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Review

Targeting CFTR Ubiquitination: Current Advances in Therapeutic Strategies for Cystic Fibrosis

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Abstract

Cystic fibrosis (CF) is a monogenic disease caused by mutations in the CF transmembrane conductance regulator (CFTR), whose folding, trafficking, and stability are tightly controlled by ubiquitination-dependent protein quality control (PQC) pathways. Although CFTR modulators have transformed CF therapy, their efficacy remains limited by persistent ubiquitination and degradation of rescued CFTR. This limitation is particularly evident in class I mutations, where premature termination codons (PTCs) reduce full-length CFTR protein production and no approved mutation-specific therapies are broadly available for canonical PTC variants. Recent advances highlight ubiquitination as a critical and druggable determinant of CFTR stability. The E3 ligase RFFL regulates peripheral CFTR PQC, restricting the stability of rescued CFTR at the plasma membrane (PM). Inhibition of RFFL, including via antisense oligonucleotides (ASO) and small molecules, enhances CFTR rescue and improves outcomes in combination with modulators and translational readthrough therapies. In parallel, deubiquitinase (DUB)-targeting chimeras (DUBTACs) have emerged as a novel modality to stabilize proteins by reversing ubiquitination. Here, we review current advances in targeting CFTR ubiquitination, with a focus on RFFL inhibition and DUBTAC-based strategies, and discuss their opportunities and translational limitations as components of next-generation CF therapies.

Keywords: CFTR; CF; ubiquitin; E3 ligase; DUBTAC

1. Introduction

CF is a monogenic disease caused by mutations in CFTR, a chloride and bicarbonate channel essential for epithelial homeostasis [1,2]. CFTR mutations are commonly grouped into functional classes according to the step of CFTR biogenesis or activity that is impaired [1,3,4]. Class I mutations, including nonsense or PTC mutations such as G542X and W1282X, reduce or abolish the production of full-length CFTR protein. Class II mutations, exemplified by F508del, which is the most common mutation in CF in Europe and North America [3,5], impair CFTR folding, domain assembly, and ER export, resulting in enhanced ER-associated degradation (ERAD). Class III mutations primarily affect channel gating, class IV mutations reduce conductance, class V mutations decrease the amount of normally processed CFTR, and class VI mutations destabilize CFTR at the PM. Although this classification is therapeutically useful, many variants display overlapping defects; therefore, mutation class should be interpreted as a dominant molecular phenotype rather than as an exclusive category [1–4].

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CFTR modulators are clinically important small molecules that improve the abundance or activity of mutant CFTR, but different modulator classes act through distinct mechanisms. Correctors, including lumacaftor (VX-809), tezacaftor (VX-661), elexacaftor (VX-445), and vanzacaftor, improve folding, domain assembly, and/or ER export of processing-defective CFTR and are clinically used as components of CFTR modulator therapies for patients with class II mutations such as F508del [3,6,7]. Potentiators, including ivacaftor (VX-770) and deutivacaftor, increase the activity or open probability of CFTR channels that have reached the PM and are clinically used to treat patients with gating and conductance defects, including class III mutations such as G551D and class IV mutations such as R117H [3,6,8]. The triple combination elexacaftor/tezacaftor/ivacaftor (ETI; Trikafta/Kaftrio) has markedly improved outcomes for many individuals with at least one F508del allele, and recent clinical studies further support the efficacy of ETI in patients with F508del-gating and F508del-residual-function genotypes [9–11]. More recently, vanzacaftor/tezacaftor/deutivacaftor (Alyftrek), a once-daily next-generation triple-combination CFTR modulator, was approved for eligible patients with responsive CFTR mutations [12,13]. These advances underscore the therapeutic value of stabilizing and activating mutant CFTR, while also highlighting the need for additional strategies for mutations that produce little or no full-length protein, including many class I mutations.

