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

Reviewer 2

January 14, 2026

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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.
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> 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.
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> 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. 636, No. 10079, pp. 1–12.

URL: https://doi.org/10.1038/s41586-025-09732-2

Abstract Summary: Precise genome-editing technologies are limited by the need to develop new therapeutic agents for each mutation, but suppressor tRNAs (sup-tRNAs) offer a general strategy for premature stop codons. This study presents prime editing-mediated readthrough (PERT), a strategy using prime editing to permanently convert an endogenous tRNA into an optimized sup-tRNA to rescue nonsense mutations in a disease-agnostic manner.

Key Methodology: Iterative screening of thousands of human tRNA variants, optimization of prime editing agents, in vitro validation in human cell models (Batten disease, Tay-Sachs disease, cystic fibrosis), in vivo delivery via AAV vectors in a mouse model (Hurler syndrome), targeted tRNA sequencing, mass spectrometry, and flow cytometry.

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

Summary

Is It Credible?

The proposal of Prime Editing-mediated Readthrough (PERT) represents a theoretically elegant advance in therapeutic genome editing. By permanently converting a redundant endogenous tRNA into a suppressor tRNA, Pierce et al. offer a solution to the delivery and toxicity bottlenecks that have historically plagued nonsense mutation therapies. The molecular engineering described is sophisticated; the iterative optimization of the tRNA-Leu-TAA family, achieving up to 35 percent protein yield in cell culture, suggests a genuine technical achievement. However, while the molecular foundation appears robust, the credibility of the *in vivo* therapeutic validation—the study's translational apex—is severely compromised by a series of documentation failures and methodological contradictions that are difficult to reconcile with the standards required for such claims.

The most concerning issues reside in the supplementary data underpinning the rescue of the Hurler syndrome mouse model. The pathology report, intended to serve as independent verification of disease rescue, contains significant document assembly errors and internal contradictions. The signature page attached to the report refers to a completely unrelated study regarding thyroid glands in CRABP-1 knockout mice, not the MPS-I model described in the article. Furthermore, while the methods claim the pathologist was blinded, the sample table within the report explicitly lists the genotypes of the animals, suggesting unblinding may have occurred. Most critically, the narrative summary of the histology results in the supplement inverts the identity of the treatment groups, stating that the "treated" mice exhibited minimal staining while citing the animal IDs of the untreated control group. Although the data tables themselves appear to support the authors' conclusions, this level of disarray in the primary evidence chain forces a skeptical reading of the *in vivo* efficacy claims.

Beyond the documentation errors, the experimental design for the animal studies lacks necessary rigor. The comparison relies solely on treated versus untreated animals, omitting a non-targeting AAV control group. Without this control, it is impossible to definitively exclude the possibility that the observed histological changes are influenced by the viral vector

or the expression of the prime editor machinery itself, rather than the specific correction of the genetic defect. This uncertainty is compounded by the failure to optimize the mouse ortholog of the suppressor tRNA. The authors acknowledge that the mouse tRNA was refractory to the optimization that worked for the human variant (p. 44), raising questions about whether the mouse model accurately predicts human efficacy. If the mouse reagent is suboptimal, the study may be underestimating potency; conversely, if the mouse biology is fundamentally different, the translation to humans remains speculative.

Finally, the safety and specificity claims, while promising, are clouded by methodological inconsistencies. The article asserts that "label-free" mass spectrometry was used to demonstrate the absence of readthrough at natural stop codons, yet the methods section describes a TMT-labeling protocol. Similarly, the manuscript vacillates between claiming n=3 biological replicates in the reporting summary and citing n=2 in multiple figure legends. While PERT is undoubtedly a compelling concept with strong initial molecular data, the cumulative weight of these reporting errors, control omissions, and inconsistencies suggests that the therapeutic validation is less definitive than claimed. The article successfully establishes PERT as a viable molecular tool, but the evidence for its robust, disease-agnostic therapeutic application is currently fragile.

The Bottom Line

Pierce et al. present a highly innovative strategy for treating genetic diseases by turning endogenous tRNAs into suppressor tRNAs using prime editing. The molecular engineering is impressive, creating potent suppressors that function at endogenous levels. However, the study's claims of *in vivo* therapeutic rescue are undermined by severe quality control failures in the pathology documentation, including the attachment of an unrelated study's signature page and internal contradictions regarding animal identities. Combined with missing experimental controls and inconsistencies in method descriptions, these errors suggest that while the technology is promising, the current evidence supporting its therapeutic reliability is significantly weaker than the narrative implies.

Specific Issues

Pathology report integrity and documentation errors: The Supplementary Note containing the histopathology report exhibits major quality control failures. The signature page provided is for a completely different study titled "Histopathologic Analysis of the Thyroid Glands of CRABP-1 knock out male mice at different age groups," dated November 2024, rather than the MPS-I study described (Supplementary Note, pp. 10, 12). Furthermore, the narrative summary of the Alcian Blue staining results explicitly inverts the treatment groups, stating that the "treated" mice had minimal staining but identifying them by the ID numbers of the untreated group (Mice 9 and 10), while the tables correctly identify Mice 1 and 7 as the treated cohort (Supplementary Note, pp. 13–15). This contradiction between the narrative and the data tables creates significant confusion regarding the primary efficacy readout.

Potential unblinding of histopathology: The methods section states that the veterinary pathologist was "blinded to the treatment conditions" (p. 18). However, Table 1 in the pathology report lists the "Investigator Animal ID & Genotype" for each sample, explicitly identifying animals as "Idua -/- +AAV" or "Idua -/- no AAV" (Supplementary Note, p. 13). If this table was provided to the pathologist as part of the submission, the blinding protocol was compromised, which affects the impartiality of the semi-quantitative scoring.

