeutics into successful clinical applications. Nanotechnology holds promise for widespread clinical applications of siRNA-therapeutics. Nanocarriers also have great potential to reduce siRNA related toxicities and prevent off-target effects in normal tissues (reviewed in detail by Jackson and Linsley, 2010) [50].

2. Nanocarriers for systemic siRNA delivery

Nanocarriers (submicron size particles ranging from 1 to 1000 nm) can overcome most hurdles that prevent the systemic use of siRNA [9,10]. Nanoparticles have been shown to carry and deliver desired cargos or payloads, such as chemotherapeutic agents, oligonucleotides, drugs, peptides, and imaging agents in *in vivo* systems. In general, the ideal nanocarrier is expected to be safe, non-toxic, biocompatible, biodegradable, and non-immunogenic, and to be able to bypass rapid hepatic or renal clearance. Furthermore, an ideal delivery system should be able to preferentially target siRNA into the tumor or preferred tissues, and allow escape of the siRNA from endosomal capsulation, releasing the payload into cytoplasm for maximal efficacy.

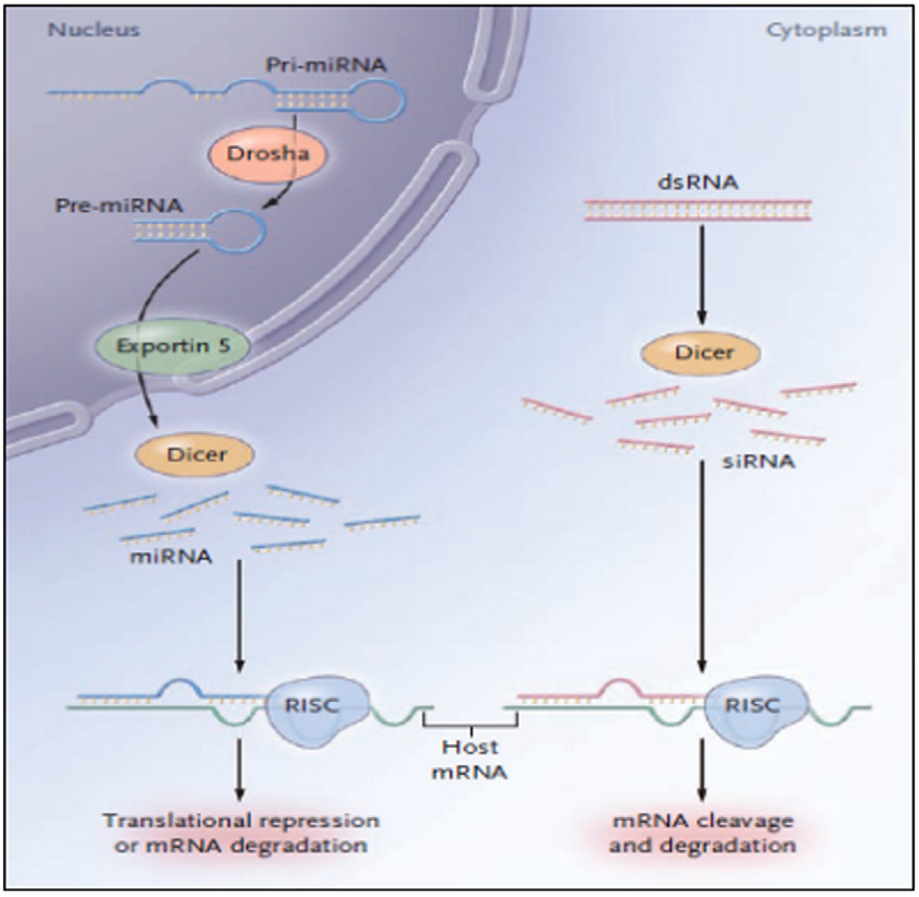

Fig. 1. The process of RNA-interference in eukaryotic cells. Long precursor microRNA (miRNA), called pri-miRNA, is cleaved by RNase III endonuclease (Drosha) into pieces of approximately 70 nucleotides each (called pre-miRNA) in the nucleus. Following transportation into the cytoplasm by exportin 5 another RNase III endonuclease (Dicer) cleaves it into mature miRNA segments. Degradation of messenger RNA (mRNA) and translational repression occurs after miRNA binds to the RNA-induced silencing complex (RISC). Cytoplasmic long double-stranded RNA (dsRNA) is cleaved by Dicer into small interfering RNA (siRNA), which is incorporated into RISC, resulting in the cleavage and degradation of specific target mRNA. Synthetic double-stranded siRNA is not processed by Dicer and directly incorporated by the RISC.
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Various nanoparticles made of biodegradable nanomaterials such as natural or synthetic lipids (*e.g.*, liposomes, micelles) and polymers (*e.g.*, chitosan, poly(lactic-co-glycolic) acid (PLGA), polylactic acid (PLA), polyethilenimine (PEI), atelocollagen), carbon nanotubes, quantum dots, gold nanoshells or iron oxide magnetic have been used for siRNA delivery [9–15]. However, each nanoparticle system has its own unique tissue biodistribution, toxicity, and tumor cell uptake based on surface charge, size and hydrophobicity. Nanoparticles with diameters <200 nm seem to passively accumulate in tumor tissues [9]. This accumulation is related to an enhanced permeability and retention effect (EPR) due to abnormal architecture of tumor vessels and gaps between endothelial cells and much larger fenestrations compared with normal tissue