WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 15, 149-162. Review Article ISSN 2277– 7105

# siRNA-BASED NANOPARTICLES: A NOVEL APPROACH FOR GENE SILENCING TO ENHANCE CANCER THERAPY

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Article Received on
15 June 2018,

Revised on 05 July 2018,
Accepted on 25 July 2018

DOI: 10.20959/wjpr201815-11778

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## ABSTRACTS

The anticancer activity of siRNA has been identified by researchers; more specifically; RNAi molecules like siRNA can silence oncogene expression that opens a new potential window in cancer treatment. It is considered a new effective theme in cancer science owing to scientists can easily design siRNA molecules to suppress any kind of oncogenic proteins that have the potential to cause cancer. In preclinical and clinical studies, various limitations have arrived on account of their delivery barriers and unfavorable pharmacokinetics in the biological environment like blood nucleases degradation and quick renal clearance. In addition with, siRNA strongly weaken their cellular internalization from hydrophilicity and negative charge of naked siRNA. To overcome these limitations, development of the appropriate delivery method is instantly required. Even as a substitute therapy of traditional chemotherapeutics, siRNA should design and draft with a safe carrier that will enhance their plasma half-life, target-wise delivery as well as their good pharmacokinetic profile in clinical applications. To account these in thought, a carton of nanoparticles (NPs) have formulated

that open a vast floor to substantially innovate the treatment strategy of cancer disease. Due to nanoparticle-based drug delivery, the treatment approaches are more reliable now than before. The purpose of this review is to pursue recent developments in siRNA-based NPs, highlights preclinical and clinical studies and limitations that should be excelled for prospective new efficient therapeutics.

**KEYWORDS:** siRNA, Nanoparticles, Cancer Therapy, Preclinical and Clinical Study, Challenges, and Future Perspective.

## BACKGROUND

To begin with, when Fire and Mello for the first time discovered that double standard RNA have the knack for silencing gene expression in the nematode Caenorhabditis elegans,[1] after that, siRNA is still one of the promising therapeutic molecules in the fastest growing oncology field for silencing of oncogenes which are overexpressed on the cancerous cells. siRNA usually inhibits the oncogenic proteins either by transcriptional or by post-transcriptional gene knockdown that are responsible for abnormal cell proliferation in cancer. Nowadays, various clinical applications proved their effectiveness in the treatment of several types of cancers with minimum toxicity and high cellular level efficacy. The key success of their effectiveness in cancer treatment relies upon their successful delivery and their unique characteristics such as address mutations by blocking mutagenic genes and downregulating cell transduction pathway in cancer physiology. As pure siRNA is not stable in the physiological environment and their inability to cross physiological barriers due to immunogenicity, nanoparticles-based drug delivery system is envisaged as the most fascinating and effectual strategy. In as much as siRNA has the wide therapeutic application of sequence-specific gene silencing rather than another mood of treatment approaches against various disease likely cancer and others.[2] The first RNA-interference based clinical data and human Phase I clinical trial data by systemic administration of targeted siRNA-based NP to a patient with solid tumors was published in 2005 and 2010 respectively whereas biological company started trading the RNAi-based therapeutics widely before that time.[3-5]

Howsoever, to overcome delivery-related problems, two types of vectors have been studied namely viral and non-viral vectors. Between them, the headway of nanoparticles are really surprising to enhance cancer treatment by the complementary base-pairing mechanism in oncology. Up to date, various methods regarding characteristics and compositions of nanoparticle have been proposed for successful delivering of siRNA into the target site. For

example, a double padlocked nanocarrier was designed against liver cancer by first conjugating Akt2-siRNA with gold nanoparticle and then, this nanoconjugate was further coated with biodegradable glycol chitosan to protect the encapsulated siRNA from gastric juices. The surface modification of this nanoconstruct with taurocholic acid facilitated apical sodium based cellular uptake and more accumulation in the liver. As-synthesized conjugation was able to enhance cellular apoptosis in liver cancer by knocking down AKT/P13K signaling.[6]

The approaches of this short review are to address the characteristics of various NPs which are being used as a gene carrier, different delivery methods, how siRNA reaches in the target tissue and what about the fate of delivered siRNA, preclinical and clinical studies, their limitations and finally future perspectives.

**Nanoparticles as a carrier for siRNA**

Even though siRNA has been studied as a popular method due to their promising anticancer properties since the last decades, it seems a burning question today pertaining their safe delivery to target tissue. Because of the limitations of naked siRNA concerning their physicochemical properties, delivery of undressed siRNA has been repealed from the oncology field.[7] However, many delivery systems have been identified over the last decades to protect siRNA from different biological environments. Among them, nanoparticles normally play a vital role in the successful delivery of nucleic acid-like short interference RNA and improve their pharmacokinetic properties for anticancer uses.[8] The main mechanism of nanoparticles is their authority to manage, manipulate and carry siRNA which are very momentous in the nanotherapies of cancer. Their supramolecular structure, nanoscale size and shape, and superficies chemistry are the key factors for keeping them top among a series of synthetic carriers. They can transgress the problems associated with traditional carriers and secure sustain and prolong release, high therapeutic window, stability in both GIT and blood, manageable toxicity, safe and target delivery, and so many. However, there are various types of NPs that are investigated to find out effective and potential nanocarriers. The stuff of nanocarriers applied to siRNA can be folded into the following categories as shown in Table 1 whereas Table 2 illustrates the advances and disadvances of those nanoparticles as a carrier of siRNA in cancer therapy.

