

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor***Controlling CRISPR-Cas9 Gene Editing**

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RNA genetic medicines,¹ in both development and clinical use, are highly selective therapeutics that recognize their cognate target DNA or RNA by base pairing. These medicines include CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats–Cas9 endonuclease), an experimental medicine that uses RNA oligonucleotides to guide the Cas9 enzyme to the target DNA sequence, and antisense oligonucleotides, which are single-stranded and suppress messenger RNA (mRNA) levels or alter mRNA splicing. Another class of RNA medicine consists of double-stranded molecules known as short interfering RNAs (siRNAs). After siRNAs are embedded in a cytosolic complex and one of the strands (the “sense” strand) is peeled away, leaving the antisense (or guide) strand exposed, they target their cognate mRNAs for degradation by the RNA-cleaving enzyme Argonaute 2 (Ago2, also a component of the complex).

Because siRNAs can durably inhibit their targets, a major concern about gene-targeted medicines is the possibility of off-target effects. For example, siRNA-loaded Ago2 can cleave target mRNAs for more than 6 months after a single subcutaneous dose of oligonucleotide.² Such durability underscores the concern about off-target effects: what if a serious adverse event develops, as a result of an off-target effect, months after the initial dosing? To address this problem, Alnylam Pharmaceuticals developed a single-stranded “Reversir” antioligonucleotide that specifically and effectively targets an siRNA guide (antisense) strand loaded into Ago2, shutting down the RNA interference response and restoring expression of the target *TTR* mRNA (encoding transthyretin)³ within a few days after subcutaneous injection into mice (Fig. 1). Similarly, Ionis Pharmaceuticals has used anti-antisense oligonucleotides to reverse the effects of a splice-

altering antisense oligonucleotide targeting the gene *SMN2* in a mouse model of spinal muscular atrophy.⁴ Together, these approaches lay a foundation to specifically counter or reverse the activity of RNA therapeutics.

CRISPR-Cas9–mediated gene editing has the potential to permanently reverse pathologic DNA mutations. However, with the permanence of gene editing comes the overarching problem of terminating Cas9 enzymatic activity after it has completed the therapeutic DNA modification, so as to avoid potential off-target editing, as well as on-target editing in nontarget tissues. Into this void step Barkau et al.,⁵ who used an antioligonucleotide approach to regulate CRISPR-Cas9 gene editing in human cells in culture.

CRISPR-Cas9 is a ribonucleoprotein (i.e., a protein–RNA complex) nuclease. The RNA component is a genetically engineered single-guide RNA (sgRNA).⁵ The sgRNA contains the guide region that base pairs with both the target DNA sequence to be cleaved and the protospacer-adjacent motif (PAM) that contains the cleavage site; it also contains four structural stem–loops that are bound by Cas9 protein and are required for correct Cas9 protein folding. Barkau and colleagues took advantage of these RNA structural requirements and targeted them individually or in combination with base-pair-complementary, single-stranded oligonucleotides, transfecting human embryonic kidney cells (of the HEK293 cell line) by electroporation. Surprisingly, targeting the obvious guide region of the sgRNA had little effect on overall Cas9 activity; likewise, individually targeting several of the sgRNA stem–loop structures was also ineffective. However, linking an anti-PAM oligonucleotide with one targeting an sgRNA structural stem–loop resulted in a significant reduction of Cas9 activity when assayed 5 days after electroporation.⁵ Because it excludes