vessels [16]. The overall leaky nature of tumor vasculature with gaps varying in size from 200 to 1200 nm (in contrast to normal endothelium with pores with 10 to 50 nm) leads to the passive accumulation of nanoparticles. Nanoparticles bigger than 100 nm in diameter are taken up by the reticuloendothelial system (RES) in liver, spleen, lung, and bone marrow, whereas smaller nanoparticles have a prolonged circulation time [9]. Also very small nanoparticles and polymers with MW < 40 kDa can be cleared by renal excretion. Physical features, charge, shape and the nature of the nanocarriers have an effect on the fate of nanoparticles. For example, negatively charged particles are cleared faster than positively charged particles and tend to be taken up by phagocytic cells and may not result in optimal loading efficiency due to the negative charge of siRNA. Overall, nanocarriers that are considered for *in vivo* systemic therapeutic siRNA applications are need to be carefully designed [8]. Table 1 summarizes some of the nanoparticles that have been used for siRNA delivery.

2.1. Liposomal nanocarriers for siRNA

Liposomal formulations have been historically the most popular delivery system and have been extensively employed to enhance the efficiency of drug delivery by systemic administration due to their high degree of biocompatibility. The ability of liposomes to deliver a variety of payloads, including chemotherapy drugs, oligonucleotides, DNA, antisense, siRNA, antigens and proteins, has made them most the successful method for delivery of therapeutic agents (recently reviewed by Petros and DeSimone, 2010) [6]. Currently there are seven FDA-approved liposomal drugs in clinic. The first FDA-approved (1995) liposomal drug was doxorubicin (Doxil). Later, FDA approved liposomal forms of chemo-agents such as daunorubicin and cytarabin and highly toxic antifungal drug amphotericin B (Abelcet, Ambisome) for the treatment of invasive fungal infections and which is widely used to treat systemic fungal disease, which is a source of major morbidity in cancer patients [6].

Liposomes are defined as unilamellar or multilamellar microvehicles consisting of a phospholipid bilayer. Phospholipids are major components of normal cell plasma membranes, which maintain the cell integrity by creating a semi-impermeable barrier by using phosphatidylcholine (PC) and sphingomyelin (SM), phosphatidylserine (PS) and phosphatidylethanolamine (PE). Phospholipids are amphiphilic molecules with the hydrocarbon tail (hydrophobic) and the polar head (hydrophilic). When exposed to water, phospholipids form a phospholipid bilayer with the hydrophobic tails facing each other and the hydrophilic heads facing water on both sides. Liposomes may contain synthetic or natural phospholipids, such as soya or egg PC, PE as well as cholesterol.

