

**Medical News & Perspectives** .....p1963

RNAi Rollercoaster Begins to Reap Therapies

**The JAMA Forum**.....p1965

Progress in Primary Care—From Alma-Ata to Astana

**Bench to Bedside** .....p1966

For Difficult-to-Model Brain Diseases, Brain Organoids Come to the Rescue

**Global Health**.....p1969

Mapping Yellow Fever Virus in Brazil

Global Hunger on the Rise as Climate Extremes Increase

Guidelines for Ending Childhood and Adolescent Tuberculosis

**News From the Food and Drug Administration** .....p1970

FDA Joins New Effort to Strengthen Medical Device Cybersecurity

Will Naloxone Become Available OTC?

Blood Group Genotyping Test Goes Beyond A, B, and O

**Medical News & Perspectives**

# RNAi Rollercoaster Begins to Reap Therapies

Bridget M. Kuehn

The 1998 discovery in the tiny nematode *Caenorhabditis elegans* that small stretches of double-stranded RNA can selectively silence genes won Craig Mello and Andrew Fire a Nobel Prize in 2006. It also helped give birth to the idea that such RNA interference (RNAi) might be used to treat genetic diseases.

After a 20-year rollercoaster and billions in investments by major pharmaceutical companies and numerous start-ups, the first RNAi therapy won US Food and Drug Administration (FDA) approval in August. Alnylam Pharmaceuticals' patisiran infusion was approved for the treatment of polyneuropathy caused by a rare, genetic disease called hereditary transthyretin-mediated amyloidosis (hATTR) in adults. Patients with hATTR, which affects about 50 000 patients worldwide, develop a buildup of abnormal transthyretin proteins in their peripheral nerves, heart, kidneys, eyes, and gastrointestinal tract. Patisiran uses small interfering RNAs (siRNA) to shut down the production of transthyretin.

"This approval is part of a broader wave of advances that allow us to treat disease by actually targeting the root cause, enabling us to arrest or reverse a condition, rather than only being able to slow its progression or treat its symptoms," said FDA Commissioner Scott Gottlieb, MD, in a statement. "New technologies like RNA inhibitors, that alter the genetic drivers of a disease, have the potential to transform medicine, so we can better confront and even cure debilitating illnesses."

**Hitting the Target**

Following the demonstration of RNAi in 1998, subsequent work in *C elegans* demonstrated that double-stranded RNA binds to a

protein complex called Dicer that chops it up into fragments, which are then assembled into an RNA-induced silencing complex (RISC). Within this complex, 1 strand of the RNA fragment is cleaved and the remaining RISC-bound RNA strand homes in on a corresponding stretch of mRNA, much like a search engine, and ultimately destroys the mRNA and prevents production of the protein it encodes. This mechanism likely evolved as a defense against viruses and jumping genes.

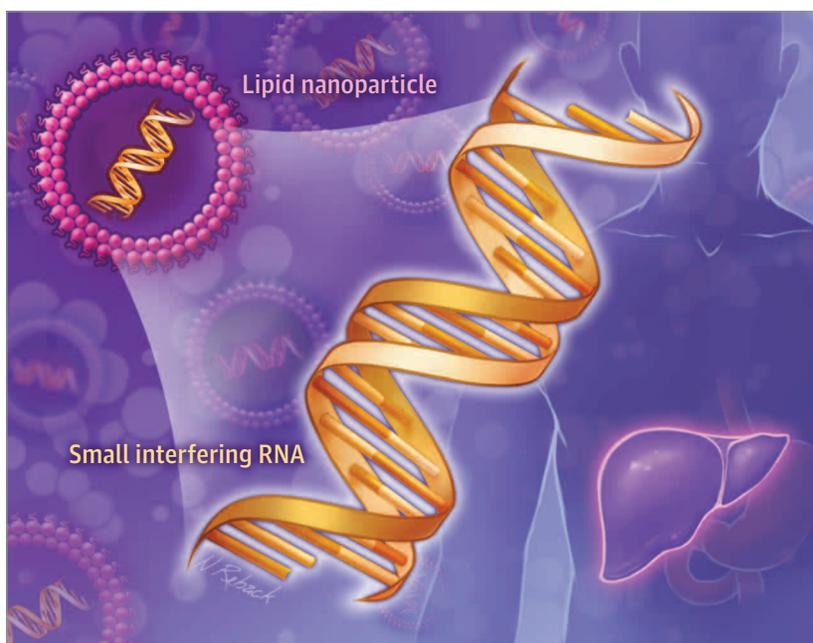
"We discovered that we could program the search engine artificially, so we could give it a search query and it would find and regulate the target information for us," Mello said.

