Beyond siRNA and mRNA: Modified tRNA and a dedicated carrier advance preclinical readthrough therapy for cystic fibrosis

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RNA medicines have matured along two routes: small interfering RNAs (siRNAs) that silence disease-causing genes and messenger RNAs (mRNAs) that direct cells to make proteins, as exemplified by the approved lipid nanoparticle (LNP) products Patisiran (siRNA), Comirnaty and Spikevax (Figure 1). Transfer RNA (tRNA) has long promised a third modality. For the ~11% of human genetic diseases caused by nonsense mutations, an engineered suppressor tRNA (sup-tRNA) can decode a premature termination codon (PTC) and restore full-length protein from the endogenous transcript.[1] Recently, Chen and colleagues confront the two obstacles that have stalled tRNA medicines—suboptimal RNA performance and vehicles borrowed from other cargos—by chemically modifying the sup-tRNA and designing a lipid nanoparticle specifically for it.[2] The advance lies less in another RNA class than in showing that effective tRNA therapy demands coordinated, cargo-specific optimization of RNA chemistry and formulation. It is nevertheless a proof of concept rather than a finished drug package.

TURNING A TRANSLATION ADAPTOR INTO A MEDICINE

Nonsense mutations convert an amino-acid codon into a PTC, truncating translation and often triggering nonsense-mediated decay (NMD). In cystic fibrosis (CF), nonsense variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene affect about 10% of patients, and although CFTR modulators such as Trikafta improve the folding, trafficking or gating of existing protein, they have little substrate to act on when a PTC blocks full-length synthesis. Sup-tRNAs offer a conceptually different solution: their anticodons are engineered to recognize a PTC and insert an amino acid, allowing the ribosome to restore full-length CFTR from the endogenous transcript.[1] It leaves DNA untouched and preserves native transcriptional regulation, and one sup-tRNA may serve every gene sharing the same PTC. The obstacles are equally clear: weak readthrough, inefficient aminoacylation, immune activation, limited persistence and poor delivery.

In a landmark 2023 study, Albers and colleagues engineered the anticodon stem and T-arm of human tRNAs so that LNP-delivered sup-tRNAs re-established CFTR expression and channel function in cell models and patient-derived nasal epithelia, with no detectable readthrough at native stop codons.[3] Those sup-tRNAs, however, were unmodified in vitro-transcribed RNAs with limited readthrough, charging and persistence, and were carried by LUNAR LNPs engineered for mRNA rather than for a compact, structured tRNA. Chen et al. upgrade both halves of the equation: N^1-methyladenosine (m^1A)-modified sup-tRNAs with higher readthrough, better aminoacylation, longer functional persistence and lower immunogenicity, paired with TTP-3, a tRNA-tailored LNP identified by screening 1,000 ionizable lipids in parallel against mRNA.

CHEMICAL MODIFICATION IMPROVES SUPPRESSOR tRNA PERFORMANCE

Chen and colleagues first addressed the cargo. Endogenous tRNAs carry modifications that shape folding, stability, decoding and synthetase recognition. Anticodon engineering disrupts these features: converting a native glycine tRNA into a suppressor cut its charging from about 84% to 30%. The standout among individually tested modifications was m^1A in the T-loop, at position 57 in the glycine scaffold and at position 58 in the arginine scaffold: an m^1A57 glycine sup-tRNA improved readthrough about 2.4-fold and restored charging to roughly 63%, while the same modification on an arginine scaffold improved readthrough up to about 10.6-fold. In CF human bronchial epithelial cells, tRNA sequencing gave an apparent half-life of about 29 days for the modified sup-tRNA versus about 12 days unmodified; signal persisted beyond 30 days in reporter cells and, after intravenous dosing, in a reporter mouse. The modified tRNAs also outlasted directly delivered reporter mRNA, provoked less interferon regulatory factor (IRF) signaling and maintained peak responses across repeated doses. More modification was not automatically better: several substitutions impaired charging or readthrough, and multiply modified designs activated nuclear factor κB.

