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siRNA nanoparticles: the future of RNAi therapeutics for oncology?

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siRNA clinical trials: state of the art

With the emergence of siRNA-based nanomedicine as a powerful therapeutic strategy for cancers, seven of these therapeutics have now advanced to clinical trials. Carriers of siRNA therapeutics include transferrin-modified cyclodextrin (CALAA-01, Arrowhead Research Corporation, CA, USA), *in vivo* jetPEI® (SNS01-T; Sevion Technologies, NJ, USA), cationic liposomes (ALN-VSP02, Alnylam Pharmaceuticals, MA, USA; TKM080301, Tekmira Pharmaceuticals, Burnaby, Canada; Atu027, Silence Therapeutics, London, UK; and DCR-MYC, Dicerna Pharmaceuticals, Watertown, MA, USA) and neutral liposomes (siRNA-EphA2-1,2-dioleoyl-*sn*-glycero-3-phosphocholine [DOPC], MD Anderson Cancer Center, TX, USA). Among those in clinical trials, the cationic lipoplex targeting *MYC1a* (DCR-MYC) is the only nanoplex that requires the Dicer enzyme in order to obtain functional siRNA.

In 2008, the transferrin-modified cyclodextrin polyplex (CALAA-01) was the first siRNA nanoparticle to be approved for cancer clinical trials. In a preclinical mouse study, a single dose of the cyclodextrin nanoplex targeting luciferase mRNA reduced its activity by 80% [1]. More importantly, melanoma patients receiving CALAA-01 in a Phase I clinical trial

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showed a 50–80% reduction in the RRM2 mRNA compared with mRNA from untreated tumor tissues [2]. Although this study showed promise, the development of CALAA-01 has been delayed and no results have been reported.

SNS01-T is a polyethyleneimine (PEI)-based (*in vivo* JetPEI) nanoparticle containing siRNAs targeting *EIF5A* and decoy plasmids expressing nonhypusuable eIF5A mutants for treatment of multiple myeloma and non-Hodgkin's B-cell lymphoma [3]. Francis and colleagues have shown that SNS01-T enhances the antitumor efficacy of lenalidomide and bortezomib in a xenograft model [3]. An open-label, Phase I/II dose-escalation study that includes 15 patients is currently underway. Sevion technologies, the manufacturer of SNS01-T, has reported no dose-limiting toxicities in four patients at a dose of 0.2 mg/kg of the nanoparticle.

Cationic lipid nanoparticles (LNPs) have effectively downregulated oncogenes in malignant hepatomas (*CDH16*, *PLK1*, *VEGF* and *MYC1a*) and prostate tumors (*PKN3*) [4]. While LNPs have a circulation half-life that is longer than 18 h [5], most lipoplexes are ultimately internalized by the liver and spleen. Although the accumulation of lipoplexes may lead to tissue toxicity in these organs at higher dosages, they appear to be well-tolerated by humans at defined dosages. In Phase I clinical trials, the maximum tolerated doses were 0.336, 0.75 and 1.25 mg/kg for Atu027, TKM080301 and ALN-VSP02, respectively. As a result, LNPs targeting *PKN3* (Atu027) and *PLK1* (TKM080301) have been advanced to Phase II trials.

Neutral DOPC liposomes have been used to target ovarian cancers (*EPHA2*, *PTK2*, and *IL8*) and melanoma (thrombin receptor) [6]. While siRNA–EphA2–DOPC inhibited tumor growth by approximately 60%, its antitumor efficacy is synergistic when used in combination with paclitaxel [7]. Importantly, the 150-μg/kg dosage of the DOPC lipoplexes used in the preclinical study is ten-times lower than those of LNPs in treating solid tumors. A low-dose treatment that maintains activity is likely to enhance the translatability of DOPC into clinical trials. This clinical trial has not yet been opened for participant recruitment.

