DISEASES AS DIFFERENT AS SPINAL MUSCULAR ATROPHY AND HYPERLIPIDEMIAS can now be treated with drugs that act on RNA. A new class of drugs — oligonucleotides — takes advantage of Watson and Crick’s base-pairing rules to target disease-related RNAs. With information from the sequencing of the human genome, it is possible to design therapeutic oligonucleotides — solely on the basis of genomic information — that will change the expression of any protein, even those not amenable to traditional approaches involving small-molecule drugs (see video).

Although the concept of using synthetic oligonucleotides to modulate RNA function was described as early as 1978,1 its realization in the form of drugs approved by the Food and Drug Administration (FDA) has required advances in genomics, chemistry, pharmacology, and drug delivery. Moreover, dozens of oligonucleotides intended to treat diseases as varied as hemophilia, amyloidosis, hemostasis, and hyperlipidemias are in clinical trials.2-9 Here, four oligonucleotides that target RNA in distinctly different ways and new directions in the field of therapeutic oligonucleotides are described.

MECHANISMS OF ACTION

Therapeutic oligonucleotides are generally 15 to 30 nucleotides in length and are designed to be complementary to a specific region of a messenger RNA (mRNA) encoding a disease-related protein or a regulatory RNA. After parenteral administration, the oligonucleotide enters a cell and binds to any complementary RNA. When designing a therapeutic candidate, the goal is to identify sequences that are highly specific for the target RNA and to avoid sequences that hybridize to unintended but homologous “bystander” RNAs. With careful design guided by bioinformatics, specific sequences can be identified such that even single members of closely related gene families can be targeted selectively.

Once the oligonucleotide drug has bound to its complementary mRNA or pre-mRNA, a series of events ensues. The outcomes depend partly on the nature of the targeted sequence and include destruction of the mRNA by means of enzymatic cleavage (which is helpful when the mRNA is mutated and encodes a pathogenic protein), a change in the pre-mRNA splicing pattern (which is helpful when the “default” splicing pattern produces a pathogenic product), or a change in the function of a regulatory RNA. The choice of strategy depends on the disease mechanism and on whether the intended outcome is gain or loss of RNA function.

Currently approved oligonucleotide drugs induce cleavage of a target mRNA or alter the splicing pattern. Cleavage-inducing oligonucleotides have structural and chemical features that recruit endogenous enzymes to the site on the target mRNA where the drug hybridizes. Oligonucleotides that alter splicing hybridize with pre-

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An illustrated glossary and a video overview of therapeutic oligonucleotides are available at NEJM.org
mRNA near a site that controls splicing; the hybridized oligonucleotide effects a change, through steric hindrance, in the action of enzymes that edit pre-mRNA.

THERAPEUTIC MECHANISMS

SPlicing to Induce Changes in Function

Only a small fraction of the genetic information in the human genome is translated into proteins. For example, DNA encoding dystrophin is dispersed across a region of 2.4 million base pairs, but dystrophin mRNA consists of approximately 14,000 bases. The process of splicing and editing pre-mRNA is a point of genetic regulation. Pre-mRNA can be edited through alternative splicing to yield different protein isoforms, which can have different — and sometimes opposing — functions. The role of alternative splicing in health and disease is becoming more widely appreciated, and mechanisms to modulate or alter the splicing process can be used therapeutically.

Exon Skipping in Duchenne’s Muscular Dystrophy

Duchenne’s muscular dystrophy is a uniformly fatal disease caused by mutations in DMD, the gene encoding dystrophin. Frameshift mutations in any of the 79 exons that produce an unstable or nonfunctional protein will cause Duchenne’s muscular dystrophy. Failure to produce dystrophin results in disconnection of the cytoskeletal elements from the sarcolemma. In patients with the disease, contractions of the muscle cause microtears in the sarcolemma, cell injury, cell death, and ultimately loss of muscle function.

