Position Statement

RNA interference gene therapy for the treatment of cardiovascular disease

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ABSTRACT

The constant progress in molecular biology and genetic engineering technologies within the field of cardiovascular pharmacology, along with the introduction of clinically applied drugs based on these advancements, compels us to reconsider our current understanding in this area. This article aims to provide an update on the underlying mechanisms, pharmacological design, and the clinical applications tested so far, and also to address the safety concerns associated with drugs that utilize RNA interference technology.

INTRODUCTION

Currently, the pharmaceutical industry is adding advanced technologies derived from molecular biology and genetic engineering, which do not act by physical block of target protein function, but seek to reduce their expression at molecular level. Frequently, terms are found referring to RNA interference (RNAi), with antisense oligonucleotides (ASO), CRISPR/Cas9 modifications. To this date, there are several drugs available, commercially based on RNAi technology that employ the normal cellular mechanism of gene expression regulation. The significance of this mechanism was reflected in the 2006 Nobel Prize awarded to Fire and Mello for the use of double-stranded RNA (dsRNA) as a gene silencing strategy. More recently, the 2024 Nobel Prize in Physiology or Medicine was granted to Ambros and Ruvkun, who discovered micro interfering RNA (miRNA) as a mechanism for gene autoregulation based on a dsRNA encoded in DNA. In brief, the RNAi technology by a small NRA molecule produces messenger RNA (mRNA) degradation in a protein, inhibiting its expression, and consequently, silencing its function.

How is protein expression inhibited? Within the human genome there are DNA regions that enable special encoding for RNAi molecules. The dsRNA synthesized in this manner is cut by the action of an RNase (DICER) into double-stranded fragments of 21-23 nucleotides, called small interference RNA or siRNA/miRNA *(Figure 1).* These fragments are assembled in a protein complex known as RISC (RNA induced silencing complex), where the two sense and antisense strands of siRNA are decoupled, as shown in *Figure 1.* Next, the antisense strand is complementarily coupled with mRNA in a region determined by the strict order in which the A, U, G, C (adenine, uracil, guanine and cytosine) nucleotides appear. This union will be the more stable the less complementarity errors there are, and vice versa, determining for the target mRNA selection to be extremely specific. The stability of this union provides the necessary time for this RISC protein complex, through an RNase, to cut the mRNA strand, disabling it to translate the protein for which it encodes.[1] This very specific capacity to control protein expression occurs independently from siRNA being generated within the cell or being introduced exogenously, as developed by the RNAi technology.

This technology provides plenty of advantages over conventional pharmacology. Among them, we can mention: 1) high specificity, avoiding the inhibition of similar proteins; 2) high intracellular stability, extending the time of action of a dose for periods from 6 to 12 months; 3) inhibition of therapy targets over which there are no available drugs or that are difficult to implement; 4) improving adherence to treatment. The growth of treatment methodologies based on siRNA as technological platform for development of drugs is reflected on the increase in the number of clinical trials, as well as the approval for commercialization by the Food and Drugs Administration (FDA) in the United States *(Table 1).*