**Table 1: Types of nanoparticles as a carrier of siRNA.**

| Type of Nanoparticles | Examples |
|---|---|
| Silica and Silica-based NP | Mesoporous Silica nanoparticles[9] |
| Metal and Metal Oxides NPs | Magnetic Nanoparticles[10] |
| | Gold NPs (AuNPs)[6] |
| Carbon-based materials | Carbon Nanotubes[11] |
| | Graphene[12] |
| | Quantum Dots[13] |
| | Carbon Dots[14] |
| Dendrimers | Polyamidoamine[15] |
| Polymers | Chitosan[16] |
| | Dextran[17] |
| | Polycations[18] |
| | Micelles[19] |
| | Beta-glucan[20] |
| Dextrin-type | Cyclodextrin[21] |
| Lipid-based NPs | Liposomes[22] |
| Others | Hydrogel[23] |
| | Semiconductor Nanocrystal[24] |

**Table 2: Advances and Disadvances of nanoparticles as a carrier of siRNA (Reproduced from ref.[24])**

| Nanoparticles | Merits | Demerits |
|---|---|---|
| Mesoporous Silica NPs | Large surface area | In vivo Toxicity |
| | Stability | |
| | Biocompatibility | |
| | Tuned biodegradability | |
| | Controllable porosity allows multifunctional and sequential delivery | |
| | Surface reactivity, and easy functionalization | |
| Magnetic Nanoparticles | Large surface area | Poor colloidal stability Limited biocompatibility and cytotoxicity Non-biodegradability |
| | Small in size, allows longer circulation and improved tissue penetration | |
| | High magnetization for remotely-controlled and fast delivery | |
| | Controlled clustering | |
| | The potential for multimodal applications (e.g. targeting, diagnostic, and therapy) | |
| Gold NPs (AuNPs) | Large surface area | The high cost of large scale production Stickiness and limited *in vivo* stability Non-biodegradability |
| | Easy synthesis, modification, and bioconjugation | |
| | Rational stability and biocompatibility | |
| | The potential for multimodal applications (e.g. targeting, diagnostic, and therapy) | |
| Carbon Nanotubes | The very precise size and shape controllability | Difficulty in production and handling Non- |
| | Water solubility and biocompatibility | |
| | Elicit negligible immune response | |

| | | |
|---|---|---|
| | Easy electrostatic interaction with nucleic acid and protection from nuclease activity | biodegradability<br>Unresolved toxic properties |
| Graphene | Large surface area | High cost and difficulty of massive production<br>Non-biodegradability<br>Increased biosafety concerns |
| | Facile synthesis | |
| | Colloidal stability | |
| | Easy surface functionalization | |
| | Good electrical and mechanical properties | |
| Dendrimers | The very precise size and shape controllability | Non-specific cytotoxicity<br>Limited release of the associated bio-actives<br>Rapid clearance |
| | Water solubility and biocompatibility | |
| | Elicit the negligible immune response | |
| | Easy electrostatic interaction with nucleic acid and protection from nuclease activity | |
| Polymers | Easy and cheap production | Limited stability<br>Synthetic polymers cause cellular necrosis and apoptosis |
| | The fine tenability of structure and properties | |
| | Simplicity for loading and complexation with nucleic acid by electrostatic interaction. | |
| | It can be tailored for a wide range of molecular weights<br>Natural polymers are nontoxic, biocompatible, and biodegradable. | |
| Cyclodextrin | Low toxicity | High-cost production<br>Concerns regarding their safety and limited solubility |
| | Act as molecular containers that can help to enhance the biological properties of loaded molecules | |
| | Lack of immune stimulation | |
| | In vivo stability due to the absence of enzyme degradation in humans | |
| Liposomes | Biocompatibility | High production cost<br>Limited instability and leakage of loaded materials<br>Low solubility<br>Rapid clearance |
| | Rapid cellular uptake | |
| | The flexibility of synthesis, modification, and formulation | |
| | Targeting and controlled release | |
| | Easy conjugation and functionalization with components such as targeting, contrast agents, probes, and fluorophores | |
| Hydrogels | Tenable synthesis and physicochemical properties | High-cost production<br>Instability |
| | Selective surface-functionalization | |
| | High degree of porosity and high loading capacity | |
| | Controlled and sustained release into the target tissues | |
| | Biocompatibility and biodegradability | |
| Quantum Dots | Size and structure-based tunable emission | Potential toxicity<br>Particle aggregation, degradation, and removal |
| | A high molar extinction coefficient | |
| | High photo and chemical stability | |
| | The potential for synergistic diagnostic and therapeutic applications | |

**Delivery of siRNA-Nanoparticles**

Today, NPs have blazoned various extraordinary features associated with siRNA delivery. For example, NPs can target specific tissue or cells, protect target gene against nuclease degradation, improve DNA stability as well as safety[25]. Due to particles size of nanoparticles (mainly from 10 to 1000 nm), shape tenability, ability to control the surface characteristics and enhanced permeability and retention (EPR) effect, NPs can easily penetrate into tumor cells and trap into them rather than normal cells to give desire therapeutic effects.[26-28] Some NPs are biocompatible, biodegradable and cationic in nature such as chitosan; that can easily conjugate with siRNA and protect naked siRNA from enzymatic degradation, immune recognition and easily cross the cell membrane compared to other carriers as well.[29] siRNA-NPs can be prevented from rapid renal excretion when the nanoparticles size and surface coating will be appropriated.[30] Nanoparticles are commonly used to carry siRNA as insertions in their core or as surface-attached molecules through covalent or non-covalent bonds. Hence, to understand the structure-activity relationships between nanoparticles and the interaction between physiological systems and siRNA-loaded nanocomplex is very crucial to identify, discover and develop an appropriate method for future procreation in siRNA-based cancer treatment. Moreover, a good explanation is required with respect to how nanoparticles bind, encapsulate or load siRNA in successful siRNA delivery to tumor tissues for the desired treatment protocol. Figure 1 below here illustrates a series of NPs to deliver siRNA with various siRNA loading methods.

