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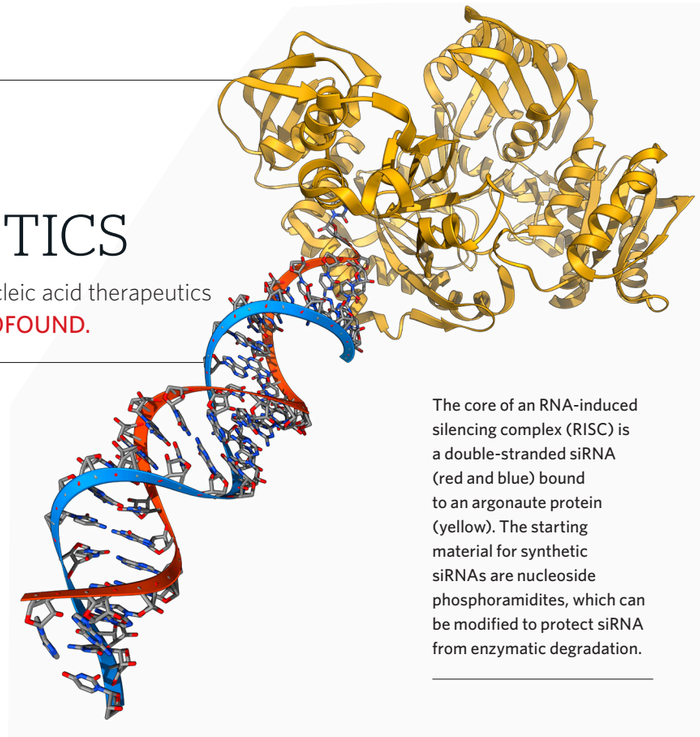
THE EXPANDING FRONTIER OF NUCLEIC ACID THERAPEUTICS

While mRNA vaccines have received intense attention of late, another class of nucleic acid therapeutics has been growing steadily in recent years. ITS IMPACT COULD BE EQUALLY PROFOUND.



The core of an RNA-induced silencing complex (RISC) is a double-stranded siRNA (red and blue) bound to an argonaute protein (yellow). The starting material for synthetic siRNAs are nucleoside phosphoramidites, which can be modified to protect siRNA from enzymatic degradation.

After decades of false starts and setbacks[1], nucleic acid-based therapeutics are now becoming regulars in the clinic[2]. The approval of mRNA vaccines for Sars-CoV-2 is perhaps the most widely recognized example, but other medicines, most prominently those based on antisense oligonuclueotides (ASOs) and short interfering RNAs (siRNAs), have garnered a string of approvals as well[3].

Nucleic acid therapeutics use engineered sequences of nucleotides to selectively modulate gene expression, allowing doctors to control the symptoms of a genetic disease without any permanent DNA modifications. Most currently approved indications are for rare diseases, but in those cases, the impacts of the therapies can be profound. For example, Biogen's nusinersen, an ASO therapy for the fatal neuromuscular condition spinal muscular atrophy (SMA), can reduce by half the likelihood of death or the need for mechanical ventilation in treated infants[4].

"This is the culmination of a long period of progress in chemistry," explains Matthew Wood, a researcher at the University of Oxford who specializes in nucleic acid therapeutics for neurological disorders. To synthesize and deliver oligonucleotides as a precisely dosed medicine was only possible after decades of fundamental research.

With many such obstacles overcome, along with a handful of regulatory approvals granted, ASO- and siRNA-based therapies have received a shot in the arm. Dozens of candidates are now in clinical trials, with more in pre-clinical testing. Researchers are also defining a clear methodology for producing new nucleic acid medicines, paving the way for the rapid development of therapies for virtually any disease with an underlying genetic cause.

The power of gene silencing

One of the most fundamental processes in molecular biology is the transcription of nuclear DNA into mRNA, which is then translated into proteins. ASOs and siRNAs both disrupt this process, effectively silencing genes without making genetic modifications. While the technologies offer a similar outcome, the mechanisms they employ are quite different.

ASO technology, which dates to the late 1970s[5], uses synthetic strands of 15 or 20 nucleotides to create a DNA or RNA[6] molecule that complements and binds to a sense strand of DNA or the mRNA directly. When an ASO binds to its target, it physically blocks or modifies protein translation. A handful of ASO drugs have been approved based on this strategy, such as Ionis's inotersen, which prevents accumulation of toxic protein clumps in the liver in a condition known as hereditary transthyretin-mediated (haTTR) amyloidosis.

siRNAs are double-stranded RNA molecules designed to complement a target mRNA sequence. Rather than binding to an mRNA directly, siRNAs are integrated into protein assembly known as the RNA-induced silencing complex (RISC). This complex uses its RNA component to recognize the target mRNA, which is subsequently destroyed by a RISC-associated enzyme.

Discovered in the 1990s, RNA interference, or RNAi, sparked great interest from drug developers for its potential to selectively suppress gene expression. It even earned the researchers behind the discovery, Andrew Fire and Craig Mello, a Nobel Prize in 2006. Yet drug developers struggled to shepherd siRNA therapies into the clinic. It wasn't until 2018 that Alnylam gained approval for the first siRNA drug, the hATTR amyloidosis therapeutic patisiran.

Since then, interest in siRNA therapies has overtaken ASOs. "If you look at efficacy, duration of effect, and safety profile, there's no question that siRNAs are way superior to antisense," says Anastasia Khvorova, an RNA therapeutics specialist at the University of Massachusetts Medical School.