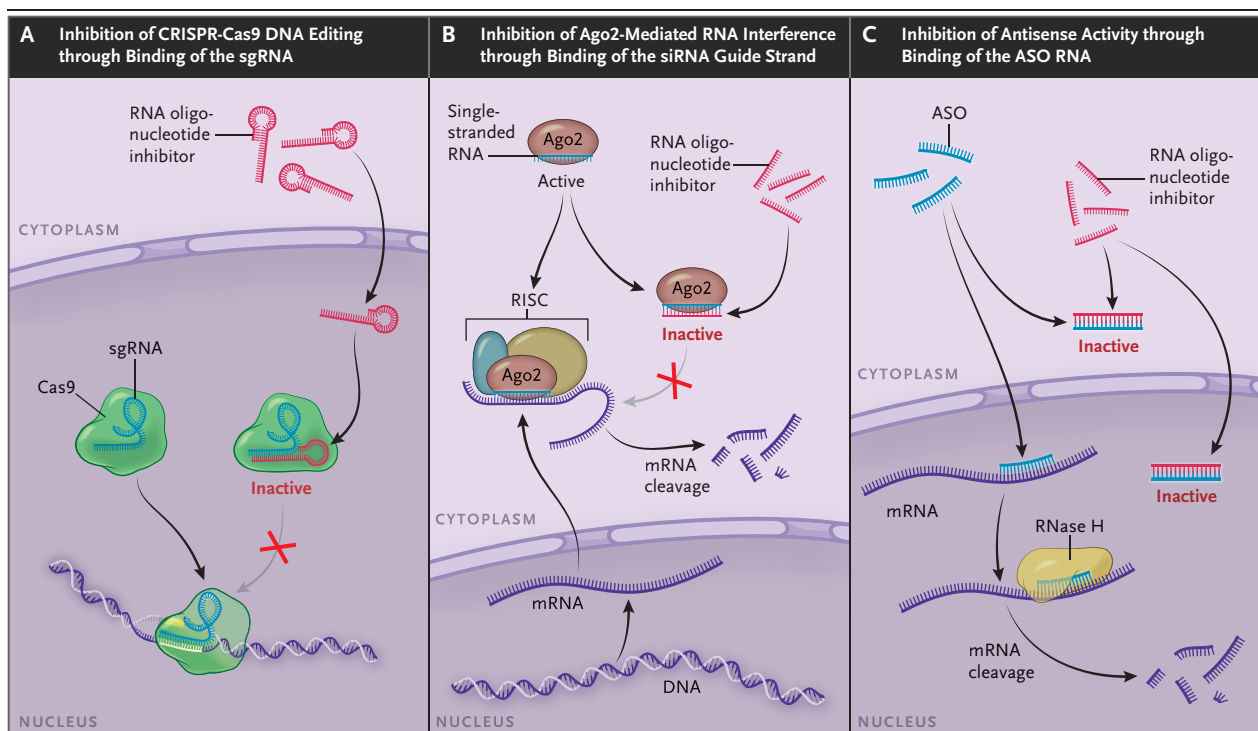


Figure 1. Regulation of RNA Therapeutics.

Antioligonucleotides down-regulate or inactivate CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats–Cas9 endonuclease) DNA editing by base pairing to the single-guide RNA (sgRNA) (Panel A). Likewise, antioligonucleotides can inhibit short interfering RNA (siRNA)–Ago2 RNA-induced silencing complexes (RISCs) from inducing RNA interference responses and thereby restore target-gene messenger RNA (mRNA) levels (Panel B). Antioligonucleotides can also inhibit antisense oligonucleotide (ASO)–mediated mRNA degradation by RNase H (Panel C).

the variable guide region sequence, this is a universal Cas9 antioligonucleotide that, in theory, can inactivate all CRISPR-Cas9 ribonucleoproteins.

This simple approach has the potential to open the door for the enhancement of the safety of clinical CRISPR-Cas9 gene-editing applications. One can imagine that after optimizing the kinetics of maximum on-target gene editing in a given tissue or ex vivo cell population, the antioligonucleotide would then be administered to shut down Cas9 activity and thereby reduce or prevent off-target DNA editing. Likewise, if the target gene need only be edited in one tissue, then preadministration of the antioligonucleotide in the nontarget tissues would prevent undesired DNA off-target editing. This, of course, depends on the oligonucleotide-delivery problem being solved,¹ but that is a topic for another day.

Barkau and colleagues have opened up a path forward to improve the safety of gene editing by Cas9 ribonucleoprotein inactivation through the

simplest of approaches: antioligonucleotides that bind to, and thereby block, the CRISPR-Cas9 sgRNA.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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