From precision to platform: suppressor tRNA therapeutics for treatment of nonsense-associated diseases

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Here, we highlight a recent advancement in the emerging field of suppressor tRNAs (sup-tRNAs) as a therapeutic platform for treating a broad range of genetic diseases caused by nonsense mutations. Fang *et al.* developed ALPHA technology that demonstrates promising *in vivo* efficacy of a protein-bound engineered alanine sup-tRNA across multiple diseases and disease inheritance patterns.

While individual rare diseases may impact only a small number of people, rare diseases are collectively very common. In fact, ~300 million people worldwide live with rare diseases, of which ~80% are caused by identified genetic variants[1]. Nonsense mutations, which result in premature termination codons (PTCs; UAA, UAG, or UGA), account for ~11% of the genetic lesions responsible for human inherited diseases[2]. The mechanism for all nonsense-associated diseases is unifying: upon translation of the premature stop, endogenous release factor proteins bind to the stop codon, terminate translation, and release a truncated nonfunctional protein (Fig. 1). There has been interest in the generation of therapeutics aimed at suppressing or "reading through" PTCs, as they have potential for helping many individuals affected by a wide range of nonsense-associated genetic disorders.

One promising strategy under investigation for addressing PTCs is suppressor transfer RNAs (sup-tRNAs)[3,4]. Sup-tRNAs are engineered tRNAs that have been modified to recognize and bind to one of the three stop codons, incorporate their associated amino acid, allowing for the continued translation of the entire transcript. The full-length protein generated can then go on to perform its intended function and, if sufficient therapeutic levels are achieved, alleviate disease-associated pathology. This approach shows promise as a therapeutic platform that can be utilized to treat many diseases caused by nonsense mutations across many genes. Development of a therapeutic platform would lower the regulatory, manufacturing, and economic burdens that are inherent to traditional personalized gene therapies and allow for the possibility of "basket" clinical trials that recruit individuals with various nonsense mutations in the same gene or even across multiple genes. Indeed, previous work by our lab demonstrated that leucine- and serine-encoding sup-tRNAs, what we call anticodon-edited (ACE)-tRNAs, are good candidates as platform sup-tRNAs due to their high suppression activity at all three stops and high likelihood of generating functional proteins due to the overall benign physicochemical attributes of leucine and serine amino acids (*e.g.* size, charge, polarity, aromaticity, *etc.*)[5].

In the current study, Fang *et al.* provide evidence for encoding alanine at PTCs as a platform therapeutic approach. This study was guided by AlphaMissense[6], which predicts that generation of alanine nonsense variants of > 66,000 pathogenic PTCs has relatively low pathogenic indices compared to other amino acid nonsense variants. This makes it an attractive therapeutic approach for multiple disease-causing mutations across several genes. However, the conversion of a human alanine tRNA to a sup-tRNA through editing of the anticodon alone results in low suppression activity that is insufficient for therapeutic development without further engineering. To circumvent this problem, the group utilized an approach previously reported to generate an efficient alanine sup-tRNA via an orthogonal tRNA scaffold, pyrrolysine (Pyl) tRNA (tRNAPyl)[7,8]. Using this concept, A1-sup-tRNA^{Ala} was derived from an evolved chimeric Phenylalanine/pyrrolysyl-tRNA scaffold (chPheT). Through further engineering of the tRNA body and co-expression with the Pyrrolysine synthetase (PylRS) tRNA-binding domain, they achieved ~78% readthrough of a UAG fluorescent PTC readthrough reporter transgene with high fidelity of alanine encoding and reasonable suppression of UGA and UAA PTCs. The resulting engineered sup-tRNA technology is called Alanine-based Programmable tRNA for Haploinsufficiency and PTC-Associated diseases, or ALPHA for short[9].

