Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

with EVs containing small interfering RNAs against MALAT1 or HDAC11 showed greater ability to clear bacteria. Conversely, PMNs treated with constructs encasing MALAT1 or HDAC11 efficiently cleared IAV and SARS-CoV-2. PMNs expressing Ehf showed greater clearance of bacteria and viruses.

Conclusions: Our findings suggest mutually exclusive roles of MALAT-1 and HDAC11 in regulating bacterial and viral clearance by airway-recruited PMNs. Expression of Ehf in airway PMNs may be a pathogen-agnostic approach to enhancing clearance by airway-recruited PMNs. Overall, our study brings proof-of-concept data for therapeutic RNA/protein transfer to airway-recruited PMNs in CF and other lung diseases and for use of EVs as a promising method for cargo delivery to these cells. It is our expectation that, by treating the immune compartment of CF airway disease, pathogen-therapies, such as antibiotics will be more effective, and epithelial-targeted therapies, such as CFTR modulators, will have greater penetrance into the cell types of interest.

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Generation of a cystic fibrosis transmembrane conductance regulator R1162X "TAG" model and comparison of readthrough to the naturally occurring R1162X "TGA"

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Background: Premature termination codon (PTC) mutations in the cystic fibrosis (CF) transmembrane conductance regulator *(CFTR)* coding sequence affect approximately 10% of patients with CF, and effective therapies for these patients remain a serious medical need. The four most common CF-causing *CFTR* PTC mutations are G542X, W1282X, R553X, and R1162X, all of which are "TGA" (opal) stop codons. Drug-induced readthrough is a promising therapeutic approach that can restore expression of full-length *CFTR* and consequently suppress nonsense-mediated messenger ribonucleic acid (RNA) decay (NMD). R1162X is thought to be more promiscuous than other PTC mutations to current readthrough molecules and is being investigated in high-throughput screening campaigns to identify novel readthrough molecules. Nucleotide sequence surrounding a PTC can affect readthrough, but how different stop codons in the same sequence context can affect readthrough is largely unexplored. We created a 16HBEge cell model of *CFTR* with a R1162X "TAG" (amber) PTC and compared it with the previously established 16HBEge cell model with the patient-relevant R1162X "TGA" (opal) mutation. These two cell models express different stop codons at the same position in *CFTR* and can be used to further explore PTC biology.

Methods: The R1162X "TAG" model was created from the 16HBE14o- cell line (wild-type [WT] *CFTR*) using prime editing. First, 144 prime editing combinations were tested in HEK-293 cells. The top eight combinations from HEK-293 cells were tested in 16HBE14o- cells in pools of 2 to 3. The top-performing combination resulted in approximately 5.5% editing efficiency in bulk and was used to generate an R1162X "TAG"–expressing heterozygous clonal cell line with high transepithelial resistance in CFTR functional assays.

Results: Preliminary studies comparing R1162X "TGA" and R1162X "TAG" showed that both had little to no function without treatment, as expected. The quadruple combination treatment of tezacaftor, elexacaftor, the aminoglycoside ELX-02, and the cereblon E3 ligase inhibitor CC-90009 [1], in addition to acute potentiation with ivacaftor in the assay, resulted in an increase in CFTR function to approximately 3% of WT function, which was not different between the R1162X "TGA" and RR162X "TAG" stop codons.

Conclusions: In summary, prime editing resulted in low-efficiency editing of the *CFTR* R1162 region using the 144 prime editing guide RNA and nicking RNA combinations tested, but a usable, heterozygous cell model expressing the R1162X "TAG" mutation was identified. Preliminary studies showed no significant difference in CFTR function between the R1162X "TAG" and "TGA" models when treated with the quadruple cocktail listed above, suggesting that the sequence context surrounding R1162X is an important component of R1162X readthrough. We expect to use the R1162X "TAG" specificity model to characterize and compare current and future readthrough agents.

Reference

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Genome-wide screen to uncover genes promoting premature termination codon readthrough

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Background: More than 2000 variants of the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) gene have been described, and at least several 100 cause CF. Nonsense variants are the second most prevalent mutation; they affect approximately 10% of people with CF and are caused by a nucleotide change that creates a premature termination codon (PTC). A ribosome encountering a PTC terminates translation, fails to displace downstream exon junction complexes, and triggers nonsense-mediated decay (NMD) of messenger ribonucleic acid (RNA), resulting in absence of functional protein and a severe CF phenotype. One of therapeutic strategies for PTC mutations is use of agents to suppress the proofreading function of ribosomes at PTCs and favor insertion of a near-cognate amino acid. Such readthrough agents (RTAs) allow translation completion (downstream to PTC), enabling production of a CFTR protein. Despite multiple screening campaigns to identify new RTAs (e.g., ataluren, negamycin, tylosin), aminoglycosides remain the most potent, but sustained exposure to aminoglycosides induces only low levels of PTC readthrough or elicits severe toxicity. Understanding of ribosome translation termination exposed the interplay between factors and revealed potential targets for pharmacological intervention, such as eRF1 and eRF3. We performed a genome-wide screen (GWS) on our readthrough reporter cell line (CFF-16HBEge CFTR G542X expressing a CFTR peptide surrounding G542X fused to green fluorescent protein (GFP) and an inducible Cas9) and have identified potential targets for readthrough therapy.

Methods: First, we used the pLVXS-sgRNA-mCherry-hyg plasmid based single guide (sg)RNA library from TakaraBio (Genome-Wide sgRNA Library System consisting of 19 114 target genes, four sgRNAs per gene, based on the Brunello Library) to produce a lentivirus (LTV) genome-wide (GW) sgRNA library. Second, we transduced our readthrough reporter cell line with the LTV GW sgRNA library at a low multiplicity of infection (~0.4) to ensure that cells carry a single sgRNA. Cells were subjected to hygromycin selection and expansion before induction of Cas 9 expression (2 days), followed by treatment with G418 (100 μM, 4 days). Third, GFP+ cells (iGFP intensity above baseline value) were fluorescence-activated cell sorted and analyzed by next-generation sequencing for sgRNA frequency. sgRNA enrichment threshold more than 100 times as great as reference (unsorted, no Cas9 induction) was used to identify genes promoting PTC readthrough.

Results: The frequency distributions of sgRNAs encoded in the TakaraBio plasmid library and the distribution observed in our LTV-transduced cells were virtually identical. In the cell-sorted population, our two positive control genes, eRF1/ETF1 and eRF3/GSTP1, were above the enrichment threshold of 100 times (4 and 3 sgRNAs >100X, respectively), validating our genomic screen system. One hundred forty different gene targets with one