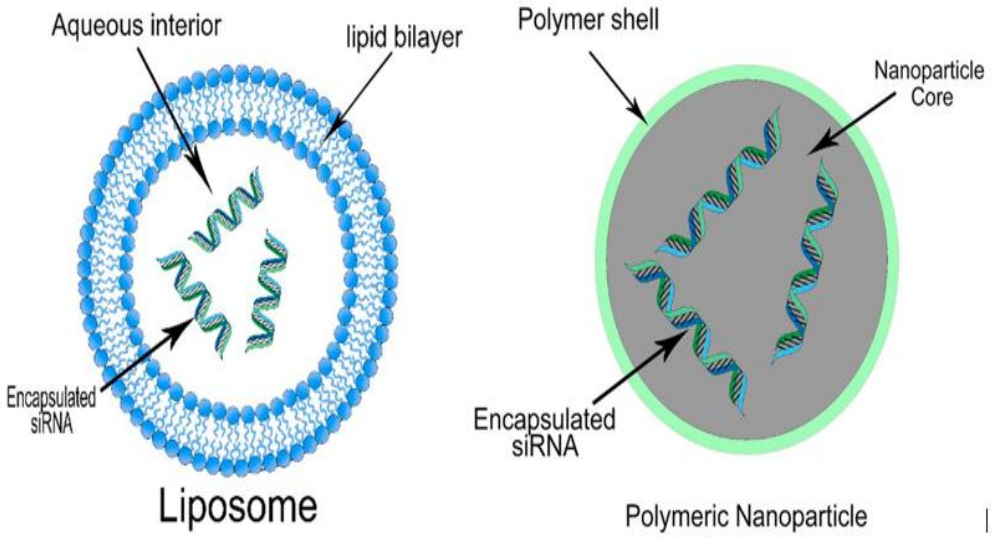


**Figure 1: Representative structures of siRNA-loaded Liposome and Polymeric Nanoparticles.[31]**

(Reproduced from ref,[31] with permission from ACS Publications 2015)

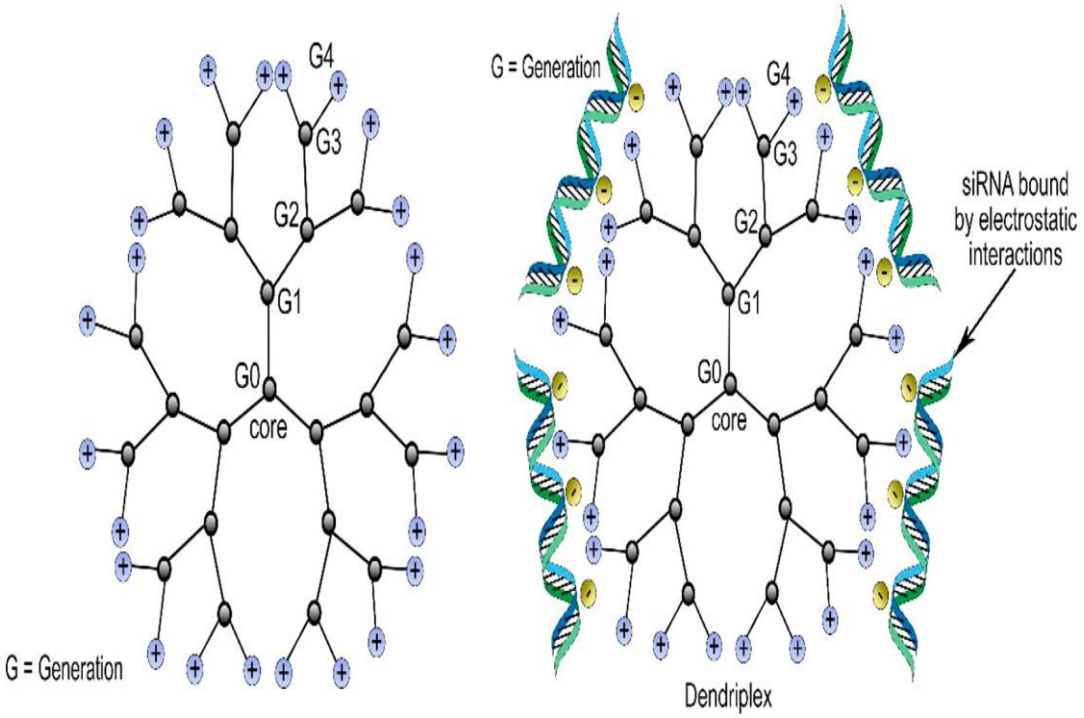


**Figure 2: Representative structures of 4th generation Dendrimer and Dendriplex[31] (from left to right)**

(Reproduced from ref,[31] with permission from ACS Publications 2015)

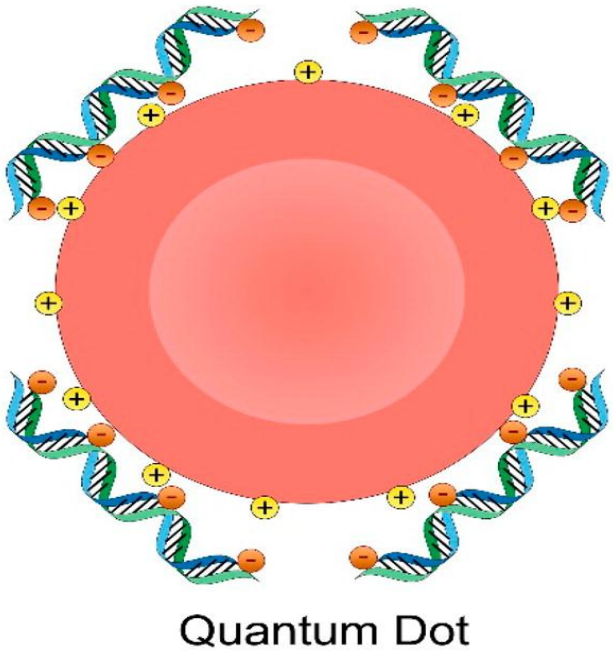


**Figure 3: Representative structures of siRNA-complexed with Quantum Dots.[31]**

(Reproduced from ref,[31] with permission from ACS Publications 2015).