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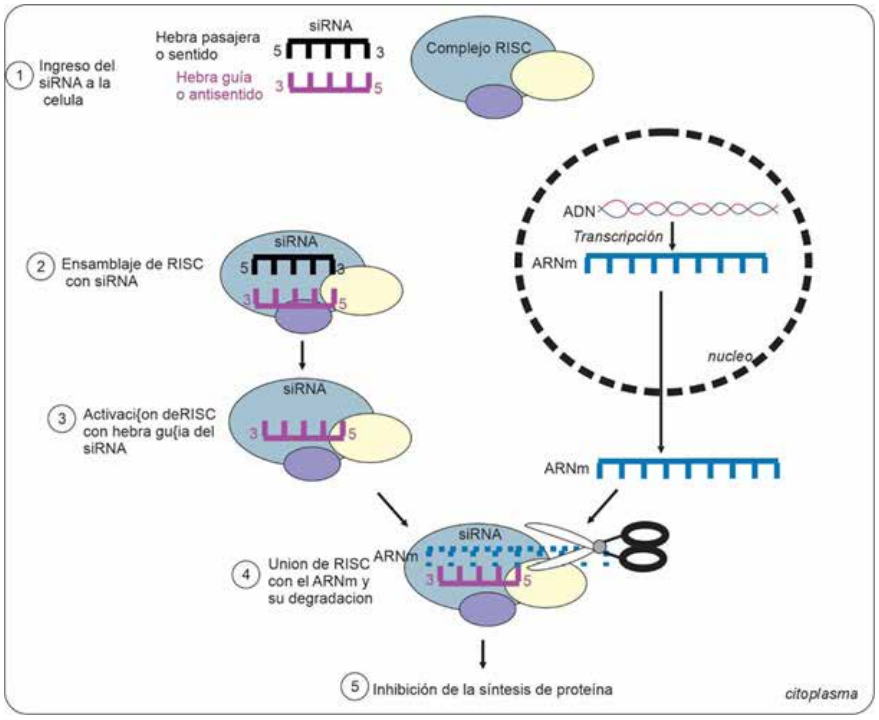


FIGURE 1.
Mechanism of action of small-interfering RNA (siRNA) molecules. **1)** siRNA enters cells by endocytosis of lipid nanoparticles or by siRNA interaction, conjugated with N-acetylgalactosamine (GalNac), which binds to the asialoglycoprotein receptor, expressed in the membrane; **2)** siRNA released in cytosol is associated with proteins to form the RNA-induced silencing complex (RISC), splitting into a single strand and activating; **3)** the strand within the complex works as a guide, so that **4)** the enzyme splits the target region in messenger RNA (mRNA), to then cut it into small fragments, preventing its translation and the encoded protein expression.

It is necessary to mention that the industry has overcome its own limitations associated to these molecules, such as stability and immunogenicity. Naked siRNA molecules in plasma are very labile, have a very low half-life (approximately 5 min), because they are degraded by RNase or because they are filtered in the kidneys. On the other hand, RNAi has to be presented to the organism in a short-strand configuration, because long-strand ones may trigger an immune response. The different chemical modifications of the nucleotide bases of RNAi enabled overcoming these hindrances, reducing toxicity in the administration and assimilation of these drugs.[1]

Another limitation has been selectively addressing RNAi toward the target organ, thus preventing its dissemination to other tissues of the body, and the generation of an undesired inhibition. As strategy, the administration of siRNA uses a local application on the desired target tissue, as for instance in the eye through intravitreal injection. For the systemic subcutaneous administration, siRNA is added to a molecule that determines a tropism to the target organ. Thus, the liver as target organ is on the cutting edge of possible targets, as comparatively, access is easier. In this case, siRNA couples with N-acetylgalactosamine (GalNac), which has a great affinity for the asialoglycoprotein receptor of great expression on the surface of hepatocytes and is mainly absent in other cells.[2] Another widely used alternative is the association with nanoparticles, as for instance lyposomal.[2] As seen in *Table 1,* most drugs approved by the FDA have proteins as target, mainly originating in the liver. In the cardiovascular area, there are only 6 treatments with siRNA bases approved by the FDA. However, between the clinical trials of phases I, II and III, more than 50 different products are being evaluated for the treatment of cardiovascular, metabolic, oncological, endocrine diseases and so on.[2,3]

In terms of cardiovascular health, the first developments were mainly addressed at the treatment of dyslipidemias. Next, we will mention the most relevant ones:

- Inclisiran: addressed at the treatment of homozygous familial hypercholesterolemia with elevated low-density lipoprotein (LDL) in plasma. siRNA inhibits the expression of protease PCSK9, which enables a greater expression of LDL receptors in the liver, favoring a reduction of cholesterol associated to lipoprotein (LDL-C) in plasma. Post-hoc studies in patients with acute myocardial infarction indicated that with the initial injection treatment, in 3 months and then every 6 months, there was an efficient and constant reduction of 40-50% of LDL-C for over a year.[4] Preliminary studies on cardiovascular risk were not completely conclusive, and encouraged the performance of more trials (ORION-4 and VICTORION-2 PREVENT), whose results on cardiovascular risk are expected for 2026.

