A Green Light for Stop Mutations

Individuals with Cystic Fibrosis (CF) continue to live longer and enjoy healthier lives (1). Before the 1980s, about half of those affected by CF did not live past their teenage years, whereas life expectancy has improved dramatically in the last three decades. The discovery of the *cftr* (cystic fibrosis transmembrane conductance regulator) gene over 30 years ago led to the identification of the structure and function of the CFTR chloride channel and, ultimately, to the era of new therapies. The new drugs available today are tailored to the different CFTR-class mutations and represent an important milestone in CF treatment, revolutionizing patient care. Over 2,000 different mutations in the CFTR gene that cause CF have been identified so far, with ~8% being nonsense mutations that result in premature cessation of translation, thereby suppressing CFTR protein synthesis (2). Premature termination codon (PTC) mutations are present in ~10% of all patients with CF, although the incidence is much higher in some populations. For example, ~85% of patients with CF of Ashkenazi Jewish descent have at least one premature stop allele (3). The new therapies aimed at correcting the basic defect have not been effective in this class of mutation.

Twenty-five years ago, the CF Research team in Birmingham, Alabama, showed that low-dose aminoglycoside antibiotics can rescue full-length CFTR expression by suppressing PTCs and thereby restore cyclic AMP-activated chloride channel activity on CF epithelia expressing class 1 mutations (4). In a double-blind, placebo-controlled, crossover study, improvements in CFTR-specific Cl^- secretion were observed when gentamicin was given intranasally to patients with CF, most of whom carried PTC on both alleles. Complete normalization of all electrophysiological abnormalities caused by the defective CFTR was achieved for 21% of patients, whereas restoration of either chloride or sodium transport was reported for 68% (5). Chronic treatment with aminoglycosides may cause renal and vestibular toxicity and is therefore contraindicated as a standard treatment for CF. Based on the results with gentamicin, an oral medication was developed to overcome the adverse effects of nonsense mutations; this worked by inducing ribosomal read-through of mRNA containing a premature stop codon and allowing for the translation of a full-length protein (6). Although phase 1 and 2 trials seemed to show promising results, phase 3 trials were inconclusive (7–9); therefore, development of the drug was not pursued for the treatment of patients with CF.

Another possible treatment for patients with CF carrying these mutations is ELX-02, an investigational aminoglycoside analog that acts by inducing read-through of nonsense mutations by interacting with the ribosome. This results in the production of full-length functional proteins. Safety and pharmacokinetics have been evaluated in a phase I study. Forskolin-induced swelling (FIS) of organoids derived from the G542X mutation treated with ELX-02 has recently been reported (10). These results support the continued investigation of ELX-02 in phase 2 clinical trials (11).

In recent years, treatments using modulators, correctors, and potentiators have been developed that are effective treatments for over 90% of patients with CF. However, only standard CF therapies are currently available for those carrying the nonsense mutations.

In this issue of the *Journal*, Mutyam and colleagues (pp. 604–616) from the CF Research group in Birmingham, Alabama, describe their finding of a new treatment for patients with premature stop mutations (12). The combined use of Galapagos/AbbVie CFTR correctors and potentiators and read-through compounds augmented the functional repair of CFTR nonsense mutations, thus indicating the potential for these novel drugs to restore function, especially to truncated W1282X CFTR. These studies used the correctors GLPG2222 C1a (derivative of C1), GLPG3221 C2a (derivative of C2), and the potentiator GLPG1837, alone or in combination with the read-through compound G418. They evaluated CFTR function using heterologous Fischer Rat Thyroid (FRT) cells, genetically engineered human bronchial epithelial (HBE) cells (16HBE14o-), and primary human cells carrying PTC mutations. They report that GLPG1837 produced a dose-dependent increase in CFTR activity that was greater than the maximum activity achieved by ivacaftor in FRT-W1282X and FRT-R1162X cells. This is similar to previous results in which GLPG1837 induced greater activity than ivacaftor in primary HBE cells derived from a patient with G551D/F508 del mutations (13). Furthermore, they show that GLPG1837+C1a+C2a restored substantial function in G542X/F508 del HBE cells and even more in cells carrying W1282X/F508 del, largely because of the corrector/potentiator effect, with no additional benefit from G418.