**Achieving Target and Releasing siRNA**

New blood vessels formation (angiogenesis) is very crucial to the newly formed abnormal cancerous cells. New blood vessels provide extra oxygen and nutrients to rapidly growing tumors, researchers have taken this advancement in the hand by discovering anti-angiogenic so that tumors can starve, even though it has its own limitations too. Recently scientists have identified that due to hypervascularization, perverse vascular structure, large windows in tumor vasculature and lack of lymphatic drainage can be exploited for selective drug targeting to solid tumors.[27] siRNA based nanoparticles can easily enter into tumor tissue because of their large fenestrations within endothelial cells whereas they can be trapped in tumor contiguity due to poor lymphatic drainage. This is called enhanced permeability and retention effect (EPR effect).[32] As they cannot penetrate into normal tissue due to the close-fitting endothelial junction, they can easily target only abnormal cells for selective target-wise drug delivery. Once entered into the target tissue, they start to accumulate and then finally release siRNA for silencing target gene to act against cancer. On the other hand, for achieving target delivery, several types of targeting ligands are being attached with siRNA-loaded nanoparticles. In these systems, when nanocomplex enter into the biological environment they find out their targeting receptors on the affected organs and then they accumulate into the target organ resulting in desirable therapeutic goal achieved. An example would be, AMD3100 assisted surface modification nanoparticles were fabricated to target CXCR4 and deliver encapsulated siRNA against liver fibrosis. As a therapeutic agent, siRNA knocked down vascular endothelial growth factor expressions whereas CXCR4 antagonist AMD3100 block the abnormal proliferation of hepatic stellate cells. The dual padlock AMD3100, and therapeutic siRNA inhibited SDF-1a/CXCR4 mediated angiogenesis and the overexpression of vascular endothelial growth factor resulting prohibition of liver fibrosis headway.[33]

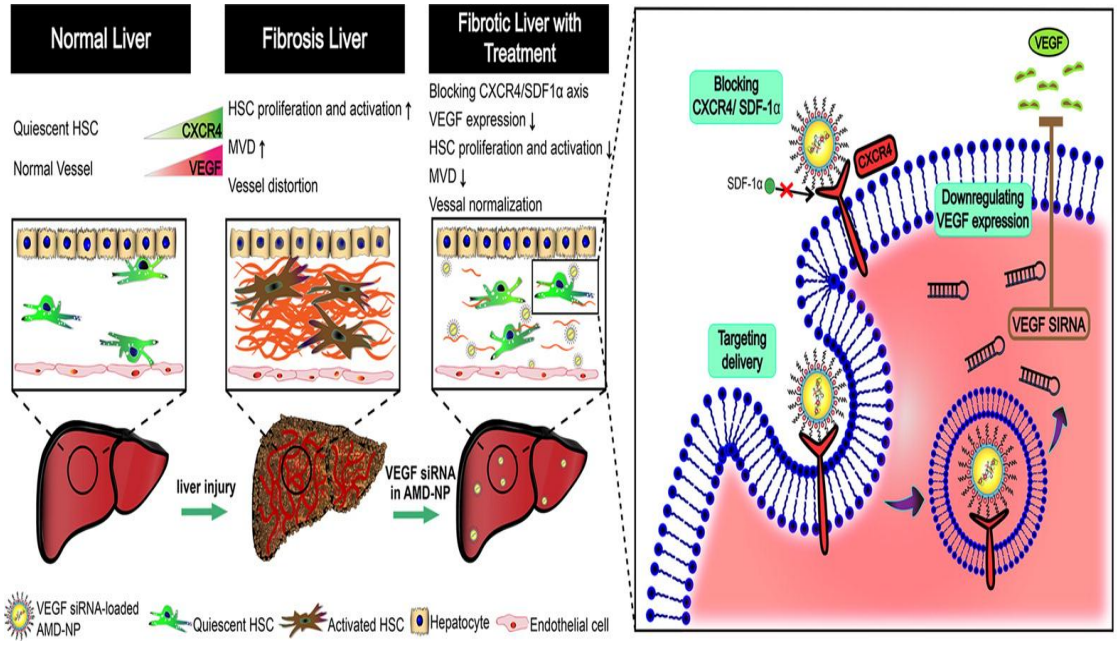


**Figure 4: CXCR4-targeted, siRNA-loaded Nanoparticles against Liver Fibrosis.[33]**

(Reproduced from ref,[33] with permission from ACS Publications 2016)

