

Putting CHIP(s) on the Table: Introducing Nitrosothiols into the Arena of CFTR Modulation

Cystic fibrosis (CF) is an autosomal-recessive, genetically based, life-limiting disease characterized by viscid secretions in multiple organ systems (1, 2). The cystic fibrosis transmembrane conductance regulator gene (*CFTR*), on chromosome 7, codes for a cAMP-regulated chloride channel on multiple epithelial surfaces, including the airways, pancreas, and intestine (1, 2). Mutations can affect the *CFTR* protein at any point, including during formation, maturation, intracellular trafficking, surface function, and denaturation (1, 2). Over 2,000 mutations have been described, with a phenylalanine deletion (genetically: c.1521_1523delCTT) resulting in misfolded protein and accelerated degradation (F508del) being the most common disease-causing mutation (1, 2). Defective *CFTR* function impairs chloride conductance, with associated defective sodium transport across epithelial surfaces resulting in the generation of viscid secretions that are the hallmark of CF (1).

Multidisciplinary center-based care combined with therapeutic advances has dramatically increased life expectancy for people with CF (PWCF); indeed, infants born today are predicted to survive for almost 50 years (2). Precision therapies capable of modulating the underlying cellular defect (*CFTR* modulators) represent the most significant therapeutic advance in CF (2). With early diagnosis through newborn screening, and the development of *CFTR* modulators, clinical care is moving away from managing downstream complications and toward targeted therapies to prevent disease progression (1–3).

The first *CFTR* modulator, ivacaftor, is a *CFTR* potentiator that improves channel gating at epithelial surfaces and potentiates chloride conductance and *CFTR* function, with resultant clinical improvement (4). Only 4% of PWCF (2) who carry “gating” mutations are responsive to ivacaftor; in these individuals a sufficient quantity of the defective *CFTR* traffics to the cell membrane (2, 4) to provide substrate for ivacaftor (1, 2, 4). Combination therapy, which involves using a *CFTR* corrector such as lumacaftor or tezacaftor to promote *CFTR* production, with ivacaftor to potentiate channel gating, has widened the range of mutations amenable to *CFTR* modulation, including F508 del (3). The clinical benefits of current combination therapy require optimization (2, 5). Although early results of triple combination therapy (two correctors and one potentiator) appear promising, the search for *CFTR* modulators suitable for all PWCF continues (2, 3, 5). Gene agnostic *CFTR* amplifiers, which increase the protein substrate for *CFTR* modulators, may widen the scope for future combination therapies (5). Even with a portfolio of promising preclinical and clinical studies, better insight into molecular pathways associated with *CFTR* trafficking may provide necessary additional therapeutic targets (2, 5).

In this issue of the *Journal*, Zaman and colleagues (pp. 765–775) describe a novel alternative to current approaches for modulating *CFTR* (6). Whereas correctors, potentiators, and

amplifiers all target the promotion of *CFTR* production (2, 5), Zaman and colleagues focus on reducing ubiquitination and proteasomal degradation, increasing *CFTR* trafficked to the cell membrane (6). The F508del *CFTR* mutation impairs protein folding and assembly, and is detected by the cells’ quality control (QC) system. Polyubiquitination of defective *CFTR* leads to its extraction from the endoplasmic reticulum (7). Detection of defective *CFTR* in the cytoplasm is performed by cHSP-70 (8). cHSP-70 interacts with the E3 ligase carboxy terminus of heat shock protein 70-interacting protein (CHIP), which in association with the E2 ligase UbcH5a effects ubiquitination and transport of misfolded *CFTR* for degradation by the cells’ QC system (the ubiquitin-proteasome system) (7, 8). Targeted therapies could inhibit ubiquitin-dependent degradation of *CFTR*, shielding it from the ubiquitin-proteasome system machinery, and CHIP inhibition has been identified as a potential pharmacological target (9) (Figure 1).