From the start, Mello said they had an inkling that this would not only be a very use-

ful tool for research but that it might also be used to silence disease-related genes.

"The worm and the human are very closely related, and so the systems that function for this type of gene expression and regulation mechanism are so ancient and highly conserved that there was considerable reason to believe that it might turn out to be relevant to potentially many other organisms," Mello said. "It was really exciting."

By 2001, Thomas Tuschl, Klaus Webber, and colleagues had confirmed that RNA interference could also be used to silence genes in mammals. Then, a flurry of animal studies demonstrated that RNA interference could be used therapeutically. Among them was a study by Judy Lieberman, MD, PhD, professor and



chair in cellular and molecular medicine at Harvard Medical School, and colleagues that showed that RNAi could be used to knock down HIV receptors or an HIV gene in vitro to inhibit HIV replication. Other studies by Lieberman and her colleagues in mice showed that injected siRNAs could **prevent** fatal autoimmune hepatitis.

"That sort of set off this whole explosion of interest in RNAi therapeutics," Lieberman said. She noted that some researchers were able to go from finding a target gene to phase 1 clinical trials in a little over a year.

Many researchers including Mello founded start-up pharmaceutical companies, and pharmaceutical giants like Roche and Pfizer also invested heavily in developing RNAi therapies. But it quickly became apparent that delivering RNAi therapies to target tissues was going to be a major hurdle.

"The RNA is often very unstable especially when you put it in with the blood or interstitial fluids, it'll be destroyed very rapidly," Mello explained. "There was really very little chance that anything that anybody was contemplating in those early days was going to work."

Adverse effects also emerged. For **example**, RNAi could knock down genes whose sequences shared nucleotide combinations with the target gene and trigger immune responses.

The snags caused both Roche and Pfizer to **scale back** RNAi research in 2010 and 2011. But Alnylam's recent success is expected to reinvestigate the field.

### Amyloidosis Approval

Alnylam focused its efforts on the liver. The liver's role as filter for the rest of the body makes it an easy target, and its role as the manufacturer of many disease-linked proteins opens the possibility of treating multiple diseases, noted Lieberman.

The company overcame the challenge of getting large, charged RNAi molecules across the cell membrane by packaging them in **lipid nanoparticles**. They also made chemical modifications to RNAi that helped avoid off-target effects. The drug knocks down the liver's production of transthyretin by about 80% to mitigate the buildup of abnormally folded transthyretin protein, which causes progressive loss of sensation and difficulties with everyday activities for patients with hATTR. This buildup of abnormal transthyretin proteins can also contribute to heart failure, arrhythmias, low blood pressure, and sudden death. Most patients survive just 5 to 10 years, noted Morie

Gertz, MD, a consultant in hematology at the Mayo Clinic in Rochester, Minnesota.

"Liver transplantation has been the only treatment that's really been available for the last 3 decades," Gertz said.

The approval of patisiran adds another option. The phase 3 APOLLO trial randomized 225 patients at 44 sites in 19 countries to receive either intravenous patisiran or placebo once every 3 weeks. **Results** published in the *New England Journal of Medicine* showed that patients in the patisiran group had statistically significant improvements in neuropathy, quality of life, gait speed, and body mass index at 18 months. Patients in the patisiran group saw a 6-point average reduction in the modified Neuropathy Impairment Score, whereas the scores of patients in the placebo group worsened by 28 points during an 18-month follow-up period.