In organoids with G542X/R553X or R1162X/R1162X mutations, enhanced FIS was observed with G418+GLPG1837+C1a+C2a, although GLPG1837+C1a+C2a alone was sufficient to improve FIS in organoids with W1282X/W1282X mutations.

The researchers from the same CF center that performed the initial research on stop mutations 25 years ago are to be commended for this work. Their results give hope to individuals with CF who carry nonsense mutations that are not responsive to modulator therapies. The goal is to maximize the effect of stop-codon suppressors on CFTR while minimizing side effects. Their results represent a first step to a brighter future for patients with nonsense mutations. Of course, these promising results need to be confirmed in the CF mouse model with a stop mutation before proceeding to human testing, but there appears to be a green light after the stop sign. ■

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References

1. Cohen-Cymberknoh M, Shoseyov D, Kerem E. Managing cystic fibrosis: strategies that increase life expectancy and improve quality of life. *Am J Respir Crit Care Med* 2011;183:1463–1471.
2. Cystic Fibrosis Mutation Database. Toronto, Ontario, Canada: Hospital for Sick Children; 2021 [accessed 2021 Feb 20]. Available from: http://www.genet.sickkids.on.ca/StatisticsPage.html.
3. Shoshani T, Augarten A, Gazit E, Bashan N, Yahav Y, Rivlin Y, *et al*. Association of a nonsense mutation (W1282X), the most common mutation in the Ashkenazi Jewish cystic fibrosis patients in Israel, with presentation of severe disease. *Am J Hum Genet* 1992;50:222–228.
4. Howard M, Frizzell RA, Bedwell DM. Aminoglycoside antibiotics restore CFTR function by overcoming premature stop mutations. *Nat Med* 1996;2:467–469.
5. Wilschanski M, Yahav Y, Yaacov Y, Blau H, Bentur L, Rivlin J, *et al*. Gentamicin-induced correction of CFTR function in patients with cystic fibrosis and CFTR stop mutations. *N Engl J Med* 2003;349:1433–1441.
6. Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, Trifillis P, *et al*. PTC124 targets genetic disorders caused by nonsense mutations. *Nature* 2007;447:87–91.
7. Kerem E, Konstan MW, De Boeck K, Accurso FJ, Sermet-Gaudelus I, Wilschanski M, *et al*.; Cystic Fibrosis Ataluren Study Group. Ataluren for the treatment of nonsense-mutation cystic fibrosis: a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Respir Med* 2014;2:539–547.
8. Shoseyov D, Cohen-Cymberknoh M, Wilschanski M. Ataluren for the treatment of cystic fibrosis. *Expert Rev Respir Med* 2016;10:387–391.
9. Konstan MW, VanDevanter DR, Rowe SM, Wilschanski M, Kerem E, Sermet-Gaudelus I, *et al*.; ACT CF Study Group. Efficacy and safety of ataluren in patients with nonsense-mutation cystic fibrosis not receiving chronic inhaled aminoglycosides: the international, randomized, double-blind, placebo-controlled Ataluren Confirmatory Trial in Cystic Fibrosis (ACT CF). *J Cyst Fibros* 2020;19:595–601.
10. Crawford DK, Mullenders J, Pott J, Boj SF, Landskroner-Eiger S, Goddeeris MM. Targeting G542X CFTR nonsense alleles with ELX-02 restores CFTR function in human-derived intestinal organoids. *J Cyst Fibros* [online ahead of print] 5 Feb 2021; DOI: 10.1016/j.jcf.2021.01.009.
11. Leubitz A, Vanhoutte F, Hu MY, Porter K, Gordon E, Tencer K, *et al*. A randomized, double-blind, placebo-controlled, multiple dose escalation study to evaluate the safety and pharmacokinetics of ELX-02 in healthy subjects. *Clin Pharmacol Drug Dev* [online ahead of print] 19 Jan 2021; DOI: 10.1002/cpdd.914.
12. Mutyam V, Sharma J, Li Y, Peng N, Chen J, Tang LP, *et al*. Novel correctors and potentiators enhance translational readthrough in CFTR nonsense mutations. *Am J Respir Cell Mol Biol* 2021;64:604–616.
13. Gees M, Musch S, Van der Plas S, Wesse AS, Vandevelde A, Verdonck K, *et al*. Identification and characterization of novel CFTR potentiators. *Front Pharmacol* 2018;9:1221.