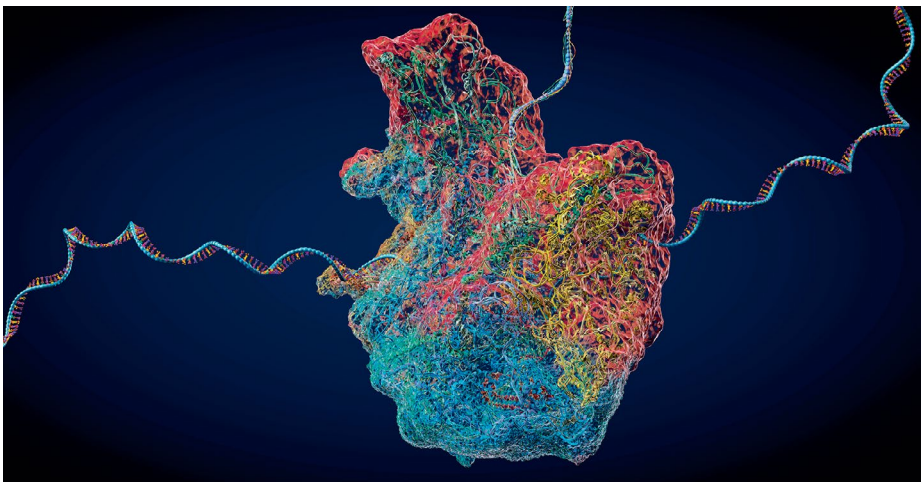
Shoot the messenger: RNA editing is here

RNA editing is rapidly gaining prominence as its transient and reversible changes promise a safer and more flexible option to reverse disease-causing mutations than DNA editing.



Scientists can fix disease-causing sequences by targeting RNA with ADARs, the body's base editing systems.

By Cormac Sheridan

Wave Life Sciences aims to be first into the clinic with RNA editing, an approach that is rapidly gaining ground. Later this year, it will file a clinical trial application for WVE-006 to treat α-1 antitrypsin deficiency (AATD). The study will mark a milestone for yet another RNA-directed therapeutic modality.

Wave is among a growing cluster of firms harnessing an endogenous enzyme, adenosine deaminase acting on RNA (ADAR), to edit mRNA transcripts. Interest in the area is exploding, as evidenced by two large deals signed late last year. One of them, between ProQR Therapeutics of Leiden in the Netherlands and Eli Lilly, builds on an existing deal they entered in 2021. The other, between London-based GSK and Wave, potentially involves RNA silencing and splicing, as well as editing. WVE-006 is the sole program disclosed so far in the alliance. Basel, Switzerland-based Roche has also become involved in ADAR-based RNA editing, through a large-scale alliance it entered in 2021 with Shape Therapeutics. Other RNA editing approaches are also in development, including Ascidian Therapeutics' novel exon-editing platform and an RNA-guided endonuclease (RGEN) technology that Rznomics, of Yongin, South Korea, has already brought into clinical development (Table 1).

ADAR-based RNA editing takes advantage of a quirk of the cell's translation machinery whereby, as a result of their structural similarities, inosine reads as guanine. This enables ADAR editors to introduce site-specific, RNA-guided adenosine-to-inosine (A-to-I) changes, opening up myriad therapeutic possibilities, including correcting disease-causing mutations, modulating gene expression or altering protein–protein interactions.

Several aspects of RNA editing make it attractive. It avoids the potential genotoxicity hazards associated with CRISPR–Cas9-based DNA editing, a tool that introduces permanent changes to the genome. Because mRNA molecules are inherently transient, the consequences of any off-target editing that may occur should be relatively minor. "It is certainly a concern, but it's orders of magnitude less, I would say, than what you've seen in the DNA editing space," says David Huss, CSO at Shape. "We don't believe we'll need that 15 years of follow-up that you need with DNA editing," says Ascidian CEO Romesh Subramanian. In addition, none of the RNA editing techniques that are in development introduces a foreign, potentially immunogenic protein, as is the case with DNA-directed CRISPR–Cas9 editing, base editing or prime editing.

Five ADAR isoforms, encoded by three different genes, have been identified in humans: ADAR1p110, ADAR1p150, ADAR2a, ADAR2b and ADAR3 (the last of which is catalytically inactive and thought to act as an inhibitor of the active form). These ADARs ordinarily act on double-stranded RNA molecules to modulate regulatory non-coding RNAs, activate antiviral immunity or suppress inappropriate innate immune responses against self transcripts, and do so in a non-specific way. "We often use the term 'ADAR is blind'," says Huss. "It doesn't recognize a sequence — it recognizes a structure." By designing guide RNAs to mimic that double-stranded RNA structure, it is possible to direct the activity of one or more ADAR isoforms to a target site on an otherwise single-stranded mRNA transcript, to obtain a desired A-to-I edit. "We invested a lot of time and effort in understanding how synthetic oligonucleotides could mimic the natural situation," says Gerard Platenburg, co-founder and CSO at ProQR, which has collaborated with Peter Beal of the University of California, Davis, a researcher who probes the structure and function of ADAR. Two key features of its guide RNA molecules are a binding domain that determines the target specificity and an editing-enabling region that pushes the targeted adenosine into the active pocket of the enzyme. The enzyme is highly active, which, given the right guide RNA design, can translate into high editing efficiencies. "We're seeing durable, consequential editing," says Paul Bolno, CEO at Wave, which has reported up to 80% editing efficiency for WVE-006 in non-human primates.

