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Review

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Emerging Cystic Fibrosis Transmembrane Conductance Regulator Modulators as New Drugs for Cystic Fibrosis: A Portrait of *in Vitro* Pharmacology and Clinical Translation

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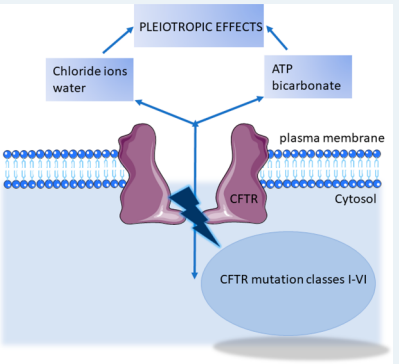
ABSTRACT: Pharmacological correction of the defective ion channel with cystic fibrosis transmembrane conductance regulator (CFTR) has become an attractive approach to therapy directed at the root cause of the life-limiting disease cystic fibrosis (CF). CFTR defects range from absence, misfolding, and resulting degradation to functional defects of the CFTR protein. The discovery and development of the CFTR potentiator ivacaftor was a major break-through in CF therapy and has triggered an enormous incentive for seeking effective modulators such as lumacaftor, tezacaftor or elexacaftor for all patients with CF. A number of emerging CFTR modulators are currently in the development pipeline, and rescue levels of CFTR protein approach a cure for cystic fibrosis. In this review, we identify and characterize all preclinical and clinical emerging CFTR modulators and discuss the *in vitro* pharmacology, looking at CFTR protein expression and chloride transport and the translation to the clinic. The new emerging CFTR modulators could offer new therapeutic solutions for CF patients.



KEYWORDS: *cystic fibrosis, ivacaftor, lumacaftor, tezacaftor, CFTR modulator, CFTR potentiators*

Cystic fibrosis is the most widespread, genetically acquired, life-shortening chronic illness affecting Caucasians today.[1] The disease causes a dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) channel dehydrating mucosal surfaces which subsequently leads to increased viscous mucus that obstructs luminal compartments in lung, pancreas, and intestine. There are approximately 2000 different mutations of the CFTR protein which are categorized into functional classes I, II, III, IV, V, and VI (Table 1, Figure 1).[2] The classic CF phenotype with pancreatic insufficiency and a nonfunctional CFTR ion channel is caused by class I, II, and III mutations; while a milder form of CF is linked to class IV, V, and VI mutations which produce a CFTR channel with partial function.[3−5] Table 1 details CFTR mutations classes and type of mutations, schematics on the effect on the CFTR function, prevalence, and examples for each class. The first drugs that target the root cause of CF are the novel CFTR modulator combinations, namely, ivacaftor with lumacaftor or tezacaftor.[6,7] CFTR modulators that increase the channel gating activity of mutant CFTR at the epithelial cell surface are referred to as potentiators, whereas CFTR modulators that improve faulty protein processing and resulting trafficking to the epithelial surface are referred to as correctors. CFTR correctors monotherapy has been shown not efficacious enough, resulting in combining correctors together with potentiators to enhance CFTR activity.

Approximately 5% of CFTR mutations are characterized by interference with the opening of the channel (class III mutations) (Figure 1, Table 1).[8] Ivacaftor is the first CFTR potentiator drug that has proven clinical efficacy resulting in a significant therapeutic benefit in CF patients bearing the G551D missense (class III) mutation.[9,10,39] Such therapeutic benefits include a significant and sustained decrease in sweat chloride levels, improvement in clinical outcomes such as a ∼10% increase in FEV_1 percent predicted, a 55% reduction in pulmonary exacerbations, weight gain, and improvement in self-reported quality of life.[11] On the basis of comparable clinical outcomes, ivacaftor monotherapy approval was later extended to include additional mutations, including G1244E, G1349D, G178R, G551S, S1252N, S1255P, S549N, S549R, and R117H.[12,13]

About 90% of patients with CF harbor the F508del-CFTR mutation on at least one allele and about 50% on both alleles. This mutation impairs the correct folding of CFTR resulting in reduced amounts of F508del-CFTR reaching the epithelial surface (class II mutation) (Figure 1, Table 1). The development of agents that effectively correct this folding mutation remains a major obstacle.