Liposomes offer several advantages as an siRNA delivery system due to their ability to 1) prevent degradation of the payload, 2) accumulate preferentially in tumor tissues (passive targeting/delivery) and deliver high concentrations of the payload, 3) specifically target siRNA to tumor cells and the microenvironment with high-affinity ligands (active targeting) and 4) provide safe and effective systemic delivery platforms in animals and humans depending on the lipid content. Furthermore, liposomal formulations have been shown to be highly effective in maintaining high plasma concentration of drugs with poor bioavailability and hydrophilic and hydrophobic features, leading to approval for cancer therapeutics by the FDA. For robust and successful siRNA delivery with lipid-based systems, optimization of lipid composition, drug-to-lipid ratio, particle size, charge, surface-targeting moieties, payload encapsulation efficiency, and the manufacturing process is required. Liposomes can be coated with PEG (polyethylene glycol) (also known as "stealth liposomes") to avoid detection and elimination by cells of the RES and have a longer circulation half-life [18,20]. In addition, synthetic phospholipids, which are conjugated to gangliosides (such as monosialoganglioside GM_1) have also been used. The PEGylation also provides a linker for the attachment of targeting ligands for specific targeting of liposomes and better interaction with cell surface receptors expressed on targeted cells. For this purpose, targeting ligands such as peptides, monoclonal antibodies (making an immunoliposome), aptamers, and chemical compounds can be linked to PEG and used for targeted delivery.

2.1.1. Cationic-lipid based liposomes for siRNA delivery

Cationic liposomes have been traditionally the most commonly used non-viral delivery systems for oligonucleotides, including plasmid DNA, antisense oligos, and siRNA/small hairpin RNA-shRNA. Cationic lipids, such as DOTAP, (1,2-dioleoyl-3-trimethylammonium-propane) and DOTMA (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl-ammonium methyl sulfate) can form complexes or lipoplexes with negatively charged DNA or siRNA to form nanoparticles by electrostatic interaction, providing high *in vitro* transfection efficiency [17,18]. Also they easily interact with negatively charged cell surfaces, facilitating delivery into

Table 1
Selected nanoparticles that have been used for siRNA delivery.

Category of particle	Type, form and composition of carrier	Natural *vs.* Synthetic	siRNA/shRNA	Comments
Lipid complex				
	Neutral-DOPC-liposomes	Synthetic	siRNA	Biodegradable, non-toxic
	Cationic liposomes	Synthetic	siRNA	ROS induction, lung toxicity, hepatotoxicity
	Lipoplexes/polyplexes (cationic lipid based)	Synthetic	siRNA	TLR4 activation, interferon type I response, hepatotoxicity
	Stable nucleic acid-lipid particles (SNALP)	Synthetic	siRNA	
Conjugated polymers	Polymer-functional peptides	Synthetic	siRNA	
	Polymer-lipophilic molecules (*e.g.* cholesterol, cationic amphiphiles)	Synthetic	siRNA	PKC inhibition, cytotoxicity
	Polymer-PEG	Synthetic	siRNA	
Cationic polymers	Chitosan	Natural	siRNA/shRNA	Biodegradable-non-toxic
	Atelocollagen	Natural	siRNA/shRNA	Biodegradable-non-toxic
	Polyethyleneimine (PEI)	Synthetic	siRNA/shRNA	Damage to cell/mitochondrial membrane, cytotoxicity
	Cyclodextrin	Synthetic	siRNA/shRNA	
	Poly-L-lysine	Synthetic	siRNA/shRNA	

cells. Cationic liposomes, while efficiently taking up and condensing siRNA, have had limited success for *in vivo* gene downregulation, perhaps because of their stable intracellular nature and resultant failure to release siRNA contents [19,20]. On the other hand, cationic lipids can destabilize and escape from the endosomal membrane and facilitate the release of siRNA into cytoplasm.

