Oligonucleotide Therapeutics — A New Class of Cholesterol-Lowering Drugs

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Not long ago, the cholesterol-lowering arena was monopolized by small-molecule drugs, most of them statins. The recent approval by the Food and Drug Administration (FDA) of alirocumab (Praluent, Sanofi/Regeneron) and evolocumab (Repatha, Amgen), monoclonal antibodies targeting proprotein convertase subtilisin–kexin type 9 (PCSK9), represented the principal fundamental advance in cholesterol-lowering therapies since the discovery of statins. Alirocumab and evolocumab are currently approved as add-on therapies to statins when statins alone are not sufficient to lower cholesterol levels; when there are unacceptable side effects, they will be used instead of statins.

Managing cholesterol levels is the standard of care for the prevention of atherosclerosis that can lead to the formation of cholesterol plaques and eventually cardiac events. PCSK9 regulates the half-life of the receptor responsible for cholesterol clearance; its silencing thus mainly enhances cholesterol clearance rather than the rate of synthesis. Modulation of PCSK9 supports highly potent lowering of cholesterol to levels unachievable by standard statin regimens.

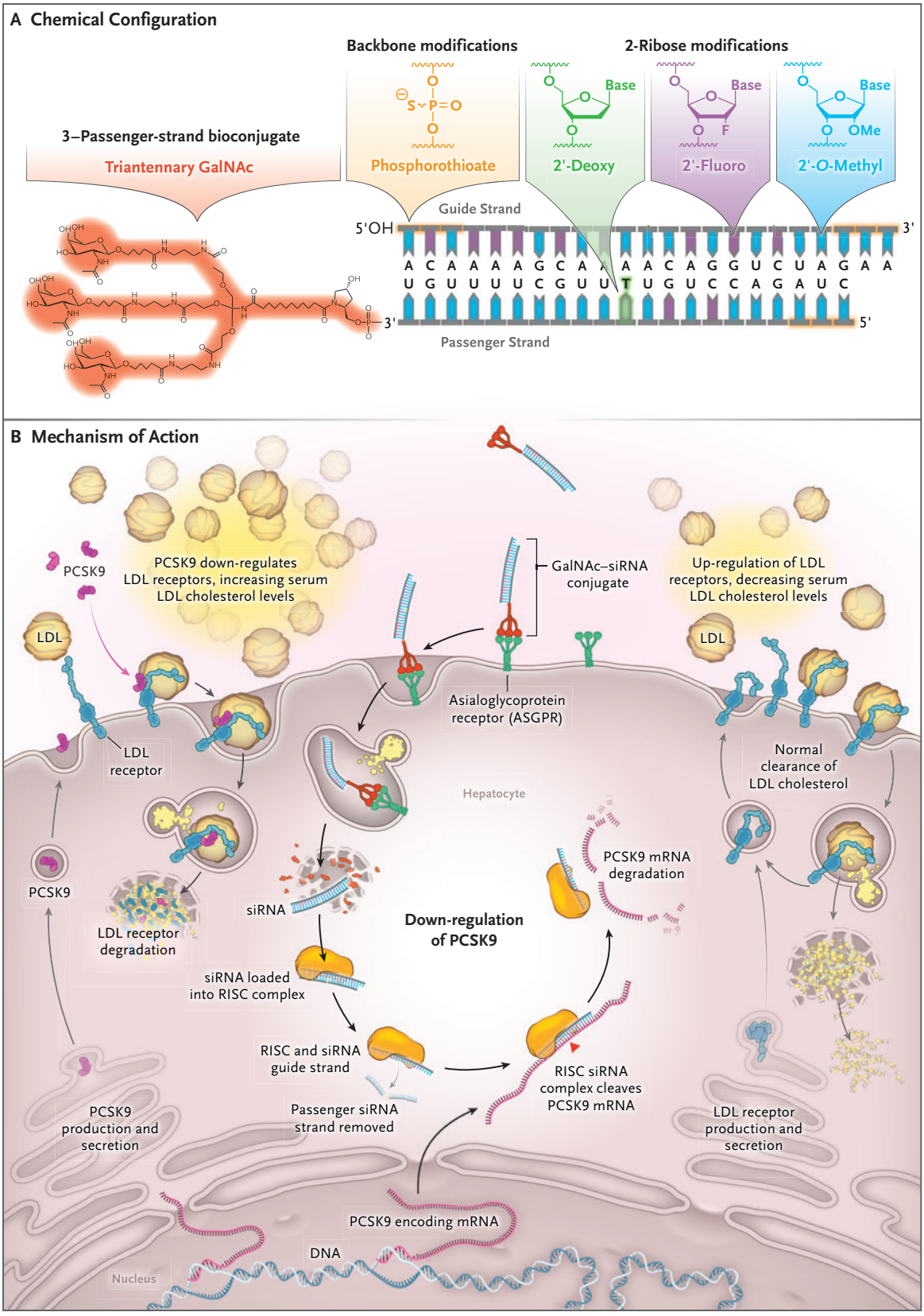
The results reported by Fitzgerald et al. in this issue of the *Journal* (pages 41–51) suggest that we may soon be able to add another drug class to our cholesterol-lowering strategies: a fully chemically modified, small interfering RNA (siRNA) conjugated to the triantennary *N*-acetylgalactosamine (GalNAc; see diagram). siRNAs are one type of oligonucleotide therapeutic, a new class of drugs that targets RNA directly (in this case, messenger RNA [mRNA]), destroying it before the protein is synthesized.