A CARRIER DESIGNED FOR tRNA RATHER THAN BORROWED FROM siRNA OR mRNA

The second half of the strategy was delivery. Using a Ugi four-component reaction, the team made 1,000 ionizable lipids and screened them in parallel for sup-tRNA and mRNA delivery in bronchial epithelial cells. Cross-cargo correlation was weak (Pearson r = 0.168 across that library, spanning roughly 7–31 $\log_2$RLU for tRNA and 2.5–18 $\log_2$RLU for mRNA): LNP structure–activity relationships are strongly cargo dependent, and an mRNA-validated carrier need not carry tRNA. Even so, the mRNA-developed lipid A10-LIN performed favorably in the sup-tRNA screen, so the screen argues for cargo-matched testing rather than mutually exclusive lipid sets. In a mucus-overlaid air–liquid-interface culture, the winner, TTP-3 (tRNA-tailored pulmonary delivery-3), outperformed MC3, the ionizable lipid in the approved siRNA drug Patisiran, and A10-LIN, a lipid developed for pulmonary mRNA delivery.[4] Design-of-experiments optimization converged on 13.5:1 ionizable-lipid:tRNA mass ratio with 60 mol% TTP-3, 10 mol% DOPE, 27.5 mol% β-sitosterol and 2.5 mol% PEG-lipid, distinct from the mRNA-optimal composition of the same lipid. After intratracheal dosing in reporter mice, TTP-3 LNPs gave the strongest pulmonary readthrough, and a Mango II aptamer (a fluorogenic RNA tag) revealed epithelial-dominant delivery reaching basal stem cells, whereas MC3 transfects endothelial cells. Nebulization retained partial activity, improved by poloxamer 188 (a surfactant that stabilizes LNPs during aerosolization), but post-aerosolization losses leave inhalation unsolved.

FUNCTIONAL RESCUE ACROSS COMPLEMENTARY CF MODELS

The optimized cargo–carrier pair was validated across a progression of CF models. In human bronchial epithelial cells carrying G542X or R1162X, modified sup-tRNAs restored mature CFTR protein; at G542X the arginine sup-tRNA outperformed the glycine version, producing the function-retaining G542R substitution. In R1162X cells, CFTR-dependent ion-channel activity improved and protein remained detectable beyond 40 days. Ribosome profiling detected no global increase in readthrough at canonical stop codons, but this aggregate readout cannot exclude low-level readthrough at individual stop codons or single off-target proteins. In R553X mice, three intratracheal doses of modified sup-tRNA in TTP-3 LNPs gave the largest increase in lung CFTR mRNA. Because CF mice do not reproduce human lung disease, rescue was assessed in intestinal organoids from R553X mice, where the same combination again produced the strongest forskolin-induced swelling. The most clinically suggestive experiment used organoids from a person with a complex CFTR genotype (two nonsense mutations, a missense mutation and a polymorphism): neither G418 nor Trikafta alone rescued function, sup-tRNA