Even so, ASOs can achieve some tasks out of reach for siRNAs. Many diseases arise through splicing errors, in which mutated genes give rise to primary RNA transcripts that skip key protein instructions or include unwanted sequence elements. A well-designed ASO can overcome this.

"You're using them to mask critical regions on an mRNA that's being processed to allow the splicing machinery to skip over or include an exon," Wood says. Nusinersen is a potent example. In that therapy, ASOs force motor neurons to produce a functional version of a protein that is absent in SMA patients.

Reliable medicines, batch after batch

The development of successful medicines requires the delivery of consistent, precisely dosed therapies, which presented another challenge for nucleic acid therapies. In the body, naturally-occurring RNAs are quickly degraded by various enzymes. To be effective, any RNA-based therapy needs to resist rapid degradation.

Due in large part to advances in nucleotide chemistry, researchers have developed an arsenal of chemically-manipulated nucleotides that help synthetic RNA resist premature destruction.

"The ribose sugar of the nucleotide is modified with different molecules that give it more stability against RNases and other enzymes to increase the RNA half-life," explains Syed Raza, manager of chemical manufacturing at Thermo Fisher Scientific. The company produces a range of such modified RNA nucleotides, including the RNA phosphoramidites that are frequently a starting material for ASO- and siRNA-based therapies.

In addition to stabilizing RNA constructs in vivo, researchers need to deliver them to the appropriate tissues. One common method is to package them within lipid nanoparticles, or LNPs, which can target liver tissues or be delivered to tissues locally; patisiran employs this approach. However, LNPs can be complex to manufacture and can elicit an immune response, among other challenges[7].

A breakthrough in delivery came when scientists at Alnylam and their collaborators identified a 'skeleton key' for efficient drug delivery to the liver: a carbohydrate called GalNAc. The molecule binds to a receptor expressed near-exclusively in liver cells. GalNAc-coupled siRNAs efficiently reach that tissue after subcutaneous or intravenous injection. "You now have a technology that could be applicable to countless liver indications, just by solving that one challenge," Wood says. Three GalNAc-siRNA drugs have already entered the clinic.

As nucleic acid therapeutics have gained momentum, the research community has converged on a robust process for developing siRNA and ASO drugs. "We're an academic lab, but within two months, I can make a clinical-quality siRNA compound," Khvorova says. Her team typically begins with software to design a few hundred candidate siRNAs. The candidates are optimized to match the gene of interest and Khvorova's team incorporates various modifications essential for RNAi to function. The candidates are then tested in vitro to identify a handful of finalists, which are then screened in animals and, depending on results, in humans.

Because manufacturing for clinical applications requires stringent quality control in starting materials, most academic researchers source their drug candidates from vendors who specialize in the manufacture of high-purity, chemically-modified oligonucleotides. Just a few companies provide phosphoramidites of appropriate quality for synthesizing these clinical-grade oligonucleotides. "Currently, there are only three companies in the whole world who are large scale manufacturers of phosphoramidites," Raza says.

In an effort to bolster drug development in this space, researchers at Thermo Fisher Scientific and the European Pharma Oligonucleotide Consortium published a consensus article in February on best practices for assessing and reporting the quality of oligonucleotide starting materials. "We have a good understanding of what the critical impurities are, and have mostly eliminated those from our processes," Raza says. For academics and those in industry, the standards should help ensure that any eventual nucleic acid drugs deliver optimal safety and efficacy, with consistent performance across manufacturing batches.

New challenges, new opportunities

Although nucleic acid therapeutics are steadily gaining momentum, roadblocks remain. Targeted delivery of RNA drugs to tissues other than the liver remains a challenge. Khvorova notes that siRNA drugs delivered directly into the cerebrospinal fluid could have a potent effect on diseases like Huntington's disease or amyotrophic lateral sclerosis. "With advanced siRNA architectures that are specific to the central nervous system, a single injection in monkeys can give you six to 12 months duration of effect," she says. Researchers are also seeking other GalNAc-like modifications that might act as effective address labels for other tissues in the body.

Pricing is another hurdle. Many current RNA drugs are priced in the mid-to-high six-figures. While recouped R&D costs account for some of the cost, Wood notes that mass production is also partially to blame. He cites reliance on a costly manufacturing process in which RNA molecules are chemically assembled stepwise on to a solid support. "Can you develop solution-phase synthesis, where you basically put all the ingredients in a bucket and they self-assemble?" he asks. "People have been working on this, and I think this is something that's going to transform the field in the next decade."

Faster, lower-cost manufacturing at an industrial scale could help nucleic acid therapeutics address common genetic disorders at a cost acceptable to insurers and health systems. It could also benefit patients with ultra-rare diseases. The so-called 'N-of-1' disorders affect extremely small patient populations, complicating the economics of standard drug pricing. There is already proof of concept. An ASO drug called milasen was developed in 2019 for a single patient with an ultra-rare neurodegenerative condition called Batten disease[8].

Khvorova sees a bright future for such bespoke therapeutics. "I'm aware of at least 20 to 25 N-of-1 programs under development right now," she says, noting that a standardized drug design framework could lower the cost of R&D to just a few million dollars. "If we're ultimately talking about treating 500 orphan disease indications at $3 million apiece, it's not that high a number." ■

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