A Report on "Prime Editing-Installed Suppressor tRNAs for Disease-Agnostic Genome Editing" by Pierce et al. (2025)

Reviewer 2

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isitcredible.com

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I am wiser than this person; for it is likely that neither of us knows anything fine and good, but he thinks he knows something when he does not know it, whereas I, just as I do not know, do not think I know, either. I seem, then, to be wiser than him in this small way, at least: that what I do not know, I do not think I know, either.

Plato, *The Apology of Socrates*, 21d

To err is human. All human knowledge is fallible and therefore uncertain. It follows that we must distinguish sharply between truth and certainty. That to err is human means not only that we must constantly struggle against error, but also that, even when we have taken the greatest care, we cannot be completely certain that we have not made a mistake.

Karl Popper, 'Knowledge and the Shaping of Reality'

Overview

Citation: Pierce, S. E., Erwood, S., Oye, K., An, M., Krasnow, N., Zhang, E., Raguram, A., Seelig, D., Osborn, M. J., and Liu, D. R. (2025). Prime Editing-Installed Suppressor tRNAs for Disease-Agnostic Genome Editing. *Nature*, Vol. 648, No. 4, pp. 191–202.

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Abstract Summary: This study presents a disease-agnostic genome-editing strategy called prime editing-mediated readthrough of premature termination codons (PERT) to permanently convert a dispensable endogenous tRNA into an optimized suppressor tRNA (sup-tRNA) to rescue nonsense mutations. The optimized sup-tRNA, installed at a single genomic locus without overexpression, showed efficient readthrough and protein rescue in human cell models of Batten disease, Tay-Sachs disease, and cystic fibrosis, and rescued disease pathology in a mouse model of Hurler syndrome.

Key Methodology: Iterative high-throughput screening of thousands of tRNA variants, prime editing optimization, fluorescence-activated cell sorting (FACS), targeted tRNA sequencing, RNA-seq, mass spectrometry, and in vivo delivery in mouse models of Hurler syndrome.

Research Question: Can prime editing be used to permanently convert an endogenous tRNA into an optimized suppressor tRNA to rescue nonsense mutations in a disease-agnostic manner?

Summary

Is It Credible?

This article introduces "prime editing-mediated readthrough of premature termination codons" (PERT), a strategy designed to treat genetic diseases caused by nonsense mutations. The authors propose a "disease-agnostic" approach by using prime editing to permanently convert a redundant, endogenous tRNA gene into a suppressor tRNA (sup-tRNA) capable of reading through premature stop codons. The headline claims are substantial: the authors report that PERT achieved "efficient readthrough" in human cells, "extensively rescued disease pathology" in a mouse model of Hurler syndrome, and did so without inducing "detected readthrough of natural stop codons" or causing significant off-target effects (p. 191).

The core efficacy claims regarding the Hurler syndrome mouse model are largely credible, though the framing is somewhat optimistic. The authors report that in vivo delivery of the prime editor resulted in "nearly complete rescue of disease pathology" (p. 192). The data support a significant therapeutic effect, with enzyme activity restored to approximately 6% of wild-type levels—well above the therapeutic threshold—and a marked reduction in tissue vacuolization (p. 200). However, the description of the rescue as "nearly complete" masks variability evident in the detailed pathology reports, where some treated animals still exhibited mild or minimal pathology in specific tissues. While the intervention is clearly potent, the absolute language obscures the fact that the rescue was not uniform across all subjects. Furthermore, there is a notable efficiency gap between the "mean editing efficiencies of ~60–80%" achieved in optimized cell culture experiments (p. 197) and the approximately 6% editing observed in the mouse cortex and heart (p. 200). While sufficient for the specific disease model tested, this drop-off presents a challenge for the broader claim that this single composition of matter is ready to treat diverse diseases

that may require higher editing thresholds.

The safety claims regarding the specificity of the suppressor tRNAs are supported by rigorous methodology but require careful interpretation. The authors state that PERT "did not induce detected readthrough of natural stop codons" (p. 191). This conclusion relies on proteomic analysis where no peptides extending past natural stop codons were found in an initial screen. However, a more sensitive targeted analysis did identify "a single peptide corresponding to potential readthrough" of a natural stop codon (p. 197). The authors discount this finding because the abundance was "not significantly different" from untreated controls (p. 197). While statistically valid, relying on a lack of statistical significance to claim an absence of biological effect is a limitation. The signal was present, suggesting that the "no detected readthrough" claim is contingent on the sensitivity and statistical power of the assay used.

Finally, the claim that PERT is a "disease-agnostic" strategy capable of addressing a vast array of mutations is supported by a high-throughput screen of 14,746 pathogenic variants. The authors report readthrough for the "vast majority" of sequences (p. 192). However, this screen measured mRNA stabilization as a proxy for functional rescue. The authors acknowledge that this metric is only "moderately correlated ($R = 0.49$)" with actual protein yield (p. 200). This means that while the mechanism engages with the target transcripts, the prediction that PERT will yield therapeutic levels of protein across all these diseases is not fully established by the data. The strategy is mechanistically agnostic, but the functional outcome appears highly context-dependent.

The Bottom Line

The development of PERT represents a credible and significant advance in therapeutic genome editing. The authors successfully demonstrate that endogenous tR-

NAs can be engineered to rescue nonsense mutations in vivo, achieving therapeutic thresholds in a relevant mouse model. However, the claims of "nearly complete" pathology rescue and "no detected" natural stop codon readthrough should be viewed with slight caution; the former masks some experimental variability, and the latter relies on statistical thresholds that may obscure low-level off-target effects. The technology is promising, but the translation from high-efficiency cell models to complex in vivo environments remains a hurdle.

