tRNA therapeutics burst onto startup scene

Companies advance tRNA therapeutics to overcome mutant stoppages in protein synthesis shared by thousands of genetic diseases and cancers.

Alltrna launched in November 2021 billing itself as "the world's first tRNA platform company." By engineering transfer RNA molecules — the cellular couriers of protein synthesis — the startup, backed by $50 million in initial financing, aims to address the mechanisms of faulty protein production that can trigger all manner of disease.

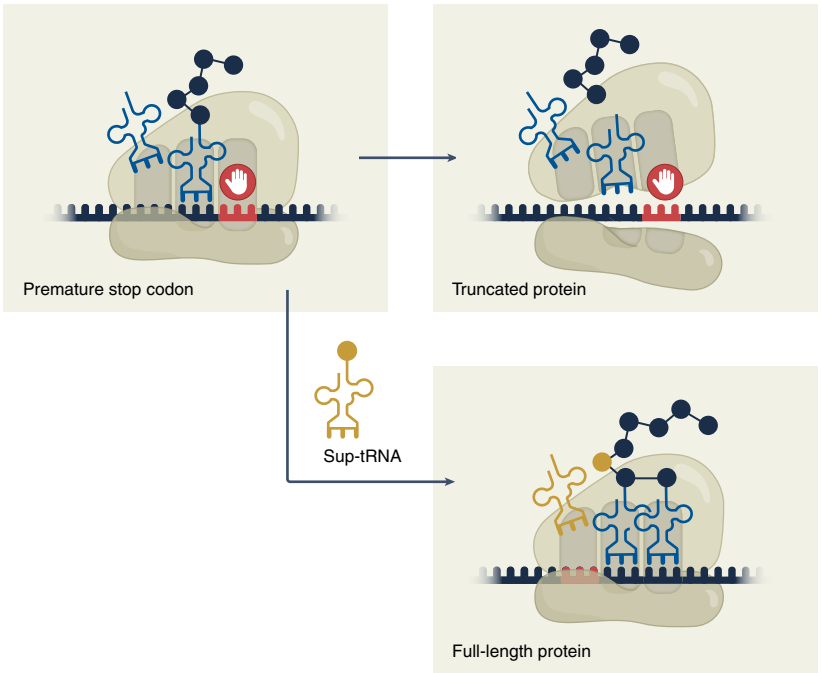
But Alltrna is hardly alone in its pursuit of tRNA-based therapeutics. ReCode Therapeutics, Shape Therapeutics and Tevard Biosciences all came before it; and the field continues to expand with the arrival of hC Bioscience, a startup that emerged from stealth mode in late February with $24 million and a plan to fight cancer and rare diseases with tRNA.

All of the companies are focused, at least in part, on designing tRNAs to bypass premature stop signals and incorporate desired amino acids instead. Such premature termination codons — which function like misplaced periods in the middle of a sentence to muddle the message encoded in messenger RNA (mRNA) — are responsible for an estimated 11% of all inherited disease. In theory then, just one 'suppressor' tRNA could conceivably remedy thousands of different rare inherited disorders, each caused by the same types of truncating 'nonsense' mutations that result in faulty gene expression.

"If it can be done safely, it really opens the door for an entire new class of therapies," says William Skach, a strategic adviser for research and drug discovery at the non-profit Cystic Fibrosis Foundation. "It unlocks an ability to meet unmet need in patient populations that otherwise are completely neglected," adds Alltrna's founding CEO and director, Lovisa Afzelius.

Yet, for all the preclinical promise of tRNA technologies, it is not yet certain that the platform will outperform small-molecule 'readthrough' drugs such as Translarna (ataluren), a treatment approved in Europe and Brazil for patients with nonsense mutation–mediated Duchenne muscular dystrophy. Plus, gene-editing strategies under development could rival tRNA drugs as well.

"We don't know the efficacy of the suppressor tRNAs in vivo yet," notes Kim Keeling, a molecular geneticist at the University of Alabama at Birmingham who continues to search for compounds with improved readthrough activity. "I'm not sure we can exclude one technology over another yet."



In tRNA medicine, a single suppressor tRNA (sup-tRNA) can read through a premature stop codon to restore production of full-length proteins.

The idea of harnessing the activity of suppressor tRNAs for correcting human disease dates back some 40 years to work from Yuet Wai Kan's laboratory at the University of California, San Francisco. There, in 1982, researchers studying β-thalassemia — a disease often caused by nonsense mutations in the hemoglobin-β gene — showed that a human tRNA designed to read through premature stop codons could help restore full-length protein production in frog eggs.

In the early 2000s, researchers at the University of Colorado at Boulder validated the approach in transgenic mice, and a group from the Ott Institute of Obstetrics and Gynecology in St. Petersburg, Russia, used mutated suppressor tRNAs to achieve low levels of disease correction in a mouse model of muscular dystrophy. But according to Ott Institute gene therapist Anton Kiselev, other therapeutic strategies — including gene replacement and readthrough drugs — were yielding better results in mice at the time, and his team, like most others in the field, dropped the tRNA approach.