Eteplirsen, an oligonucleotide agent approved by the FDA, induces the skipping of exon 51. Eteplirsen functions by hybridizing to a site within exon 51, thereby sterically blocking the splicing machinery from binding and forcing it to “skip” the exon to correct the frameshift mutation (Fig. 1). In most cases, the resulting in-frame mRNA is translated into dystrophin, and as a result the more distal, downstream exons are read in frame by the cell’s translational machinery.

The therapeutic potential of the exon-skipping approach is supported by the natural history of patients with Becker’s muscular dystrophy, who have in-frame mutations in central exons of DMD that lead to a shortened albeit functional form of dystrophin and a milder form of the dystrophic phenotype. The goal of treatment with exon-skipping agents such as eteplirsen is to convert severely damaging mutations in DMD exons into in-frame deletions by excluding exon 51 in order to mimic a milder phenotype (resembling Becker’s muscular dystrophy). Approximately 13% of boys with muscular dystrophy have mutations amenable to the skipping of exon 51. Drugs intended to target other, less commonly mutated exons are in development, but designing clinical trials that are powered to demonstrate statistical differences in smaller subpopulations of patients will be difficult, if not impossible. With tailored approaches such as these, the question of how to demonstrate evidence of therapeutic benefit remains a challenge.

Treatment of patients with exon 51–related Duchenne’s muscular dystrophy with a weekly intravenous infusion of eteplirsen yielded slight increases in the shortened mRNA encoding DMD and in the expression of dystrophin. There was some preservation of 6-minute walk distance in those receiving treatment as compared with historical controls. Although the interpretation of the results is difficult because of the modest extent of restoration of dystrophin, the small size of the trial, and the modest effect on outcome, the FDA approved eteplirsen on the basis of stipulations that both doses and patient numbers would be increased in subsequent trials. One reason for the weak activity of eteplirsen is that most of the drug is rapidly excreted through glomerular filtration and only a small fraction of the remainder is taken up by skeletal muscle. Indeed, delivery to target tissues after systemic delivery has been a key challenge in the development of oligonucleotide drugs.

Exon Inclusion in Spinal Muscle Atrophy

Spinal muscle atrophy is an autosomal recessive disease caused by mutations in SMN1 that result in loss of SMN1 protein function. Spinal muscle atrophy is manifested as muscle weakness, with the most severely affected infants never gaining head control. Degeneration of motor neurons in the ventral horn is followed by loss of respiratory function and death. SMN2 is a paralogous gene (i.e., it shares a
Figure 1. Exon Skipping Induced by Eteplirsen in Duchenne’s Muscular Dystrophy.

Shown is the transcript for dystrophin in the nucleus of a muscle cell with an indicated frameshift mutation upstream from exon 51 (Panel A). If uncorrected, the nonsense mutation leads to the creation of a nonproductive protein or to nonsense-mediated decay of RNA. The administration of eteplirsen and its internalization by the cell allow the oligonucleotide to hybridize to a site in exon 51 that causes the spliceosome to skip exon 51 and read the transcript in frame to produce a shorter but functional dystrophin-like protein (Panel B). DMD denotes the gene encoding dystrophin.
“common ancestor” gene with SMN1), with a coding region that would be identical to that of SMN1 if not for a single nucleotide change in exon 7 that causes the exon to be skipped. The SMN2 protein product is translated without the part encoded by exon 7; it is short-lived (i.e., degraded rapidly) and therefore nonfunctional.18 However, some fraction of the SMN2 mRNA is translated into SMN protein. This observation, taken together with the fact that there can be multiple copies of SMN2 (because it is subject to copy-number variation), each contributing to SMN levels, explains the variability in the severity of the condition.

If the fraction of SMN2 that is translated into a functional protein could be increased with the use of oligonucleotides to induce the expression of exon 7, a sufficient amount of functional SMN protein could be formed to rescue neuronal function19-21 (Fig. 2). By synthesizing a number of antisense sequences in areas in the intronic region 3′ to exon 7, an oligonucleotide (nusinersen) was identified that effectively forced inclusion of exon 7.22 When hybridized to its target site, nusinersen blocks the splicing signal that causes the exclusion of exon 7. Consequently, the mRNA is processed to include the formerly missing exon 7 of SMN2. The SMN2-related SMN protein, now fully functional, can serve as a substitute for the SMN that is missing in these patients.

