

THERAPEUTICS

A tale of two drugs

A rare genetic disease became a focus for two pharmaceutical companies that specialize in RNA therapies. Now, treatments for other hereditary conditions are within the sights of the industry.

BY LIAM DREW

In 2018, US and European regulators approved two RNA-targeted drugs for the rare genetic disease hereditary transthyretin (hATTR) amyloidosis, offering hope to around 50,000 people with the condition worldwide.

The disease is caused by mutations in the gene that encodes transthyretin, a protein in blood that is made by the liver. Such mutations lead to a decrease in the solubility of transthyretin, so that, instead of being transported through the body, the protein forms clumps around nerve fibres and in the cardiovascular system, progressively eroding tissue function.

The drugs that were approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) enter liver cells to thwart transthyretin production. Each binds to the messenger RNA that encodes the mutated protein. But these drugs are otherwise distinct, and represent the two most advanced strategies for targeting RNA therapeutically.

One of the drugs, inotersen (Tegsedi), is an antisense oligonucleotide (ASO). Typically, ASOs are short single strands of modified DNA that are designed to bind to a specific mRNA. They thereby suppress that mRNA's ability to guide protein synthesis, and often lead to destruction of the mRNA.

ASOs were discovered more than 40 years ago, and the developers of inotersen, Ionis Pharmaceuticals, founded in 1989 and based in Carlsbad, California, led the long process of

chemically modifying DNA to convert ASOs into functional drugs. Inotersen was only the fifth medication in this class to gain clinical approval.

The other approved treatment for hATTR is patisiran (Onpattro), which was developed by Alnylam Pharmaceuticals in Cambridge, Massachusetts. Patisiran comprises a short stretch of double-stranded RNA known as a short interfering RNA (siRNA) and is the fruit of Nobel-prizewinning research that showed plants and animals use a mechanism called RNA interference (RNAi) to control the abundance of mRNA. In RNAi, cells produce siRNAs that are complementary to sections of specific longer mRNAs. When an siRNA binds to its target mRNA, it catalyses the destruction of that molecule.

Alnylam was founded in 2002 with the goal of hijacking this pathway to make RNA therapies. Patisiran is the first RNAi-based drug to be approved.

When recounting the histories of their respective companies, Stanley Crooke, founder and chief executive of Ionis, and Kevin Fitzgerald, senior vice-president of research at Alnylam, tell similar stories. Biological technologies, they say, go through three phases during development. First is what Fitzgerald calls the "hope and promise" phase, in which investors bet big on the technology. But this is almost always followed by a phase of slow progress and growing scepticism, during which funding is harder to come by and developers must innovate to deliver on initial promises.

The third phase — if reached — begins with

the first promising data in human trials and proceeds to drugs getting approved for clinical use. According to Crooke, when this happens, people start to argue over who came up with the technology in the first place.

RNA therapies seem to be securely in the third phase. What remains to be seen is how widely they will affect clinical practice.

FIRST, THE LIVER

To work, RNA therapies must meet a number of criteria. Most importantly, ASOs and RNAi drugs should be stable enough to persist in the body for a useful length of time — around a day — to allow for a reasonable dosing regimen. On administration, they must reach their target organs, enter the target cells and then bind tightly to target mRNAs. The ASOs that existed when Ionis was founded in 1989 had none of these properties. And the first human trials in the 1990s revealed that they had serious side effects. In 2002, the development of RNAi drugs was facing many of the same issues.

In 1998, Ionis got clinical approval for fomivirsen (Vitravene) — an ASO based on early chemistry that treated CMV retinitis in people with AIDS. Even so, throughout the 1990s the company focused mainly on modifying DNA for use in ASOs.

By tweaking the chemical structure of DNA, researchers were able to make ASOs from synthetic analogues of DNA that retained the molecule's crucial property of binding to specific RNA targets, on the basis of Watson-Crick base-pairing, but that otherwise behaved differently from natural DNA. Gradually,



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Ellie Darby-Prangnell has a genetic condition called spinal muscular atrophy.

molecules emerged that were more resistant to nucleases, enzymes that form part of the body's mechanism for degrading stray nucleic acids. This

drastically increased the biological half-life of ASOs. Other improvements included binding to target RNAs more tightly and generating fewer inflammatory side effects.