Research progress into tRNA therapeutics stalled. Attention shifted to retooling tRNAs to incorporate non-standard amino acids as a way of making recombinant protein therapeutics with new properties. And early patents around the therapeutic use of suppressor tRNA were never licensed or developed further.

The technology back then just "wasn't ready," says Charles Link, an early patent holder who now serves as chief medical officer and executive chairman of Syncromune, an immuno-oncology startup. "At the time, it was hard to conceive of how you could get effective enough delivery and high enough amounts" of suppressor tRNA expression to bring about meaningful clinical benefit.

Even as recently as 2014, when researchers in Portugal showed that suppressor tRNAs held potential for treating or preventing hereditary cancer syndromes caused by nonsense mutations, few investors or academics seemed to take notice. According to Carla Oliveira, a cancer geneticist at the i3S–Institute for Research and Innovation in Health in Porto who led the work, interest in her team's results was "marginal."

That soon began to change — although, for most companies, designing suppressor tRNAs was not the initial focus. Shape Therapeutics, for example, was founded on the work of Prashant Mali, a bioengineer at the University of California, San Diego, who had described and patented two ways of targeting point mutations found in RNA. One involved suppressor tRNAs, an approach the company is now pursuing for the treatment of Rett syndrome, a neurodevelopmental disorder caused by nonsense mutations in the *MECP2* gene. However, it was the other technology, one involving adenosine deaminase enzymes for editing RNAs, that has long been Shape's primary interest.

tRNAs were not initially Tevard's priority either. The company's origins trace back to a platform-agnostic desire to "reverse" Dravet syndrome — hence the name, which is Dravet spelled backward. And early considerations centered around base editing, which explains why Harvard University's David Liu, a pioneer of that technology, is a scientific advisor. But a 2017 encounter between Jeff Coller, an RNA biologist now at Johns Hopkins University, and Harvey Lodish, a cell and molecular biologist at the Whitehead Institute, changed the company's thinking. (Both are Tevard co-founders, along with CEO Daniel Fischer and board chair Warren Lammert, who launched the company for highly person reasons: both men have daughters with Dravet syndrome.)

Coller had shown that the balance between codon abundance in a particular gene transcript and the concentrations of corresponding tRNAs in the cell — a concept known as codon optimality — can greatly change the expression levels of proteins. He and Lodish realized that Tevard could harness that knowledge to develop what it calls 'enhancer' tRNA therapeutics for Dravet syndrome, a rare form of epilepsy mostly caused by heterozygous loss-of-function mutations in the sodium channel gene *SCN1A*. With a cocktail of three enhancer tRNAs, Fischer claims that the company can approximately double the productivity of the working copy of *SCN1A* in affected cells without dramatically altering the expression of other, non-target genes.

Because that enhancer therapy harnesses the potential of the functional gene copy, it could conceivably help all people with *SCN1A*-mutant Dravet syndrome, regardless of the specific defect in the other gene copy. But, as it turns out, both Fischer's and Lammert's daughters, like approximately 25% of all patients with Dravet, harbor premature stop mutations in their *SCN1A* genes — which makes them candidates for a suppressor tRNA approach as well. Tevard is advancing both strategies in partnership with Zogenix, a company that specializes in epilepsy drugs and that will soon be part of Brussels-based UCB under a $1.9 billion buyout plan announced in January.

To enable its suppressor tRNA program, Tevard licensed intellectual property connected to a 2019 paper from Christopher Ahern and his former postdoc John Lueck, who had built a library of hundreds of anticodon-edited tRNAs, dubbed ACE-tRNAs, each capable of suppressing premature termination codons and faithfully incorporating desired amino acids instead. "We covered every known tRNA that could be used as a suppressor in human disease," says Ahern, a molecular physiologist at the University of Iowa in Iowa City who now advises Tevard. (Ahern is also a scientific cofounder of hC Biosciences, which licensed his patents for other applications.)

Ahern and Lueck, in collaboration with Skach from the Cystic Fibrosis Foundation, also showed that ACE-tRNAs prompt only low levels of normal stop readthrough, thereby helping to alleviate one of biggest safety concerns associated with the therapeutic strategy. Other groups have since corroborated this finding. Researchers at the University of Massachusetts Medical School in Worcester, for example, found in mouse models that suppressor tRNAs could fix Hurler syndrome, a lysosomal storage disease caused by nonsense mutations in the *IDUA* gene, with minimal errant translation elongation.

Arcturus Therapeutics, which is co-developing suppressor tRNA drugs with biochemist Zoya Ignatova from the University of Hamburg in Germany, has supporting unpublished data of its own. "We don't actually get a lot of aberrant elongation of random proteins," says Arcturus CSO and COO Pad Chivukula.