Treatment with nusinersen led to production of a functional SMN protein, rescue of motor neurons, and more normal neuronal development.23 The chemistry of nusinersen includes the methoxyethyl modification of the 2’ position of the ribose sugars that increases affinity to its cognate sequence. That modification, combined with the replacement of the phosphodiester linkages between ribose sugars with phosphorothioate linkages, confers nuclease resistance, which reduces susceptibility to nuclease degradation, increasing tissue half-life such that nusinersen can be administered infrequently.

Successful clinical trials in which four loading doses were used during the first month of treatment followed by maintenance doses every 4 months thereafter produced statistically significant increases in the motor function of infants5 and older children. This use of nusinersen was recently approved by the FDA.23

**CLEAVAGE-BASED MECHANISMS FOR THE DESTRUCTION OF mRNA**

Several oligonucleotide drugs in clinical trials and three FDA-approved drugs use sequence-driven cleavage mechanisms to reduce levels of a disease-related mRNA and its protein product. Two drugs, mipomersen and inclisiran, use different cleavage mechanisms to modify cholesterol disposition. The two drugs each target a different gene product, each of which is important in hypercholesterolemia, underscoring the versatility of oligonucleotide therapeutic agents.

The common factor in each mechanism — as a result of the hybridization of each oligonucleotide drug to the target — is the activation of endogenous enzymes, which results in cleavage of the targeted mRNA at the site of hybridization.

Mipomersen is a single-stranded oligonucleotide with a sequence that is complementary to a portion of the RNA encoding apolipoprotein B, a component of low-density lipoprotein (LDL) cholesterol that is produced in the liver. The 20-mer oligonucleotide is chemically modified to increase its affinity for RNA and its stability. The central portion of the sequence of mipomersen has DNA-like properties, and when mipomersen hybridizes to the pre-mRNA for apolipoprotein B, the presence of the DNA–RNA heteroduplex attracts and activates RNase H, which cleaves the mRNA in the heteroduplex (Fig. 3). Cleavage renders the apolipoprotein B mRNA inactive, thereby reducing the amount of apolipoprotein B that is produced. As a result, the export of very low-density lipoprotein cholesterol from the liver is reduced and, ultimately, circulating levels of LDL cholesterol are diminished, even in patients for whom statin therapy is ineffective.24-27 The FDA approved mipomersen in 2013 for the treatment of homozygous hypercholesterolemia.

Inclisiran is an experimental therapeutic agent that induces cleavage of the mRNA encoding proprotein convertase subtilisin–kexin type 9 (PCSK9), an enzyme that negatively regulates levels of the LDL receptor (LDLR).24,28 Persons with naturally occurring genetic variants that reduce the activity of PCSK9 have increased LDLR levels, reduced LDL cholesterol levels, and reduced cardiovascular risk as compared with persons who do not have these variants.29 Inclisiran cleaves and inactivates PCSK9 mRNA, which has the effect of decreasing levels of the PCSK9.
Nonsense or frameshift mutations in \(\text{SMN1}\) lead to nonsense-mediated decay of RNA, and no functional protein is created (Panel A). Pre-mRNA for \(\text{SMN2}\) has a natural variant in exon 7 (in red) that results in the exclusion of exon 7 from 90% of mature \(\text{SMN2}\) mRNAs. After treatment with nusinersen (Panel B), the oligonucleotide hybridizes to a site within just 3’ of the boundary of exon 7, which forces the inclusion of exon 7 into the mRNA and thereby increases the amount of full-length \(\text{SMN2}\) produced by \(\text{SMN2}\), rescuing the cell from \(\text{SMN}\) deficiency.