The siRNAs consist of two strands, guide and passenger. The guide strand carries the sequence information necessary for target-gene recognition, while the passenger strand serves as a prodrug that supports the geometry required for loading into the RNA-induced silencing complex (RISC). When siRNAs are introduced into the cells, the guide strand enters the RISC and reprograms the powerful natural mechanism RNA interference (RNAi), silencing genes on demand. The loaded RISC has a long half-life, and as few as 100 to 200 loaded RISC complexes per cell are sufficient to eliminate expression of the targeted gene (see diagram). The importance of this fundamental mechanism is well recognized — indeed, the two scientists who discovered it, Craig Mello and Andrew Fire, were awarded the Nobel Prize in 2006.

The early enthusiasm for the promise of RNAi therapeutics was quickly dampened when several nonoptimized compounds failed or showed minimal clinical efficacy in early clinical trials. After a decade of chemical optimization,[1] however, inclisiran has now been demonstrated to have extremely potent and durable effects: a single subcutaneous injection can lower a patient's low-density lipoprotein cholesterol level for 6 months. Although, like the new monoclonal antibodies, such oligonucleotides are injectable, their potency and relatively infrequent administration requirements (every 2 to 4 weeks for monoclonal antibodies and every 6 to 12 months for siRNAs) make them attractive alternatives to statins, which must be taken daily. Initially, RNAi therapeutics, too, are likely to be used when statins alone fail to lower a patient's cholesterol level efficiently enough or when their use causes unacceptable side effects, but the use of both new classes of cholesterol-lowering agents may expand with growing clinical experience.

Facing page: Inclisiran Chemical Configuration and Mechanism of Action.

Inclisiran is a fully chemically modified siRNA conjugated to the triantennary GalNAc. Inclisiran is made from one 2′-deoxy, eleven 2′-fluoro, and thirty-two 2′-*O*-methyl modified nucleotides. Termini of the du-plex are modified with phosphorothioates, and the 3′ end of the passenger strand is functionalized with triantennary GalNAc (Panel A). When inclisiran is delivered to the hepatocytes through GalNAc interactions with the ASGPR receptor, the guide strand of the duplex enters the RISC complex, hybridizes to the PCSK9 mRNA and cleaves it (red triangle), preventing protein production. Down-regulation of PCSK9, a protein involved in low-density lipoprotein (LDL) receptor degradation, results in the up-regulation of LDL receptor levels on the surface of the hepatocytes, supporting more efficient clearance of LDL cholesterol from the bloodstream (Panel B).



Inclisiran is a fully chemically stabilized duplex RNA, targeting 3′ UTR of the PCSK9 mRNA (see diagram).[2] Five types of modifications are used to make this compound based on natural RNA: phosphorothioate, 2′-deoxy, 2′-fluoro-RNA, 2′-*O*-methyl-RNA, and triantennary GalNAc. Unmodified siRNAs are highly unstable owing to the high susceptibility of ribonucleotides to exonuclease and endonuclease degradation. The combination of the 2′-fluoro and 2′-*O*-methyl modifications allows for substantial compound stabilization without compromising the ability of siRNAs to enter RISC, the protein machinery responsible for fine-tuning transcriptional profiles inside cells. The terminal phosphodiester linkages are modified with phosphorothioates, which provide additional stabilization against exonuclease degradation and potentially contribute to the compound's cellular internalization.

There is one deoxythymidine included in the middle of the sense strand at a position corresponding to the RISC cleavage site, which might improve guide-strand loading, accelerate the degradation of the released passenger strand, or both. The 3′ end of the passenger (nonactive) strand is conjugated to a triantennary GalNAc. This conjugate is specifically recognized by the asialoglycoprotein receptor (ASGPR) that is highly expressed on the surface of liver hepatocytes, the cell type primarily responsible for cholesterol clearance. Although triantennary GalNAc is a primary means for hepatocyte delivery and uptake[3] (described by Levin in this issue of the *Journal* [pages 86–88]), the structure and chemical composition of the oligonucleotide plays an essential role in potency, and it is the key to the extraordinarily long activity of inclisiran.