Building a smarter RNAi

Exciting breakthroughs in RNAi designs hold significant promise in advancing the field. With the packaging RNA (pRNA) derived from the Φ29 bacteriophage, Guo's group has made thermodynamically stable RNA nanoparticles [8]. These polygonal (e.g., hexamers) or branched RNA nanoparticles are fabricated by complementary base pairings from different motifs of pRNA structures. siRNAs or miRNAs can be fused onto the pRNA, and the terminal ends of the RNA oligonucleotides can be chemically conjugated with ligands (aptamers, folates or RGD peptides) or chemotherapeutics [8]. The gene-silencing efficacy of RNA nanoparticles has not yet been studied *in vivo*, but specific accumulation in tumors and enhanced pharmacokinetics have been achieved in mouse models.

Rolling-cycle transcription has been utilized in order to synthesize long RNA hairpins that self-assemble into high-molecular-weight sponge-like microspheres [9]. A microsphere contains approximately 500,000 copies of hairpin RNA, which require enzymatic cleavage by the Dicer enzyme for their incorporation into RISC. Although delivery of the 2-μm-sized microsphere is difficult *in vivo*, PEI has been used to condense the RNA microspheres to

homogeneous nanoplexes of 200 nm. Intratumoral injection of these PEI- encapsulated nanoplexes results in dose-dependent luciferase silencing *in vivo*. RNA nanoparticles and RNA microspheres have the advantage of being nanoscale sizes and having predefined structures and low immunogenicity, thereby showing considerable promise as valuable therapeutic modalities.

Shot on goal: targeted nanoparticles

In order to target the cancer cells preferentially, siRNA nanoparticles have been formulated with ligands that are specific to the receptors expressed by the tumors and/or angiogenic vessels [10]. For example, aptamers that bind to specific molecular targets have been covalently conjugated to siRNAs, noncovalently linked to siRNAs through a linker or conjugated with polycations in order to form nanoplexes with siRNA [11]. RNA aptamers against HER2, BAFF, transferrin receptor, and PSMA have been identified in order to enhance the selectivity of aptamer-guided nanoparticles in the treatment of cancers. Alternatively, Gilboa and colleagues have adapted a chimeric RNA molecule that combines an siRNA inhibiting *MTOR* and an aptamer recognizing the 4-1BB receptors expressed by T lymphocytes [12]. The 4-1BB aptamer–siRNA chimera mediated the inhibition of *MTOR* in CD8⁺ T cells, leading to enhanced memory responses and vaccine-induced antitumor immunity in tumor-bearing mice. Notably, the effective dose of the targeted siRNA that resulted in protective immunity was ten- to 50-fold lower than that of the nontargeted siRNA-delivery systems.

In addition to aptamer-targeted siRNA conjugates, Lieberman's group has developed a fusion protein (F5-P) containing protamine and single-chain fragmented antibodies against HER2 [13]. Systemically administered F5-P–siRNA nanoplexes targeting *PLK1* effectively reduced tumor growth and prevented metastasis in HER2⁺, but not HER2[−] breast cancer models. Notably, in both immunodeficient and immunocompetent mice, there was no noticeable cytokine induction or cytotoxicity at a dose of 2 mg/kg of siRNA. These studies undoubtedly provide clinically relevant evidence to support the effectiveness of targeted nanoparticles.

1 + 1 > 2: siRNA/chemotherapy combinations

Several studies have shown that efficient silencing of a single gene can only partially inhibit tumor progression [7,10]. Indeed, cross-talk between oncogenic pathways or compensatory activation of signal transduction in malignant cell lines treated with chemotherapy are commonly observed [14]. As a result, siRNA nanoplexes have been administered sequentially with conventional chemotherapeutics in order to block multiple signaling networks simultaneously [3,7]. For example, Kiwada and coworkers demonstrated that siRNA-directed silencing of antiapoptotic *BCL2* sensitizes colorectal cancer against a prodrug of 5-fluorouracil [15].

Cancer cells that survive and proliferate after a first course of chemotherapy may develop resistance to anticancer drugs. The identification and characterization of molecular targets associated with drug-resistant pathways are essential for the development of combinatorial

therapies. Several promising targets associated with the drug-resistant pathways, such as *ABCC1* and *BCL2*, have been exploited with RNAi [16].