David Corey, a pharmacologist at the University of Texas Southwestern Medical Center in Dallas, says that scepticism about ASOs grew during the 1990s, but at the start of the 2000s, the science suddenly became convincing. Corey attributes this to two major factors: the development of innovative classes of ASO, and the realization that researchers needed to focus on where ASOs accumulate in the body — the liver.

Alnylam discovered that the same was true of siRNA therapies, and therefore focused its attention on developing two strategies for getting these drugs into liver cells.

The first approach involves packaging siRNAs into vesicles comprising lipid nanoparticles, which mimic naturally occurring lipid droplets that are taken up by liver cells through the low-density lipoprotein (LDL) receptor. The second requires conjugating siRNA to a small molecule called *N*-acetylgalactosamine (GalNAc) that binds to and enters liver cells through a receptor that is selectively and highly expressed on such cells, a tactic that Ionis also used to further hone ASO delivery to the liver.

RNA therapies do not yet work as orally administered pills. They must be given by subcutaneous injection or, for lipid-nanoparticle formulations, by intravenous infusion — a process that requires a health-care professional. But the drugs now act for weeks or months, potentially compensating for these more cumbersome administration routes. Focus on the liver yielded the second ASO to gain FDA approval — 14 years after the first. Mipomersen (Kynamro), developed by Ionis, treats homozygous familial hypercholesterolaemia, an inherited disease that elevates cholesterol levels in the blood by reducing the production of a protein called apolipoprotein B in the liver. This was a very important drug, says Corey, because its intended effects on apolipoprotein B and cholesterol were directly observed in humans.

Although the main goal was to treat the genetic condition, Ionis saw mipomersen as a drug that might ultimately be used to tackle cholesterol levels in the general population. It touted the ASO as a future competitor of the cholesterol-lowering statin atorvastatin (Lipitor): infrequent, long-lasting injections that would replace daily pills.

But mipomersen can lead to liver toxicity and serious cardiovascular side effects. In 2012, it was refused a licence by the EMA, and clinicians in the United States favoured lomitapide (Juxtapid), a small-molecule drug developed by Aegerion Pharmaceuticals in Cambridge,

Massachusetts, that lowers apolipoprotein B levels by a different mechanism.

Side effects and ease of administration are important factors in drug development. People with conditions such as hATTR amyloidosis and other rare genetic disorders, for whom there might be no other treatment options, are often more willing to tolerate drugs with side effects. They also visit doctors regularly, which could explain why they are often amenable to more-involved drug-delivery routes.

Inotersen and patisiran are far from being perfect drugs. For example, inotersen is more conveniently given by subcutaneous injection, but recipients must be monitored for a decrease in blood platelet counts — a serious potential side effect. Patisiran has a better side-effect profile, but requires intravenous administration in a clinic, and those given the drug must take steroids to suppress infusion-related reactions. The relative success of these drugs will not depend simply on their efficacy, but also on patients' experiences of taking them.

MASS APPEAL

Using RNA therapies to treat genetic liver conditions seems to be an approach that works. In May, Ionis gained approval from the EMA (but not the FDA, when it considered the application in August 2018) for its third liver-acting ASO, volanesorsen (Waylivra), to treat a hereditary disorder that elevates levels of fats called triglycerides in the blood. And in August, the FDA granted givosiran priority-review status. The drug is an siRNA developed by Alnylam for acute hepatic porphyria, another inherited liver disease.

But researchers in the field hope that liver-targeting RNA therapies will soon be used to treat more-common disorders. There are more than ten ongoing phase III clinical trials of such therapies for liver-related diseases. Ionis, for example, has developed candidate ASO-based treatments for thrombosis, which target a blood-clotting factor made in the liver, as well as an ASO that aims to decrease levels of apolipoprotein A — high levels of which are a risk factor for cardiovascular disease, and for which no lowering strategy is available.

It is probable, however, that a GalNAc-conjugated siRNA called inclisiran will be the first RNA therapy to hit the mass market. Developed initially by Alnylam for familial hypercholesterolaemia, in 2013, it was licensed to The Medicines Company in Parsippany, New Jersey, with a view to using it in people with high cholesterol levels not caused by genetic conditions. In 2018, The Medicines Company sold off its other assets to focus on inclisiran exclusively.

The drug inhibits the synthesis of PCSK9, an enzyme that regulates the uptake of LDL cholesterol by liver cells, such that lowering PCSK9 expression reduces LDL cholesterol levels in the blood (high LDL levels are another risk factor for heart disease). A phase III trial of the drug in people who had experienced a heart attack or stroke showed a roughly 50%

decrease in LDL cholesterol levels.