Nitric oxide (NO) synthases are abundant in the respiratory epithelium and are essential for the formation of stable bioactive S-nitrosothiols (SNOs), which are important for cellular signaling, -NO transfer, and regulation of protein function (10). Dysregulation of SNO formation is implicated in multiple respiratory diseases (10), including CF. Nitrosylation inhibits E3 ligases (11) and increases *CFTR* maturation (12) and chloride transport (13) in CF airway epithelial cells. Zaman and colleagues hypothesized that SNOs could inhibit *CFTR* proteasomal degradation through S-nitrosylation-dependent inhibition of CHIP. They showed CHIP expression in primary cells and bronchial cell lines, with dose-dependent S-nitrosylation of CHIP after treatment with S-nitrosoglutathione (GSNO), which reduced CHIP expression and activity. This led to a reduction in CHIP-*CFTR* and to decreases in *CFTR* ubiquitination, allowing F508 del *CFTR* to escape the endoplasmic reticulum QC system and traffic to the cell membrane. This reveals, for the first time, the mechanism behind the effect of S-nitrosylation on *CFTR* expression (Figure 1). Of all SNOs tested, S-nitrosoglutathione diethyl ester (GNODE) showed the highest effect on *CFTR* maturation, but in the current study this was tested only in cell monolayers. To progress toward *CFTR*-modulating therapies, cell-permeable GSNO analogs, including GNODE, should be used to test whether these findings can be replicated in differentiated pseudostratified epithelium. The bioavailability and bioactivation of SNOs may be lower in pseudostratified epithelium, potentially reducing the overall effect of GSNO on *CFTR* expression (14).

Modulation of the QC system may increase *CFTR* trafficked to the cell surface (6, 7), but the impact on *CFTR*-dependent chloride transport and later denaturation requires further investigation both *in vitro* and *in vivo* (2, 5). Previous *in vivo* studies using SNOs in CF focused on their antiinflammatory, smooth-muscle relaxant and bronchodilatory potential (15). A randomized trial of inhalational