AATD is an ideal test bed for RNA editing therapies. Patients have a high prevalence of a single point mutation in the AAT protein that prevents its export from the liver, where it is produced, and into blood. The mutation results in a glutamic acid residue at position 342 in the protein being replaced with a lysine, leading to build-up of misfolded AAT in the liver and to lung damage resulting from excessive neutrophil elastase activity, which freely circulating AAT normally holds in check.

Korro Bio is also pursuing the indication and has set an ambitious target for its program, in terms of AAT plasma concentration. "For us, normal is 20 micromolar and above," says CEO Ram Aiyar. "That's what we're shooting for."

Table 1 | Selected RNA editing companies

Company	Technology	Delivery	Therapeutic Focus	Status
Rznomics (Yongin, South Korea)	RNA-guided endonuclease reprogramming and editing technology based on the *Tetrahymena* group I intron, which selectively replaces human telomerase reverse transcriptase (hTERT) RNA sequence with RNA encoding herpes simplex virus 1 thymidine kinase, to sensitize cancer cells expressing hTERT to ganciclovir	Replication-defective adenovirus vector	Human telomerase reverse transcriptase-positive hepatocellular carcinoma	Phase 1/2
Ascidian Therapeutics	Trans-splicing pre-mRNA exon editing technology comprising a synthetic RNA sequence containing the exons of interest as well as molecular cues, including donor and acceptor sites, to ensure the corrected RNA sequences are included in the emerging mRNA transcript during pre-mRNA processing by the spliceosome; the editing components are first encoded in a recombinant DNA molecule, which can be delivered to the target cells in an AAV vector or a lipid nanoparticle	AAV vector initially, but other delivery methods may also be possible	Ocular disease, including a lead program in ABCA4 retinopathy; neurological and neuromuscular disease	Preclinical
EdiGene	Leaper technology, which uses covalently closed circular RNA molecules to recruit endogenous ADAR to make targeted A-to-I conversions; I is then translated as guanine	AAV	Ophthalmology, CNS disease	Preclinical
Korro Bio	Oligonucleotide Promoted Editing of RNA (OPERA), which uses a synthetic guide oligonucleotide to direct ADAR to make targeted A-to-I edits	Oligonucleotide	α-1 anti-trypsin deficiency; diseases of the eye, liver and CNS	Preclinical
ProQR Therapeutics	Axiomer platform, which generates single-stranded 'editing oligonucleotides'; these recruit and direct ADAR to make targeted A-to-I conversions	Oligonucleotide	Diseases of the liver and CNS	Preclinical
Shape Therapeutics	ADAR-based editing using guide RNAs designed by combining screening with machine learning	Tissue-specific AAV vectors	CNS, ocular, liver and rare diseases	Preclinical
Wave Life Sciences	AIMers, A-to-I base editing oligonucleotides, which recruit ADAR to correct single-base mutations in RNA transcripts	Oligonucleotide	Liver, lung and CNS diseases	Preclinical

CNS, central nervous system. Sources: Company websites

For delivering ADAR-directed therapies, some developers favor naked oligonucleotides, which can be formulated and administered like antisense oligonucleotide drugs, These companies include ProQR, Wave, Korro Bio and EdiGene. Although this approach means constant redosing, as is the case with gene silencing approaches, it adds a layer of safety, as the drug can be withdrawn should any problems emerge.

How companies choose to modify the oligonucleotide backbone to enhance stability and potency could become a differentiator. Korro plans to work with established chemistries because it plans to tackle highly prevalent conditions. "To do that, you need a drug product that can get through regulatory systems, that can get through manufacturing systems and you need to have at least an understanding of safety," says Aiyar. Wave has reported that backbone modifications of its molecule using a combination of phosphorothioate and phosphoryl guanidine (PN) had substantial effects on their potency and efficiency. Phosphorothioate modifications are routinely used in oligonucleotide-based drugs, but PN chemistry is newer. "We have clinical data on PN modification," Bolno says. Beijing-based EdiGene has developed covalently closed guide RNAs with enhanced potency and efficiency. Shape, in contrast, has opted to encode its ADAR-targeting RNA in a recombinant DNA molecule, which it packages in an adeno-associated virus (AAV) vector. As is the case with AAV-based gene therapy, this construct persists as an extrachromosomal episome and is transcribed into an active RNA molecule continuously.

