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LECTURE

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RNAi technique, how far is it from pediatrics?

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Abstract

The new technology of ribonucleic acid interference (RNAi) or small/short interfering RNA (siRNA) can be used to reduce expression of genes in a sequence specific manner, and thereby can treat various diseases caused by expression or overexpression of genes. Phase 1 and phase 2 clinical studies on application of this technology to treat diseases have demonstrated efficacy and safety of this approach in a few specialties/subspecialties. However, no clinical trials have been reported in the fields of pediatrics. This article aimed to describe very briefly what the RNAi technique is, examples of demonstration of the efficacy and safety of RNAi techniques in a few different fields of clinical medicine, and to encourage pediatricians and pediatric researchers to actively participate in studies on this new therapeutic approach for treatment of various pediatric diseases.

KEYWORDS
clinical application, gene silencing, lipid lowering therapy, RNA interference (RNAi), small interfering RNA (siRNA)

1 | INTRODUCTION

Clinical medicine has already stepped into the era of applying the new technology of ribonucleic acid interference (RNAi) or small/short-interfering RNA (siRNA) for the treatment of human diseases.[1-4] The RNAi-based therapeutic approaches can effectively and safely treat very common diseases, such as hyperlipidemia, cancers, macular degeneration, and some very rarely seen diseases, such as pachyonychia congenital (a disabling plantar keratoderma) and transthyretin amyloidosis. The aim of this lecture is to briefly review the encouraging advances in the applications of this new technology in the adult medical disciplines including internal medicine, oncology, dermatology, and ophthalmology. With the awareness of this technology, pediatric researchers are likely to study this technology, promote and accelerate studies on the application of RNAi technology for the treatment and prevention of pediatric diseases.

2 | WHAT IS RNA INTERFERENCE TECHNIQUE?

The RNA interference (RNAi) derived from observation that small or short double stranded RNA (siRNA) molecules, 19–22 base pairs in length, can interfere with the process of gene expression by degrading the mRNA of a gene in a sequence-specific manner.[5-7] The consequence of the interference is apparently reducing the expression of a particular gene, which is also described as down-regulation of gene expression. If the gene expression is reduced by greater than 70%, the term "gene silencing" is applied. Therefore, RNAi has gene-silencing or gene inhibitory effect, and such effect has enormous therapeutic potential.[5-7] The RNAi-based therapeutic approaches have the advantages of specificity, potency, and versatility.[5]

To silence a gene, an siRNA of specific sequence which is complementary to the sequence of a certain segment of the target mRNA should be designed and synthesized. In most cases, the synthesized siRNA (naked siRNA) is packaged/encapsulated physically (eg, in liposomes or lipid nanoparticles) or conjugated or complexed chemically in a certain way so that the siRNA can be delivered to the sites or cells where they can act on the target mRNA without being degraded by ribonucleases (RNases). The naked siRNA can be directly injected into the tissue topically if this is needed and appropriate.[8]

A wide variety of diseases and conditions have been studied in vitro and in vivo for the possibilities of treating by the application of the RNAi technology. Such diseases/syndromes include cancers,

hyperlipidemia, viral infections, sepsis, and many others. Single gene diseases and diseases caused by mutated single nucleotide polymorphisms[9] and those caused by mutated or normal but overexpressed genes should be the prioritized candidate diseases for which the RNAi-based new therapeutics should be considered for development. Huntington disease[10] and hemophilia[4] may be among the best examples of such diseases.

Single gene diseases are caused by abnormalities/mutation of a single gene. Reducing or abolishing the expression of the abnormal genes may be possible to have certain therapeutic effects, that is, thus reducing the severity of the disease, improving or curing the patients' condition. Such approaches might prevent the disease from its onset if the expression of the gene is terminated before the disease develops. For such single gene diseases, an extremely important therapeutic approach is to down-regulate the expression of the pathogenic genes.

Another important context where the approaches of down-regulation or knocking down of gene expression are needed is that in certain diseases or conditions overexpression of some genes is central to the pathophysiology. A typical example of such conditions are factors such as vascular endothelial growth factors (VEGF), epidermal growth factor receptor (EGFR) gene. In many malignant tumors, inhibition of expression of genes of such factors has become extremely important parts of anticancer treatment.[11,12] The RNAi technique can reduce the expression of these factors sequentially. Therefore, down-regulation of the expression of such factors applying the RNAi technique will become a very important part of the anticancer treatment.