**Figure 2.** Exon Switching Induced by Nusinersen in Spinal Muscular Atrophy.
Figure 3. RNase-Mediated Cleavage of Apolipoprotein B mRNA.

A hepatocyte translates the mRNA for apolipoprotein B (APOB) into the protein component of low-density lipoprotein (LDL) cholesterol. After treatment with mipomersen, the oligonucleotide hybridizes to the mRNA, and the DNA portion of the oligonucleotide attracts and activates RNase H, which recognizes the RNA–DNA heteroduplex and cleaves the APOB mRNA, reducing the output of very low-density lipoprotein (VLDL) cholesterol and ultimately the levels of LDL cholesterol in circulation.
and therefore increasing both LDLR levels and the clearance of LDL cholesterol and reducing circulating levels of LDL cholesterol. Inclisiran is being tested in late-stage clinical trials (e.g., NCT03397121, NCT03400800, NCT03705234, and NCT03399370).

Inclisiran cleaves mRNA through a mechanism distinct from that of mipomersen. Inclisiran is a double-stranded, small interfering RNA (siRNA). One of the RNA strands of inclisiran is complementary to a portion of PCSK9 mRNA. Once inclisiran enters the cell, the complementary strand (or guide strand) is loaded into the RNA-induced silencing complex (RISC), a protein complex that displays the strand to the intracellular milieu. Once a near-perfect complementary sequence (within an mRNA molecule) hybridizes with part of the guide strand, an enzyme that is part of RISC cleaves the mRNA. The mRNA cleavage products cannot be translated, and PCSK9 protein levels are thereby reduced (Fig. 4). Inclisiran is chemically modified to increase its stability against endogenous nucleases, conferring a durable effect and months of therapeutic activity after each dose. In fact, single doses of inclisiran have shown pharmacologic activity for more than 3 months in clinical trials, permitting dosing as infrequently as quarterly or even twice a year.

Drugs that trigger RNase H–mediated and RISC-mediated cleavage (inotersen and patisiran, respectively) are being used in the treatment of transthyretin (TTR) amyloidosis. In this form of amyloidosis, mutations in TTR induce misfolding of the protein product, which results in the formation of amyloid deposits in multiple tissues, including peripheral neurons and the heart. Both drugs produce dose-related reductions in circulating TTR levels that ameliorate the disease state, and both have been approved by the FDA. In phase 3 trials, these drugs were associated with limiting the progression of neuropathy and improving quality-of-life measures.

**Challenges for Oligonucleotide Therapeutic Agents**

Similar to other new technological developments in medicine, including monoclonal antibody therapy and gene therapy, the field of oligonucleotide-based treatment has overcome a number of challenges during a period of maturation. Challenges related to chemistry and manufacturing have been addressed. Synthetic sources of oligonucleotide precursors have reduced the costs of manufacture, and chemical modifications to oligonucleotides have improved resistance to metabolism by nucleases and produced more favorable pharmacokinetic profiles.

Despite considerable progress, two major hurdles stand in the way of widespread application of oligonucleotide therapeutics: drug safety and delivery. The administration of oligonucleotides has been associated with the activation of innate immunity through interactions with toll-like receptors (TLRs). Some oligonucleotides bind to TLRs and induce immune responses similar to those induced by viral and bacterial RNA and DNA. Different members of the TLR family are activated by single-stranded, DNA-like oligonucleotides (e.g., RNase H–dependent and splice-skipping oligonucleotides), and different sequence
Proprotein convertase subtilisin–kexin type 9 (PCSK9) down-regulates levels of the low-density lipoprotein (LDL) receptor (LDLR) on the cell surface. Inclisiran, conjugated to trinary N-acetylglucosamine residues, binds to the asialoglycoprotein receptor on the surface of hepatocytes and is internalized. When the guide strand for inclisiran is loaded into RNA-induced silencing complex (RISC), the complex scans mRNAs for homologous sequences. Hybridization with the mRNA for PCSK9 induces mRNA cleavage, thus inhibiting the expression of PCSK9 protein. Reducing the negative regulator of PCSK9 increases the levels of LDLR, thereby enhancing clearance of LDL cholesterol from circulation.
motifs have been identified as agonists of TLR family members. These immunostimulatory effects can be minimized by avoiding these sequence motifs and using chemical modifications.\textsuperscript{34-38}