Toxicity of cationic lipids is the major issue following systemic administration preventing them from being a major candidate for siRNA delivery (Table 1). The use of cationic liposomes in *in vivo* mouse models elicited dose-dependent toxicity and pulmonary inflammation, hepatotoxicity and a systemic interferon type I response, attributed in part to the activation of TRL4 [19–21]. Cationic lipids also activate the complement system and cause their rapid clearance by macrophages of the RES. It was also demonstrated that cationic lipids are highly toxic to macrophages and other immune cells (ED50 < 50 nm/L) [22]. Different lengths of hydrocarbon chains can also influence the cytotoxicity of cationic lipids [24]. Cationic lipid-induced toxicity was more pronounced with the multivalent cationic liposome (Lipofectamine®) than with the monovalent cationic lipids, such as DOTAP [20]. This effect was not observed in mice treated with neutral or negatively charged particles. Toxicity of cationic lipids is linked to induction of reactive oxygen species (ROS) and increased intracellular calcium levels [19]. In addition, DOTAP-based particles accumulate near the vasculature and are preferentially taken up by the liver and spleen, limiting their effectiveness in systemic or anti-tumor therapy [17]. Overall, although cationic lipid-based delivery systems offer some advantages as a siRNA delivery system, potential toxicities need to be addressed before their translation in clinical trials. Careful selection of lipids and formulation strategies may help reduce the potential toxicities.

2.1.2. Neutral lipid-based nanoliposomes for siRNA delivery

To eliminate toxicity and overcome potential issues observed with other carriers including cationic liposomes and negatively charged carriers, we have developed neutral 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC)-based nanoliposomes [17,25–31]. DOPC-nanoliposomes incorporating siRNA targeting either EphA2, FAK, neuropilin-2, IL-8, TMRRS/ERG, EF2K or Bcl-2 demonstrated remarkable anti-tumor efficacy in orthotopic and subcutaneous xenograft tumor models of various cancers, including ovarian [17,25,27,29], colon, [26], breast [28], pancreatic [30], prostate cancer [31], and melanoma [32], suggesting a potential use of this approach in human trials. DOPC-nanoliposomes incorporating siRNA, at a dosing schedule of 150 μg/kg, resulted in substantial and robust reductions in the expression of the target genes/proteins (EphA2, FAK, neuropilin-2, IL-8, EF2K, and Bcl-2) in mice bearing various human cancers [17,25–31]. Systemic intravenous (i.v.) administration of a single dose of DOPC-siRNA (approximate mean size 65 nm) inhibited target protein expression for more than 3 days in tumors in mice [17]. These nanoliposomes were able to deliver siRNA *in vivo* into tumor cells 10- and 30-fold more effectively than did cationic liposomes (DOTAP) and naked siRNA, respectively [17,25]. More importantly, DOPC-based neutral-lipid nanoliposomes do not seem to cause any detectable distress and toxicity and were found to be safe in mice and non human-primates after single and repeated intravenous administration of liposomes for 4 weeks [17,25–28]. Lack of induction of pro-inflammatory cytokines, such as interferon (IFN) and IL-2 after i.v. DOPC-liposomal siRNA injections and reactive oxygen species, which are often induced by cationic lipids in lung, as well as increased (~10-fold) tumor tissue accumulation compared with cationic liposomes may contribute to the safety profile in animals. DOPC-siRNA formulation toxicity in normal cells including fibroblast, bone marrow and hematopoietic cells may contribute lack of toxicity, making them highly attractive nanocarriers for systemic delivery. Using different cancer targets in a variety of preclinical tumor models in mice, we have further demonstrated the efficacy of the neutral DOPC-nanoliposomal delivery system. As shown in Fig. 2, using DOPC-nanoliposomes, we demonstrated that *in vivo* therapeutic silencing of Bcl-2 by NL-Bcl-2 siRNA inhibited *in vivo* tumor growth of ER(−) MDA-MB-231 and ER(+) MCF tumor xenografts in nude mice and significantly enhanced the efficacy of chemotherapy [28]. These findings also indicated that neutral DOPC-siRNA-based therapy can be effectively combined with other conventional anti-cancer therapies, such as chemotherapy, to enhance the efficacy of conventional drugs.