Infectious diseases caused by a variety of infectious agents including bacteria, fungi, mycoplasma, chlamydiae, rickettsia, or parasites can be treated by a reduction in expression of the pathogens' vital genes. Such strategies may lead to termination or abolition of the infections caused by any of these agents. Of course, one infectious agent may have more than one vital gene. Therefore, designing of the siRNA targeted at more than one vital gene may provide very strong inhibitions of proliferation of these infectious agents. Among the infectious diseases, more than 10 viruses have been targeted for developing RNAi-based therapeutic approaches[5] although clinical studies have been performed for a limited number of viruses.

It is very clear that the mechanism of RNAi or siRNA technique can be mediated by altering expression of abnormal endogenous genes or genes of infectious agents that have entered (invaded) into human body. However, for diseases caused by the absence of genes that normally present in human body, this approach is not useful. However, siRNAs or RNAi technique has been proven to be a very good therapeutic approach to diseases that needs silencing of certain genes, preferably the single gene diseases and diseases caused by a few genes expression or overexpression.

Six years ago, there were only 3 published reports on clinical application of siRNA technique,[13] and in one of them, the subjects were actually healthy volunteers. But now, much more clinical trials are underway but no phase III trials have been published.

3 | EXAMPLES OF SUCCESSFUL CLINICAL APPLICATION OF RNA INTERFERENCE TECHNIQUE

3.1 | Example 1, lipid-lowering therapy

Treating diseases with RNAi or siRNA technique was a dream more than a decade ago, but recent reports are very encouraging. This sequence-specific gene-silencing technique has unique advantages as compared with some other therapeutic approaches (some of which were also recently developed and some others have been well recognized through decades of clinical application).

One of the most striking success is in the treatment of adult patients with hyperlipidemia who are at high risk of developing cardiovascular events.[1,2] Statins and fibrates have been the mainstay of lipid-lowering treatments during the past decades. However, some cases are unresponsive to the present therapies. Also, statins can have severe side effects, such as myalgia and rhabdomyolysis, elevated glycated hemoglobin A1c, and even hepatotoxicity at larger doses.[14-16] Also, the medications require frequent administration of the drug that may reduce the compliance with the therapy. There was a high proportion of patients' discontinuation of the therapy within the first year of treatment.[17] Thus, opportunity exists for researchers to develop better and more ideal therapeutic approaches to the treatment for hyperlipidemia.

Discovery of the important role of proprotein convertase subtilisin/kexin type 9 (PCSK9) in transportation and metabolism of low-density lipoprotein cholesterol (LDL-C) provided the possibility for treating hyperlipidemia by the application of gene-silencing technique. PCSK9 is an enzyme that exists in human body, and it can cause increased levels of LDL-C by degrading the receptor of LDL-C. On the other hand, loss-of-function mutation of the gene of PCSK9 led to decreased levels of LDL-C, which suggested that reduction or inhibition of PCSK9 might also be associated with reduced levels of LDL-C.[18] Studies demonstrated that direct inhibition of PCSK9 by using monoclonal antibodies against PCSK9 could significantly reduce the levels of LDL-C.[19,20] RNAi or siRNA is an attractive method to reduce the expression of PCSK9 by knocking down of its gene. siRNA targeting PCSK9 gene significantly reduced the levels of both LDL-C and PCSK9. The phase I and phase II clinical studies[1,2,21] on siRNA targeting PCSK9 showed that the effect of inclisiran, an siRNA targeting PCSK9, was potent and sustainable (Table 1). In the phase II study,[2] a multicenter, double-blind, placebo-controlled, multiple ascending-dose trial, at day 180 the mean reductions in LDL cholesterol levels from baseline were 35.5%-52.6%. The incidence of serious adverse events (11% vs 8% in inclisiran and placebo groups) was not significantly different. In the patients who received two doses of 300 mg inclisiran, the mean reduction in LDL cholesterol was 64.2 mg/dL at day 180 and 47.2% lower than at baseline at day 240. Two patients died late in the study, both in inclisiran group. One had long-standing vasculopathy and frequent angina and died of cardiac arrest; the other died of fistula and sepsis after having percutaneous repair of thoracic aortic aneurysm in the study course.