Peter Wijngaard, chief development officer at The Medicines Company, says the drug's appeal is that it needs to be given only twice a year, which guarantees six months of lower cholesterol after each physician-administered dose. The company is preparing to file an application for FDA approval later this year. In the long term, if the drug is successful in people at high risk of a cardiovascular event, it could be used in prevention and as an alternative to statins, Wijngaard adds.

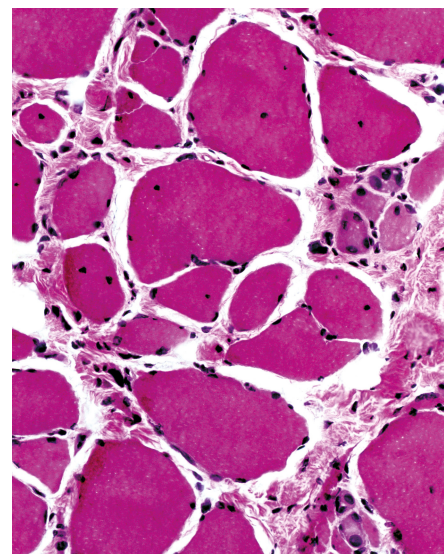
BEYOND THE LIVER

Blocking the translation of mRNA into protein is the most straightforward and common application of RNA therapies. Indeed, catalysing mRNA degradation is the only mechanism of action of siRNA-based drugs. But ASO technology has another string to its bow.

When an mRNA is transcribed from a gene, it contains both introns and exons. Exons are stretches of mRNA that dictate the sequence of the resultant protein. Introns are stretches that are excised before protein synthesis begins, in a process called splicing. ASOs that bind to an unprocessed mRNA can influence how it is spliced into a mature mRNA.

Two drugs that alter RNA splicing have now been approved by the FDA, and, notably, neither targets the liver. One, aimed at skeletal muscle, was controversially approved for treating Duchenne muscular dystrophy (DMD). The other is RNA therapy's first blockbuster drug — a highly effective treatment for spinal muscular atrophy that operates in the central nervous system, a tissue that has become the field's second frontier.

DMD is caused by mutations in the gene that encodes the protein dystrophin, which protects muscle fibres from the damaging effects of contraction. When a mutation causes the loss of functional dystrophin,



Light micrograph of tissue affected by Duchenne muscular dystrophy, in which some of the muscle fibres (dark purple) have been destroyed.

muscles progressively deteriorate and then die. Dystrophin mRNA typically comprises 79 exons. Importantly, even if an exon is missing, functional dystrophin can still be made. ASOs can be used to treat some DMD-causing mutations that render the mRNA downstream of the mutations unreadable, such that no functional protein is formed.

In the early 2000s, geneticists showed that ASOs binding to dystrophin mRNA could influence splicing in such a way that an exon containing a harmful mutation was left out of the mature mRNA (A. Aartsma-Rus *et al.* *Neuromusc. Disord.* **12**, S71–S77; 2002). Known as exon skipping, this resulted in the downstream mRNA no longer being unreadable and in functional dystrophin being formed.

Consequently, two groups of researchers set to work on developing exon-skipping ASOs for DMD. One team, led by geneticist Annemieke Aartsma-Rus at Leiden University Medical Center in the Netherlands, tested a compound in nearly 300 people, but it failed to reliably improve clinical outcomes.

The second team — led by researchers from Sarepta Therapeutics in Cambridge, Massachusetts — showed that its drug, eteplirsen (Exondys 51), raised dystrophin levels in recipients, but only by about 0.28%. The drug was, however, approved in a contentious FDA hearing in which family members of people with DMD gave impassioned pleas in favour of the drug and the chairperson overruled the recommendation of an expert panel. The EMA, when presented with the same data, refused to approve the application.

The field now anxiously awaits completion of an obligatory post-approval clinical trial that must show improvements in participants' symptoms. Aartsma-Rus worries that the FDA's decision might backfire: if the follow-up trial fails, regulators could hesitate to approve future drugs solely on the basis of them increasing protein levels, even when those increases are large.

She is working on the next generation of ASOs. "Exon skipping still has a place," she says, "but there's room for improvement." Aartsma-Rus's optimism stems from the fact that eteplirsen is an early type of ASO that doesn't contain the chemical modifications that have subsequently improved ASO efficiency. But targeting muscle tissue remains a challenge.

