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suppressor tRNA drugs with biochemist Zoya Ignatova from the University of Hamburg in Germany, has supporting unpublished data of its own.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.

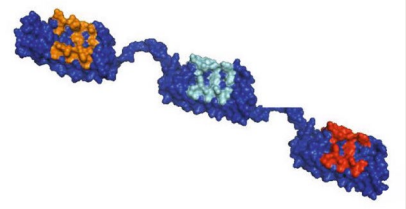
All of the companies in the therapeutic tRNA space hope to capitalize on technological progress made with other types of genetic medicines, including mRNA vaccines, virus-mediated gene replacement therapies and CRISPR-based gene-editing therapeutics. But they will also face many of the same issues associated 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.

DARPins score against COVID-19



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In February, Novartis and Molecular Partners applied for Emergency Use Authorization (EUA) for a new class of multispecific biologic for treating COVID-19. The partners filed for approval with the US Food and Drug Administration on the basis of positive phase 2 trial results obtained with the multivalent DARPin ensovibep. Of 407 symptomatic patients with SARS-CoV-2 infection, those receiving a single infusion had lower viral loads after eight days than those on placebo. The drug also reduced the risk of hospitalizations by 78%. DARPins (designed ankyrin repeat proteins) are protein scaffold biologics, engineered to have advantages over conventional antibodies. Each module is a tenth the size of a monoclonal antibody, and it is possible to string DARPins with peptide linkers to form agents that bind multiple targets with high affinity and specificity. Ensovibep contains three covalently attached domains that bind to the receptor-binding domain of the SARS-CoV-2 spike protein. This trispecific structure, the company believes, will retain binding ability even when there are changes in the viral spike protein — the site used by the virus to enter host cells and whose mutation can lead to viral escape. This is welcome news for Molecular Partners, whose DARPin targeting vascular endothelial growth factor for macular degeneration, abicipar pegol, was rejected in 2020 by the US FDA due to concerns over intraocular inflammation. Ensovibep will face competition from other antivirals; in December the FDA granted EUA to Pfizer's antiviral treatment Paxlovid, the orally available protease inhibitor nirmatrelvir given with ritonavir to slow its breakdown.

Published online: 16 March 2022
https://doi.org/10.1038/s41587-022-01266-6