TABLE 1 Comparison of efficacy, safety, and clinical utility of three lipid-lowering therapies

Lipid-lowering therapy	Amplitude of LDL-c reduction (%)	Route of administration	Frequency of administration	Durability of lipid-lowering effect	Major adverse effects	Potential long-term adverse effects
Statins	40%-70%	Oral	Daily	Short	Rhabdomyolysis, elevated glycated hemoglobin (Hb)A1c	Elevation of LDL-C after a longer period use
Monoclonal antibodies against PCSK9 Evolocumab Alirocumab	50%-55%	Subcutaneous injection	Once or twice monthly	Up to 1 month	Injection site reactions	Antidrug antibody formation that may reduce efficacy
Inclisiran (siRNA)	41%-55%/d120; 27%-47%/d240	Subcutaneous injection	Once every 3 months	Up to 5 months	Injection site Reaction; 3 cases had transient elevation of liver enzymes	One case had antidrug antibody before treatment

These two deaths seemed not to be related to the therapeutic agent. The smaller sample size and the shorter follow-up period could not rule out serious side effects that are not frequently seen or side effects that might emerge after a longer or very long period of follow-up.

3.2 | Example 2, treatment for transthyretin amyloidosis

Transthyretin amyloidosis (TA) is a relatively rare disease resulting from the deposition of transthyretin amyloid in peripheral nerves and the heart. Potent antitransthyretin small-interfering RNA, ALN-TTR01 and ALN-TTR02, showed a rapid, dose-dependent, and durable effect of lowering transthyretin levels in both patients with TA (n = 32) and healthy volunteers (n = 17). Therefore, these phase I studies established the proof of concept for RNAi therapy targeting messenger RNA transcribed from a disease-causing gene.[3]

In these clinical studies, there are raised concerns about possible adverse effects of RNAi techniques that may include immune activation by siRNAs, "off-target effects," which might include the degradation of mRNAs irrelevant to the intended target for the treatment and needed for normal physiological processes.[5,7] Further studies and longer observations will answer all the questions concerning safety of the RNAi agents.

3.3 | Example 3, cancer treatment

In a single-dose, placebo-controlled phase I trial for locally advanced pancreatic cancer, Golan et al[22] found that in 12 of 15 patients an siRNA drug against KRAS (G12D) in combination with chemotherapy could reduce progression of the cancer. In addition, 7 of 10 patients had decreased levels of the tumor marker CA19-9. The median overall survival was 15.12 months, and 18-month survival rate was 38.5%.

In a phase I clinical study,[23] Atu027, a liposomal siRNA that silences expression of protein kinase N3 in the vascular endothelium, was well tolerated in the patients with advanced solid tumors. Forty-one percent of patients had stabilization of the tumors for at least 8 weeks. No serious adverse events were found in the patients receiving the treatment.

In another human phase I clinical trial,[24] detection of drug in tumor biopsies and siRNA-mediated mRNA cleavage in the liver were observed in 41 cases with advanced cancer with liver metastasis who were treated with ALN-VSP, a lipid nanoparticle (LNP) formulation of siRNAs targeting VEGF and kinesin spindle protein (KSP). This study showed pharmacodynamics, targeted down-regulation, and antitumor activity, including complete regression of liver metastases in endometrial cancer. It was also shown that biweekly intravenous administration of ALN-VSP was safe and well tolerated. The data of this study confirmed the proof of concept for RNAi therapeutics in humans and form the basis for further development of such therapeutic agents for cancer. This study demonstrated the safety, pharmacokinetics, RNAi mechanism of action, and clinical activity with a novel first-in-class LNP-formulated RNAi therapeutic in patients with cancer. The advantages of RNAi technology to facilitate specific multitargeting, high specificity, and safety will promote increasingly active development of RNAi-based therapeutic agents in oncology.

3.4 | Example 4, treatment for infectious diseases

A large field of studying infectious disease involves RNAi technology that has its unique advantages, especially for pathogens for which there are neither effective and safe therapeutic approaches nor effective vaccine. Respiratory syncytial virus (RSV), ebola virus, and multidrug-resistant bacteria should be prioritized candidates.