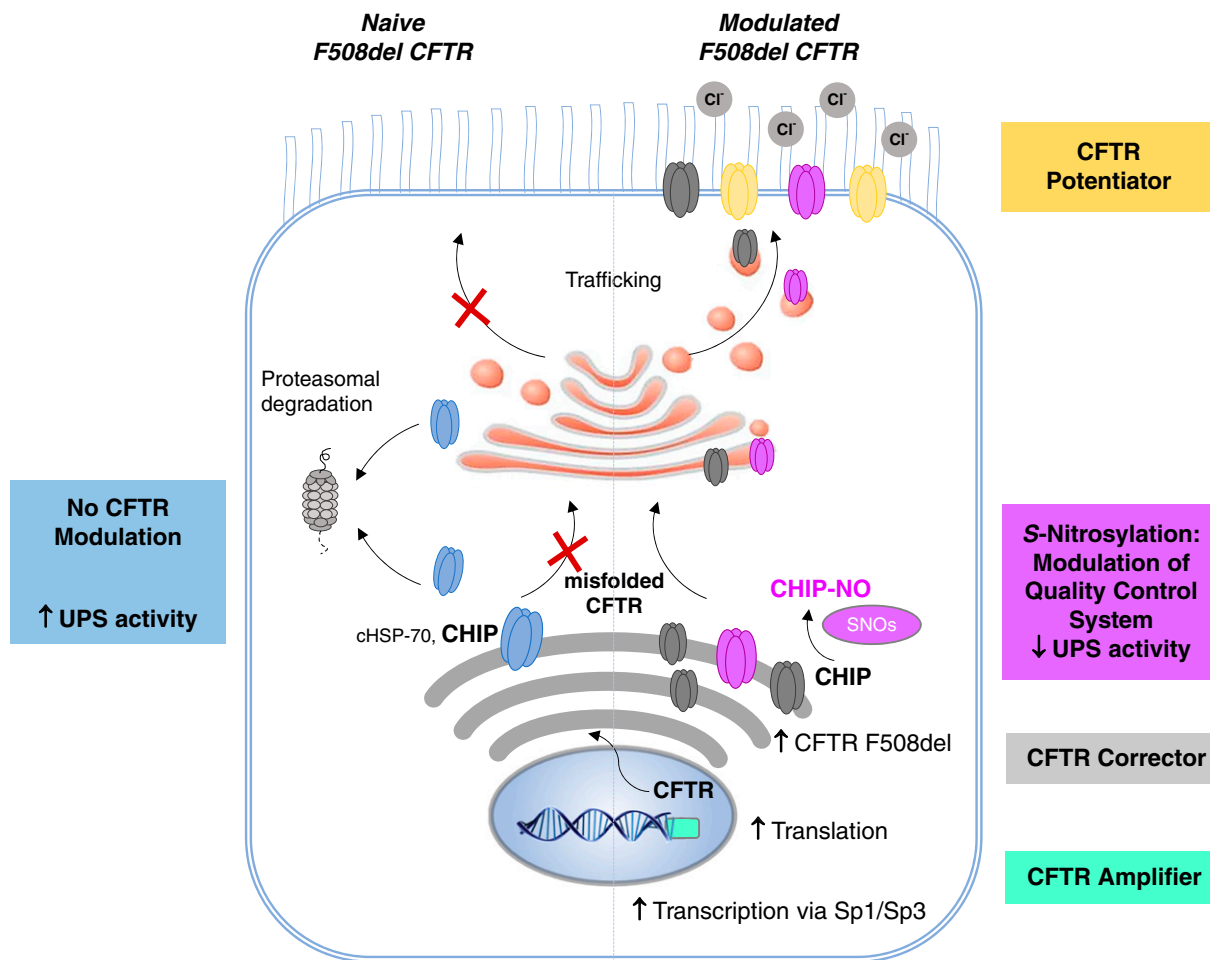


Figure 1. Modulation of the cystic fibrosis transmembrane conductance regulator (CFTR): through the action of the CHIP (carboxy terminus of heat shock protein 70–interacting protein), misfolded CFTR (F508del) is targeted toward proteasomal degradation (left). CFTR correctors enhance CFTR transcriptional and translational output, allowing for some CFTR to be trafficked to the apical surface. Here, CFTR potentiators enhance the channel gating, resulting in chloride conductance (right). In the future, CFTR amplifiers could increase the CFTR substrate available for CFTR correctors. Alternatively, nitrosylation of CHIP (nitrosothiols) leads to reduced CHIP expression and activity, allowing misfolded CFTR to escape the quality control system, enhancing CFTR maturation. The addition of CFTR potentiators may provide further benefits. CHIP-NO = nitrosylated CHIP; cHSP-70 = cytosolic heat protein-70; Cl⁻ = chloride anion; SNO = S-nitrosothiols; UPS = ubiquitin-proteasome system; Sp1/Sp3 = transcription factors specificity protein 1/ specificity protein 3.

GSNO demonstrated tolerability, but only modest effects on oxygen saturation were seen (15). Although Zaman and colleagues focus on the potential of GSNO as an inhalational agent, all current CFTR modulators are delivered orally to permit systemic CFTR modulation (3, 4). CF is a multisystem disease and the systemic effects of CFTR modulators contribute significantly to their clinical benefits (1–5). Future studies are needed to investigate pharmacological modifications of QC systems and their effects on proteasomal degradation of CFTR, which may differ depending on cell- and tissue-dependent expression (7). With nitrosylation of CHIP being dose dependent (6), optimizing therapeutic ranges across multiple cell types and individuals will be challenging. Furthermore, any adverse effects of nitrosative stress (excessive NO/reactive oxygen species) that can contribute to NO-induced neurotoxicity (11) must be identified.

This study highlights a novel therapeutic target, nitrosylation of CHIP, with the potential to provide an innovative means of modulating CFTR. Selective modification of the QC machinery

remains an attractive approach. Further exploration of therapeutic targets in the pathway identified by Zaman and colleagues may lead to the generation of systemic therapies that benefit PWCF. In combination with other CFTR modulators, this could further optimize clinical benefits in the future (Figure 1). Zaman and colleagues may have brought a potential new player to the table, and further study will determine what impact this has on those already there. ■

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