2.2. Solid lipid-based systems (SNALPs and SLN)

Solid lipid-based technologies have also been developed for systemic delivery of siRNA. These positively charged carriers include stable nucleic acid-lipid particles (SNALPs) and solid-lipid nanoparticles (SLN) [23,24]. SNALPs consist of a lipid bilayer containing a mixture of cationic and fusogenic lipids that enable the cellular uptake and endosomal release of siRNA. SNALPs are also stabilized by coating with a diffusible polyethylene glycol–lipid (PEG–lipid) conjugate that provides a neutral and hydrophilic exterior. SNALP can successfully deliver intravenously administered siRNA (2.5 mg/kg) into monkeys with marked inhibition of apolipoprotein B protein for up to 11 days [23]. SNALP was tested in a human Phase I clinical trial in hypercholesterolemia as a cholesterol lowering therapy. SLNs are reconstituted from natural components of protein-free LDL and used to deliver siRNA. SLNs are composed of cholesteryl ester, triglyceride, cholesterol, dioleoyl phosphatidyl ethanolamine (DOPE), and 3-beta-[N-(N′,N′-dimethylamino ethane) carbamoyl]-cholesterol (DC — cholesterol). An efficient target gene silencing and serum stability with a minimal level of cytotoxicity can be achieved by these carriers [24]. Although these formulations can be easily adjusted for use in the treatment of cancer, toxicity issues related to their cationic lipid-based structure remain as an important obstacle with respect to these nanoparticles.

2.3. Lipidoid nanoparticles

Lipidoid particles are lipid-like delivery molecules that utilize cholesterol and PEG-coated lipids for delivery of specific siRNAs. To improve SNALP-mediated delivery, Akinc et al. developed a chemical method to allow the rapid synthesis of a large library of lipidoids and tested their efficacy in siRNA delivery [33]. One of the leading lipidoid-based siRNA formulations showed a 75%–90% reduction in ApoB or FVII factor expression in hepatocytes in nonhuman primates and mice [33]. This formulation provides gene silencing at lower doses of siRNA than those required by the original SNALP formulation, resulting in reduced toxicity.

2.4. Lipophilic siRNA conjugates

Lipophilic siRNA conjugates such as cholesterol conjugates have also been used for siRNA delivery. Cholesterol-siRNA preassembled with high-density lipoprotein was 8–15 times more effective than cholesterol-siRNA in silencing target protein expression *in vivo*. Following intravenous administration in mice, target gene silencing was shown to be dependent on the nature of the carbon chain of the fatty acids [34]. In this study, however, the conjugates were still administered at a relatively high dose of 50 mg/kg, requiring a significant improvement in efficacy and safety profile, and more efficient methods for siRNA delivery (Table 1).

3. Clinical applications of siRNA-nanotherapeutics

siRNA-based therapies have quickly moved into the clinic especially for diseases requiring localized or topical delivery, including age-related macular degeneration, diabetic macular edema, and respiratory virus infection and pachyonychia congenital [7]. The first clinical trial involving siRNA began in 2004 for the treatment of acute macular degeneration [6]. Later, several clinical trials based on systemic delivery of siRNA-therapies have progressed into the clinic and are currently