Respiratory syncytial virus is a virus for which there are neither effective antiviral agents nor vaccine for prevention. A randomized, double-blind, placebo-controlled phase II clinical trial[25] has demonstrated that ALN-RSV01, a synthetic siRNA targeting mRNA of RSV encoding the RSV nucleocapsid N protein, was well tolerated in healthy male adult volunteers with low serum neutralizing antibody against RSV. In this study, its safety profile was similar to that of placebo, and it significantly reduced the rate of RSV infection by 38.1%. ALN-RSV01 had a significant antiviral effect against human RSV. This study established a unique proof of concept for RNAi therapeutic agent.

The clinical application of ALN-RSV01 was tested in 2 phase II studies[26,27] for the possibility of reducing bronchiolitis obliterans syndrome (BOS) caused by RSV infection. In the phase IIa

randomized, double-blind, placebo-controlled trial in lung transplant recipients[26] with RSV respiratory tract infection, aerosolized ALN-RSV01 was administered daily for 3 days. The incidence of new or progressive BOS was found to be significantly reduced in ALN-RSV01 recipients. ALN-RSV01 was well tolerated, with no drug-related serious adverse events.

In the phase IIb clinical study,[27] 87 patients with locally confirmed RSV infection who had received lung transplantation were randomized to receive either ALN-RSV01 or placebo. Of these cases, 77 were confirmed by central laboratory to be RSV infected. ALN-RSV01 was found to be safe and well tolerated. At day 180, in ALN-RSV01-treated patients compared with placebo group, there was a trend toward a decrease in new or progressive BOS (13.6% vs 30.3%, $P = .058$). Treatment effect was enhanced when ALN-RSV01 started <5 days from symptom onset. There was no significant impact on viral parameters or symptom scores.

The results of these phase II studies demonstrated that ALN-RSV01 was safe and well tolerated and, importantly, reduced the risk of BOS after RSV infection in lung transplanted recipients.

3.5 | Example 5, treatment for ophthalmologic diseases

In a prospective, phase I, multicenter clinical trial,[28] the safety, tolerability, and pharmacokinetics of a single intravitreal (IVT) injection of PF-04523655, a 19-nucleotide siRNA, targeting the RTP801 gene in

TABLE 2 Possible pediatric target diseases/conditions of RNAi-based therapeutics

Subspecialty	Fields of diseases/conditions	Name of diseases/conditions/pathogens
Infectious diseases	Viral infectious diseases	Respiratory syncytial virus Ebola virus/Marburg virus Epstein-Barr virus[a] Dengue fever Epidemic Japanese encephalitis B, West Nile virus encephalitis, and other viral encephalitis Viral hepatitis A, B, and E Human parvovirus B19 Human herpes virus types 6, 7, and 8 Enterovirus 71 infection (hand-foot and mouth disease)[b] Coxsackievirus (CoV) serotypes causing severe clinical diseases such as CoV A9, A16, B2, 4, 5 Hemorrhagic fever and renal/lung syndrome (caused by Hantaan virus) Rabies
	Bacterial and other infectious diseases caused by pathogen	Multidrug-resistant bacteria, including MRSA, tuberculosis, vancomycin-resistant enterococcus, and other bacteria Meningococcal meningitis Plague Cholera Severe infections caused by other pathogens such as fungi, parasites, protozoa
Hereditary diseases	Autosomal dominant and recessive disorders	Osteogenesis imperfecta congenital Colon multiple polyps Renal diabetes Thalassemia alpha Hereditary hemorrhagic telangiectasia Neurofibromatosis Congenital blepharoptosis
	Single gene diseases	Hemophilia Gaucher's disease Familial hypercholesterolemia Fanconi's anemia Hurler's syndrome Hunter's syndrome
Malignant neoplasms	Hematologic malignancies	Leukemia, all types Lymphomas, all types malignant histiocytosis
	Solid tumors	Brain tumors including ependymoblastoma, gliocytoma, multiforme glioblastoma, malignant schwannoma, neuroblastoma, nephroblastoma, soft tissue sarcoma (rhabdomyosarcome), hepatoblastoma, embryonic cancers
Diseases of other organ systems	Cardiology endocrinology, ...	Hyperlipidemia, Kawasaki disease, autoimmune diseases, ...

[a]Includes chronic active EBV infection and EBV-induced hemophagocytic syndrome.
[b]Although there are vaccines against enterovirus 71 in China, effective and safe therapeutic agents are still in urgent need during epidemics.