The encouraging first-generation corrector, lumacaftor, increases CFTR function to ∼25% of normal CFTR activity, when used in combination with ivacaftor *in vitro* by restoring the F508del-CFTR folding.[11] In patients, this combination led

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Table 1. CFTR Gene Mutation Classes Divided into Six Categories Based on Pathogenic Mechanism[a]

CFTR Class	Type of mutation	Effect on protein	Representative cellular structure where defect occurs	CF phenotype	Example	Prevalence within CF cohort
I	Protein synthesis	Complete loss of protein function		pancreatic insufficiency	3659delC; 621 + 1G→T 1078delT; 1717-1G→A;	22%
II	Maturation processing	Defective regulation processing		pancreatic insufficiency	R560T, N1303K G85E, F508del, I507del,	88%
III	Ion channel gating	Defective protein regulation		pancreatic insufficiency	G551S, G970R, G1244E, S1251N, G178R, S549N, S549R, G551D, S1255P, G1349D	6%
IV	Ion channel conductance	Defective protein conductance		milder disease form	R347P, R334W, R117H	6%
V	Reduced protein	Reduced protein synthesis		milder disease form	A455E, 2789 + 5G→A, 3849 + 10KbC→T	5%
VI	Reduced membrane stability	Impacted surface retention		milder disease form	120del23, N287Y	0.5%

[a]Figures adapted with permission from refs 2, 8, and 36−38. Copyright Science 2019; Wiley 2017; Mary Ann Liebert, 2015; and Elsevier, 2014, respectively.

