

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Editing tRNA Genes to Broaden Nonsense Therapeutics

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Premature termination codons (PTCs) are the result of **nonsense variants** that occur across thousands of genes. PTCs underlie diseases affecting virtually every organ system and therefore appear to demand gene-specific therapeutic approaches (Fig. 1). However, all nonsense-associated diseases share a common mechanism: the premature termination of protein synthesis (translation). With this unifying mechanism, platform therapeutics that “read through” PTCs have been of keen interest. Encouraging, then, is a recent study reported by Pierce, Erwood, and colleagues.¹ They present a strategy using **prime editing** to generate “suppressor” **transfer RNAs** (sup-tRNAs) (see Key Concepts) that read through PTCs for the development of a disease-agnostic therapeutic approach.

A COMMON CAUSE IN DISPARATE RARE DISEASES


Nonsense variants are single-nucleotide substitutions that change a sense codon (i.e., a codon encoding an amino acid) into one of three stop codons (TGA, TAG, or TAA). Natural termination codons halt translation at the end of all open reading frames. PTCs result in truncated non-functional protein, which is the shared mechanism for 10 to 15% of all monoallelic diseases. Although each of these approximately 1000 diseases is classified as rare, together they affect hundreds of millions of persons through more than 7500 known nonsense variants.²


MAKING SENSE OUT OF NONSENSE

The idea of using sup-tRNAs as a nonsense therapeutic was described in 1982 by Temple and colleagues.³ It involves the generation of sup-tRNAs, in which the anticodon of a human tRNA is altered to recognize and read through one of the three PTCs: instead of terminating translation, the sup-tRNA introduces an amino acid, resulting in a full-length protein. Interest in advancing sup-tRNA therapeutics lay dormant



KEY CONCEPTS

Prime editing  A precise genome-editing technique that directly writes new genetic information into DNA. It can generate targeted insertions, deletions, or base changes without creating double-strand breaks. Prime editing is mediated by a fusion enzyme (comprising a nickase and reverse transcriptase) complexed with a single engineered RNA, which directs the complex to the target sequence and provides the template for de novo DNA synthesis.

Transfer RNA (tRNA)  An adaptor molecule that recognizes a particular codon in messenger RNA (mRNA) and transfers a specific amino acid to the carboxy terminus of the growing polypeptide. The specificity of a particular tRNA is determined by its anticodon. Charged tRNA (i.e., tRNA linked to an amino acid) enters the ribosome and binds the complementary codon in mRNA. After transfer of the amino acid to the nascent polypeptide chain, the free tRNA vacates the ribosome, which then positions the next codon so that it is available for translation by an incoming tRNA.



An illustrated glossary is available at [NEJM.org](https://www.nejm.org)



for decades because of the presumption that their suppression of PTCs would result in toxic effects owing to increased read-through of natural termination codons, generating aberrant C-terminal extensions throughout the proteome. This concern was tempered when it was shown that highly active sup-tRNAs (expressed from plasmid DNA) for all three stop codons resulted in only minimal transcriptome-wide read-through of natural termination codons in cultured cells.⁴ It was subsequently shown that lipid nanoparticle-assisted delivery of a sup-tRNA as RNA to mouse lung epithelial cells did not result in rates of natural termination codon read-through exceeding basal levels.⁵ Of note, these and other studies^{6,7} showed