TABLE 3 The general processes of studies on RNAi technique for the treatment of a certain disease

General processes	Notes
1. Choosing the target gene to silence and define the target sequence of mRNA	Largely need to obtain from literature
2. Synthesizing the siRNA candidate agent with one of its strand having the sequence complementary to the target mRNA sequence	Facility to synthesize RNA is required
3. Confirmation of the gene-silencing effects of the synthesized siRNA candidate agent in in vitro experiments	
4. Choose or create animal model of the disease of interest	
5. In vivo confirmation of the gene-silencing effect of the siRNA at both mRNA and protein	
6. Ruling out of "off-target" effect, immune activation, any other toxic or adverse effects of the siRNA candidate agent in vitro and in vivo	
7. Consider the first-in-human clinical study to confirm the safety, tolerability, and efficacy to some extent.	
8. Obtain evidences for postsilencing therapeutic effects (such as effects on tumor growth, invasiveness, and metastasis if the target disease is a cancer).	
9. Development of siRNA packaging or delivery system and their application	
10. Efficacy, safety, delivery efficiency, convenience, etc., should be confirmed in human studies, that is, phase I, II, and III clinical trials.	

patients with neovascular age-related macular degeneration (AMD) were evaluated. A single intravitreal (IVT) injection of PF-0523655 $\leq$3000 μg seemed to be safe and well tolerated in eyes with neovascular AMD.

A multicenter, open-label, prospective, randomized, comparator (ranibizumab)-controlled exploratory study[29] enrolled 151 patients with subfoveal choroidal neovascularization secondary to neovascular AMD. The patients were randomized to 1 of 5 treatment groups with an equal ratio. The combination of targeted drug with ranibizumab led to an average gain in best corrected visual acuity, which was higher than with ranibizumab monotherapy. No safety concerns were identified.

Benitez-Del-Castillo et al[30] described a phase I and 2 phase II clinical trials for evaluating the safety and efficacy of topically applied SYL1001, a novel short-interfering RNA for the treatment of dry eye disease (DED). In 156 healthy subjects and patients with DED, there was a decrease in visual analog scale scores in the SYL1001 group as compared with placebo group ($P = .013$). Ocular surface disease index scores in all dosage SYL1001 groups were found to be significantly reduced compared with their baseline levels ($P < .01$). The conjunctival hyperemia significantly improved after instillation with SYL1001 1.125% compared with placebo ($P < .05$).

Although none of the above-mentioned clinical studies are phase III trials, the results of those phase I and phase II trials have well documented that the RNAi-based therapeutic agents were safe, well tolerated, and quite effective, especially those developed for the treatment of hyperlipidemia, cancer, and RSV infection. Not only have these drugs shown excellent therapeutic effects but also these agents have potential superior efficacy or advantages over the current standard therapies. These encouraging preliminary in-human studies apparently signal the arrival of a new era of applying the RNAi-based therapeutic agents for the treatment of many diseases and syndromes. Some of these conditions are intractable, and for some, there are no specific effective therapies.

4 | HOW ARE STUDIES ON RNA INTERFERENCE GOING IN PEDIATRICS?

Specialties/subspecialties such as internal medicine, oncology, infectious diseases, and ophthalmology are leading the way in the application of RNAi technology. A number of studies have been performed in pediatrics.

PubMed literature search can indicate to some extent the status of study on a special topic or in a specified field. If a literature search is conducted by using the search terms "RNAi OR RNA interference OR siRNA OR small interfering RNA OR short interfering RNA" without any limits, the total number of retrieved articles could be around 128 751; however, if "Pediatric OR Children" is added into the "Affiliation," the total number of articles was 957. If "clinical trials" is chosen from "article types," only 4 articles were listed. On screening of the 4 abstracts, only one clinical trial was identified, which was a phase II clinical trial on RSV infection. The authors are pediatricians, but the subjects were healthy adult volunteers.[25] If "China" is added into the "Affiliation," the number of retrieval is 254, and none of them is clinical trial. In comparison, pediatric researchers have performed fewer studies in this area. RNAi is a very promising novel technique for the treatment of various diseases. No clinical studies on RNAi in pediatrics have been reported.