ERAD and peripheral quality control (periQC) represent spatially and mechanistically distinct proteostasis pathways that can act sequentially on mutant CFTR. ERAD operates at the ER, where misfolded or incompletely assembled CFTR is recognized; ubiquitinated by ER-associated E3 ligases, including CHIP, RNF5, and RNF185 [14–16] and cytosolic E3 ligases UBE3C and HERC3 [17,18]; retrotranslocated; and degraded by the proteasome [19,20]. By contrast, periQC acts after CFTR has escaped the ER and reached post-ER compartments, including the PM and endosomes; aberrant CFTR conformers are ubiquitinated at or after the cell surface by E3 ligases CHIP, RFFL and RNF34 and are routed toward lysosomal degradation [21–24]. This distinction is important because modulator-rescued F508del-CFTR can reach the PM but remains conformationally metastable. Thus, periQC can remain active even after corrector treatment and can limit the durability of pharmacological rescue.

The development of therapeutic agents targeting CFTR misfolding has progressed from first-generation correctors and potentiators to highly effective triple combinations and newer investigational modulators. In addition to approved combinations, recent studies have identified novel pharmacological correctors and alternative approaches to enhance CFTR function, including tricyclic pyrrolo-quinoline correctors and peptide-based strategies that improve CFTR gating and airway physiology [25,26]. Nevertheless, rescued F508del-CFTR remains more susceptible to ubiquitination-dependent periQC than wild-type CFTR [21,22]. Thus, targeting ubiquitination represents a critical strategy to stabilize rescued CFTR and maximize the efficacy of CF pharmacological therapy [27].

G542X is the second most common CF-causing mutation [3,5,28] and is classified as a class I mutation. This mutation introduces a PTC, leading to truncated protein production and nonsense-mediated mRNA decay (NMD), thereby resulting in little or no full-length CFTR protein [29]. Currently approved CFTR modulators, including Trikafta/Kaftrio and Alyftrek, require the presence of a CFTR protein product to exert their activity and are therefore largely ineffective for canonical class I mutations in the absence of sufficient readthrough or transcript correction [3,12,13,30,31]. Translational readthrough-inducing drugs (TRIDs), including aminoglycosides such as gentamicin and synthetic aminoglycosides such as ELX-02, as well as non-aminoglycoside compounds such as ataluren/PTC124 and other experimental small molecules, have been explored as approaches to restore full-length CFTR protein [32–35]. Experimental studies using G542X-CFTR and related nonsense variants have demonstrated that readthrough can generate full-length CFTR with

residual channel function, and that this readthrough-derived CFTR can be further enhanced by CFTR correctors and potentiators [31,33,36,37]. However, readthrough efficiency and fidelity vary according to the PTC identity and sequence context, and the resulting CFTR proteins may exhibit structural heterogeneity because near-cognate tRNAs insert different amino acids at the PTC [33–35]. Importantly, even when full-length CFTR is generated, its functional rescue is limited by rapid degradation [38].

2. RFFL-Mediated Ubiquitination and Its Therapeutic Targeting

The E3 ubiquitin ligase RFFL (CARP2) has emerged as a key regulator of CFTR periQC [23]. RFFL mediates ubiquitination of conformationally defective CFTR at the PM and endosomes, promoting lysosomal degradation. This mechanism is therapeutically important because rescue by modulators does not completely normalize CFTR conformation [39]. Instead, pharmacologically corrected CFTR often remains metastable and susceptible to peripheral ubiquitination-dependent removal [39,40]. Thus, RFFL can function as a post-rescue checkpoint that limits the durability of modulator responses (Figure 1).