"It's the first therapy that shows actual improvement in [polyneuropathy] in some patients," said Joel Buxbaum, MD, emeritus professor of molecular medicine at Scripps Research Institute in La Jolla, California. "Most of the other studies have demonstrated a reduction in the rate of progression rather than objective improved function."

But he cautioned that it is uncertain how sustained patisiran's effects will be because of the relatively short duration of the study. He noted that about 20% of patients who receive a liver transplant see early improvements, but in most recipients the procedure arrests progression of the neuropathy rather than producing better neuronal function. After a liver transplant, patients exhibit considerable life extension, but by living longer, they experience other complications such as cerebrovascular events, seizures, dementia, migraine, or eye disease related to ongoing production of transthyretin in tissues outside the liver.

"I don't suspect patisiran will be any better than liver transplant for those complications," he said. "There is no evidence that the present formulations will penetrate the cerebral spinal fluid or the eye."

An **antisense oligonucleotide therapy** and **small molecule drugs** that stabilize transthyretin are also being investigated for treating hATTR. In fact, the antisense oligonucleotide therapy inotersen was **approved** by the FDA in October, and Buxbaum expects that small molecule tafamidis will also be approved. However, it's uncertain how to judge whether an individual patient will respond to any one of the oligonucleotide or transthyretin-stabilizing drugs or to all of them, Buxbaum

said. He noted there are no biomarkers to help guide physicians to the drug most likely to help their individual patients. "The individual patient and physician have to choose among 6 current therapies—perhaps more in the future," Buxbaum said. "It is not a problem if you are paying 10 cents a pill and you can have an answer in three weeks, but if you are paying \$400 000 a year [for patisiran] and cannot tell whether the drug is working for 6 or 12 months, the situation is more complicated." According to a recent Institute for Clinical and Economic Review **analysis**, current list prices for inotersen and patisiran exceed long-term cost-effectiveness thresholds.

### Beyond the Liver

Mello is confident that the next several years will yield more, and potentially better RNAi therapeutics. He noted that the RNAi technology in patisiran is about a decade old, and improvements in the chemistry of RNAi therapeutics continue.

"The current chemistries mean that gene knock down can last for 6 months after a single injection," Lieberman said. "The new chemistry is about 50 times more efficient than the old chemistry. You only need a fraction of a milligram of RNA per kilogram."

For example, subsequent drugs in Alnylam's pipeline have 2 major improvements, Lieberman explained. One is that the RNAi is conjugated to a sugar that is recognized by a receptor almost exclusively on liver cells. They've also modified the RNA to bind more tightly to RISC. These next generation delivery methods avoid some of the immune-triggering properties of lipid nanoparticles, noted Lieberman in a recent **review** article.

A potential challenge ahead for RNAi therapies, particularly now that the drugs have become longer lasting, will be ensuring their safety. Mello noted this is always a concern with a new class of drugs.

"It's quite potent so, the dosing is quite low, and I think so far the profile looks pretty clean from a toxicity perspective," he said.

The next RNAi therapeutics are likely to be diseases that affect the liver or involve proteins produced in the liver, said Lieberman. For example, Alnylam has RNAi **drugs** in late-stage clinical trials for hypercholesterolemia and hepatic porphyrias.

"In the liver, I think the delivery problem has been solved," Lieberman said.

But delivery beyond the liver remains a major challenge. There are ambitious efforts under way to tackle this challenge. Among the

most promising are approaches that attach the RNAi to a molecule called an aptamer that binds specific cell-surface receptors allowing entry, according to Lieberman's review. One group is exploring the utility of conjugating RNAi to fatty acids used by neurons to silence the gene associated with Huntington disease.

