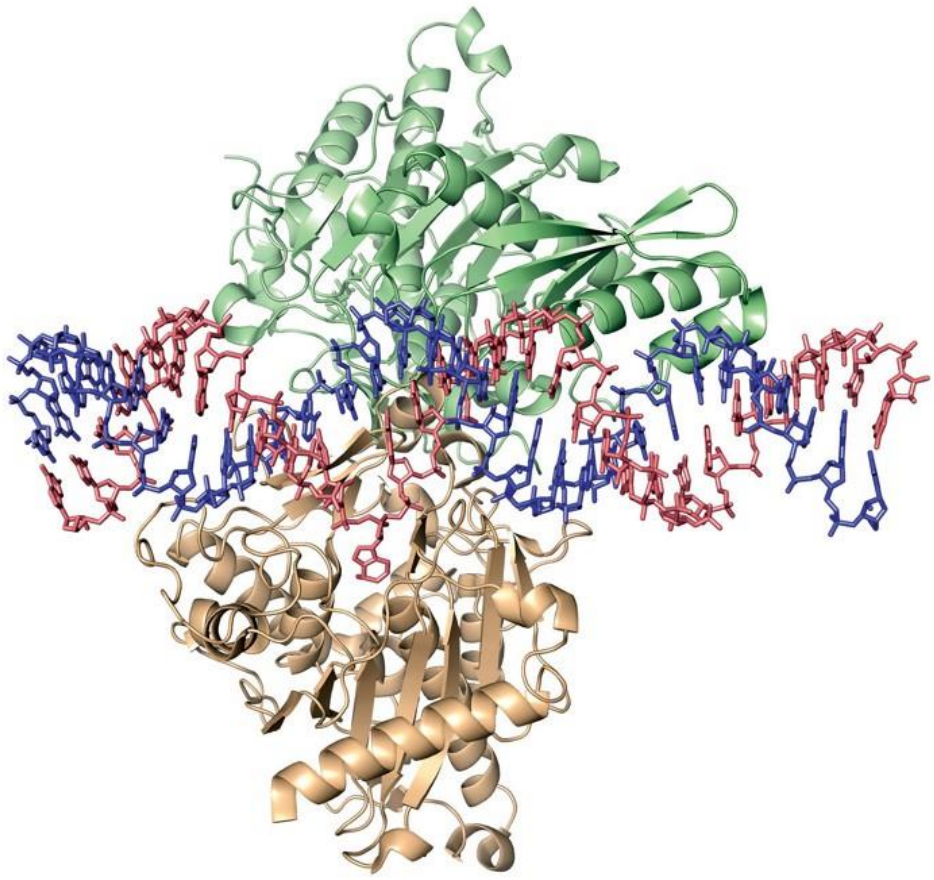
RNA-editing race intensifies as Big Pharma buys in

Investment in the nascent technology, which has seemingly endless applications, is invigorating the field

by Ryan Cross



A crystal structure showing a synthetic guide RNA (blue) directing the ADAR enzyme (green and tan) to make an edit on messenger RNA (red). (Peter Beal)

For small biotech companies hoping to strike a deal with larger drug developers, there's no greater destination than the J.P. Morgan Healthcare Conference. In early January 2020, leaders from the start-up Shape Therapeutics made the annual pilgrimage to this mecca of biotechnology networking in San Francisco to make a pitch: What if you could edit someone's genetic code without ever touching their DNA?

The biotech industry is awash in companies using tools like CRISPR gene editing to fix or turn off problematic DNA. If gene editing works, it could provide a one-and-done cure. But some

researchers are worried that if CRISPR slips up and cuts DNA at the wrong site, the damage could also be permanent. “Targeting DNA has a lot of all-or-nothing consequences,” says David Huss, head of research at Shape.

At the conference, Huss explained to potential partners that Shape’s solution was to edit RNA instead of DNA. Our cells constantly produce short-lived RNA molecules that convert the DNA code into functional proteins. Incredibly, our bodies have already evolved an ingenious tool for editing RNA: an enzyme called ADAR—adenosine deaminase acting on RNA. The enzyme converts select adenosine (A) bases, one of four letters that compose the messenger RNA (mRNA) code, into another base that the cell interprets as guanosine (G). Shape was founded in 2018 on the basis of academic work showing that synthetic molecules called guide RNAs could recruit ADAR and direct it to make these A-to-G edits at precise sites.