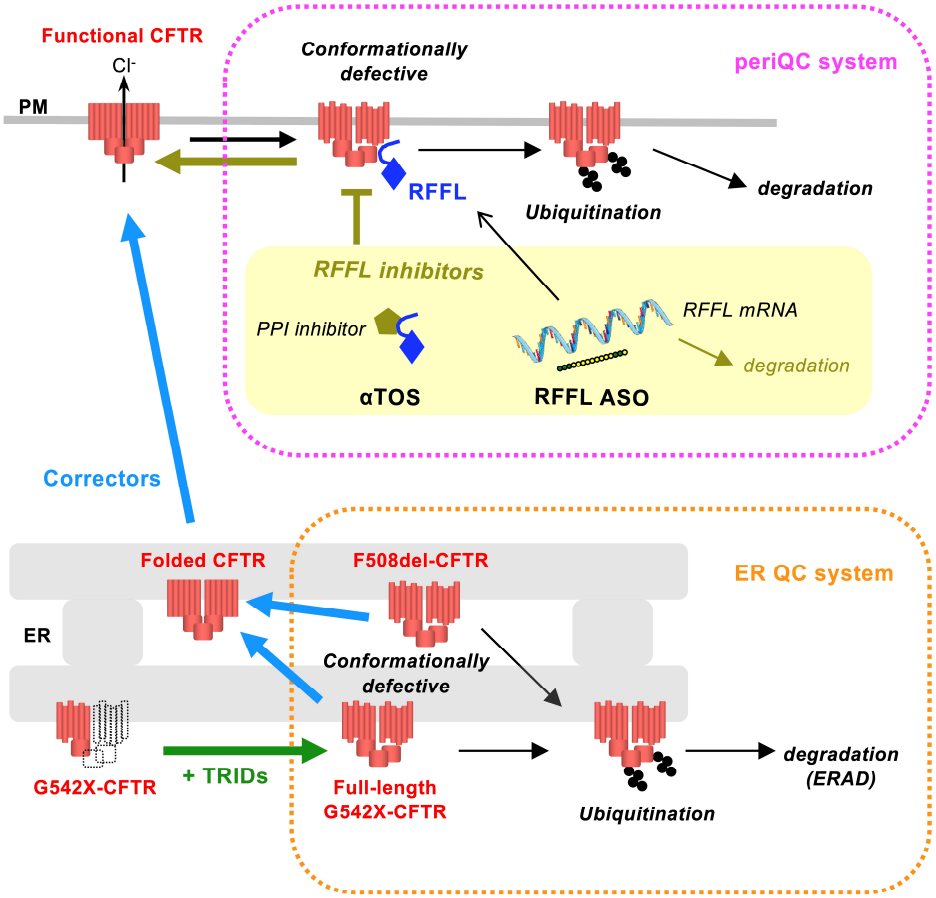


Figure 1. Therapeutic strategies for class I and class II CFTR mutants targeting the RFFL E3 ligase. F508del-CFTR, the most common CF-causing mutant, and the full-length G542X-CFTR protein generated by TRID-mediated readthrough are subject to ER-associated ubiquitination and degradation. CFTR correctors, including those used in Trikafta/Kaftrio, largely overcome these defects by improving protein folding and trafficking. However, both rescued F508del-CFTR and readthrough-derived full-length G542X-CFTR remain susceptible to RFFL-mediated periQC, resulting in ubiquitination-dependent removal from the PM. RFFL inhibition using approaches such as αTOS or RFFL-targeting ASOs prevents CFTR elimination from the PM and enhances the therapeutic efficacy of Trikafta/Kaftrio and/or TRID-based treatments.

Genetic evidence strongly supports the therapeutic relevance of RFFL. Ablation of RFFL significantly enhances CFTR modulator efficacy in F508del-CFTR mouse organoids, demonstrating that suppression of RFFL-mediated ubiquitination improves functional rescue in a physiologically relevant model [41]. Genetic ablation of RFFL increases cell surface F508del-CFTR levels and enhances functional rescue in airway epithelial models and organoids. Moreover, RFFL inhibition potentiates CFTR modulator efficacy, indicating that RFFL acts downstream of folding correction to limit F508del-CFTR stability.

ASO-mediated knockdown (KD) of RFFL represents a promising therapeutic strategy. RFFL ASOs provide sustained suppression of RFFL expression and significantly enhance endogenous F508del-CFTR function in primary bronchial epithelial cells [42]. Compared with siRNA approaches, ASOs exhibit improved durability and translational feasibility (Figure 1).