- Olpasiran, leposidaran and zerlasiran: addressed at reducing the levels of lipoprotein (a) or Lp(a), a special type of LDL associated to atherosclerotic cardiovascular diseases (ASCVD) and valve stenosis expressing apolipoprotein A (Apo-A). Hyperlipidemia as a risk factor in ASCVD corresponds to more than 20% of the population in the US, and is primarily determined by genetic alterations of the Apo-A gene, so it does not respond to conventional therapies or changes in lifestyle. Silencing Lp(a) with leposidaran achieved a 90% reduction in up to one year after the last doses. With olpasiran, patients received 4 doses every 3/4 months, achieving reductions in the same range.[5] There are other drugs based on siRNA against Lp(a) in earlier clini-

TABLE 1.
Outline of approved siRNA-based pharmacological treatments, and/or in different phases in clinical trials.

Name	Protein/gene	Pathology	Formulation	Approval/state
Patisiran	Transthyretin*	Hereditary transthyretin amyloidosis	Lipid nanoparticles	2018
Inclisiran	PCSK9	Heterozygous familial hypercholesterolemia (HeHF)	siRNA-GalNAc	2020-2021
Olpasiran	Lipoprotein A	ASCVD with high Lp(a)	siRNA-GalNAc	2024 Phase III
Lepodisiran	Lipoprotein A	Cardiovascular disease, elevated Lp(a)	siRNA-GalNAc	2024 Phase II/III
Zerlasiran	Lipoprotein A	Cardiovascular disease, elevated Lp(a)	siRNA-GalNAc	2024 Phase II
Zodasiran	ANGPTL3	Mixed hyperlipidemia, elevated LDL	siRNA-GalNAc	2024 Phase II
Plozasiran	APOC3	Mixed hyperlipidemia, elevated TG-VLDL	siRNA-GalNAc	2024 Phase II/III
Zilebesiran	Angiotensinogen	Hypertension	siRNA-GalNAc	2024 Phase II/III
Vutrisiran	Transthyretin	Hereditary transthyretin amyloidosis	siRNA-GalNAc	2022/2025

* Drug studied to evaluate the treatment of amyloidosis in the central nervous system.

cal phases, such as zerlasiran, that tested in patients with ASCVD in 3 and 6 months, according to 2024 June reports, produced a 90% reduction in Lp(a).[6]

- ***Zodasiran and solbinsiran:*** addressed at favoring plasma clearance of lipoproteins rich in triglycerides (TG) through peptide ANGPTL3 silencing (angiopoietin-like peptide). This peptide inhibits the lipoprotein lipase (LPL), so its inhibition favors lipase action and reduces TG levels in blood. Phase II clinical studies for zodasiran showed, in patients with hyperlipidemia, that with 2 doses, one basal on day 1 and then 3 months later, a 56% reduction was achieved in TG in 6 months. Epidemiological studies with loss of function in this protein show a reduction in cardiovascular disease risk, as well as variable percentages of TG and cholesterol reduction in blood.[7] This RNAi target is still being researched in other phase II clinical trials (PROLONG-ANG3, NCT05256654). Although the preliminary studies in animals and humans showed efficacy in the management of dyslipidemias also for solbinsiran, its cardiovascular consequences have not yet been studied.[8]

- ***Plozasiran:*** it is designed to inhibit the liver synthesis of apolipoprotein C3 (APOC3), a protein that acts by decreasing the activity of lipoprotein lipase (LPL), and therefore, by reducing the plasma clearance of lipoproteins rich in triglycerides, thus favoring its accumulation in circulation. In clinical studies made in individuals with dyslipidemias, the administration of this molecule led to a 77% reduction in plasma levels of triglycerides after two doses, an effect that remained in 3 and 9 months of follow-up.[9] In patients with familial chylomicronemia syndrome, the treatment was associated to an effective control of the disease and a significant decrease in the risk of pancreatitis.[10] Currently, the clinical trials in phase III: SHASTA-3 (NCT06347003) and SHASTA-4 (NCT06347016), with the aim of evaluating its efficacy and safety in the context of severe hypertriglyceridemia.[11] Nevertheless, to this moment no studies have been developed exploring its impact on global cardiovascular risk.