Potential Issues

Interpretation of safety data regarding natural stop codon readthrough: The article makes a strong safety claim that its prime editing-installed suppressor tRNA (PERT) strategy "did not induce detected readthrough of natural stop codons" (p. 191). This conclusion is based on a proteomic analysis correctly restricted to the 4,036 human genes that terminate with a TAG stop codon, as this is the only stop codon recognized by the specific suppressor tRNA used in the experiments. However, the interpretation of the data warrants nuance. An initial analysis found "no peptides from translation past the NTC for any TAG-terminated protein," but a more sensitive targeted analysis subsequently identified "a single peptide corresponding to potential readthrough of an NTC" (p. 197). The authors dismiss this finding because its abundance was "not significantly different (adjusted P value > 0.05)" between treated and untreated cells. While this is a common approach, a finding of no statistical significance is not definitive evidence of no biological effect, particularly in the absence of a power analysis to confirm that the experiment was sensitive enough to detect a small but potentially meaningful increase in readthrough. This shifts the claim from an absence of detection to an absence of a statistically significant increase, a distinction that may be important for a comprehensive safety assessment.

Framing of therapeutic efficacy in the Hurler syndrome mouse model: The article reports "nearly complete rescue of disease pathology" in a mouse model of Hurler syndrome following PERT treatment (p. 192). This conclusion is supported by a substantial reduction in histological pathology scores compared to untreated animals and by the achievement of mean IDUA enzyme activity of approximately 6% of wild-type levels (pp. 192, 200). As the authors note, this level of enzyme restoration is well above the ~1% therapeutic threshold previously established for this disease. However, the claim of "nearly complete rescue" may be debatable, as the article's own detailed pathology report indicates that minimal to mild pathology remained

in some tissues for at least one of the three treated animals (Supplementary Note, p. 54). While the therapeutic benefit is clearly substantial, there is a potential tension between the strong framing of the outcome and the variability present in the underlying data.

Generalizability of high-throughput screening results: The article's claim of broad applicability across many diseases is supported by a high-throughput screen of 14,746 pathogenic premature termination codons (PTCs), which found an "average readthrough score of 69 $\pm$ 30%" (p. 200). However, this score measures the stabilization of mRNA transcripts, not the production of functional protein. The article acknowledges that this mRNA-based proxy is only "moderately correlated ($R = 0.49$)" with actual protein yield in a validation experiment using 15 CFTR mutations (p. 200). A correlation of this magnitude ($R^2 \approx 0.24$) indicates that the mRNA score explains only about a quarter of the variance in protein yield, making it a relatively weak predictor of the ultimate functional outcome. While the screen successfully demonstrates broad mechanistic engagement of the suppressor tRNA with thousands of PTCs, the weak correlation suggests that protein-level rescue is likely to be far more variable and potentially lower than the high average mRNA score might imply.

Discrepancy between in vitro and in vivo editing efficiency: The article reports a significant drop in editing efficiency between experiments in cultured cells and those in a living animal. The optimized prime editing strategy yielded "mean editing efficiencies of ~60–80% in HEK293T cells" (p. 197), whereas the mean efficiency in the target tissues of the mouse model was approximately 6% (p. 200). The article acknowledges this gap, stating that "editing efficiency would benefit from further optimization in mice" (p. 200). Although the authors demonstrate that this lower in vivo efficiency was sufficient to produce a therapeutic effect in the Hurler syndrome model, the more than tenfold decrease in performance represents a key translational challenge for the technology.

Presentation and clerical issues: Several minor issues related to presentation and clerical accuracy are present. First, the article describes its off-target screen as a "genome-wide unbiased method" (p. 197), which could be viewed as an overstatement given that the screen is based on a computational prediction algorithm and uses an artificial lentiviral context. The authors do, however, transparently describe the methodology. Second, a central performance claim that "Top-performing PE3 strategies yielded mean editing efficiencies of ~60–80% in HEK293T cells" (p. 197) is supported by incorrect cross-references (Supplementary Fig. 8c and Extended Data Fig. 7e); the data supporting this claim are located elsewhere in the article (Fig. 4c and Supplementary Fig. 10a). Third, the Reporting Summary states that P values will be given as "exact values whenever suitable" (p. 34), but the text frequently uses thresholds such as "$P < 0.05$" or "$P > 0.05$" (pp. 197, 199), an inconsistency in reporting style.

Future Research

High-sensitivity safety profiling: Future work could employ long-term in vivo studies with highly powered proteomic analyses specifically designed to investigate the "single peptide" signal detected in this study. Rather than relying on snapshot comparisons of abundance, longitudinal studies could determine if low-level readthrough of natural stop codons accumulates over time or leads to subtle deleterious effects in tissues with high metabolic activity, thereby rigorously testing the safety limits of the suppressor tRNAs.

Protein-level functional screening: To better substantiate the "disease-agnostic" potential of PERT, researchers could develop high-throughput screening methods that measure protein function or yield directly, rather than relying on mRNA stability as a proxy. Since mRNA scores only weakly correlate with protein levels, a library of reporter constructs fused to diverse pathogenic sequence contexts could help define the specific rules governing which mutations will achieve therapeutic protein levels, moving beyond simple readthrough detection.

Optimization of in vivo delivery and editing: Future research could focus on bridging the efficiency gap between the ~80% editing seen in vitro and the ~6% observed in vivo. This might involve optimizing the delivery vectors for specific tissues or refining the prime editing machinery to function more robustly in the physiological environment of the mouse brain and liver, ensuring that the technology can reach therapeutic thresholds for diseases requiring higher levels of correction than Hurler syndrome.

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