NEIL WEBB

DRUG DISCOVERY

Pharma's RNA roller coaster

After a groundswell of hype and a sceptical backlash, the pharmaceutical industry is learning how to leverage RNA interference in the clinic.

BY MICHAEL EISENSTEIN

Fortunes can shift precipitously in the drug-discovery world. At the start of the twenty-first century, all eyes were focused on a powerful gene-silencing technology called RNA interference (RNAi), and many companies saw the almost limitless potential of harnessing the tool to manipulate genes implicated in diseases. By 2006, Andrew Fire and Craig Mello, the biologists who discovered RNAi, had received the Nobel Prize in Physiology or Medicine, and large pharmaceutical companies were pouring billions of dollars into RNAi start-ups.

Just four years later, this exuberance had given way to despair — and the money was drying up. "People started giving up hope," says John Maraganore, chief executive of Alnylam Pharmaceuticals — one of the first biotechnology companies to pursue RNAi therapy. "They started thinking that drugs would never come out of this, and the pharmaceutical industry left the space." The problem was not the mechanism behind RNAi, which quickly became a central element of the genetics-research toolbox, but rather the difficulties associated with safely delivering intact molecules of gene-modulating RNA to target tissues.

Even as investors fled, a small number of companies soldiered on, shifting strategies and therapeutic targets, and finally notching up some victories in clinical trials. In 2018, Alnylam, which is based in Cambridge, Massachusetts, netted the first approval for an RNAi therapy in both the United States and Europe. That drug, patisiran (Onpattro), is used to treat hereditary transthyretin (hATTR) amyloidosis, a build-up of the protein amyloid in nerves and organs. Several other biotech companies have also got RNAi candidate drugs in their pipelines, and the despair from a decade ago is all but forgotten. "There's now a sense of destiny in the field," says Douglas Fambrough, chief executive and co-founder of Dicerna Pharmaceuticals in Cambridge. Such confidence was made possible only after learning some costly and difficult lessons — a body of knowledge that has helped to distinguish the true potential of RNAi therapy from the hype that accompanied its birth.

BILLION-DOLLAR BABY

RNAi-therapy researchers know that many existing medicines received approval only after the technologies that underpin them took a serious tumble. Gene therapy, for example, has a troubled history, in which the death of a teenage boy during a clinical trial in 1999 derailed the field for years; the first such product to receive approval from the US Food and Drug Administration (FDA), voretigene neparvovec (Luxturna), developed by Spark Therapeutics in Philadelphia, Pennsylvania, to treat a rare form of vision impairment, did not receive approval until 2017. Even monoclonal antibodies struggled to receive regulatory approval, with the first generation of mouse-derived molecules generating unwanted immune responses in people. Only after the development of 'humanized' monoclonal antibodies did the technology take off in the clinic. "There were multiple moments where people gave up hope," says Maraganore. "But now monoclonals are the largest class of pharmaceutical medicines."

The instant appeal of RNAi therapy is understandable. In 1998, Fire, then at the Carnegie Institution for Science in Washington DC, and Mello, at the University of Massachusetts in Worcester, demonstrated that they could efficiently and selectively dial down the expression of various genes in the worm *Caenorhabiditis elegans* by injecting small quantities of short

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interfering RNA (siRNA) molecules[1], which comprise paired strands of RNA. Over the next several years, researchers began to understand the molecular mechanisms that underlie RNAi and the design principles for generating siRNAs that inhibit gene expression effectively. After a team led by gene-therapy researcher Mark Kay at Stanford University in California demonstrated[2] the first use of RNAi in mice in 2002, the clinical potential of this method was immediately apparent.

By May 2003, US business magazine *Fortune* was calling RNAi "biotech's billion-dollar breakthrough", and a handful of companies had staked their worth on the technology, including Alnylam, founded the previous year by several RNA biologists, and Sirna Therapeutics, launched in San Francisco, California, in 2003 by Fambrough and other investors. Although there were further contenders, these two companies controlled a formidable array of intellectual property that pertained to the clinical application of RNAi, and big pharma was eager to write them cheques to get a place at the table. In 2006, the same year in which Mello and Fire received the Nobel prize, drug company Merck in Kenilworth, New Jersey, bought Sirna Therapeutics for more than US$1 billion in cash, and Alnylam lined up a formidable array of partnerships with larger firms. "They did these big deals with Takeda, Roche and Novartis," says Ted Tenthoff, managing director at investment bank Piper Jaffray in New York City. "The value of these deals was probably on the order of billions of dollars."

CRASH ON DELIVERY

Despite the unbridled enthusiasm of investors, early efforts to move RNAi therapies through clinical trials demonstrated that important challenges remained. "When we were doing our initial studies, the big question was what would be the time frame in which one could develop a clinically relevant delivery approach that would work safely in humans," recalls Kay. The most pressing issue was working out how to get siRNAs into the correct cells in the body, and in an intact state. "The delivery technology just wasn't there," says Ritu Baral, managing director at Cowen, an investment bank in New York City. "You would give somebody RNAi and their enzymes would just rip the thing to shreds immediately, before it got near where it needed to go."