Other clinical trials and treatments are addressed directly to solving cardiovascular disease primarily:

- ***Patisiran and vutrisiran:*** addressed at treating fatal nerve disease secondary to hereditary transthyretin amyloidosis (ATTR). siRNA inhibits the expression of the transthyretin (TTR) enzyme of liver synthesis, which by being silenced in ATTR, leads to the accumulation of amyloid deposits in key organs, such as the central nervous system, peripheral nervous system, and the heart. Patisiran (Apollo-B study) reduced 81% of TTR in plasma and heart wall thickness, along with contractility improvement in patients.[12] It was the first drug of this nature to be approved by the FDA in 2018. Another medication in the same line, revusiran, was prematurely suspended in phase III.[13] Recently, in March 2025, the anti-TTR, siRNA-based therapy was approved by the FDA for the treatment of cardiomyopathies. The drug called vitrisiran, tested in the HELIOS-B study, was injected 1 every 3 months during 36 months, achieving reduction in death and recurrence of cardiovascular events among other indices.[14]

- ***Zilebesiran:*** addressed at the reduction of blood pressure by angiotensinogen (AGT) expression inhibition, which prevents the synthesis of angiotensin II and aldosterone in a sustained manner, leading to a sustained decrease in blood pressure. This molecule has been evaluated within the framework of the KARDIA clinical trials program. In individuals with moderate hypertension, zilebesiran achieved a reduction of up to 90% in the plasma levels of AGT, accompanied by a 12-14% decrease in systolic blood pressure.[15] Recent results of the KARDIA-2 study, presented

in the European Meeting on Hypertension and Cardiovascular Protection, showed that in non-controlled hypertensive patients under standard antihypertensive treatment, the administration of a single dose of zilebesiran induced an additional reduction of between 7 and 18 mmHg in systolic blood pressure, an effect that remained for a period of up to 6 months.[16] Currently, there is an ongoing study, the KARDIA-3, with the aim of assessing its efficacy in other hypertensive populations.

The safety and effectiveness at cardiovascular level of RNAi treatments seem to still be an unsolved question. These drugs, strategically addressed to a target only related to the pathology to be treated, present a high index of safety.

In general, phase I and phase II clinical trials showed a good safety profile, and the mass administration of vaccines based on these technologies have proven that the minimization of immunogenicity has been optimized. However, given how recent this development is in the pharmaceutical industry, it is necessary to keep a continuous long-term monitoring. In this regard, some clinical trials with this type of drugs were interrupted, in spite of efficiently achieving target protein silencing, due to the emergence of unexpected adverse effects, such as it occurred with teprasiran and revusiran.[13,17] The causes included from inappropriate dosing and suboptimal formulations, to using delivery systems (like lipid nanoparticles) that caused inflammatory responses, or the lack of a significant clinical efficacy.

Although currently, most approved or advanced therapies with cardiovascular effects are addressed to rare or hereditary diseases, or with pharmacological vacuums, as a position statement about them it is possible to say that siRNA-based pharmacological therapy offers an optimistic outlook, given the great advantage of specificity and posology. The advantage of being administered spread out over time allows for better adherence, because of the long-duration effect of siRNA. The pharmacological use of RNAi technology is currently a reality of exponential expansion, seeking to reach target organs other than the liver. The growing interest in the translation of experimental results obtained in basic investigation will allow to satisfy vacant pharmacological areas, addressing these technologies directly at the primary causal proteins, linked to the development and progression of cardiovascular diseases.

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