Inclisiran has now been demonstrated to have extremely potent and durable effects: a single subcutaneous injection can lower a patient's low-density lipoprotein cholesterol level for 6 months. Such oligonucleotides' potency and relatively infrequent administration requirements make them attractive alternatives to statins.

A previous version of the same technological concept was used for the design of revusiran,[4] an experimental drug evaluated for the treatment of transthyretin-associated (ATTR) amyloidosis with cardiomyopathy involvement. To achieve efficient gene silencing, revusiran required weekly administration with cumulative yearly exposure of 25 g. In October 2016, Alnylam announced the termination of a phase 3 clinical trial of revusiran, primarily because of a significant imbalance in mortality rates between the drug and placebo groups.

So what is the mechanism behind the significant differences in potency and duration of effect observed between revusiran and inclisiran? The chemical differences between the two are limited: inclisiran incorporates four additional phosphorothioates, providing both strands with 5′ terminal stabilization, and it includes about half the number of 2′-fluoro-RNA modifications (replacing them with 2′-*O*-methyl-RNA modifications), further enhancing stability. The majority of the injected dose is delivered to the liver hepatocytes, and only a relatively small fraction becomes biologically active and is loaded into the RISC immediately. The loaded RISC has a half-life of weeks, which may be further enhanced in a context of fully stabilized siRNAs. In addition, the oligonucleotides trapped in the endosomes may serve as an intracellular depot of the drug, slowly releasing it over time.

The pharmacokinetic properties of an oligonucleotide are largely defined by its chemical and structural architecture — chemical modifications of sugars, bases, and the phosphate backbone; single-stranded or duplex structure; and the presence or absence of a targeting ligand. The inclisiran results offer hope that similar chemical architecture can be used to silence other therapeutically relevant targets with similar durability. They thus provide clinical validation for GalNAc–siRNA conjugates as a new class of investigational drugs for diseases with liver involvement. With the demonstration of

a 6-to-12-month duration of effect, oligonucleotide therapeutics might become competitive not only with biologics, but also with orally administered drugs, an outcome that was unforeseeable only a short while ago. Of course, the future of inclisiran and oligonucleotide therapeutics is completely dependent on the demonstration of their safety.

Conjugated oligonucleotides are chemically defined "large small molecules" that can be synthesized in an advanced oligonucleotide lab within a day. They are assembled from standard building blocks, phosphoramidites, in an automatic fashion using solid-phase chemistry. The cost of oligonucleotides is driven mainly by the cost of its precursors and is expected to be in the low hundreds of dollars per gram on a commercial scale. With a yearly dose of 300 to 500 mg, the manufacturing cost for this class of drugs is on par with that of small-molecule drugs and is probably much lower than that of monoclonal antibodies. Although the manufacturing cost accounts for only a minority of the initial market price of the drug, the relative simplicity of the manufacturing process and the room-temperature stability of the dried oligonucleotides, which can be brought to solution at the point of care, might eventually make this class of therapeutics widely available for a broad population.

Disclosure forms provided by the author are available at NEJM.org.

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A View from the Edge — Creating a Culture of Caring

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In 2008, an occult adenoma in my liver ruptured, and I effectively bled to death in my own hospital. I lost my entire blood volume into my abdomen, triggering what's known in trauma as the Triad of Death — a kind of suicidal spiral of the blood in which it becomes too acidic and too cold to clot. I would receive more than 26 units of blood products that night — packed red cells, platelets, cryoprecipitate, fresh frozen plasma. I would go into multisystem organ failure, my liver and kidneys would shut down, I would be put on a ventilator, have a stroke and a complete hemodynamic collapse. The baby I was 7 months pregnant with would not survive, but I would — thanks to the incredible skill and grace of the teams of professionals who cared for me.

My recovery involved five major operations including a right hepatectomy. I had to relearn to walk, speak, and do many other things I had taken for granted. But in the process, as a patient, I learned things about us — physicians and other medical professionals — that I might not have wanted to know. I learned that though we do so many difficult, technical things so perfectly right, we fail our patients in many ways.

As a patient, I was privy to failures that I'd been blind to as a clinician. There were disturbing deficits in communication, uncoordinated care, and occasionally an apparently complete absence of empathy. I recognized myself in every failure.

When I overheard a physician describe me as "trying to die on us," I was horrified. I was not trying to die on anyone. The description angered me. Then I cringed. I had said the same thing, often and thoughtlessly, in my training. "He was trying to die on me." As critical care fellows, we had all said it. Inherent in that accusation was our common attribution of intention to patients: we subconsciously constructed a narrative in which the doctor–patient relationship was antagonistic. It was one of many revelatory moments for me.

I heard my colleagues say