Another problem was that these initial RNAi therapies were often mistaken by the body's immune system as the remnants of infectious agents, which would trigger side effects. "Experiments in animal models miss the fact that the human immune response is much more sensitive," says Maraganore. "We had to learn more about how these molecules behaved in a human setting." The first clinical programmes, which targeted infectious diseases and eye disorders, yielded little progress — a stagnation that made RNAi therapy's early backers increasingly nervous. In the late 2000s, that concern reached a crisis point.

Baral puts it bluntly: "Big pharma lost its nerve." In 2010, Roche and Novartis, both in Basel, Switzerland, began to pull back, terminating their partnerships with Alnylam. The following year, Pfizer in New York City and Abbott in Abbott Park, Illinois, ended their independent RNAi drug-discovery programmes. Merck scaled back its RNAi efforts, but held out for a few more years before selling its Sirna Therapeutics assets to Alnylam in 2014 for less than 20% of the price it had paid in 2006. "The progress towards clinical-stage programmes wasn't as fast as the field had collectively thought it was going to be," says James Hamilton, vice-president of clinical development at Arrowhead Pharmaceuticals, an RNAi company in Pasadena, California. More importantly, the bad news had a ripple effect on media coverage of the industry, creating a narrative in which RNAi had failed broadly as a clinical tool.

The RNAi companies that survived this time did so through a combination of determination and sacrifice. Alnylam maintained a financial lifeline through a few committed investors and by licensing its technologies, but it also had to make some tough decisions. "We had to do two rounds of lay-offs around that time to preserve enough capital to get the data that we ultimately needed to convince investors," says Maraganore. The company's competitors, in the meantime, made double-or-nothing bets. At Arrowhead, for example, Hamilton and his colleagues took advantage of big pharma's flight by convincing investors to back acquisition of the RNAi portfolios being ditched by other companies — from Roche in 2011 and Novartis in 2015. "We were a new company focused purely on RNAi after the Roche transaction, whereas it was just a piece of what we were doing before," says Hamilton.

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BACK ON TRACK

The irony is that the exodus occurred just as clinical RNAi efforts were starting to bear fruit. Long-standing researchers remained confident, even as scepticism swelled. "We always had good reason to believe that we could make it work," says Fambrough, who launched Dicerna in 2007, shortly after Merck purchased Sirna Therapeutics. The clinical-trials data were finally beginning to justify this confidence. A 2010 study clearly demonstrated that siRNA could effectively silence specific genes in humans[3], and by 2013, Alnylam had published clinical-trials data that demonstrated patisiran's safety and efficacy[4].

By this time, researchers at such companies had learnt important lessons about working with RNAi technology in people. For example, it became clear that almost all siRNA-based drugs administered into the bloodstream tend to accumulate in the liver. RNAi companies therefore turned their focus to conditions that could be treated by targeting this organ, such as hereditary transthyretin amyloidosis. It also became clear that making chemical modifications to RNA can greatly improve its performance as a drug. "They enhance the stability of the molecule, and reduce the immunogenicity," explains Kay, who notes that other types of modification can improve the efficiency with which therapeutic RNA molecules are incorporated into the cellular machinery that underpins RNAi. "We didn't understand a lot of stuff back in 2001 or 2002."

Biologists Craig Mello (left) and Andrew Fire discovered RNA interference.

Robust solutions to the problem of delivering RNAi therapies to target tissues have also emerged. Patisiran is encapsulated by lipid nanoparticles, an approach that was sufficiently safe and effective for delivery to the liver to win regulatory approval. But Alnylam and other companies have shifted to an alternative delivery strategy, in which siRNAs are chemically coupled to the sugar *N*-acetylgalactosamine (GalNAc), which binds strongly to a receptor that is expressed abundantly in the liver. "GalNAc delivery to the liver is better than lipid nanoparticles in every way," says Fambrough. "There's less toxicological burden, it's easier to make, it lasts longer and it's easier to administer." Ease of administration is a crucial advantage: GalNAc enables the subcutaneous injection of RNAi therapies, rather than requiring their intravenous delivery, as do lipid-based formulations.

Even with these advances, the past five years have seen major setbacks.

Alnylam's first experiment with GalNAc-mediated RNAi delivery, revusiran, a treatment for hereditary transthyretin amyloidosis, ended abruptly in 2016 after a phase III trial in people with amyloidosis-associated heart disease showed that there were more deaths in people who received the treatment than

ALNYLAM PHARMACEUTICALS

A researcher in the lab at Alnylam — the first company to get approval for an RNA-interference therapy.

in those who took a placebo. The underlying cause of the deaths, which were mostly heart-related, remains unclear, but revusiran's formulation seems to have contributed. "It required very high dose levels to achieve reduced gene expression," says Maraganore. "The data seem to suggest that metabolites of that drug given at those doses might have been poorly tolerated in that very frail population."