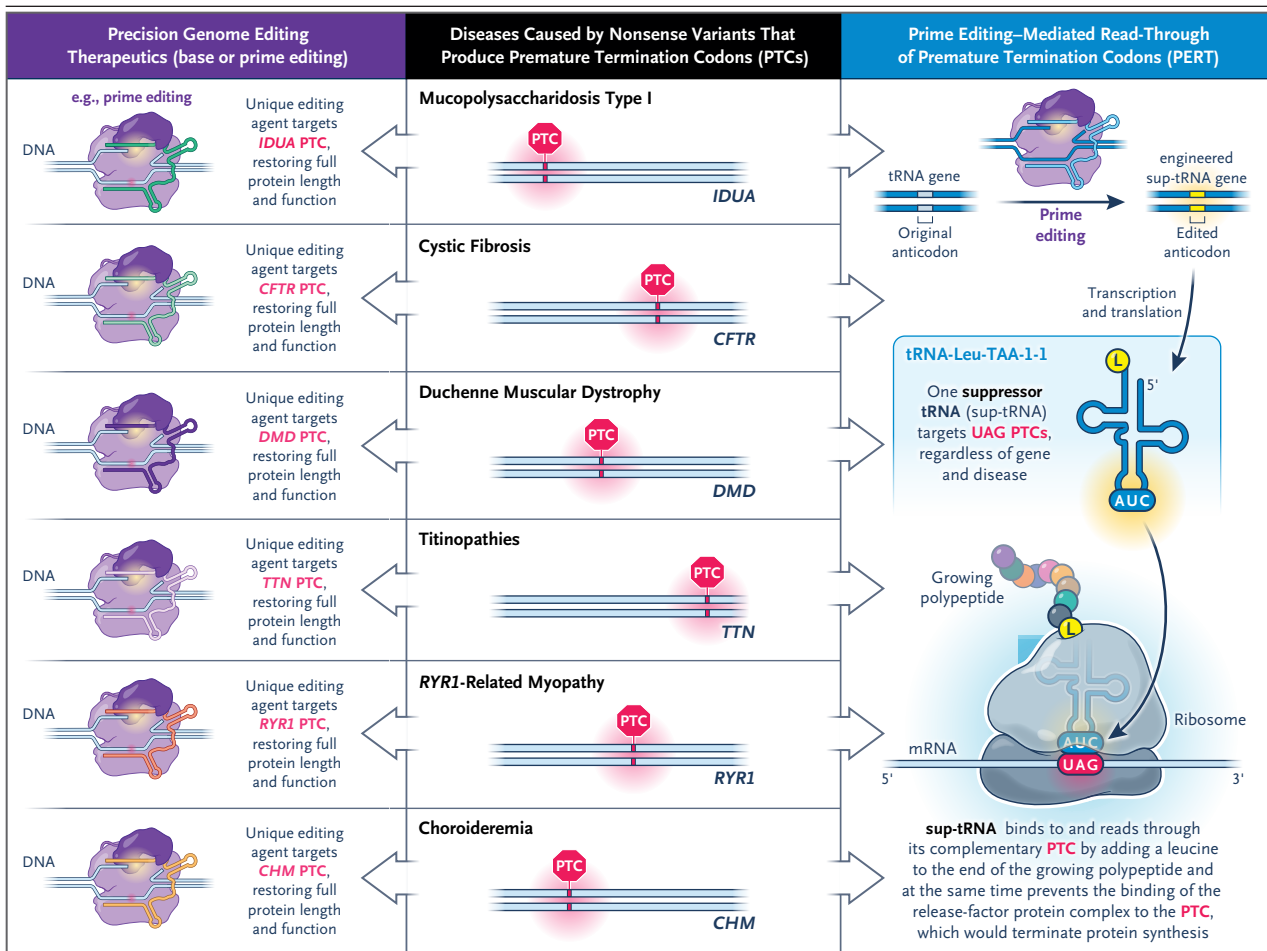


Figure 1. Variant-Specific Genome Editing versus Disease-Agnostic Prime Editing to Generate a Suppressor tRNA.

Base editing and prime editing can be used to permanently fix pathogenic variants but require the development of a distinct drug to correct each pathogenic variant. Suppressor transfer RNAs (sup-tRNAs) in theory can support a disease-agnostic therapeutic strategy that is independent of the affected gene or resulting disease but must be provided for the lifetime of the patient. Using prime editing to permanently convert a dispensable genomic tRNA gene into an engineered sup-tRNA supports a one-time, disease-agnostic therapeutic approach with a single composition of matter that could be implemented for the treatment of diverse disorders caused by nonsense variants.

that sup-tRNAs suppressed disease-causing PTCs to an extent consistent with a potentially therapeutic effect.

TWO APPROACHES TO GENETIC THERAPY

Total gene replacement approaches are variant-agnostic but require the development of a unique therapeutic for every affected gene and delivery to the relevant tissues. Base editing, a form of precision gene editing that irreversibly changes single nucleotides, also requires diverse sets of therapeutics tailored to individual genetic variants.⁸ Nonetheless, a recent study that used nonviral

delivery of a base-editing therapy in vivo to hepatocytes supported a treatment effect in an 8-month-old patient who received a diagnosis at birth of neonatal-onset carbamoyl-phosphate synthetase 1 deficiency resulting from a nonsense variant.⁹ This study highlights the ability to rapidly develop corrective and apparently safe precision gene-editing therapies for patients with ultrarare or even unique pathogenic variants. However, current regulatory, manufacturing, and economic considerations are hurdles to adapting this approach to the vast genetic diversity of patients with nonsense variants. Enter sup-tRNAs.