Another reason to be hopeful is the success of a blockbuster drug for spinal muscular atrophy (SMA), a genetic condition that affects motor neurons. It progressively diminishes a person's ability to move and, in children, can fatally disable the muscles that control breathing.

The condition occurs when a person inherits two faulty copies of the gene *SMN1*. But the human genome contains another version of this gene, *SMN2*, that is expressed at a much lower level. The protein encoded by *SMN2*

can, however, substitute functionally for that encoded by *SMN1*. Nusinersen (Spinraza), an ASO developed by Ionis, binds to *SMN2* mRNA to promote the retention of an exon that increases the production of SMN2 protein to such an extent that it can compensate for the absence of SMN1. Frank Bennett, co-founder of Ionis, who leads its drug-development programme for SMA, had concerns about tackling nervous-system disorders using ASOs.



Logan Velchansky (front) began treatment with Spinraza soon after it was commercialized.

Although he knew that the drugs worked well in neural tissue, he also knew that they did not cross the blood–brain barrier. To treat such a condition, ASOs would have to be given by lumbar puncture — an invasive procedure that it was hard to imagine subjecting patients to on a weekly basis.

But when Ionis began to test ASOs in the central nervous system (CNS) of rodents, monkeys and then humans, it discovered that the drugs stayed active for a long time. In people, nusinersen is effective when administered every 4–6 months. And trials of it were a success. Some of the children showed improvement and started to hit developmental movement milestones. Those who began the treatment before their symptoms appeared fared even better.

Bennett and his collaborator Adrian Krainer, a splicing researcher at Cold Spring Harbor Laboratory in New York, received a 2019 Breakthrough Prize for their work on the drug. Crooke says, "If I'd done nothing except Spinraza in my career, I'd be happy."

NEXT STEPS

Such is the lasting effectiveness of ASOs in neurons that tackling CNS disorders represents a major goal for RNA-therapy researchers. Ionis has gained positive phase I/II trial results in people with Huntington's disease and similar phase I results in those with familial amyotrophic lateral sclerosis (also known as familial motor neuron disease). It has also launched ASO programmes for other neurological diseases. In April 2018, biotechnology

company Biogen in Cambridge, Massachusetts, invested US\$1 billion in Ionis's neuroscience programme. And a year later, Regeneron Pharmaceuticals of Tarrytown, New York, backed Alnylam's eye and CNS RNAi programme to the tune of \$800 million, with Alnylam set to develop siRNA-carrying molecules analogous to GalNAc for use in those tissues. This work forms part of a dynamic ecosystem of drug development that involves at least 18 companies that are participating in ongoing clinical trials of more than 50 RNA-targeting drugs.

As well as increasingly eyeing up common disorders, ASO developers are helping to tackle extremely rare conditions. An initial promise of ASOs was that, after a suitable chemical structure had been achieved, subsequent ASO drugs based on the same structure — varying only by nucleotide sequence — would have a similar pharmacology. Each fresh sequence would have its own off-target effects, but its well-characterized basic structure would enable pharmacologists to predict tissues and cells that the drug would target, and how often a dose would need to be administered. (By contrast, for small-molecule drugs, these parameters must be determined from scratch.)

Such cumulative knowledge is useful for developing any drug, but it could be transformative for extremely rare conditions. For example, ASOs tailored to specific genetic mutations are being used by doctors at Boston Children's Hospital, Massachusetts, to treat a nine-year-old girl with the hereditary neurodegenerative condition Batten disease, and by neurologists at Columbia University Irving Medical Center in New York City to treat a 26-year-old woman with a rare form of familial amyotrophic lateral sclerosis.

Assessing drug effectiveness in trials involving only one patient is challenging — unless there are very clear effects. But they are helping to establish a framework for developing therapies for extremely rare genetic conditions.

RNA therapies stand on the cusp of a period that will define their wider adoption. There will be further cases in which RNAi- and ASO-based treatments go head-to-head, although Aartsma-Rus says that it won't be "as simple as one is always better than the other". The specifics of each condition and its treatment strategy will determine which will be the best therapeutic agent.

There is also increasing success using gene therapy, as well as the excitement that swirls around the gene-editing technology CRISPR–Cas9. Crooke sees that as a perfect example of the early wave of hope that fresh technologies attract, which isn't to say that it won't become an influential clinical tool — just that there is much work still to be done. "There will be competition for all these drugs," says Crooke. "That's the nature of these things." ■

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