**Understanding the Fate of Delivered siRNA**

To reveal a biostable, efficient and non-toxic transfection method for siRNA-based NP applications, it is one of the momentous concerns among researchers to study an intracellular destiny of siRNA. Many methods have identified for these purposes. For example, the fluorescence resonance energy transfer (FRET) is one of them that uses up-conversion fluorescent nanoparticles as a source of energy donor to figure out the intracellular fate of siRNA.[32] In addition, FRET-based imaging approach was established to surveillance of siRNA integrity.[34] For instance, a fluorescent dye when conjugated with polymeric NPs can easily target hepatic parenchymal cells with the ability to investigate the mechanisms of organ-selective uptake, distribution, gene silencing activity and clearance using biophotonic approaches.[35] To approach it from another angle, a red fluorescent protein (RFP) was coated by chitosan NPs where the optimum size of the conjugate was 7-23 nm for delivering siRNA into the cell.[36] Alabi et al. studied a Förster resonance energy transfer (FRET)- labeled siRNA probes that could be used to track the formation, stability, and disassembly of NP-siRNA complex in both extracellular and intracellular environments.[37] Overall, such developed tools and assays are not only benefits the transfection potency of siRNA but also to detect, quantify and analyze the fate of intracellular and extracellular siRNA. Furthermore, researchers can conjecture about the fate of their delivering siRNA-based nanoparticles by means of the cellular uptake, distribution, metabolism, and excretion according to their

delivering strategies. For instance, previously described Kang et al., claimed that their fabricated siRNA-NP can absorb from small intestine and uptake by the liver through apical sodium bile acid transporting system due to the attachment of bile acid on the surface of siRNA-NP. To confirm their hypothesis, they labeled as-prepared siRNA-NP with Rhodamine B and measured the amount of siRNA-NP in hepatic circulation via relative fluorescence intensity; an enhancement of liver uptake was performed.[6]

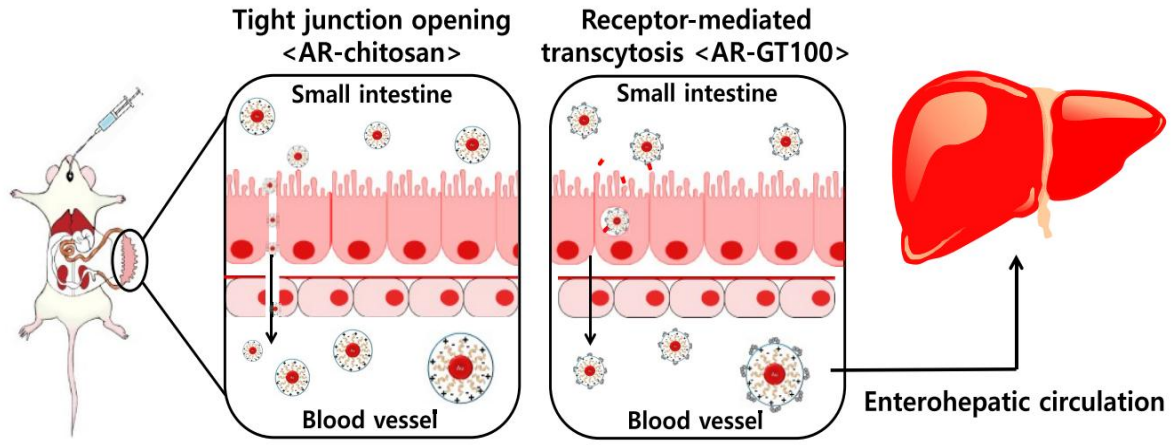


**Figure 5: Oral Absorption, Cellular Uptake and Target-ability of siRNA-loaded Nanoparticles against Liver Metastases.[6]**

(Reproduced from ref,[6] with permission from ACS Publications 2017)

**Preclinical Studies**

siRNA encapsulated by NPs is a novel concept for delivering nucleic acid as therapeutics against targeted cancerous cells or tissue. From the very past to the running century, various preclinical studies associated with cancer have been conducted to find out effective and possible inflictions of siRNA which are shown in the following table 3.

**Table 3: Preclinical data of Nanoparticle-based siRNA delivery.**

| Nanocarriers | Targeted Cell Lines | Tissues | References |
|---|---|---|---|
| PEI-PEG | SGC7901 | Gastric Cancer Cells[38] | [37] |
| Poly-L-lysine nanoshells | H1299 | Lung Cancer Cells[39] | [38] |
| Single-walled Carbon Nanotubes | MCF7 | Breast cancer Cells[40] | [39] |

***In Vitro* Studies**

It is crucial to understand how NPs behave in the biological environment, more specifically, in targeted cells or tissues that mainly depend on different physical properties of NPs and/or various characteristics of the cellular membrane. NP-Cell interaction can cause toxicity. If it

shows an extreme level of toxicity, then the carrier of siRNA should be abdicated [41]. Researchers identified and developed chitosan nanoparticle as low toxic, biodegradable and biocompatible to be used as a siRNA vehicle. Chitosan-siRNA has the ability to silence a gene in both CHO K1 and HEK 293 cells lines where chitosan-tripolyphosphate NP that coated siRNA is comparatively more active than chitosan-siRNA complexes[42]. Even, the hydrophobically modified glycol chitosan (HGC) can be used as optimize and efficient intracellular tumor-targeting drug delivery carrier through their enhanced permeability and retention (EPR) effect. HGC has its own sound landmarks including enhanced distribution in the whole cells, fast cellular uptake, low toxicity, and biocompatible.[43] To reduce toxicity, safe and efficient therapeutic application, it is fateful to comprehend endocytosis, exocytosis and clearance mechanism of NPs from NP-Drug conjugates.[28] NPs usually enter the cell via four types of pathway such as clathrin/caveolar-mediated endocytosis, phagocytosis, macropinocytosis, and pinocytosis whereas NPs exit the cell via three types of pathway such as lysosome secretion, vesicle-related secretion, and non-vesicle-related secretion. In addition, the selection of siRNA-backbone, biodegradable nanoparticles, surface modifications of nanoparticles, systemic delivery and so many issues should be addressed before designing a potential research scheme considering siRNA-based therapy.