The discoveries leading up to ADAR-based RNA editing are hardly new. Aiyar credits in particular the research of Brenda Bass of the University of Utah and Kazuko Nishikura of the Wistar Institute. "You've got to give credit where credit is due," he says. A rudimentary version of ADAR-based RNA editing that worked in vitro and in *Xenopus* oocytes was described almost 30 years ago by scientists at Ribozyme Pharmaceuticals (which then became Sirna Therapeutics, before its acquisition by Merck).

Around the same time, Bruce Sullenger and Nobel laureate Thomas Cech described a totally different scheme, using engineered ribozymes — RNA molecules with catalytic activities — for RNA-guided trans-splicing of targeted RNA sequences in vitro and in *Escherichia coli*. "These catalytic RNAs don't come from mammalian systems," says Sullenger, who is at Duke University. "In evolution, a lot of the [catalytic] functions have been replicated by proteins." Sullenger and colleagues took this concept further in pursuit of a potential therapy for sickle cell disease, but that work suffered from the freeze on genetic research that followed Jesse Gelsinger's death during a gene therapy clinical trial at the University of Pennsylvania in 1999.

Seong-Wook Lee, a former postdoctoral researcher in Sullenger's lab, continued the work in his native South Korea, and Rznomics (to which Sullenger is an adviser) has emerged to take it into the clinic but with the emphasis switched to cancer. Rznomics' lead program, RZ-001, for which it has received FDA clearance to conduct a US clinical trial for liver cancer, is designed to sensitize to ganciclovir cancer cells that overexpress human telomerase reverse transcriptase. It has engineered a trans-splicing ribozyme — derived from the *Tetrahymena* group I intron — to disrupt hTERT transcripts by inserting an RNA sequence encoding herpes simplex virus thymidine kinase, which metabolizes ganciclovir from its prodrug to its active, cytotoxic

form. A phase 1/2 trial in South Korea is already underway.

The trans-splicing approach to editing mRNA exploits another quirk of evolution. In human cells, the spliceosome — the RNA–protein complex that supports pre-mRNA processing within the nucleus — only conducts cis-splicing: it removes introns and splices together exons from a single transcript. However, the same structure is also able to support trans-splicing, or the end-to-end joining of two different RNA molecules. The phenomenon occurs in nature in ascidians, a class of marine invertebrates. Ascidian Therapeutics has adapted the trans-splicing mechanism to replace disease-containing exons from target pre-mRNA molecules with wild-type exons that encode highly specific binding domains to ensure they are incorporated into the mature transcript. "We essentially recruit the spliceosome machinery to our molecule," says head of research Robert Bell. By engineering RNA molecules (which are encoded in a DNA viral vector) to bind the spliceosome more tightly and by producing them in excess, the system leads its RNA molecules to outcompete the host cells' mutated exons for access to the spliceosome. As a result, the corrected protein is produced at the appropriate physiological concentration, as it is still limited by the amount of endogenous healthy exons that are produced by the cells' transcriptional machinery.

Unlike ADAR-based editing, which is limited to single-base A-to-I substitutions, both Ascidian's and Rznomics' approaches enable large-scale changes to be introduced in a mature mRNA transcript. "ADAR is like fixing typos. The splicing approaches are rewriting paragraphs," says Sullenger. Ascidian's approach, says Subramanian, is particularly suited both to correcting mutations in large genes that exceed the packaging limit of AAV vectors and to replacing large stretches of genetic code in which multiple disease-causing mutations are present in the patient population. Its lead program is in development for ABCA4 retinopathy, a form of inherited vision loss caused by a lack of functional ABCA4 (ATP-binding cassette subfamily A member 4), which is responsible for removing toxic vitamin A derivatives from retinal photoreceptors. Ascidian's therapy replaces 22 of the 50 exons present in *ABCA4* mRNA. If it works, it could address about 60% of the 2,000 disease-causing mutations in the ABCA4 protein known from the human population. The therapy, which is delivered to photoreceptors in an AAV vector by injection into the subretinal space, has already been administered to non-human primates with high rates of transduction, resulting in editing efficiencies of up to 38%. "In that area, we are transducing the vast majority of photoreceptors, well above 50%," says Bell.

Even if ADAR-based editing firms are limited to making single-base changes, the resulting biological consequences can be large. Modulating the levels of a transcription factor by increasing its half-life by even 10% can have a significant effect, Aiyar says. Introducing a dominant negative mutation to a protein can also have an outsized effect, Platenburg says. With the notable exception of AATD-directed therapies, most ADAR-based programs remain under wraps for now. But as they start to near the clinic, that will change — and the specific possibilities associated with RNA editing will start to become clearer.

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