Importantly, RFFL inhibition is highly relevant for class I CFTR mutations. TRIDs can generate full-length CFTR; however, the rescued protein is often unstable and rapidly degraded. RFFL-mediated ubiquitination limits the functional rescue of TRID-generated full-length CFTR, whereas RFFL inhibition significantly enhances its stability and activity [38]. These findings identify RFFL as a key bottleneck in class I CF mutation therapy. This point is especially relevant for G542X-CFTR and related class I mutants. Experimental studies have shown that readthrough-induced full-length CFTR can retain function and may respond further to CFTR modulators, supporting the concept that Trikafta- or Alyftrek-like modulator combinations can act on the translated product once sufficient protein is generated [31,33,36–38]. However, because the readthrough-derived protein is expected to be structurally heterogeneous and conformationally fragile, its removal by RFFL-dependent periQC may substantially blunt therapeutic benefit. Therefore, a triple-combination strategy consisting of a readthrough agent, a highly active modulator combination, and RFFL inhibition represents a compelling precision-medicine framework for class I CFTR mutations [38] (Figure 1).

In addition to genetic approaches, small-molecule targeting of RFFL has also been reported. Alpha-tocopherol succinate (αTOS) was identified as an inhibitor of RFFL-substrate interaction, leading to stabilization of CFTR at the PM [43] (Figure 1). This study provides proof-of-concept that pharmacological disruption of RFFL-mediated ubiquitination is feasible. In addition, RFFL inhibition reduces proinflammatory interleukin-8 (IL-8) production under chronic obstructive pulmonary disease (COPD)-relevant stress conditions, suggesting anti-inflammatory benefits beyond CFTR stabilization [41].

3. DUBTACs and CFTR: A Complementary Strategy to Counteract Ubiquitination

While inhibition of E3 ligases such as RFFL prevents ubiquitination, an alternative approach is to actively remove ubiquitin from target proteins since protein ubiquitination is a reversible process, and conjugated ubiquitin can be removed by a family of DUBs. DUBTACs have emerged as a powerful technology to achieve targeted deubiquitination and protein stabilization. The DUBTAC concept was first established as a general strategy for targeted protein stabilization by inducing proximity between a deubiquitinase and a substrate [44]. Although DUBTACs have not yet been directly applied to CFTR in clinical situations, their mechanism provides a compelling framework for CF therapy. Most CF-associated CFTR mutants are heavily regulated by ubiquitination at multiple stages, including ER and PM mediated by several E3 ligases. Thus, CFTR represents an ideal target for DUBTAC-based stabilization, as this approach directly counteracts ubiquitination-dependent degradation and complements strategies that inhibit E3 ligases (Figure 2).