The proinflammatory nature of single-stranded phosphorothioate oligonucleotides stymied some early therapeutic programs. Injection-site reactions were commonly observed after subcutaneous injections of antisense drugs, including mipo-mersen and the splice-skipping drug candidate drisapersen.\textsuperscript{39,40} Constitutional symptoms such as fever, chills, and rigors have been associated with high doses of phosphorothioate oligonucleotides.\textsuperscript{41,42} The response of adaptive immunity has been more muted. Treatment with some sequences of single-stranded phosphorothioate oligonucleotides has been associated with weak titers of antidrug antibodies.\textsuperscript{40}

The use of some siRNA therapeutics in clinical trials may be associated with another liability: inflammatory responses to the lipid nanoparticle formulations used to promote the uptake of siRNAs. Lipid nanoparticles are known to induce a complex antiviral-like response of innate immunity.\textsuperscript{5,44} To diminish the immunostimulatory effects of the formulations, siRNAs in lipid nanoparticles have been administered in combination with antihistamines, nonsteroidal antiinflammatory drugs, and glucocorticoids.\textsuperscript{3,4,31}

Other challenges for single-stranded phosphorothioate oligonucleotides include renal accumulation and a rare but notable reduction in platelet count.\textsuperscript{30,40} Single-stranded, phosphorothioate-modified oligonucleotides are generally protected from glomerular filtration because they are bound to plasma proteins. However, the small unbound fraction is readily reabsorbed by renal proximal tubular cells. Mild, low-molecular-weight proteinuria and, in rarer instances, glomerular nephritis have been reported in some patients after treatment with some phosphorothioate oligonucleotides.\textsuperscript{30,40,45} An analysis of data on renal function from a database of approximately 2400 patients suggested no clinically meaningful changes in levels of protein, creatinine, or plasma urea across multiple sequences.\textsuperscript{46} In a phase 1 trial, the administration of a single-stranded phosphorothioate oligonucleotide at a dose of 5 mg per kilogram of body weight per week, modified with locked nucleic acids, produced acute tubular necrosis.\textsuperscript{47,48} This toxic effect has not been observed with other oligonucleotide drugs and appears to be sequence-related but nevertheless has led regulatory authorities to recommend increased surveillance for renal toxicity in clinical trials involving phosphorothioate-modified oligonucleotides.

At least three phosphorothioate oligonucleotides have produced marked thrombocytopenia in small subgroups of patients in clinical trials. These events occurred in three unrelated indications with no overlap in oligonucleotide sequences. Platelets dropped precipitously to class 4 thrombocytopenia in 3% of patients receiving long-term treatment (14 to 26 months) with drisapersen at a dose of 6 mg per kilogram per week\textsuperscript{40,45} but the condition has also been observed in patients receiving treatment with phosphorothioate-modified sequences for triglyceridemias and transthyretin amyloidosis.\textsuperscript{30,49}

All observed toxic effects are dose-related. Some adverse effects may be minimized with newer versions of oligonucleotide drugs in which the chemistry has been improved or the delivery made more efficient in an effort to reduce doses.

Delivery remains one of the greatest challenges to more widespread application of oligonucleotide therapeutics. Because oligonucleotide drugs have molecular weights in the range of 5 to 15 kDa and are hydrophilic in nature, their ability to penetrate cell membranes is limited, thus diminishing access to their site of activity in cytoplasmic or nuclear compartments. The first successful delivery strategy for an oligonucleotide agent was intravitreal administration of fomiversen; this was approved in 1998 for the treatment of cytomegalovirus retinitis, which was followed more recently by nusinersen, which is locally administered through intrathecal injection for spinal muscle atrophy.