### *In Vivo* Studies

Up to date, hundreds of research study has been reported regarding in vivo pharmacological activities of siRNA-based nanoparticles, among them, in vivo anticancer properties of siRNA is ranked at the top in several respects. Howsoever, to appraise *in vivo* gene silencing activity of siRNA, Chen et al., 2010, delivered siRNA via LPH nanoparticle formulation modified with tumor-targeting scFv into B16F10 lung metastasis bearing mice. The result of western blot analysis and immunostaining method concerning an evaluation of gene silencing activity revealed that the GC4-targeted siRNA-based nanoparticles significantly inhibited lung metastasis of murine B16F10 melanoma.[41] Moreover, for evaluation of toxicity associated with NPs, C57BL/6 mice were used to examine the levels of proinflammatory cytokines and hepatotoxicity markers in the serum. In the same study, siRNA encapsulated LPH nanoparticle did not increase IL-6, IL-12, and IFN-γ levels in comparison with control group even aspartate aminotransferase and alanine aminotransferase like hepatotoxicity markers remained the same as untreated mice. In another study, Medarova et al. designed a nanoparticle probe targeting Bcir5 (anti-apoptotic gene) that encodes survivin (MN-NIRF-siSirvivin). In human neoplasm, survivin (a member of IAP family) shows tumor-restricted

expression as a winsome therapeutic target. In the in-vivo model, real-time PCR analysis exhibited a specific gene silencing characteristics of survivin with remarkable progress in tumor-associated level of apoptosis and necrosis.[43] Furthermore, in case of solid tumors, siRNA-based nanoparticle not only knocks down the oncogenic expression but also reduce the tumor size in a dose and time-dependent manner. A recent study suggests that siRNA-loaded nanoparticles can dramatically decrease the size of prostate tumors even very close to fully cure. In details, intravenous injection of 10mg siRNA with a significant nanocarrier can reduce 92% of tumor size in a prostate tumor-bearing mice.[44] siRNA-NPs does not limit their administration through injection routes, nanoparticles prove their potency to carry and deliver siRNA via the oral route. More promisingly, AuNP-siRNA/glycol-chitosan-taurocholic acid nanoparticle for specific delivering of Akt2 siRNA against colorectal liver metastasis was successfully developed for oral administration in which biofunctional glycol chitosan-taurocholic acid conjugates enclosed AuNP-siRNA to protect Akt2 siRNA from GI degradation. Hence, Active transportation has been taking place via enterocytes that increased selective accumulation in cancerous cells. After oral administration (animal model), low production of Akt2 had been appeared by means of initiation of apoptosis in cancerous cells.[6]

**Clinical studies**

The successful *in vivo* result of siRNA-NPs enables them to enter into various clinical phase trials. Here in table 4, we described an overview of various siRNA based nanoparticle systems which are already introduced in clinical trials against various types of cancer.

**Table 4: Clinical Trials data of siRNA-Nanoparticles against several types of cancer treatment.[44,45,49]**

| Drug | Target | Disease | Clinical Trials |
|---|---|---|---|
| CALAA-01 | RRM2 | Solid Tumors | I |
| Atu027 | PKN3 | AST, | I |
| | | PDC | Ib/IIa |
| ALN-VSP02 | KSP and VEGF | AST with liver involvement | I |
| ALN-VSP03 | KSP and VEGF | Solid Tumors | I |
| TKM 080301 | Polo Kinase 1 | STLI, | I |
| | | Neuroendocrine Tumor, | I/II |
| | | Adrenocortical- Carcinoma | I/II |
| siRNA-EphA2-DOPC | EphA2 | Solid Tumor | I |
| siG12D LODER | KRASG12D | PDAC | I |

RRM2: M2 subunit of ribonucleotide reductase, KSP: kinesin spindle protein, VEGF: vascular endothelial growth factor AST: advanced solid tumors, PDC: pancreatic ductal carcinoma, PDAC: pancreatic ductal adenocarcinoma, STLI: solid tumor with liver involvements, KRASG12D: K-ras G12D mutant, PKN3: Protein Kinase N3

**Limitations Associated with siRNA-NPs**

Although it has been reported that plasmatic enzyme degradation problem of undressed siRNA is solved by the encapsulation of an inert nanoparticle,[8] there are still some challenges in nano-based siRNA delivery system. It is also a burning question today to exhibit immune, a toxic free, biodegradable and biocompatible siRNA-NPs delivery system for gene silencing to enhance cancer therapy. For example, the siRNA-loaded nanocarriers have to pass not only the biological barriers in the mucosa but also the cellular and humoral feature of the immune system to accumulate in the target site. At this time, anionic blood serum proteins activate these particles hence, they are responsible for opsonization by immune cells where reticuloendothelial system actuates monocytes and macrophages to trap and degrade intravenously injected particles (>100 nm in diameter). In contrast, nanostructures, which are <4 nm in diameter, mainly responsible for rapid renal clearance from the human body within a few hours of administration. Thus, the proposed optimal size of nanoparticles should be 5-100 nm in diameter. Tanaka et al. (2010) claimed that NPs are closely related to systematic toxicity, especially in the liver[46]. Besides, variations in the specificity of siRNA, uncontrolled off-target effects, poor patient compliance, repetitive dosing, unwanted side effects, dose-dependent toxicity, severe toxicity to non-targeted tissues and resistance to nuclease degradation would be considered before designing siRNA based anticancer study. However, the heterogeneity of tumors confirms unavoidable resistance to some RNAi, owing to factors such as ethnicity, somatic mutations, and germline single nucleotide polymorphisms.[47]