Several groups are also working to develop RNAi therapies for cancers. A potential advantage of RNAi therapies for cancer

is the possibility of targeting multiple genes in a single therapy, Lieberman said. Using RNA aptamers that target specific tumor cell receptors to induce cell death, she and her colleagues have shown in animal studies that RNAi therapies could be designed to spare normal cells from toxic effects unlike many existing chemotherapies.

"We basically showed that we could target cancer without any detectable uptake

into normal cells and effectively kill the tumors," Lieberman said. In the meantime, leaders in the field are celebrating the approval of the first RNAi therapy and hope it will open the door to more.

"I think finally the promise of this type of therapeutic is really going to be realized over the next several years," Mello said. ■

**Note:** Source references are available through embedded hyperlinks in the article text online.

## The JAMA Forum

# Progress in Primary Care—From Alma-Ata to Astana

Dave A. Chokshi, MD, MSc; Louise Cohen, MPH

At the 1978 International Conference on Primary Health Care, world leaders and health experts convened by the World Health Organization (WHO) and UNICEF (United Nations Children's Fund) endorsed the [Alma-Ata Declaration](#) "to protect and promote the health of all the people of the world." The declaration identified primary care as the cornerstone of healthy, thriving communities; the foundation for integrating the full spectrum of health and social services to improve health outcomes; and the key to sustainable, accessible, and equitable health systems.

Forty years later, however, despite some progress, much of this vision remains to be realized. Too many patients' first interaction with the health system is still for catastrophic care, the disease burden continues to shift from infectious diseases to noncommunicable diseases, and there is a growing impetus to improve value in health care delivery. Primary care helps address these challenges by focusing on prevention, chronic disease management, and what matters the most to each patient.

Now is an opportune time to revitalize a commitment to primary care. As leaders reconvene in Astana, Kazakhstan, for the [Global Conference on Primary Health Care](#), an expected focus on [universal health coverage](#) is appropriate. But 2 additional priorities are fundamental to strengthen primary care in the United States: investing in primary care infrastructure and evolving primary care around a renewed repertoire of relationships that promote health.

## Investing in Primary Care

Today, as in 1978, the global community grapples with providing basic, essential health

care—especially for the most underserved among us, who are often at the margins of society and the center of health tragedies. In the United States, burgeoning health care costs are increasingly recognized as untenable.



At the time of the Alma-Ata Declaration, on a per capita basis, health care spending in constant dollars was \$2627 in 1978 and more than tripled to \$10 348 in 2016. Of this spending, primary care only receives approximately [7 cents on the dollar](#), according to one estimate—although part of the problem is that [there is no uniform definition of primary care spending](#), and it is not well-studied.

Some states have taken action to enhance primary care funding. Starting in 2010, [Rhode Island](#) required that commercial plans increase spending on primary care by 1% per year, so that by 2014, 10.5% of total spend-

ing would be on primary care—through payments supporting quality and efficiency, such as incentives tied to Patient-Centered Medical Home recognition. [During this period](#), Rhode Island was the only state in New England to increase the supply of primary care physicians per capita, while spending by commercial health insurers grew more slowly compared with other states in the region. Oregon and now Delaware have followed suit.

Shifting resource allocation to primary care would help change the core infrastructure of the health system. Fully 11% of adults in the US have gone without care because of cost, but this number is doubled for people at the lower end of the income scale. [Uncertainties for community health centers](#) and other safety-net facilities (including public health care systems) exacerbate this situation.

Reducing barriers to accessing primary care—particularly limiting out-of-pocket costs—would help ensure that no one forgoes essential care because of cost. Also, funds to incentivize physicians, nurses, and allied health professionals to enter and stay in primary care careers would invigorate the workforce and create a more stable health system.

Resources also could help expand integrated behavioral health services, linkages to public health and social service organizations, additional home-based services, and telehealth services. For instance, the Netherlands has invested in a [national system](#) of nurse telephone triage with the ability to invite a patient to visit a physician in real time, arrange a physician home visit, or call for an ambulance. The nodes of the system are organized regionally by primary care physician cooperatives, facilitating rapid clinical advice while linking patients back to more longitudinal care.