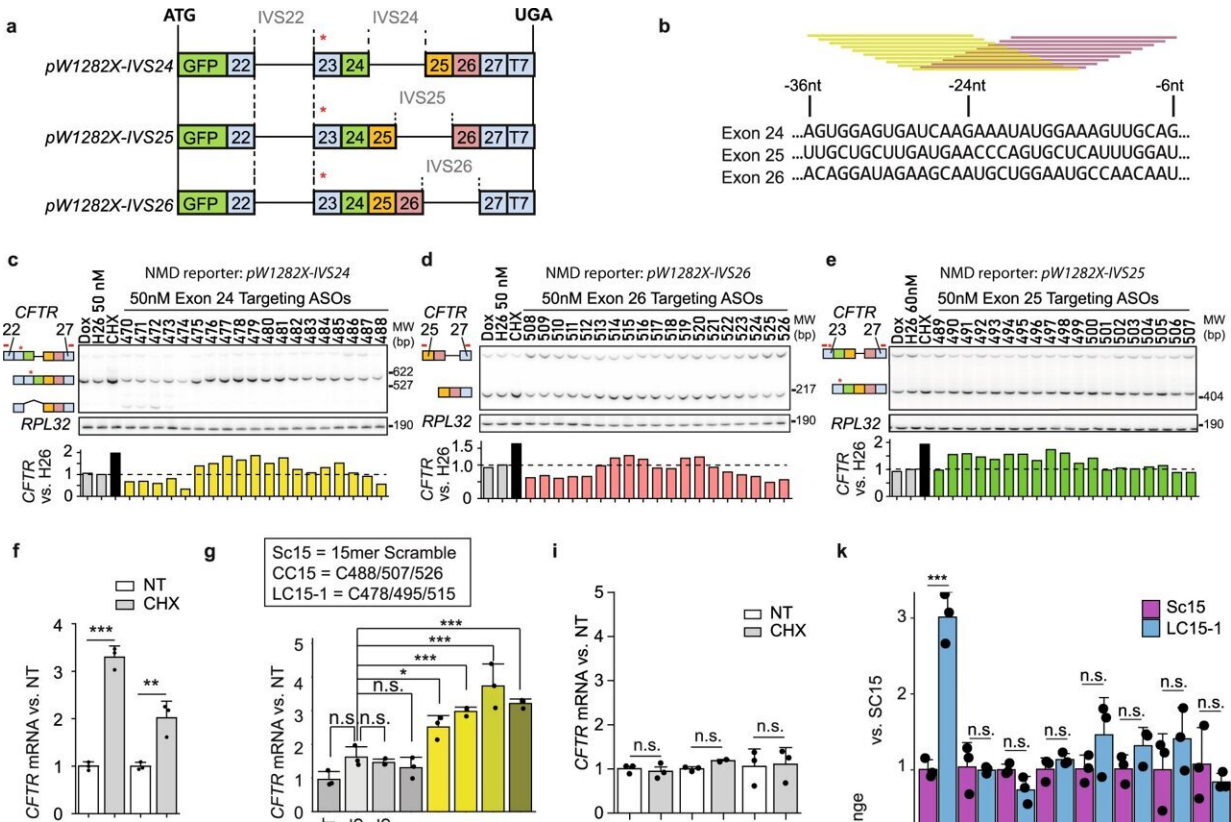


A new treatment approach for cystic fibrosis

July 14 2022



Identification of NMD-inhibiting ASOs and assessment of their specificity. **a** Schematic of NMD reporters. The numbers show the *CFTR* exons in the NMD reporters. The red asterisk (*) indicates the location of the W1282X mutation. **b** Schematic of ASO screening. 19 MOE-PS-modified 15-mer ASOs (yellow and magenta bars) were designed to cover the presumptive EJC binding sites on exons 24, 25, and 26 at 1-nt resolution. U2OS cells stably expressing each NMD reporter were transfected with individual ASOs targeting EJC binding regions on *CFTR* exon (c) 24, (d) 25, or (e) 26, respectively. Reporter mRNA levels were measured by radioactive RT-PCR, using primers (red bars above the target exon) listed in Supplementary Table 5. **f** Effect of cycloheximide on *CFTR* expression

in 16HBE-W1282X and DLD1-W1282X cells. **g** Effect of the 15-mer ASO cocktail LC15-1 on *CFTR* expression in 16HBE-W1282X cells. **h** Comparison between the effects of LC15-1 or LC18 on *CFTR* expression in 16HBE-W1282X cells. **i** Effects of cycloheximide on *CFTR* expression in DLD1-WT, 16HBE-G551D, and 16HBE-F508del cells. **j** *CFTR* mRNA levels in DLD1-WT, 16HBE-G551D, and 16HBE-F508del transfected with Sc15 or LC15-1 at a nominal total concentration of 120 nM. **k** Endogenous NMD-sensitive mRNA levels in 16HBE-W1282X cells treated with cycloheximide, 120 nM Sc15 or LC15-1. All mRNA levels in (**f**)–(**k**) were measured by RT-qPCR; *CFTR* mRNA levels were measured using forward and reverse primers targeting exon 22 and exon 23, respectively. *RPL32* served as internal reference for all panels except **h**, in which *HPRT* served as internal reference. NT = No treatment; Dox: doxycycline 1 $\mu\text{g}/\text{mL}$; Sc15/18 = 15/18-mer Scramble ASO; CC15 = ASO cocktail C488/C507/C526; LC15-1=ASO cocktail C478/C495/C515; LC18 = 18-mer ASO cocktail C24/25/26; CHX = 1-h incubation with 100 $\mu\text{g}/\text{mL}$ cycloheximide. Data are represented as mean values \pm SD. All data points represent independent biological replicates. **c–e** ($n = 1$). **f–k** ($n = 3$ for all treatments, except $n = 2$ in LC18-mer 120 nM in **h**). For all statistical tests, n.s. $P > 0.05$, * P

Citation: A new treatment approach for cystic fibrosis (2022, July 14) retrieved 15 February 2024 from <https://medicalxpress.com/news/2022-07-treatment-approach-cystic-fibrosis.html>

This document is subject to copyright. Apart from any fair dealing for the purpose of private study or research, no part may be reproduced without the written permission. The content is provided for information purposes only.