The fact that normal stop signals are less affected by suppressor tRNAs and remain largely error-free can likely be explained by their genomic context. Codons found at the ends of open reading frames, as well as their neighboring genomic motifs, have been fine-tuned by evolution to favor translation termination — whereas nonsense mutations, many of which arise spontaneously to cause disease, are buttressed by a genetic architecture that drives continued protein synthesis.

"It's all about ribosome behavior at the stop codons," says Rachel Green, a ribosome biologist at Johns Hopkins who chairs the scientific advisory board for Alltrna. Interactions between neighboring genomic motifs and RNA-binding proteins that alter the kinetics of how ribosomes ratchet along their templates should mean that, as Green puts it, "a suppressor tRNA is more likely to read through a bad stop codon than a good stop codon."

Still, any sidestepping of normal stop signals — even at low levels — could be dangerous if it triggers the production of toxic proteins. So Lueck, now at the University of Rochester Medical Center, has continued to validate the technology, starting in human lung cells harboring three different nonsense mutations linked to cystic fibrosis. Next, he plans to test the approach in mouse and pig models of the disease. He's fairly confident the strategy will be effective. "There's nothing in my lab that I found that says this will not work." But, Lueck notes, "we need to know if it's going to be safe."

To overwrite wayward stop signals, Lueck and Ahern have largely focused on manipulating just the anticodon portion of tRNAs — the section at the base of the L-shaped molecule that pairs with the corresponding codon on mRNA. But others are now tinkering with the entire structure and finding, as Ignatova did, that changes to other parts, including stem and loop domains that stabilize binding to the elongation factors that facilitate protein synthesis, can enhance suppression activity. According to Ignatova, this helps to "trick" the premature stop codon into accepting an amino acid–carrying tRNA instead.

All of the companies in the therapeutic tRNA space hope to capitalize on technological progress made with other types of genetic medicines, including mRNA vaccines, virus-mediated gene replacement therapies and CRISPR-based gene-editing therapeutics. But they will also face many of the same issues associated with efficiently and safely bringing these treatments to patients. "The biggest challenge is delivery," says Leslie Williams, co-founder, president and CEO of hC Bioscience. As with other types of genetic cargoes, adenoviral vectors and lipid nanoparticle carriers remain the delivery systems of choice for most therapeutic tRNA companies, with some academic work on DNA plasmid-based administration as well.

Compared with other types of genetic medicines, however, tRNA therapeutics do offer some key advantages, experts say. "This nonsense suppression strategy is universal for premature stop codons," says Qing Xia, a chemical biologist at Peking University in Beijing and the founder of QiXia Decode Therapeutics, a startup focused on using engineered tRNA–enzyme pairs for treating muscular dystrophies and cancers caused by nonsense mutations. (According to Xia, her startup has raised approximately $16 million to date.) Plus the tRNAs themselves

are small, so they will not bump up against size limits that can preclude viral delivery of some complete genes or CRISPR enzymes, she notes.

Suppressor tRNAs should also return protein activity to normal levels but not induce overexpression that can be problematic with some finely tuned ion channels, kinases or tumor suppressors. David Huss, CSO of Shape Therapeutics, thus describes the technology as the "Goldilocks" solution to diseases like Rett syndrome, in which too much expression of the target protein can be toxic to neurons.

"You are not going to overshoot the amount of protein because you're just correcting at the RNA level," he says. (That's not necessarily desirable in all disease contexts, however. Lung cells, for example, can handle high levels of the cystic fibrosis transmembrane conductance regulator, which explains why ReCode Therapeutics elected to prioritize an mRNA therapeutic for cystic fibrosis, rather than a suppressor tRNA candidate that the company had also been working on. "mRNA just gave a better [disease] rescue," says ReCode co-founder and R&D vice president Michael Torres.)

Most tRNA-focused companies have a lead disease indication in mind, but the universal nature of premature stop codon correction means that they could consider running so-called basket trials, in which patients are selected on the basis of having pathogenic nonsense mutations, regardless of the exact disease manifestation. "It just seems like the right way to go about rare disease clinical trials when you have drugs that target shared molecular pathways," says P.J. Brooks, deputy director of the Office of Rare Diseases Research at the US National Center for Advancing Translational Sciences.

This strategy is common in oncology, but has been used sparingly for rare diseases. Although companies have run trials that enroll patients with many different kinds of epilepsies, say, or muscular dystrophies — diseases that share core sets of symptoms — researchers have struggled with how best to design trials and select clinical endpoints when patients share molecular commonalities but show disparate outward signs of illness.

Such basket trials would only be for suppressor tRNAs geared at treating diseases caused by premature stop codons, however. And Ahern predicts that the field of tRNA therapeutics will quickly move beyond that category of illness — into enhancer tRNAs for genetic diseases of heterozygosity, and much more. "The suppressors are going to be the gateway to seeing tRNAs being worked on as potential clinic therapeutics," he says. "But I think it's really just the beginning." ❐

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