**Experts Opinion**

Due to the advancement of siRNA technology from mere academic discovery to a potential class of treatment modality against human diseases, it is now easier today to treat critical diseases more specifically like cancer. Even though it is a new technique, the conjugates of siRNA-NPs have extended its hand to many other complex diseases as a therapeutic and becoming more popular day by day because of these conjugates are economic, fast, convenient as well as efficient. To be more specific we would like to cite anticancer activity

of nano-based siRNA delivery, they have the ability to cross biological barriers, a target-wise accumulation that can differentiate cancerous cell from the normal one and so many. However, this novel approach has proved its significant effects in preclinical studies by means of in vitro and in vivo studies, now some of them are gone into clinical trials as described before. Despite all advantages, it has been claimed that NP carriers have their own limitation too, related to immunogenicity and toxicity. In addition, the total mechanism of RNAi (siRNA) is not completely clear even it is tenacious to figure out siRNA regulatory network rather than a handful isolated target[48]. Moreover, the researchers should be more watchful about patient compliance concerning repeated dosing for cancer therapy. To recapitulate, as it is a novel approach for gene silencing in cancer therapy, nano-based siRNA can be the pioneer of new treatment protocols for cancer as it is expected.

**Authors Contribution:** SMSS and MN designed this study. SMSS and LAS wrote the manuscript draft which was read and edited by MN. All authors read and approved the final version of the manuscript.

**FUNDING:** "This research received no external funding.

**CONFLICT OF INTEREST**

The authors report that they have no conflicts of interest. The authors alone are responsible for the content and writing of this article.

**REFERENCES**

1. Fire, A. *et al.* Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature*, 1998; 391-806.
2. Tokatlian, T. & Segura, T. siRNA applications in nanomedicine. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol*, 2010; 2: 305–315.
3. Whelan, J. First clinical data on RNAi. *Drug Discov. Today*, 2005; 10: 1014–1015.
4. Castanotto, D. & Rossi, J. J. The promises and pitfalls of RNA-interference-based therapeutics. *Nature*, 2009; 457: 426–433.
5. Davis, M. E. *et al.* Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature*, 2010; 464-1067.
6. Kang, S. H. *et al.* Oral siRNA Delivery to Treat Colorectal Liver Metastases. *ACS Nano*, 2017; 11: 10417–10429.

7. Chen, S. H. & Zhaori, G. Potential clinical applications of siRNA technique: Benefits and limitations. *Eur. J. Clin. Invest,* 2011; 41: 221–232.
8. Resnier, P., Montier, T., Mathieu, V., Benoit, J.-P. & Passirani, C. A review of the current status of siRNA nanomedicines in the treatment of cancer. *Biomaterials,* 2013; 34: 6429–6443.
9. Hom, C. *et al.* Mesoporous silica nanoparticles facilitate delivery of siRNA to shutdown signaling pathways in mammalian cells. *Small,* 2010; 6: 1185–1190.
10. Mykhaylyk, O. *et al.* Magnetic nanoparticle formulations for DNA and siRNA delivery. *J. Magn. Magn. Mater,* 2007; 311: 275–281.
11. Varkouhi, A. K. *et al.* SiRNA delivery with functionalized carbon nanotubes. *Int. J. Pharm,* 2011; 416: 419–425.
12. Ren, L., Zhang, Y., Cui, C., Bi, Y. & Ge, X. Functionalized graphene oxide for anti-VEGF siRNA delivery: preparation, characterization and evaluation in vitro and in vivo. *RSC Adv.,* 2017; 7: 20553–20566.
13. Lin, G. *et al.* Quantum Dots-siRNA Nanoplexes for Gene Silencing in Central Nervous System Tumor Cells. *Front. Pharmacol,* 2017; 8: 182.
14. Wang, Q. *et al.* Fluorescent carbon dots as an efficient siRNA nanocarrier for its interference therapy in gastric cancer cells. *J. Nanobiotechnology,* 2014; 12: 58.
15. Hong, C. A., Son, H. Y. & Nam, Y. S. Layer-by-layer siRNA/poly(L-lysine) Multilayers on Polydopamine-coated Surface for Efficient Cell Adhesion and Gene Silencing. *Sci. Rep.,* 2018; 8; 7738.
16. Chen, M. *et al.* Chitosan/siRNA Nanoparticles Encapsulated in PLGA Nanofibers for siRNA Delivery. *ACS Nano,* 2012; 6: 4835–4844.
17. Krishnamachary, B., Penet, M. F. & Bhujwalla, Z. M. Acid-degradable dextran as an image guided siRNA carrier for COX-2 downregulation. *Theranostics,* 2018; 8: 1–12.
18. Zuckerman, J. E., Choi, C. H. J., Han, H. & Davis, M. E. Polycation-siRNA nanoparticles can disassemble at the kidney glomerular basement membrane. *Proc. Natl. Acad. Sci. U. S. A.,* 2012; 109: 3137–3142.
19. Christie, R. J. *et al.* Targeted Polymeric Micelles for siRNA Treatment of Experimental Cancer by Intravenous Injection. *ACS Nano,* 2012; 6: 5174–5189.
20. Cohen, J. L. *et al.* Peptide- and Amine-Modified Glucan Particles for the Delivery of Therapeutic siRNA. *Mol. Pharm,* 2016; 13: 964–978.
21. Chaturvedi, K. *et al.* Cyclodextrin-based siRNA delivery nanocarriers: a state-of-the-art review. *Expert Opin. Drug Deliv,* 2011; 8: 1455–1468.