The authors reinforce the potential of sup-tRNAs as a therapeutic platform, demonstrating that the ALPHA system can effectively suppress PTCs across various diseases, each characterized by distinct correction thresholds and inheritance patterns. Previous studies have highlighted the *in vivo* effectiveness of sup-tRNAs in treating metabolic disorders like Mucopolysaccharidosis type I (MPS I), where even a minimal restoration of functional protein can alleviate phenotypic symptoms[10,11]. In this study, the authors have shown biochemical, histological, behavioral, and gross anatomical improvements across four different mouse models of nonsense-associated diseases. These encompassed MPS I (Hurler syndrome; autosomal recessive), Alport syndrome and Duchenne muscular dystrophy (DMD; both X-linked), and familial adenomatous polyposis (FAP; autosomal dominant). The findings of this study provide further support for the potential of sup-tRNA therapies, including ALPHA technology,

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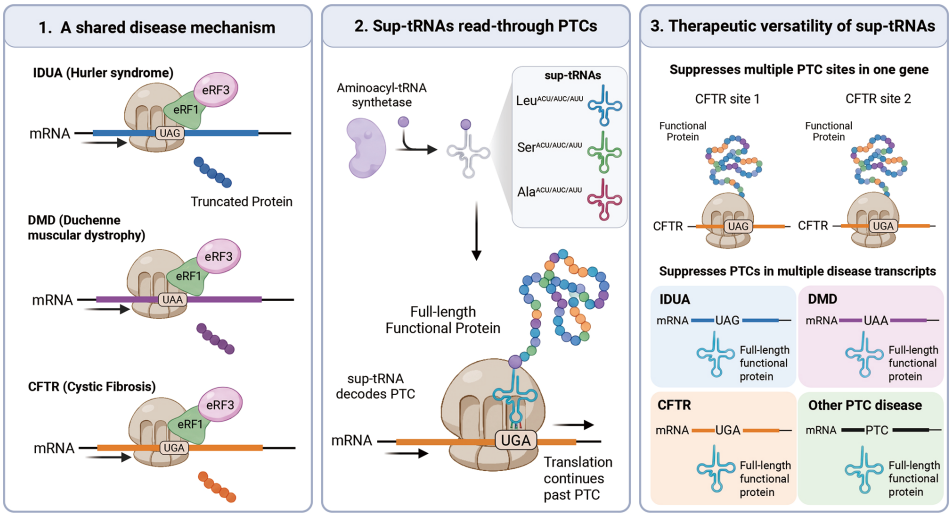


Fig. 1 Sup-tRNAs as a therapeutic platform. 1 Nonsense-associated diseases share a common disease mechanism. **2** The mechanism of sup-tRNAs, including those that have been used in a "platform approach" (Ser[5], Leu[5,11], Ala[7,9]). **3** The therapeutic versatility of sup-tRNAs for mutations in the same gene or across different genes.

to alleviate disease burden across a range of genetic disorders with differing therapeutic correction windows[12].

As with traditional gene therapies, ensuring durability and safety is crucial. For sup-tRNAs, genomic integration or editing represents the most durable option for correction, similar to the recently introduced prime editing-installed suppressor tRNA strategy, or PERT[11]. Nonetheless, Feng *et al.* observed sustained nonsense suppression for at least 13–16 weeks using ALPHA delivered via AAV9. In the MPS I mouse models, studies on glycosaminoglycan (GAG) accumulation in the urine showed an increase at the 16-week mark compared to the 8-week midpoint. Similarly, in the Alport syndrome mice, the albumin-to-creatinine ratio began to rise around the 13–16 week timeframe and continued to increase until the 22-week endpoint[9]. These findings suggest that the durability of the ALPHA sup-tRNA delivered via AAV9 might necessitate eventual redosing, which may be hindered by immune responses triggered by the initial treatment. While the reason for waning therapeutic benefit was not discussed, it may be due to the turnover of ALPHA-AAV9-transduced cells. Importantly, the study demonstrated that AAV9-administered ALPHA was both safe and well-tolerated at therapeutic doses in rats and mice.

However, given that > 30% of individuals have existing antibodies to AAV capsids[13] and repeated AAV administration will trigger an immune response in humans, further investigation into non-viral nucleic acid delivery mechanisms is warranted and actively ongoing. Currently, sup-tRNAs are being delivered using lipid nanoparticles and minimal DNA vectors[14-16]. Ultimately, the development of effective sup-tRNA therapies will require continued advances in sup-tRNA engineering as well as delivery technologies, with delivery likely needing to be tailored to the target tissue. However, this approach offers a promising path toward expanding therapeutic options for the millions of individuals affected by nonsense-associated genetic disorders.

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COMPETING INTERESTS

John D. Lueck has intellectual property considerations around the use of suppressor tRNAs as therapeutics with no other disclosures to report.

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ADDITIONAL INFORMATION

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