Omission of non-targeting controls in vivo: The *in vivo* efficacy study compares PERT-treated mice only against untreated controls (p. 10). The study lacks a control group injected with a non-targeting AAV or vehicle. This omission makes it difficult to rigorously exclude non-specific effects of AAV transduction or Cas9/PE expression on the histological phenotypes, such as inflammation or cellular morphology, independent of the specific genetic correction.

Methodological contradiction in proteomic analysis: There is a direct contradiction regarding the mass spectrometry methodology used to assess safety. The main text states that "label-free quantitative mass spectrometry" was performed (p. 7), whereas the Methods section explicitly describes a protocol involving "labelling with tandem mass tags (TMTs)" (pp. 16–17). These are distinct workflows, and this discrepancy creates uncertainty regard-

ing how the safety data regarding natural stop codon readthrough was generated.

Contradiction in biological replicate reporting: The Reporting Summary claims that "All experiments were conducted at least three times with biological replicates" and specifies n=3 for validation cell culture experiments (p. 35). However, multiple figure captions for quantitative data (e.g., Fig. 2d, Extended Data Figs. 2a, 3a, 4c) explicitly state "n = 2 independent biological replicates" (pp. 5, 20, 23). This inconsistency misrepresents the statistical robustness of the in vitro optimization data.

Constraints on "disease-agnostic" scope: The article frames PERT as a "disease-agnostic" strategy (p. 1), but the authors acknowledge significant biochemical constraints. Specifically, they were unable to generate effective suppressor tRNAs for TAA stop codons, which are the most stringent (pp. 3, 11). Additionally, the strategy is currently limited to inserting specific amino acids (primarily Leucine), which may not restore function for all proteins. These limitations narrow the universal applicability implied by the title.

Reduced efficiency in MMR-proficient cells: The study reports high editing efficiencies in MMR-deficient HEK293T cells but acknowledges that editing was "substantially less effective" in MMR-proficient HeLa cells (p. 45). The authors speculate this is due to DNA repair surveillance of Pol III genes (pp. 45–46). This discrepancy suggests that the high efficiencies reported may not be achievable in all clinically relevant, DNA-repair-competent cell types.

Translational uncertainty regarding mouse orthologs: The authors report that the optimization strategy successful for the human tRNA failed to yield comparable improvements for the orthologous mouse tRNA (p. 44). They acknowledge this "complicates pre-clinical evaluation" and raises questions about the predictive value of the mouse model (p. 44). This failure implies that the specific engineered mutations may not be universally transferable across species or contexts.

Data presentation and completeness issues: There are several gaps in the presentation of the *in vivo* data. The study reports treating both homozygous and heterozygous littermates but only presents efficacy data for the homozygous mice (p. 10; Supplementary Note, p. 14). Additionally, while IDUA enzyme restoration is reported in peripheral tissues, verification of vector presence was incomplete. Anti-GFP immunostaining to track the vector was de-

tected in the brains of all three treated mice, but in the peripheral tissues of only one of these mice (Supplementary Note, pp. 13–14), leaving a gap in confirming broad vector delivery to the periphery. Similarly, the pathology report lacks IHC scores for IDUA in peripheral tissues, despite the sample table indicating these tissues were submitted (Supplementary Note, pp. 13, 16). Finally, the sample sizes for the *in vivo* study are small (n=3 treated, n=2 untreated controls), which limits the statistical power of the findings (p. 9; Supplementary Note, p. 15).

Technical constraints and metric limitations: The claim of broad efficacy relies on a "readthrough score" from a pooled screen, which the authors admit is only moderately correlated ($R = 0.49$) with actual protein yield (pp. 10, 43). Furthermore, the optimization was performed in HEK293T cells, which are triploid for the target locus, meaning "endogenous" expression represents up to three genomic copies, potentially inflating the perceived potency per allele. The authors also note that while endogenous leader sequences are generally best, there is significant variability, with some endogenous leaders failing to support robust activity (p. 4).

Future Research

Rigorous replication of in vivo histopathology: The histopathological analysis of the Hurler syndrome model requires replication with strict adherence to blinding protocols and accurate documentation. Future work must ensure that the pathologist is blinded to the genotype and treatment status of the samples, and the resulting report must be correctly assembled and free of narrative contradictions regarding treatment groups.

Inclusion of appropriate in vivo controls: Future therapeutic validation studies should include a control group treated with a non-targeting AAV delivering the prime editor components without the specific sup-tRNA edit. This is essential to decouple the therapeutic effects of the sup-tRNA from the physiological impacts of viral transduction and exogenous protein expression.

Expansion of sup-tRNA capabilities: Research is needed to overcome the current inability to target TAA stop codons. This may involve screening additional tRNA backbones or engineering the anticodon loop and modification status more aggressively to enhance decoding of this stringent stop codon. Additionally, expanding the repertoire of sup-tRNAs to insert amino acids other than Leucine, Arginine, and Serine would enhance the disease-agnostic potential of the platform.

Investigation of MMR evasion in Pol III genes: Given the reduced editing efficiency in MMR-proficient cells, future research should investigate the specific mechanisms by which DNA repair machinery surveils Pol III genes. Developing prime editing strategies that can effectively evade or temporarily inhibit these repair pathways in competent cells is crucial for clinical translation.

Clarification of safety methodologies: Future studies must resolve the ambiguity between label-free and TMT-based mass spectrometry methods. A side-by-side comparison or a clearly defined protocol is necessary to provide high-confidence data regarding the lack of readthrough at natural termination codons.

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