Pediatrics is a major discipline of the clinical medicine, and many of the pediatric diseases are actually ideal candidates for RNAi-based therapeutics. Hyperlipidemia does exist in adolescents and could be treated like in the studies described above. Also, malignant neoplasms such as leukemia and lymphomas are among common pediatric diseases and potential targets. Pediatric diseases that should be prioritized for the consideration of using RNAi-based technology to treat are listed in Table 2.

Undoubtedly, many pediatric diseases desperately need novel, safe, and effective treatments. Pediatricians need to study new techniques and methods for the treatment of pediatric diseases. There are difficulties and barriers in studying and applying an entirely new

technique, including manpower, funding, space, and equipment. However, it is possible to overcome the difficulties if applicability and importance of the technique is demonstrated. Table 3 lists an outline of general processes of studies on RNAi technique for the treatment of a certain disease.

A number of useful questions could be raised by pediatricians or pediatric researchers who are interested in learning and studying RNAi technology and its application in the treatment for pediatric diseases. I sincerely hope our readers who are already familiar with RNAi technology, working on RNAi research and clinical application could kindly answer some or all of the questions in the form of Correspondence (Letter to the editor) in this journal or any other way preferred. RNAi technology holds great promise and could be more widely learned, studied, and applied in the various fields of our pediatrics!

Questions about learning, studying, and applying RNAi technology:

1. RNAi technology applies high-level molecular biological techniques. Is it very difficult to learn? Is it possible for a pediatric clinician with a background in laboratory studies to learn this technology?
2. Is there any textbook or procedural manual on RNAi technology? Are there any training courses for major theories and laboratory test procedures for RNAi?
3. Which laboratories/research institutes would accept trainees for RNAi techniques? How long are the courses? How much training fees will be charged?
4. If the training courses are outside China, what requirements the trainees should meet?
5. What major equipment and instruments are necessary for an RNAi research laboratory?
6. Does RNAi research work need expensive reagents and other consumable laboratory utilities?
7. Do RNAi experiments need to use any extraordinarily hazardous (to the researchers and work environment) reagents or substances?
8. Are there any risks to the laboratory personnel who carry out studies on RNAi technique?

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CONFLICT OF INTEREST

The author has no conflict of interest to declare.