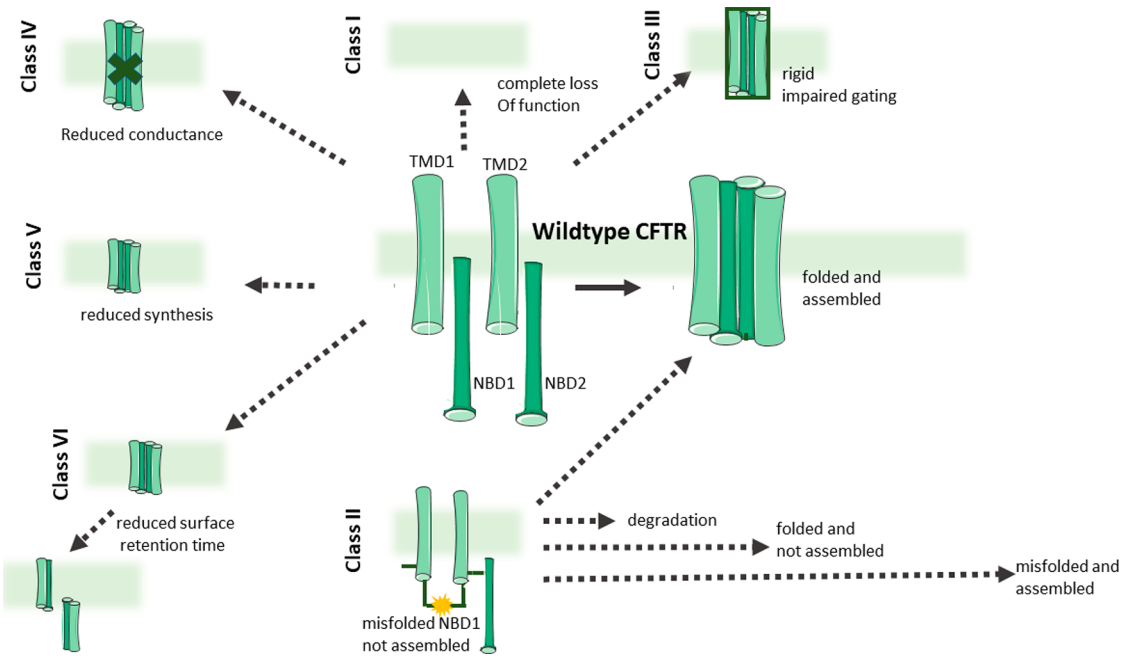

Figure 1. Disease causing mutation subclasses in cystic fibrosis and different paths to correct CFTR folding. The dysfunctional CFTR mutation at the cell surface does not equate function. Depending on the mutation subclass multiple modes of rescue are possible. Figure adapted with permission from ref 22. Copyright Elsevier 2017.

to modest improvements in lung function and a reduction in exacerbations in patients that are homozygous for the F508del mutation; it was ineffective in heterozygous patients.[14,15] Furthermore, undesirable drug interactions of lumacaftor such as strong cytochrome P450 3A induction resulting in a reduction in therapeutic efficacy of cytochrome P450 3A substrates (e.g., ivacaftor) contributed to the development of tezacaftor (VX-661).[16−18] The combination of ivacaftor−tezacaftor was efficacious in F508del heterozygous patients with CFTR residual function as well as displaying comparable clinical efficacy outcomes in terms of FEV_1 and fewer drug−drug interactions and unwanted side effects.[15,19]

Current strategies focus on new CFTR correctors that stabilize the CFTR protein to help overcome its folding defects and increase functional CFTR proteins on the cell surface. Encouragingly, a number of next-generation correctors are currently in the pipeline for preclinical models and early phase clinical trials (Table 2, Figure 2).

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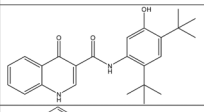
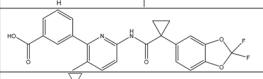
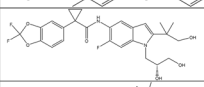
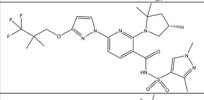
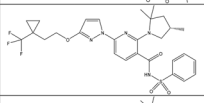
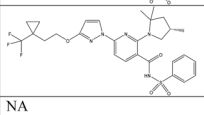
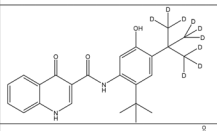
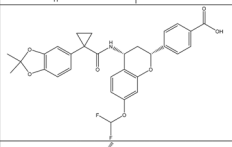
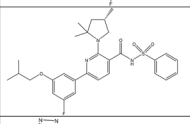
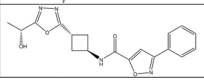
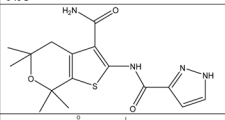
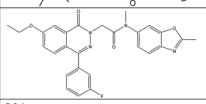
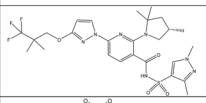
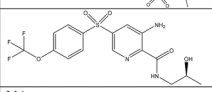
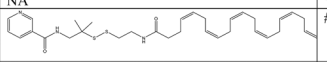
Table 2. CFTR Modulators Developed for Cystic Fibrosis

Name	Clinical Stage	Mode of Action	Structure	Ref
Ivacaftor (VX-770)	Approved	Potentiator		#
Lumacaftor (VX-809)	Approved (+ivacaftor)	Corrector		#
Tezacaftor (VX-661)	Approved (+ivacaftor)	Corrector		#
Elexacaftor (VX-445)	Preregistered (+tezacaftor+ ivacaftor)	Corrector		#
Bamocaftor (VX-659)	Phase 3 (+tezacaftor+ ivacaftor)	Corrector		#
Bomacaftor potassium	Phase 2	Corrector		#
ABBV-2737	Phase 2	Corrector	NA	*
Deuterated ivacaftor (VX-561, CTP656)	Phase 2	Potentiator		[25]
Galicaftor (GLPG-2222)	Phase 2	Corrector		#
Olacaftor (VX-440)	Phase 2	Corrector		#
PTI-428	Phase 2	Amplifier		#