Systemic delivery to most organs and tissues, with the exception of the liver, has proved to be challenging. After intravenous or subcutaneous delivery, phosphorothioate oligonucleotides and siRNAs in lipid nanoparticles are taken up primarily by nonparenchymal cells and to a lesser extent by hepatocytes. N-acetylgalactosamine (GalNAc) conjugated to oligonucleotides binds to the asialoglycoprotein receptors (ASGPR) on hepatocytes to transport and release the oligonucleotides into the intracellular compartment.\textsuperscript{50} This receptor-mediated uptake allows for dosing that is lower than that required for the therapeutic delivery of unconjugated oligonucleotides.\textsuperscript{8} Both single-stranded and double-stranded oligo-
nucleotides can be delivered to hepatocytes with the use of GalNAc conjugates. ASGPR-directed delivery to hepatocytes is being used in the experimental treatment of diseases related to hepatocyte-derived protein products and liver diseases. Delivery to other cell types, such as muscle cells, can be accomplished by targeting antibodies or antibody fragments against cell-surface proteins known to be involved in intracellular transport.51

Polymeric nanoparticles have been used to deliver oligonucleotides, but the efficiency with which the liver clears plasma of nanoparticles makes it difficult to direct delivery to other tissues and organs. The technology has not progressed beyond early clinical studies with an siRNA payload.52

In nature, the transfer of genetic information between cells is mediated by membrane-bound nanovesicles or exosomes, which bud from some cell types. Exosomes range from 20 to 200 nm in diameter and are known to move between cells, delivering mRNA, microRNA (miRNA), and proteins that modulate neighboring cell function.53-55 Early studies involving harvested exosomes loaded with synthetic miRNAs exploit exosome membrane proteins and sugars for delivery to distal sites.56-59 The growing body of information on naturally circulating exosomes, their membrane composition, and the membrane-associated proteins will lead to better delivery systems.

### Future Directions

The field of RNA-targeting therapeutics is currently dominated by oligonucleotides that act through cleavage mechanisms and splice modulation. However, appreciation for other RNA-based mechanisms is growing (Table 1).

Argonaute 2 (Ago2) is one of the key enzymes in RISC and is responsible for RISC-mediated cleavage of mRNA targets. However, when Ago2 is loaded with an RNA that can hybridize in the vicinity of the promoter region of a gene, the Ago2-bound RNAs can activate transcription, which can result in a paradoxical increase in mRNA expression in a process known as RNA activation (Fig. 5).61-63 This mechanism is used in a drug in a phase 1 trial (NCT02716012) of hepatocellular carcinoma to increase the expression of CCAAT/enhancer-binding protein α (C/EBPα), which is associated with the differentiation of hepatocytes.64

One endogenous regulatory process for RNA editing that can be exploited for therapeutic use is the enzymatic conversion of adenosine to inosine.65,66 Because the nucleobase inosine is recognized as guanine, adenosine-to-inosine editing has the potential to change the amino-acid composition of a protein by altering the reading of a single codon or by introducing or negating a termination codon. Enzymes called ADARs (adenosine deaminase acting on RNA) have affinity for specific sites on RNA that induce adenosine-to-inosine editing in the presence of specific oligonucleotide guide strands. Therapeutic uses of the process are being explored.67

Other forms of oligonucleotide-directed editing that are moving toward clinical applications are variations on CRISPR (clustered regularly interspaced short palindromic repeat) technology. CRISPR–Cas9 systems are designed to use a

### Table 1. Current and Potential Mechanisms of RNA-Based Therapies.

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<td>Editing of RNA</td>
<td>Single-stranded oligonucleotide structured to attract adenosine deaminase or RNA coadministered with exogenous Cas enzymes</td>
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guide RNA (sgRNA) to direct editing by an exogenous endonuclease, Cas9, to target sites in DNA for correction of mutations in the genome or to deactivate mRNA using sgRNAs designed to hybridize to mRNAs and other exogenous Cas enzyme variants.