In order to incorporate both chemotherapeutic agents and siRNA into a nanoparticle, the carrier often comprises hydrophobic and cationic components. Liposomes have been used to encapsulate doxorubicin in order to increase tumor penetration and to prolong the clinical therapeutic effect. Doxorubicin or paclitaxel encapsulated into liposomes can form nanoplexes with siRNA by the addition of polyelectrolytes [16]. For example, Hammond's group employed layer-by-layer technology in order to deposit poly-L-arginine and siRNA-targeting *ABCC1* on liposomes containing doxorubicin [17]. Inhibition of *ABCC1* restored the chemosensitivity of drug-resistant MDA-MB-468 xenografts against doxorubicin, leading to 90% tumor regression by this combination therapy compared with the saline control group.

For polymer-based delivery systems, several groups have utilized poly(ϵ -caprolactone), β -cyclodextrin, poly(lactic-co-glycolic acid) (PLGA) or mesoporous silica [16] in order to incorporate hydrophobic drugs. While these polymers are modified with polycations (e.g., PEI, poly-L-lysine, or poly[2-aminoethylethylene phosphate]) in order to bind siRNA, PEG is frequently formulated as the hydrophilic block in order to aid the formation of a micellar structure. Farokhzad and colleagues combined PEG-PLGA and cationic lipidoids in order to form nanoplexes with cisplatin and siRNA-targeting DNA repair pathways. After 12 intratumoral injections, regression of tumors, with all mice surviving for at least 50 days, was observed in a prostate xenograft model [18]. By contrast, no mice survived beyond 30 days in the saline- or cisplatin-treated control groups. Notably, the codelivery strategy in the above studies was significantly more effective than the sequential treatment of siRNA nanoplexes and anticancer drugs in the suppression of tumor growth [17,18].

Pearls in the sand

The selection of the carriers for *in vivo* use is usually based on the screening of silencing activity *in vitro*. Nevertheless, our and other groups have observed that this approach often fails to identify the most effective carriers [19,20]. In order to improve translatability, correlations among the physicochemical properties and the efficacy of siRNA complexes have emerged as promising strategies to replace trial-and-error approaches with a rationally based protocol. For example, binding interactions between carriers and siRNAs have been studied quantitatively with dye exclusion assays, gel electrophoresis, isothermal titration calorimetry and nuclear magnetic resonance spectroscopy [21]. While the electrostatic interaction has been shown to play an important role in initiating nanoplex self-assembly, Chou and colleagues have established that hydrogen bonding between histidine-containing peptides and siRNA enhances the stability and biological activity of nanoplexes in serum [21]. Although further binding studies will be required in order to correlate them with silencing activity *in vivo*, it is anticipated that the identification of key biophysical features will aid in the development of more effective carriers.

Characteristics of binding interactions should not be studied alone, but rather paired with the distribution of nanoplexes and the profile of payload release in the target tissues. Anderson's

group has described a means of determining the disassembly of siRNA nanoplexes with fluorescence resonance energy transfer (FRET)-labeled siRNA [22]. Although this method is currently limited to *in vitro* studies, it has shown that a therapeutic window exists between balanced stability and the disassembly of the nanoplex. Kataoka's group has utilized intravital real-time confocal laser scanning microscopy in order to investigate the spatial and temporal behavior of nanoplexes in the tumor xenograft [23]. This approach, in combination with FRET-labeled siRNA with appropriate filters, may be used to monitor the release of siRNA in living animals in order to facilitate the future development of nanocarriers.

Conclusion

With its high payload and its specific silencing activity, siRNA nanomedicine is particularly suitable for personalized medicine. Over the past decade, the development of nanocarriers has been focused on increasing their stability and tumor targeting and reducing their cytotoxicity. Nevertheless, the evaluation of carriers remains time consuming and cost ineffective. In this article, we provided an update of the current progress of siRNA nanomedicine in clinical trials and discussed several versatile nanoplexes in preclinical studies. We also emphasized the importance of establishing systematic correlations between carrier–siRNA complexation, unpackaging and efficacy of gene inhibition. In addition to improved specificity and penetration within the tumor, the further development of siRNA therapeutics will depend on a greater understanding of the molecular gene targets and synergistic antitumor interactions between chemotherapy and siRNA.

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Biographies

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