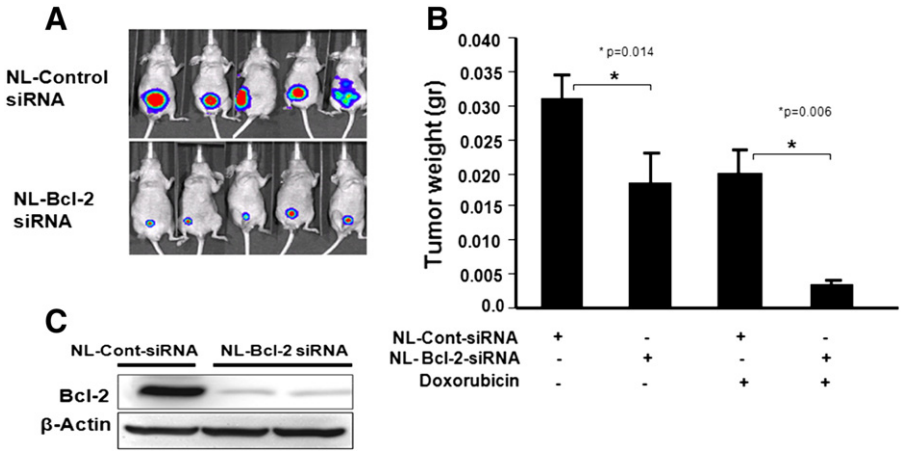

Fig. 2. *In vivo* therapeutic silencing of Bcl-2 by NL-Bcl-2 siRNA inhibits *in vivo* tumor growth of ER(−) MDA-MB-231 xeonografts in nude mice (Tekedereli& Ozpolat, 2013). A) Mice were orthotopically (into mammary fat pat) injected with luciferase expressing MDA-MB-231 cells. About 2 weeks after tumor cell injection mice were imaged using IVIS-Xenogen live imaging system for tumor growth. Mice bearing tumors with equal size were randomly assigned into control and treatment groups (n = 5 mice per group). Mice received either NL-Bcl-2 siRNA or NL-control siRNA treatments (0.15 mg siRNA/kg or 4 μg/mouse, i.v., twice a week) from tail vein for 4 weeks (total of 8 injections). After 4-weeks of treatments mice were imaged by IVIS live-imaging system and tumors were removed and weighted. B) *In vivo* silencing of Bcl-2 by intravenously administered NL-Bcl-2 siRNA enhances the antitumor efficacy of chemotherapy. Mice were treated with NL-Bcl-2-siRNA or NL-control siRNA (0.15 mg siRNA/kg, i.v., twice a week) and also received doxorubicin (4 mg/kg, i.p, once a week) for 4 weeks. The tumor weights after 4 weeks of treatments were measured. Mice that received NL-Bcl-2 siRNA had significantly inhibited tumor weight compared with the control group that was treated with NL-Control siRNA and enhanced the anti-tumor efficacy of doxorubicine. C) Bcl-2 protein expression after 4 weeks of treatments in MDA-MB-231 tumors. After sacrificing mice tumors were removed (48 h after the last NL-siRNA injection) and tumor lysates were analyzed for Bcl-2 expression by Western blot. NL-Bcl-2 siRNA treatment was well tolerated and did not have weight lose in mice, compared to those who received NL-control siRNA.

being tested in Phase I/III clinical trials for viral diseases (hepatitis B) and acute renal failure. Currently, there are eight clinical trials in solid tumors and chronic myeloid leukemia (CML) in the United States (Table 2). The first demonstration of the siRNA-mediated effect in a clinical trial for human solid tumor (melanoma) was started in 2008; this study targeted ribonuclease reductase (RRM2) using a cyclodextrin-based polymer conjugated siRNA [35] following a study in non-human primates [36]. Preliminary data showed that in general this approach was well tolerated although dose limiting toxicity was observed in several patients. Based on their safety profile of DOPC-based nanoliposomal siRNA in non-human primates, Phase I clinical trial is being started to target EphA2 in solid tumors in ovarian cancer patients at the MD Anderson Cancer Center (Table 2). Overall, available data indicate that different preclinical cancer models provide proof-of-principle for the translation of siRNA-based nanotherapies into clinic.