The basic concept used is the same as that for other oligonucleotide drugs: the sequence of the oligonucleotide agent (in this case, sgRNA) hybridizes to a specific region and then attracts an effector to the region of hybridization. CRISPR systems use an exogenous enzymatic entity that

Figure 5. Increasing Protein Expression by Increasing DNA Transcription.
When a double-stranded (ds) RNA loads into Argonaute 2 (Ago2), RNA helicase A (RHA) unwinds, and one strand (designated as the passenger strand) is shed from the Ago2 complex. The loaded Ago2 is transported into the nucleus, where it can interact with RHA. The Ago2–RHA complex then scans for sequences with complementarity. Once associated with DNA, the Ago2–RHA complex attracts the polymerase-associated factor 1 (PAF1) complex, which together form the RITA (RNA-induced transcriptional activation) complex, which in turn attracts and activates RNA polymerase II and results in increased expression of that mRNA.
is coadministered with the sgRNA. The requirement for the intracellular delivery of a bacterial enzyme (Cas9) is a major challenge for the technology and is complicated by the presence of preexisting antibodies to the bacterial enzyme.

Oligonucleotide-directed editing of RNA and DNA has advantages and disadvantages. As a treatment, RNA editing is more druglike than DNA editing, with effects reversing after the exogenous editing oligonucleotides are metabolized and eliminated from the body. As a result of this reversibility of pharmacologic activity, any unintended effects would be reversible as well. Although genome editing at the DNA level has the advantage of being potentially permanent and curative, it also carries the risk of making unintended genomic changes permanent and heritable. The reversibility of pharmacologic activity associated with RNA-mediated mechanisms may be an advantage in some cases.

Another promising area of therapeutic research for oligonucleotides pertains to regulatory RNAs. These RNAs, once considered the “dark matter” of the genome, are transcribed but not translated into protein. Some intronic and intergenic sequence information is transcribed into RNAs with regulatory functions, including miRNAs and long noncoding RNAs.

MicroRNAs are regulatory RNAs that load into RISC, inhibiting the function of complementary mRNAs by blocking translation or by cleaving the mRNA through an RISC-based mechanism analogous to that used by siRNAs. MicroRNAs fine-tune gene expression, and each miRNA may reduce the expression of numerous mRNAs. Because miRNAs negatively regulate expression of their target genes, inhibition of the miRNA function will increase the expression of the miRNA-targeted mRNAs. There are multiple miRNA inhibitors in clinical development, including one inhibitor that has an antiviral mechanism.

An miRNA that supports the replication of lymphocytes is miR-155. Clinical trials of an inhibitor of miR-155 for the treatment of cutaneous T-cell lymphoma are in progress (e.g., NCT02580552 and NCT03713320). Some disease states are associated with reductions in miRNA expression, and in such cases, synthetic miRNA supplementation can be therapeutic. For example, in various fibrotic conditions, miR-29 expression is reduced; miR-29 down-regulates the expression of multiple collagens, of elastins, and of TGF-β, and administration of synthetic miR-29 can reduce fibrogenesis. Clinical trials with subcutaneously administered synthetic miR-29 are in progress to assess its role in scarring (e.g., NCT02603224 and NCT03601052).

Long noncoding RNAs have multiple regulatory functions in the transcription and translation of mRNAs. To date, these RNAs have not been targeted for therapeutic activity in clinical trials. As the understanding of the role that various RNAs play in cellular regulation and disease progression increases, these long noncoding RNAs will represent potential targets for oligonucleotide-based RNA-targeting drugs.

A new appreciation for the role of RNAs as regulators of gene expression and for the role of RNA splicing in health and disease renders the concept of using Watson–Crick base-pairing rules to design new therapeutic agents even more attractive today than in the 1980s, when the idea was first described. The maturation of the technology over the past several decades has made it possible for RNA-targeting oligonucleotides to fulfill the promise of using genomic information in drug design.

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

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TREATING DISEASE WITH OLIGONUCLEOTIDES