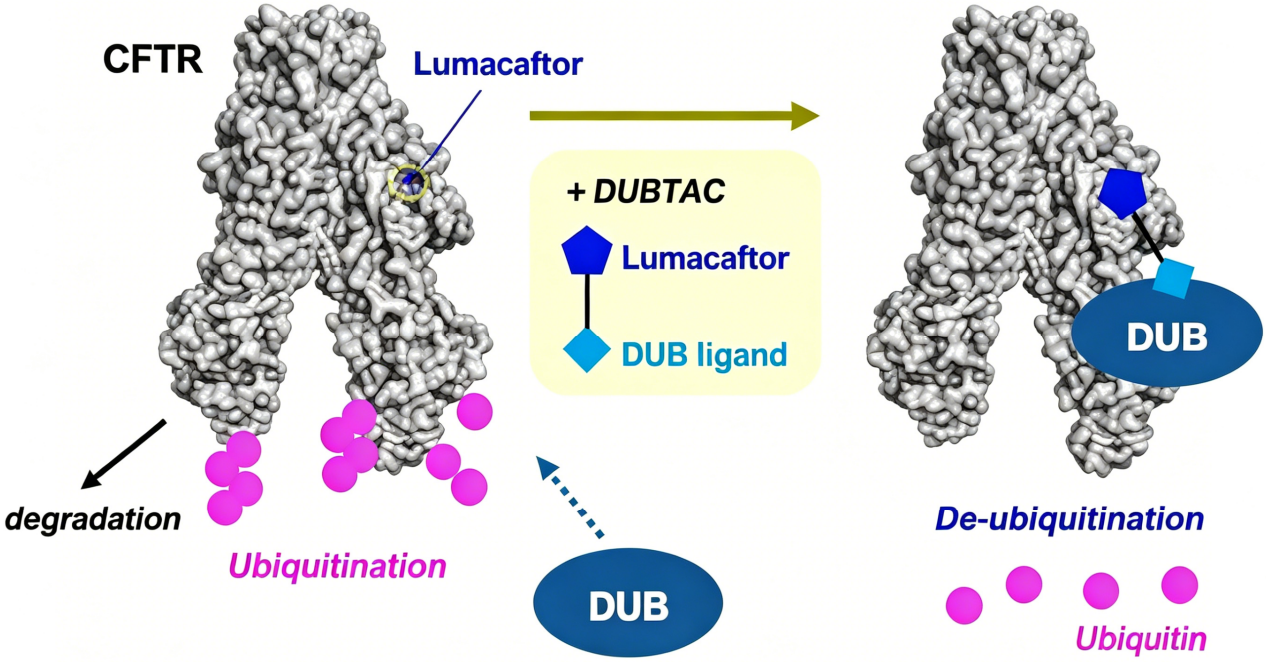


Figure 2. Potential mechanism of action of DUBTAC-based strategies for CF treatment. Although endogenous DUBs may interact with conformationally defective CFTR under certain conditions, such interactions are not necessarily efficient, selective, or sufficient to counteract CFTR ubiquitination and degradation at the ER and PM. DUBTACs are designed to overcome this limitation by inducing proximity between a DUB and CFTR, thereby promoting CFTR deubiquitination and stabilization. The CFTR–lumacaftor complex model was generated based on PDB: 7SVR. Since the specific ubiquitination sites of CFTR and the types of ubiquitin modifications involved remain to be fully elucidated, the figure illustrates a hypothesized model of CFTR ubiquitination in the cytoplasm.

To date, DUBTACs utilizing ligands for OTUB1, USP1, USP7, and USP28, as well as the CFTR ligand lumacaftor, have been developed, and their ability to inhibit F508del-CFTR degradation has been demonstrated in cultured cells [44–48]. These approaches have also yielded partial improvements in F508del-CFTR channel function. However, the overall efficacy of CFTR-targeted DUBTACs remains limited. Moreover, USP1, USP7, and USP28, commonly used in current DUBTAC designs, are predominantly localized in the nucleus [44–46], which may limit their suitability for targeting CFTR. Therefore, the identification of more appropriate DUBs for CFTR-directed DUBTACs will be essential. At the same time, the development of improved DUB ligands, currently a major limiting factor in DUBTAC design, is expected to enhance the overall effectiveness of this approach.

Several criteria should guide the selection of CFTR-compatible DUBs. First, the DUB should be accessible to CFTR at relevant subcellular sites, including the ER membrane, cytosol-facing surfaces of endosomes, and the PM-proximal cytosol. Second, it should remove ubiquitin linkages that direct CFTR to proteasomal or lysosomal degradation without broadly perturbing unrelated ubiquitin-dependent signaling. Third, it should have a ligandable pocket or surface that allows development of a selective DUB recruiter. Fourth, the DUB should not require nuclear enrichment or cell-cycle-regulated complexes that would restrict access to epithelial CFTR. In this regard, cytosolic or membrane-associated DUBs, including endogenous CFTR-associated candidates such as USP10, may represent useful starting points because USP10 has been reported to deubiquitinate CFTR and promote endocytic recycling in airway epithelial cells [49]. More broadly, systematic DUB screens, proximity labeling of CFTR interactomes, and ubiquitin-chain linkage analyses will be important for identifying DUBs that are both mechanistically compatible and therapeutically safe.

For class I CFTR mutations, DUBTACs could be positioned downstream of readthrough in a manner conceptually analogous to RFFL inhibition [38]. Once a readthrough compound generates full-length CFTR and modulators such as Trikafta/Kaftrio or Alyftrek restore folding and channel activity, DUBTAC-mediated deubiquitination could further stabilize the rescued protein by directly opposing ubiquitin-dependent degradation. In this context, DUBTACs and RFFL inhibition should be viewed as complementary rather than competing strategies: the former promotes ubiquitin removal from rescued CFTR, whereas the latter limits ubiquitin addition by a key peripheral E3 ligase. Importantly, DUBTAC-based approaches may synergize with existing therapies, as stabilization through deubiquitination could enhance modulator efficacy and improve functional rescue of TRID-generated CFTR in class I mutations.