Scientists estimate that A-to-G editing could fix mutations responsible for nearly 50% of genetic diseases. “We have a tool that can be applied to so many diseases that we couldn’t possibly do them all ourselves,” Huss says. When Shape executives pitched their RNA-editing technology to the Big Pharma company Roche, the two teams clicked, says Sylke Poehling, head of therapeutic modalities at Roche.

It was the first, and last, time that leaders from the two companies met in person. But as the pandemic unfolded, videoconferencing between Copenhagen, Denmark, and Seattle kept the connection strong. In August 2021, Shape and Roche formed a pact to develop RNA-editing therapies for multiple conditions, including Alzheimer’s and Parkinson’s diseases. Two weeks later, the Dutch biotech firm ProQR Therapeutics announced it had struck its own RNA-editing partnership with Eli Lilly and Company to develop therapies for liver and nervous system diseases.

RNA editing, recently considered a niche research field, is now an area of focus for at least a dozen companies. Although most of these companies’ initial sights are set on treating genetic diseases, many are already dreaming up more creative applications of RNA editing, such as introducing mutations to disrupt interactions between proteins. “The field has really started to mature over the past few years, and it is really picking up steam incredibly quickly,” says Andrew C. Adams, vice president for new therapeutic modalities at Lilly. “We would like to be one of the first to push the science forward from a large pharma perspective.”

Making the edit

In contrast to DNA’s iconic double-stranded helix, RNA molecules are often depicted as single strands of nucleotides floating inside cells without any obvious structure. But sometimes a portion of an RNA strand can loop back and pair with itself, forming a double-stranded landing pad for ADAR, explains Peter Beal, who studies the enzyme at the University of California, Davis, and is working with ProQR on RNA editing therapies. At that site, ADAR converts an adenosine into an unusual base called inosine—what scientists call an A-to-I edit. When translating mRNA to make proteins, our cellular machinery then interprets the inosine as the more common base guanosine, making the net result of ADAR’s reaction an A-to-G edit.

Scientists have devised several methods for controlling where this editing takes place, but the general idea is to create a landing pad that draws ADAR to the right spot on an RNA strand. Some firms rely on the length of their guide RNA to form a large double-stranded segment of RNA that attracts ADAR. Others add a sequence to the guide RNA that folds into a 3D structure called an ADAR-recruiting domain to attract the enzyme.

Sometimes there are multiple adenosines in the landing pad, and to control which one ADAR edits, scientists design their guide RNAs so that a cytidine sits directly across from the target adenosine. Cytidine and adenosine don't normally pair, and the mismatch creates a bulge in the double-stranded RNA that ADAR preferentially targets. Beal and his colleagues recently discovered that using a certain artificial nucleotide in the guide RNA instead of the mismatched cytidine strengthens the RNA's interaction with ADAR and improves editing.

Beal is excited to study how adding artificial nucleotides or chemical modifications to other parts of the guide RNA could improve editing even further. "It is going to keep us busy for a while," he says. The success of RNA-editing therapies will hinge on designing better guide RNAs, an area that stands to benefit from more than 4 decades of research on antisense oligonucleotides, which are synthetic single-stranded RNA molecules that bind to mRNA to stop or alter protein production.

We have a tool that can be applied to so many diseases that we couldn't possibly do them all ourselves.
David Huss, head of research, Shape Therapeutics

There are already multiple approved antisense oligonucleotide drugs and several firms developing new ones. Daniel A. de Boer, CEO of ProQR, which is developing antisense oligonucleotide therapies, views RNA editing as "a new application of an existing modality," which he says helps make it a less risky investment. "All the lessons we've learned from single-stranded oligonucleotides can be applied" to RNA editing, he adds. Paul Bolno, CEO of Wave Life Sciences, which has several antisense oligonucleotide programs in clinical trials, agrees. "This is just a natural extension to the work we've been doing," he says. Lilly and Roche have research programs focused on antisense oligonucleotides as well.

Most firms, including ProQR and Wave, are planning to administer their guide RNAs just as they would with an antisense oligonucleotide therapy: directly into the eye for vision diseases, intravenously for liver diseases, and into the spinal fluid for brain diseases. Unlike CRISPR, which in theory makes a lifelong change to DNA, RNA editing would last only as long as the guide RNA molecules remain in the body, likely on the order of weeks to months. "The reversible nature of RNA editing is a feature rather than a bug," Lilly's Adams says. "It takes away some of the concerns we might have with a permanent edit."