SUP-TRNAS AS PLATFORM THERAPEUTICS

Pierce et al. harnessed prime-editing technology to convert a genomically encoded human tRNA into a sup-tRNA. They call this approach prime editing–mediated read-through of premature termination codons” (PERT).¹ Optimization of sup-tRNA activity compensated for limited allelic representation (of the edited tRNA gene) and supported therapeutically meaningful rescue of endogenous protein function. In agreement with previously published studies, Pierce et al. confirmed that a gene encoding a tRNA that is normally charged with leucine was a good candidate target for the suppression of PTCs at different positions across several genes.^{10,11} A key aspect of this gene-agnostic approach is that leucine is not necessarily the “normal” amino acid at the relevant position (corresponding to the PTC) in the protein, so the corrected protein must be able to tolerate leucine at this position.¹⁰ This approach has the potential to drastically consolidate therapeutic entities and thus the regulatory approval and manufacturing requirements that would be needed for the treatment of persons with diverse nonsense-associated diseases. Furthermore, because the sup-tRNA is generated from an edited tRNA gene in the genome, therapy would be expected to be durable, to the extent that sufficient numbers of edited cells persist.

PERT PROOF OF PRINCIPLE

Pierce et al. used several HEK293T cell lines engineered to harbor PTCs in *TPP1*, *HEXA*, or *NPC1* (genes that, when pathogenically variant, cause Batten disease, Tay–Sachs disease, and Niemann–Pick disease type C1). HEK293T cells have high transfection efficiency and, in any case, are cells in a dish: the challenge to efficient delivery posed by a complex body of cells (in the form of tissue or an organ system) was not present. After PERT, these cell lines showed restoration of full-length protein expression — either the native protein sequence for *TPP1* and *HEXA* or a leucine-substituted missense variant for *NPC1* — with varying levels of rescue. Furthermore, the authors observed rescue of full-length CFTR expression from 15 distinct pathogenic CFTR PTC variants, although they did not assess functional rescue.

They tested PERT in vivo using a mouse model of the human lysosomal storage disease mucopolysaccharidosis type I (MPS-I, commonly known

as Hurler syndrome), which is caused by a lack of the protein α -L-iduronidase. This disease mouse model has a stop codon in the gene encoding α -L-iduronidase that is similar to the most common PTC that causes Hurler syndrome. Treatment of several affected tissues in this model organism was accomplished by packaging the prime-editing machinery into two separate adeno-associated viral vectors (both AAV9); these vectors preferentially transduce the machinery into cells of the central nervous system, heart, skeletal muscle and liver in mice after intravenous injection. Rescue of α -L-iduronidase function exceeded the therapeutic threshold, although longitudinal phenotypic studies were not performed.

Could PERT be a “one-for-all” silver bullet? No. It has the potential to be a “some-for-many” therapeutic. There would need to be at minimum three separate therapeutic entities to target each of the three stop codons. The editing of other tRNA genes would probably be necessary to rescue the expression or functionality of proteins that cannot tolerate the sup-tRNA–mediated introduction of leucine.¹⁰ Nevertheless, the approach reduces the number of prime-editing PTC therapies from thousands to a handful.

One could envisage basket clinical trials in which patients are selected on the basis of having a specific pathogenic nonsense variant, regardless of the gene and disease. A demonstration of effective and safe treatment of skeletal-muscle features of nonsense-associated Duchenne muscular dystrophy could provide the impetus for similar treatment of nonsense-associated limb-girdle muscular dystrophies, titinopathies, and other myopathies without requiring separate clinical trials for each. Such a pathway could accelerate access to and reduce costs of treatments for a broad range of patients, especially those with orphan diseases.

CHALLENGES TO THE SUP-TRNA APPROACH

As with all gene-therapy approaches, a major hurdle in the path of the approach described by Pierce et al. is efficient delivery to the affected tissue. To date, prime-editing machinery has been successfully delivered in vivo with the use of viral and nonviral methods. A further consideration when using the PERT approach is that the limit in dose administration per cell is effectively 2 (1 per allele), with some diseases probably requiring the

ability to “turn it up to 11” for therapeutically meaningful recovery of protein function, if the functionality of the protein product is subpar or if its production or stability is compromised as compared with its wild-type counterpart. However, the threshold for correction of the α -L-iduronidase protein for treatment of Hurler syndrome is only approximately 1% of wild-type activity,¹² a level that can feasibly be reached with the use of PERT. It is widely accepted that CFTR recovered function of more than 10% is required for treatment of cystic fibrosis, which would probably require relatively greater levels of sup-tRNA. This level could be achieved through exogenous delivery of sup-tRNAs.^{5,10} Although Pierce et al. found that mice could tolerate conversion of an endogenous tRNA to a sup-tRNA, it remains to be determined whether human tissues would be similarly tolerant.

Regulatory and economic realities have disincentivized clinical research into the treatment of rare genetic diseases. It is exciting to consider that sup-tRNAs and PERT could change these realities by providing a common treatment for diverse diseases.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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