22. Xia, Y., Tian, J. & Chen, X. Effect of surface properties on liposomal siRNA delivery. *Biomaterials*, 2016; 79: 56–68.
23. Sarett, S. M., Nelson, C. E. & Duvall, C. L. Technologies for controlled, local delivery of siRNA. *J. Control. Release*, 2015; 218: 94–113.
24. Draz, M. S. *et al.* Nanoparticle-mediated systemic delivery of siRNA for treatment of cancers and viral infections. *Theranostics*, 2014; 4: 872–892.
25. Dizaj, S. M., Jafari, S. & Khosroushahi, A. Y. A sight on the current nanoparticle-based gene delivery vectors. *Nanoscale Res. Lett.*, 2014; 9: 252.
26. Drummond, D. C., Meyer, O., Hong, K., Kirpotin, D. B. & Papahadjopoulos, D. Optimizing Liposomes for Delivery of Chemotherapeutic Agents to Solid Tumors. *Pharmacol. Rev.*, 1999; 51: 691 LP-744.
27. Greish, K. Enhanced permeability and retention of macromolecular drugs in solid tumors: A royal gate for targeted anticancer nanomedicines. *J. Drug Target*, 2007; 15: 457–464.
28. Babu, A. *et al.* Nanoparticles for siRNA-based gene silencing in tumor therapy. *IEEE Trans. Nanobioscience*, 2016; 15: 849–863.
29. Ragelle, H., Vandermeulen, G. & Préat, V. Chitosan-based siRNA delivery systems. *J. Control. Release*, 2013; 172: 207–218.
30. Haussecker, D. The Business of RNAi Therapeutics in 2012. *Mol. Ther. Nucleic Acids*, 2012; 1: e8–e8.
31. Adesina, S. K. & Akala, E. O. Nanotechnology Approaches for the Delivery of Exogenous siRNA for HIV Therapy. *Mol. Pharm.*, 2015; 12: 4175–4187.
32. Jiang, S. & Zhang, Y. Upconversion nanoparticle-based FRET system for study of siRNA in live cells. *Langmuir*, 2010; 26: 6689–6694.
33. Liu, C. H. *et al.* Dual-functional nanoparticles targeting CXCR4 and delivering antiangiogenic siRNA ameliorate liver fibrosis. *Mol. Pharm*, 2016; 13: 2253–2262.
34. Järve, A. *et al.* Surveillance of siRNA integrity by FRET imaging. *Nucleic Acids Res.*, 2007; 35: e124–e124.
35. Press, A. T. *et al.* Cell type-specific delivery of short interfering RNAs by dye-functionalised theranostic nanoparticles. *Nat. Commun*, 2014; 5: 5565.
36. Liu, Y. *et al.* Chitosan-coated red fluorescent protein nanoparticle as a potential dual-functional siRNA carrier. *Nanomedicine*, 2015; 10: 2005–2016.
37. Alabi, C. A. *et al.* FRET-labeled siRNA probes for tracking assembly and disassembly of siRNA nanocomplexes. *ACS Nano*, 2012; 6: 6133–6141.

38. Mohammadi, M. *et al.* Single-walled carbon nanotubes functionalized with aptamer and piperazine-polyethylenimine derivative for targeted siRNA delivery into breast cancer cells. *Int. J. Pharm.*, 2015; 485: 50–60.
39. Adjei, I. M., Sharma, B. & Labhasetwar, V. Nanoparticles: Cellular Uptake and Cytotoxicity BT - Nanomaterial: Impacts on Cell Biology and Medicine. in (eds. Capco, D. G. & Chen, Y.) 73–91 (Springer Netherlands). doi:10.1007/978-94-017-8739-0_5, 2014.
40. Nam, H. Y. *et al.* Cellular uptake mechanism and intracellular fate of hydrophobically modified glycol chitosan nanoparticles. *J. Control. Release*, 2009; 135: 259–267.
41. Chen, Y., Zhu, X., Zhang, X., Liu, B. & Huang, L. Nanoparticles Modified With Tumor-targeting scFv Deliver siRNA and miRNA for Cancer Therapy. *Mol. Ther.*, 2010; 18: 1650–1656.
42. Park, N. O. J.-H. Endocytosis and exocytosis of nanoparticles in mammalian cells. *Int. J. Nanomedicine*, 2014; 9: 51–63.
43. Medarova, Z., Pham, W., Farrar, C., Petkova, V. & Moore, A. In vivo imaging of siRNA delivery and silencing in tumors. *Nat. Med.*, 2007; 13: 372.
44. Young, S. W. S., Stenzel, M. & Jia-Lin, Y. Nanoparticle-siRNA: A potential cancer therapy? *Crit. Rev. Oncol. Hematol*, 2016; 98: 159–169.
45. Ozcan, G., Ozpolat, B., Coleman, R. L., Sood, A. K. & Lopez-Berestein, G. Preclinical and clinical development of siRNA-based therapeutics. *Adv. Drug Deliv. Rev.*, 2015; 87: 108–119.
46. Tanaka, T. *et al.* Sustained Small Interfering RNA Delivery by Mesoporous Silicon Particles. *Cancer Res.*, 2010; 70: 3687 LP-3696.
47. Pecot, C. V, Calin, G. A., Coleman, R. L., Lopez-Berestein, G. & Sood, A. K. RNA interference in the clinic: challenges and future directions. *Nat. Rev. Cancer*, 2010; 11: 59.
48. Xin, Y. *et al.* Nano-based delivery of RNAi in cancer therapy. *Mol. Cancer*, 2017; 16: 134.
49. U.S. National Library of Medicine, *ClinicalTrials.gov* Direct Link: *https://clinicaltrials.gov/show/NCT00004451*.