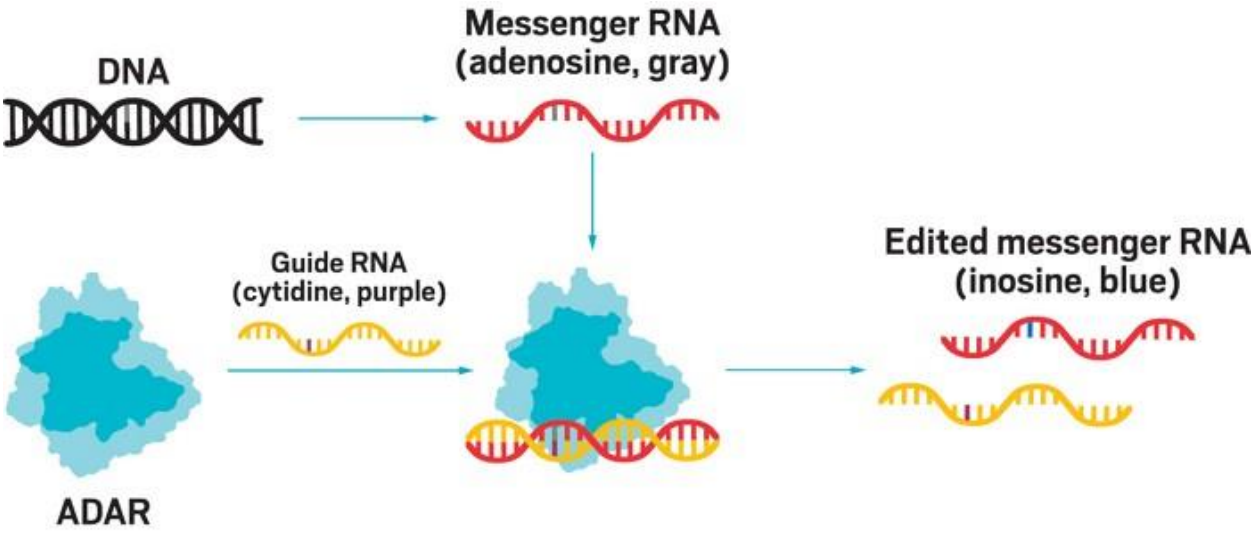
Two companies, EdiGene and Shape, stand out for their focus on an approach that would make RNA editing more permanent but without the risk of irreversible DNA damage that comes with gene editing. Both firms plan to use adeno-associated viral vectors to deliver into the body a set of DNA instructions that would allow cells to perpetually make the guide RNA. This would turn RNA editing into a form of gene therapy—an attractive feature to Roche, which owns the gene

therapy firm Spark Therapeutics. “What we liked about the Shape technology is that you don’t cut the DNA,” Poehling says, “but you still have a permanent effect, so you don’t need redosing.”

Endless applications

Most companies are planning to assess their RNA-editing technology in genetic diseases first. “I think that is the best test of the technology in a clinical setting,” Lilly’s Adams says. “There are a lot of potential diseases that we can make interesting medicines for with A-to-G switches.” Shape has disclosed a list of more than three dozen disease-implicated genes that it thinks could be addressed with its RNA-editing technology. As an example, Huss points to a relatively common mutation in the kinase LRRK2 that is linked to genetic forms of Parkinson’s disease.

At least three companies—ADARx Pharmaceuticals, Korro Bio, and Wave—are using RNA editing to tackle alpha-1-antitrypsin deficiency. They want to reverse the effects of a mutation that causes the alpha-1-antitrypsin protein to misfold, aggregate in the liver, and damage the organ. Even if they are successful, these companies could face competition from many other drug developers trying to develop treatments for the same disease with nearly every approach imaginable, including small-molecule drugs, protein replacement, and even gene editing.



Several companies are developing therapies that use a guide RNA to direct an enzyme called ADAR to edit messenger RNA. A cytidine in the guide RNA that sits across from an adenosine in messenger RNA coaxes ADAR to swap that adenosine for an inosine. The cell’s protein-making machinery interprets inosine as guanosine, making the end result an A-to-G edit.

While fixing genetic mutations is an obvious starting point for the technology, some people, including Korro CEO Ram Aiyar, are more excited by RNA editing’s more creative applications, in which cleverly swapping out an amino acid in the right place can change a protein’s function and treat disease. “That’s the reason I joined the company, to be completely honest,” Aiyar says. “The indication space is as vast as small-molecule drug development.” Roche’s Poehling

says that an “obsession” with fixing point mutations “might hold people back from really seeing the true potential of the technology.”