4. Tumor-targeting nanoparticles

Targeted delivery (active delivery) of therapeutics and diagnostics into tumor cells and/or tumor-vasculature is recognized as a powerful approach for treatment of cancer. Targeted drug delivery systems expand the therapeutic windows of drugs by increasing delivery to the target tissue and reducing side-effects. Traditionally, this concept was proven to work by using tumor cell specific antibodies [37]. Tumor-targeting nanocarriers can accumulate in tumor tissues (about 10–100 fold) compared with passive delivery depending on the approach used [17]. In general, high-affinity ligands are attached to the exterior surface to increase the delivery of siRNA or particular payload. Studies demonstrated that functional peptides, lipophilic molecules, PEG, and aptamers can be used for tumor-targeting. Most common approaches for targeting have been folate receptor (FR) alpha, transferrin receptor, and AlphaVBeta3/5 integrin receptors, and prostate specific membrane antigen (PSMA) [38–45]. FR-alpha is a highly selective tumor marker that is overexpressed in about 90% of ovarian and nasopharyngeal cancers and to some extent in other cancers including breast cancers [38]. In addition, αVβ3 and αVβ5 are specifically expressed on angiogenic tumor epithelium and in some primary and metastatic cancers. For instance, DOPC-liposomes conjugated with folate or RGD showed better and longer *in vivo* target silencing and demonstrated remarkable antitumor efficacy compared with non-tumor targeting DOPC-liposomes in two different ovarian cancer models. These tumor-targeting particles also significantly enhanced *in vitro* siRNA (Alexa555 or Cy3 labeled) uptake about 5–8 fold into receptor positive ovarian cancer cells while a single dose *in vivo* i.v. administration significantly increased the siRNA uptake (at least 10-fold) into tumors in mice (Dr. Ozpolat's unpublished observation). Similar targeting strategies can be done by coating liposomes with specific antibodies [46]. The antibodies or peptides can be directed against various receptors or surface antigens, including antibodies against the transferrin receptors [47]. These strategies have been successfully applied for receptor-specific

Table 2
Current clinical trials of siRNA based therapeutics.

Target gene	Carries	Condition	Phase
EphA2	Neutral (DOPC) liposomes	Advanced cancers	Phase 1
Immunoproteasome β-subunits LMP2, LMP7 and MECL1	Dendritic cells	Metastatic melanoma Absence of CNS metastases	Phase 1
Ribonucleotide reductase-M2 (RRM) (CALAA-01)	Transferrin receptor-targeted PEGylated cylodextrin-polymer	Solid tumors phase 1	Phase 1
KRAS-G12D (siG12D LODER)	Polymer	Pancreatic cancer	Phase 2
PLK1 (TKM-080301)	Lipid nanoparticles	Colorectal, pancreas, gastric, breast, ovarian cancer types with hepatic metastases	Phase 1
pbi-shRNA STMN1 LP	Lipoplex	Solid tumors (intratumoral)	Phase 1
KSP and VEGF (ALN-VSP)	SNALP	Liver cancer and solid tumors	Phase 1
PKN3 (Atu027)	Liposome	Solid tumors	Phase 1

delivery of chemotherapy agents, radiopharmaceuticals, imaging contrast agents, peptides, and siRNA, suggesting that they can enhance the efficacy of siRNA by increasing the concentration of siRNA in tumors at relatively lower doses than those of untargeted nanocarriers [46–49]. In conclusion, studies suggest that tumor-targeted carriers incorporating siRNA, drugs or imaging agents can significantly improve therapeutic efficacy and reduce payload related toxicities.

5. Conclusion and future prospects

Nanocarriers hold great potential for cancer therapy, diagnosis and imaging. In addition to the delivery of wide variety of anti-cancer agents, they seem to be the best candidates for the administration of siRNA-based therapeutics and are currently being tested in human clinical trials. Pharmacokinetic, pharmacodynamic parameters and most importantly the toxicity/safety profiles of various potential siRNA delivery systems should be well defined and considered in future studies to develop highly effective and safe delivery systems for clinical applications. In the last decade several promising nanocarriers including neutral-lipid based nanoliposomes have shown promise as highly effective and safe delivery systems for systemic use of siRNA therapeutics. To further enhance the antitumor efficacy of these carriers, tumor-targeting versions of these carriers that express high affinity ligands are required for increased tissue accumulation and reduced side effects. Although not novel, a PEGylated version of these nanocarriers may improve the stability, enhance circulation time and result in less frequent injections. In conclusion, siRNA-based therapeutics offer great hope for Phase I–III clinical trials for the treatment of various cancers by targeting signaling pathways and oncogenes that promote cell proliferation, cell cycle progression, invasion/metastasis and resistance mechanisms in tumors. In the next decade we will most likely witness broad applications of this therapeutics not only in cancer but also in other diseases alone and in combination with standard care.

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