Safety concerns also led Arrowhead to take a dramatic decision in 2016, when it pulled the plug on a trio of clinical programmes on the basis of a tentative link between their RNAi delivery strategy and excessive deaths in non-human primates. "It was a tough business decision at the time, but I think, in retrospect, it was the right decision," says Hamilton. He notes that the company has since turned to a GalNAc-based strategy that enables more efficient and durable reductions in gene expression, with no safety concerns emerging from studies in animals and people.

A BETTER TOMORROW

In 2018, patisiran broke away from the pack to win regulatory approval on the strength of a pivotal trial that showed it had a good safety profile and led to meaningful improvement in the health and quality of life of people with hereditary transthyretin amyloidosis. The decision vindicated researchers who had stuck by RNAi, and particularly those at Alnylam. "It was a moment like no other — once in a lifetime — when you've been involved in 16 years' worth of effort to bring an important innovation forward for patients, and finally have it approved," says Maraganore.

Rival firms such as Arrowhead and Dicerna also benefited from having a first-in-class drug on the market. "This is the first safety package the FDA has thoroughly vetted and passed," says Baral. "And it shows investors that there are markets that one can go after with this technology where there is a likely return on investment." It should also be noted that, despite its proven safety and efficacy, patisiran employs the relatively outdated lipid-nanoparticle delivery system, which requires intravenous delivery and steroids to manage the side effects from infusion. This could give RNAi therapies that use GalNAc-based delivery, which can be achieved with a minimally invasive subcutaneous injection, a chance to shine even more brightly. Indeed, Alnylam is conducting phase III trials of vutrisiran, a GalNAc-modified version of patisiran that can be administered subcutaneously at a low dose every three months, rather than every three weeks.

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Several RNAi programmes for liver conditions are also under way, including the development of Alnylam's givosiran, which helps to prevent the production of neurotoxic metabolites of haem, a molecule found in red blood cells, that accumulate in people with acute hepatic porphyria. In a 2019 trial, givosiran reduced the frequency of the painful and debilitating neurological symptoms of porphyria attacks by 74%, and the drug is awaiting regulatory approval. Dicerna is performing phase II testing of DCR-PHXC, an siRNA-based drug for primary hyperoxaluria. In this condition, the liver generates excessive levels of a compound called oxalate, which accumulates in the kidneys and can lead to organ failure. And since retooling its delivery strategy, Arrowhead has moved ARO-AAT, a potential treatment for α1-antitrypsin (AAT) deficiency, into a pivotal phase II/III trial. This lung and liver disorder arises from a mutation that causes the AAT enzyme to misfold and then aggregate in cells, and unpublished phase-I data have demonstrated the capacity of ARO-AAT to efficiently decrease production of the mutated protein. "Even at low doses, we were seeing mean serum reductions of 60–70%," says Hamilton.

Buoyed by these successes, the RNAi field is looking beyond the liver. There is particular interest in hitting a tricky target: the central nervous system (CNS). Although getting RNA across the blood–brain barrier remains impractical, progress towards another class of RNA-based therapy has demonstrated the feasibility of repairing neurological damage through direct injections of such drugs into the cerebrospinal fluid. The strategy requires the targeted delivery of single strands of RNA, known as antisense oligonucleotides, to affected tissues. But, in contrast to siRNAs, which undergo processing by enzymes in cells and then modulate gene expression as part of a larger inhibitory protein complex, antisense oligonucleotides inhibit gene expression by directly binding to target messenger RNAs.

Both Alnylam and Dicerna are aggressively pursuing RNAi drugs for conditions that affect the CNS, including Huntington's disease and Alzheimer's disease, and Fambrough is optimistic about the prospects of this line of research. Unlike therapies that target the liver, which rely on a receptor expressed by the organ to access liver cells, he notes that therapeutic RNAs can penetrate neurons without the need for GalNAc modification once they have been delivered into the CNS.

These ambitious efforts are being made possible by fresh investment from big pharma — perhaps the surest sign that RNAi therapy has regained its stride. In October 2018, for example, Eli Lilly in Indianapolis, Indiana, proffered an upfront investment of $100 million to support Dicerna's work in CNS and other non-liver conditions, and Johnson & Johnson in New Brunswick, New Jersey, purchased the rights to Arrowhead's hepatitis B RNAi programme in a deal that could net the biotechnology company up to $1.6 billion. And in April, Regeneron Pharmaceuticals in Tarrytown, New York, and Alnylam embarked on a $1-billion collaboration to develop RNAi therapies for use in the CNS, eye and liver. After almost two decades of development, and with one drug across the regulatory finish line — and several other candidates close behind — hopes are high that these investments might finally unlock RNAi's clinical potential. "I think we've pretty much put the old scepticism and demons to bed," says Fambrough. ■

Michael Eisenstein *is a freelance journalist based in Philadelphia, Pennsylvania.*

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