Roche and Shape want to use RNA editing to selectively disrupt the interactions between two proteins, something that is difficult for conventional small molecules to do. The approach could allow drug developers to target proteins that are so fundamental that you don’t want to block them entirely. Although the companies are not disclosing specific targets, Huss says that Shape has focused on two classes of enzymes: secretases and kinases.

Kinases activate or inactivate other proteins by adding phosphate groups to specific amino acids on the proteins. Secretases cleave proteins at specific sites to alter their function. Instead of directly targeting a kinase or secretase, RNA editing could change a single amino acid on a protein the enzyme acts on. For example, γ-secretase cleaves more than 90 proteins, including one whose cleavage is linked to Alzheimer’s disease. In theory, RNA editing could remove a cleavage site on that Alzheimer’s-implicated protein without disrupting γ-secretase’s other functions.

Transcription factors, a class of proteins that turn genes on or off, are also difficult to target with small-molecule drugs. In a recent presentation to investors, Wave’s chief technology officer, Chandra Vargeese, described how the company used RNA editing to change a key amino acid to block the interaction between the transcription factor Nrf2 and its repressor Keap1, proteins implicated in inflammation and oxidative stress. A single edit in either protein could prevent their interaction and increase protective anti-inflammatory and antioxidant genes, Vargeese said.

Shape and Wave have also independently discussed using RNA editing to decrease gene expression, modify protein stability, or alter the process of RNA splicing. Some researchers are working on using RNA editing to change the structure of sodium channels responsible for pain. Lilly’s Adams even suggests that RNA editing could be used to “engineer in protective mutations” to prevent a disease, although this is not the company’s initial focus. “In the long term there is a lot of really cool stuff you can potentially do with editing, but we want to cross the more proximal bridges first.”

Refining the edit

For now, companies developing RNA-editing therapies are offering more promises than data. It will likely be at least a couple of years before any company has clinical data on RNA editing, although ADARx Pharmaceuticals may have the most aggressive timeline. CEO Zhen Li says her company could begin clinical trials as soon as the second half of 2022, depending on the results of an ongoing toxicology study.

Some companies developing RNA-editing therapies are holding back their excitement until they can evaluate data from their own studies. “The clinical applicability remains to be seen,” says Judith van Deutekom, chief scientific officer at Vico Therapeutics, which is developing RNA-editing therapies for brain diseases. “It is still an early technology, and we still need to learn a lot about it.”

For example, the field is grappling with the limitations of relying on ADAR. The enzyme is picky about its surroundings, and certain bases preceding or following the target adenosine can increase or decrease the likelihood that ADAR makes the edit. Scientists also can't say for sure if our cells routinely make enough of the enzyme for RNA-editing therapies to be effective. And there's the risk that guide RNAs may overwork ADAR and distract it from its normal duties. "Those are the things we will need to measure," EdiGene's CEO, Dong Wei, says.

Studies by academic scientists suggest that guide RNAs typically spur low levels of editing in cells. Companies say they've boosted editing efficiency. Wave, for instance, says it can edit 50% of a target mRNA in monkeys, but the results are yet to be published in peer-reviewed journals.

Improving the therapies will rely heavily on the development of better guide RNAs. Shape is taking a high-throughput screening approach and testing 250,000 to 500,000 guide RNAs with unique sequences that target a single adenosine to determine which designs edit most efficiently and specifically. It then feeds these results back into its machine-learning programs to "learn more and more about what makes a good guide RNA," Huss says.

Even as the field works out the kinks of A-to-G editing, some researchers are trying to expand the base-editing alphabet. For instance, more than 10% of genetic diseases are caused by mutations in which an errant uridine base in mRNA prevents cells from fully making a protein. In 2011 at the University of Rochester Medical Center, Yi-Tao Yu discovered a way to convert those errant uridines into a similar base called pseudouridine, which restored protein production. The approach uses a guide RNA to direct an enzyme called pseudouridine synthase to make the edit at specific sites.

Although Yu's work was first published a decade ago, he recently received a surge of interest in the technology and was contacted by four venture capital firms during the pandemic. Much to their chagrin, ProQR had already licensed the technology, and de Boer says the firm's uridine-editing programs are only about a year behind their adenosine-editing programs. Yu credits the COVID-19 vaccines with spurring interest in the technology. "Because of the success of the mRNA vaccines, I guess RNA editing is getting hot," Yu says. "People just wanted to find any RNA project going on."

Chemical & Engineering News
ISSN 0009-2347