REFERENCES

1. Fitzgerald K, White S, Borodovsky A, et al. A highly durable RNAi therapeutic inhibitor of PCSK9. *N Engl J Med*. 2017;376:41-51.
2. Ray KK, Landmesser U, Leiter LA, et al. Inclisiran in patients at high cardiovascular risk with elevated LDL cholesterol. *N Engl J Med*. 2017; 376:1430-1440.
3. Coelho T, Adams D, Silva A, et al. Safety and efficacy of RNAi therapy for transthyretin amyloidosis. *N Engl J Med*. 2013; 369:819-829.
4. Pasi KJ, Rangarajan S, Georgiev P, et al. Targeting of antithrombin in hemophilia A or B with RNAi therapy. *N Engl J Med*. 2017; 377:819-828.
5. Uprichard SL. The therapeutic potential of RNA interference. *FEBS Lett*. 2005;579:5996-6007.
6. Karagiannis TC, El-Osta A. RNA interference and potential therapeutic applications of short interfering RNAs. *Cancer Gene Ther*. 2005;12:787-795.
7. Deng Y, Wang CC, Choy KW, et al. Therapeutic potentials of gene silencing by RNA interference: principles, challenges, and new strategies. *Gene*. 2014;538:217-227.
8. Gavrilov K, Saltzman WM. Therapeutic siRNA: principles, challenges, and strategies. *Yale J Biol Med*. 2012;85:187-200. PMID: 22737048.
9. Aagaard L, Rossi JJ. RNAi therapeutics: principles, prospects and challenges. *Adv Drug Deliv Rev*. 2007;59:75-86.
10. Harper SQ. Progress and challenges in RNA interference therapy for Huntington disease. *Arch Neurol*. 2009;66:933-938.
11. Son S, Kim N, You DG, et al. Antitumor therapeutic application of self-assembled RNAi-AuNP nanoconstructs: combination of VEGF-RNAi and photothermal ablation. *Theranostics*. 2017;7: 9-22.
12. Liu YC, Ma WH, Ge YL, et al. RNAi-mediated gene silencing of vascular endothelial growth factor C suppresses growth and induces apoptosis in mouse breast cancer in vitro and in vivo. *Oncol Lett*. 2016 Nov;12:3896-3904.
13. Chen SH, Zhaori G. Potential clinical applications of siRNA technique: benefits and limitations. *Eur J Clin Invest*. 2011 Feb;41:221-232.
14. Ito MK, Maki KC, Brinton EA, Cohen JD, Jacobson TA. Muscle symptoms in statin users, associations with cytochrome P450, and membrane transporter inhibitor use: a subanalysis of the USAGE study. *J Clin Lipidol*. 2014;8:69-76.
15. Cholesterol Treatment Trialists' (CTT) Collaboration. Protocol for analyses of adverse event data from randomized controlled trials of statin therapy. *Am Heart J*. 2016;176:63-69.
16. Clarke AT, Johnson PC, Hall GC, Ford I, Mills PR. High dose atorvastatin associated with increased risk of significant hepatotoxicity in comparison to simvastatin in UK GPRD cohort. *PLoS One*. 2016;11: e0151587.
17. Zhang H, Plutzky J, Skentzos S, et al. Discontinuation of statins in routine care settings: a cohort study. *Ann Intern Med*. 2013;158:526-534.
18. Horton JD, Cohen JC, Hobbs HH. PCSK9: a convertase that coordinates LDL catabolism. *J Lipid Res*. 2009;50(Suppl):S172-S177.
19. Reyes-Soffer G, Pavlyha M, Ngai C, et al. Effects of PCSK9 inhibition with alirocumab on lipoprotein metabolism in healthy humans. *Circulation*. 2017;135:352-362.
20. Nicholls SJ, Puri R, Anderson T, et al. Effect of evolocumab on progression of coronary disease in statin-treated patients: the GLAGOV randomized clinical trial. *JAMA*. 2016;316:2373-2384.
21. Fitzgerald K, Frank-Kamenetsky M, Shulga-Morskaya S, et al. Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, phase 1 trial. *Lancet*. 2014;383: 60-68.
22. Golan T, Khvalevsky EZ, Hubert A, et al. RNAi therapy targeting KRAS in combination with chemotherapy for locally advanced pancreatic cancer patients. *Oncotarget*. 2015;6:4560-4570.

23. Schultheis B, Strumberg D, Santel AJ, et al. First-in-human phase I study of the liposomal RNA interference therapeutic Atu027 in patients with advanced solid tumors. *Clin Oncol*. 2014;32: 4141-4148.
24. Tabernero J, Shapiro GI, LoRusso PM, et al. First-in-humans trial of an RNA interference therapeutic targeting VEGF and KSP in cancer patients with liver involvement. *Cancer Discov*. 2013; 3:406-417.
25. DeVincenzo J, Lambkin-Williams R, Wilkinson T, et al. A randomized, double-blind, placebo-controlled study of an RNAi-based therapy directed against respiratory syncytial virus. *Proc Natl Acad Sci USA*. 2010;107:8800-8805.
26. Gottlieb J, Zamora MR, Hodges T, et al. ALN-RSV01 for prevention of bronchiolitis obliterans syndrome after respiratory syncytial virus infection in lung transplant recipients. *J Heart Lung Transplant*. 2016;35:213-221.
27. Zamora MR, Budev M, Rolfe M, et al. RNA interference therapy in lung transplant patients infected with respiratory syncytial virus. *Am J Respir Crit Care Med*. 2011;183:531-538.
28. Nguyen QD, Schachar RA, Nduaka CI, et al. Phase 1 dose-escalation study of a siRNA targeting the RTP801 gene in age-related macular degeneration patients. *Eye (Lond)*. 2012;26:1099-1105.
29. Nguyen QD, Schachar RA, Nduaka CI, et al. Evaluation of the siRNA PF-04523655 versus ranibizumab for the treatment of neovascular age-related macular degeneration (MONET Study). *Ophthalmology*. 2012;119:1867-1873.
30. Benitez-Del-Castillo JM, Moreno-Montañés J, Jiménez-Alfaro I, et al. Safety and efficacy clinical trials for SYL1001, a novel short interfering RNA for the treatment of dry eye disease. *Invest Ophthalmol Vis Sci*. 2016;57:6447-6454.

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