4. Future Perspectives and Challenges

Targeting CFTR ubiquitination represents a promising therapeutic approach; however, several challenges remain. First, specificity is a critical issue. Ubiquitination pathways regulate numerous substrates, and non-selective modulation may lead to unintended effects. Development of CFTR-specific targeting strategies such as selective E3 ligase inhibition, CFTR-directed DUB recruitment, or mutation-context-specific combinations will be essential. Second, long-term modulation of PQC pathways may impact cellular proteostasis. Chronic inhibition of PQC components such as RFFL may lead to accumulation of conformationally abnormal proteins or altered endosomal signaling, even though RFFL knockout does not appear to cause overt phenotypic abnormalities in available preclinical models [50]. Third, delivery remains a major challenge, particularly for ASO- and DUBTAC-based therapies. Efficient targeting of airway epithelial cells, appropriate duration of action, and minimizing systemic exposure will be critical for clinical application. Notably, RFFL ASO can be administered to airway epithelia in saline without transfection reagents [42], highlighting its potential advantage for local airway delivery.

DUBTAC technology is at an especially early stage. Although DUBTACs offer conceptual advantages by stabilizing endogenous proteins rather than globally inhibiting degradation, several barriers must be overcome before clinical translation. These include efficient intracellular delivery to airway epithelial cells, favorable pharmacokinetics and tissue distribution, avoidance of excessive or non-selective stabilization of off-target proteins, compatibility between the selected DUBs and the ubiquitin-chain topology on CFTR, and the potential immunological or inflammatory consequences of long-term ubiquitin remodeling. The current reliance on nuclear DUBs such as USP1, USP7, and USP28 also underscores the need to identify cytosolic or membrane-associated DUB recruiters better matched to CFTR biology [51–53].

Another key challenge is the identification of optimal DUBs for CFTR targeting. Although OTUB1, USP1, USP7, and USP28 have provided proof of concept in other systems [44–48], it remains unclear which DUBs can most effectively counteract CFTR ubiquitination in the context of ER PQC and periQC. Ideally, CFTR-compatible DUBs should be present at relevant subcellular sites, retain catalytic activity near CFTR, recognize or process the appropriate ubiquitin-chain types, and exhibit sufficient substrate selectivity to avoid broad proteome-wide effects. Systematic evaluation of DUB–CFTR compatibility will therefore be required to advance DUBTAC-based CF therapies.

Finally, it is important to acknowledge that enthusiasm for RFFL-targeting strategies should be balanced by potential conflicts and translational uncertainties. One of the authors is a co-inventor on a patent related to RFFL ASO, and this relationship is disclosed below. Accordingly, conclusions regarding RFFL inhibition should be interpreted in light of the

current preclinical evidence base, and independent validation in additional models will be important.

Despite these challenges, emerging evidence indicates that modulation of ubiquitination can markedly enhance CFTR rescue. Integrating CFTR modulators with ubiquitination-targeted strategies, such as E3 ligase inhibition and DUBTAC-mediated stabilization, represents a promising avenue for next-generation CF therapies.

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Conflicts of Interest: T.O. is a co-inventor on a patent (WO2026/049020) related to RFFL ASO. Y.K. declares no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ASO	Antisense oligonucleotide
αTOS	α-Tocopherol succinate
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
COPD	Chronic obstructive pulmonary disease
DUB	Deubiquitinase
DUBTAC	Deubiquitinase-targeting chimera
E3 ligase	Ubiquitin E3 ligase
ER	Endoplasmic reticulum
ERAD	Endoplasmic reticulum-associated degradation
IL-8	Interleukin-8
KD	Knockdown
NMD	Nonsense-mediated mRNA decay
PeriQC	Peripheral quality control
PM	Plasma membrane
PQC	Protein quality control
PTC	Premature termination codon
TRID	Translational readthrough-inducing drug

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