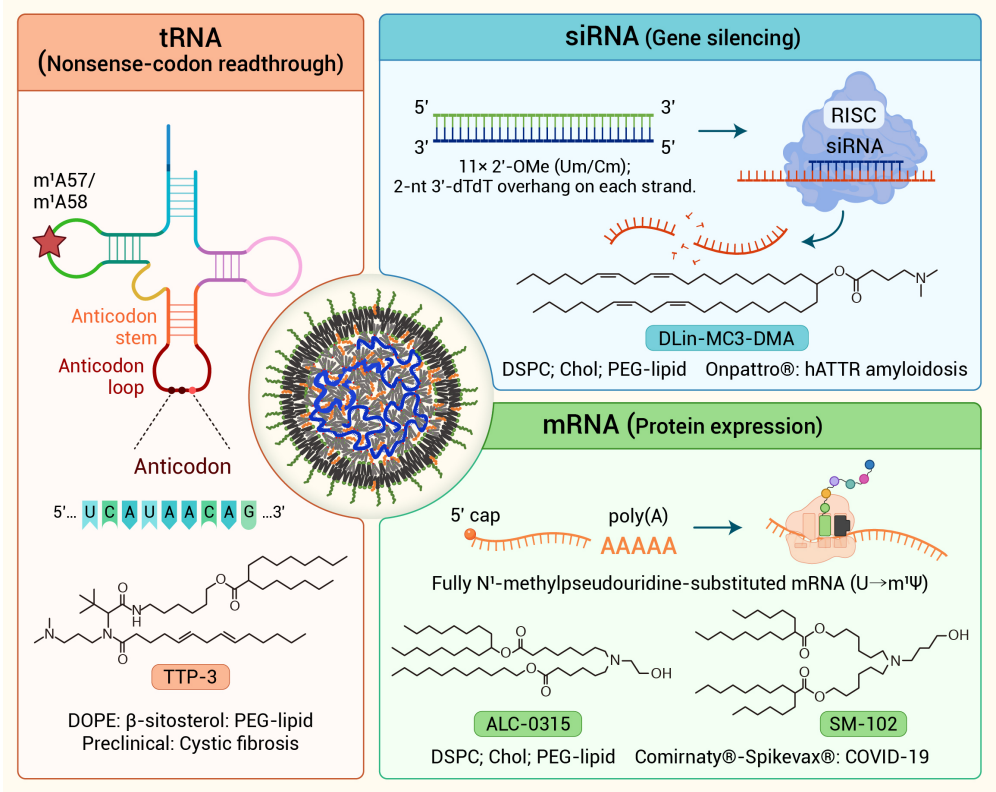

Figure 1. Representative applications of lipid nanoparticles for nucleic acid therapeutics A lipid nanoparticle (LNP) is built from an ionizable lipid, a helper phospholipid, a sterol (cholesterol or a phytosterol such as β-sitosterol) and a PEGylated lipid. The same architecture has been used to encapsulate three classes of RNA cargo: small interfering RNA (siRNA) for gene silencing (for example, Patisiran), messenger RNA (mRNA) for protein replacement and vaccination (for example, Comirnaty and Spikevax), and engineered suppressor transfer RNA (sup-tRNA) for readthrough of premature termination codons (PTCs), as delivered by the cargo-tailored LNP TTP-3 (tRNA-tailored pulmonary delivery-3) discussed here. Abbreviations: LNP, lipid nanoparticle; mRNA, messenger RNA; PEG, polyethylene glycol; PTC, premature termination codon; siRNA, small interfering RNA; sup-tRNA, suppressor transfer RNA; TTP-3, tRNA-tailored pulmonary delivery-3.

alone gave only a modest, nonsignificant improvement, but sup-tRNA plus Trikafta restored CFTR function. The biology is complementary — sup-tRNAs rebuild full-length protein and Trikafta assists its folding, trafficking and activity — providing a rationale to extend modulators to genotypes they cannot currently serve.

BENCHMARKING GAPS AND REMAINING TRANSLATIONAL BARRIERS

The most conspicuous gap is benchmarking against normal CFTR rather than against current therapy, such as modulator-treated F508del-CFTR.[5] Wild-type anchors are present — wild-type cells provide the functional and transcriptomic comparators, in which transcript recovery reaches >10% of wild-type levels, and mouse lung CFTR mRNA is plotted against a wild-type group — but no F508del-on-Trikafta arm appears in the cells, mice or organoids, the immunoblot panels report only untreated and treated mutant cells, the patient-derived organoids lack both a healthy control and an F508del homozygous comparator, and no primary human bronchial or nasal cultures with relevant genotypes are reported. Without a modulator-treated arm, how much sup-tRNA therapy would add to current therapy cannot be judged, and the rescue is best read as encouraging rather than benchmarked.

Several caveats also temper the clinical reading. The human data come from immortalized 16HBEge lines and intestinal organoids, whose predictive value for human lung disease is uncertain because the R553X mouse lacks the characteristic airway pathology. The biodistribution mapping relies on a sup-tRNA carrying a Mango II aptamer that lowers readthrough activity by 30–40%, so cell-type targeting is inferred from a functionally attenuated surrogate. Tolerability was encouraging — transaminases were unchanged and pulmonary inflammation was transient, dose-dependent and largely resolved by day 14 — but these are single-dose data at 1–10 mg/kg, and repeated-dose immunogenicity of the LNP itself, anti-PEG responses and biodistribution beyond the lung remain uncharacterized. The complementary effects observed with Trikafta are mechanistically plausible but were demonstrated in a single complex genotype; formal synergy and generalizability to other nonsense genotypes and modulators remain unestablished.

OUTLOOK

Sup-tRNA therapy is codon-directed rather than gene-directed: one molecule may rescue the same PTC class across disorders, and arginine-to-UGA mutations alone account for an estimated 24% of known pathogenic PTCs. The study therefore offers more than a CF candidate: a modular framework in which tRNA sequence, inserted amino acid, modification, delivery lipid, formulation, route and partner drug are optimized as connected variables. The most valuable next experiments are the missing comparators: modulator-treated arms in patient-relevant airway cells and in the organoid model, and repeat-dose immunogenicity and biodistribution studies showing whether sup-tRNA delivery adds to current modulator therapy. The consequential challenge is whether cargo-specific design principles generalize into modular LNP families serving siRNAs, mRNAs and structured tRNAs without sacrificing tissue selectivity. The 1,000-lipid screen generated the multidimensional structure–formulation–activity datasets needed for AI-guided LNP design, a capability now maturing for mRNA and potentially extensible to structured RNA cargos — although such models will extrapolate poorly unless trained on structured-RNA cargos and in vivo endpoints. By moving tRNA from a passive biological adaptor toward an engineerable therapeutic, this work widens both the vocabulary of RNA medicine and the design space of nonviral delivery.

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All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.