Name	Clinical Stage	Mode of Action	Structure	Ref
QBW251	Phase 2	Potentiator	NA	*
VX-152	Phase 2	Corrector	NA	*
GLPG-1837	Phase 2	Potentiator		[39]
FDL-169	Phase 1/2	Corrector		*
PTI-801	Phase 1/2	Corrector	NA	*
PTI-NC-733	Phase 1/2	Triple combination of Amplifier, Corrector and Potentiator	NA	*
Elexacaftor (VX-445)	Phase 1/2	Corrector		#
ABBV-2451	Phase 1	Potentiator		[39]
ABBV-3067	Phase 1	Potentiator	NA	*
FDL-176	Phase 1	Potentiator	NA	*
GLPG-2851	Phase 1	Corrector	NA	*
GLPG-3221	Phase 1	Corrector	NA	*
PTI-808	Phase 1	Potentiator	NA	*
CAT-5571	Preclinical	Novel mode of action		#, [34-35]
PTI-130	Preclinical	Amplifier	NA	*
1016048 (Vertex)	Preclinical	Modulator	NA	*
FD2052160	Preclinical	Corrector	NA	*

[a]Cortellis database. Accessed on 20/09/19. [b]Integrity database. Accessed on 25/07/19. NA = structure has not yet been disclosed.

Presently, elexacaftor (VX-445) and bamocaftor (VX-659) are two new compounds being tested in phase 3 trials. Both drugs are next-generation CFTR correctors designed to restore F508del-CFTR protein function in patients with CF when administered with tezacaftor and ivacaftor.[20] Furthermore, positive data has been reported in CF patients who are heterozygous for the F508del CFTR mutation or have one minimal function (MF) mutation (F508del-MF).[20,21] In the F508del-MF group, treatment with either ivacaftor + tezacaftor + elexacaftor or bamocaftor resulted in mean absolute improvements in ppFEV1 of 13.8 and 13.3 percentage points, respectively ($P < 0.001$). In the F508del cohort, patients received standard ivacaftor−tezacaftor treatment. In this cohort the addition of elexacaftor and bamocaftor resulted in an 11.0-point and 9.7-point rise in the percentage of predicted FEV1 ($P < 0.001$), respectively. The side effects most commonly observed were increased sputum production, pulmonary exacerbations, hemoptysis, and pyrexia. Generally speaking, the double or triple combination regimens were well tolerated across all tezacaftor trials. On the basis of these results, the triple combination of elexacaftor + tezacaftor + ivacaftor was submitted for global regulatory approval in 2019. The submission is a major step toward the goal of bringing treatments to the largest remaining group of people with CF that still do not have an approved medicine yet, as well as toward providing significantly enhanced benefits to patients that are homozygous for the F508del CFTR mutation.

Olacaftor (VX-440) a next generation corrector was assessed in a phase 2 trial, randomized, double blind, placebo, and active-controlled study designed to evaluate the safety and tolerability of VX-440 in triple combination with tezacaftor and ivacaftor in patients with CF who are heterozygous for the F508del mutation and a MF CFTR mutation not likely to respond to tezacaftor and/or ivacaftor therapy (F508del-MF), or who are homozygous for the F508del mutation (ClinicalTrials.gov Identifier: NCT02951195) .

Four novel correctors have been developed using high throughput screening.[22] If a corrector is additive to a second corrector these synergistic correctors are referred to as C1 and C2 correctors, respectively.[22] Correctors Galicaftor (GLPG-2222) and GLPG-2851 (C1 correctors) are additive to ABBV-2737 (GLPG-2737) and GLPG-3221 (C2 correctors). Galicaftor shares structural similarities with lumacaftor and tezacaftor (namely a 2-phenyl substituted chromane analogue) and proved to be more potent *in vitro*. In patients homozygous for F508del CFTR mutation (FLAMINGO trial) or heterozygous for F508del and an additional gating mutation, (ALBATROSS trial), two placebo-controlled, phase 2a studies

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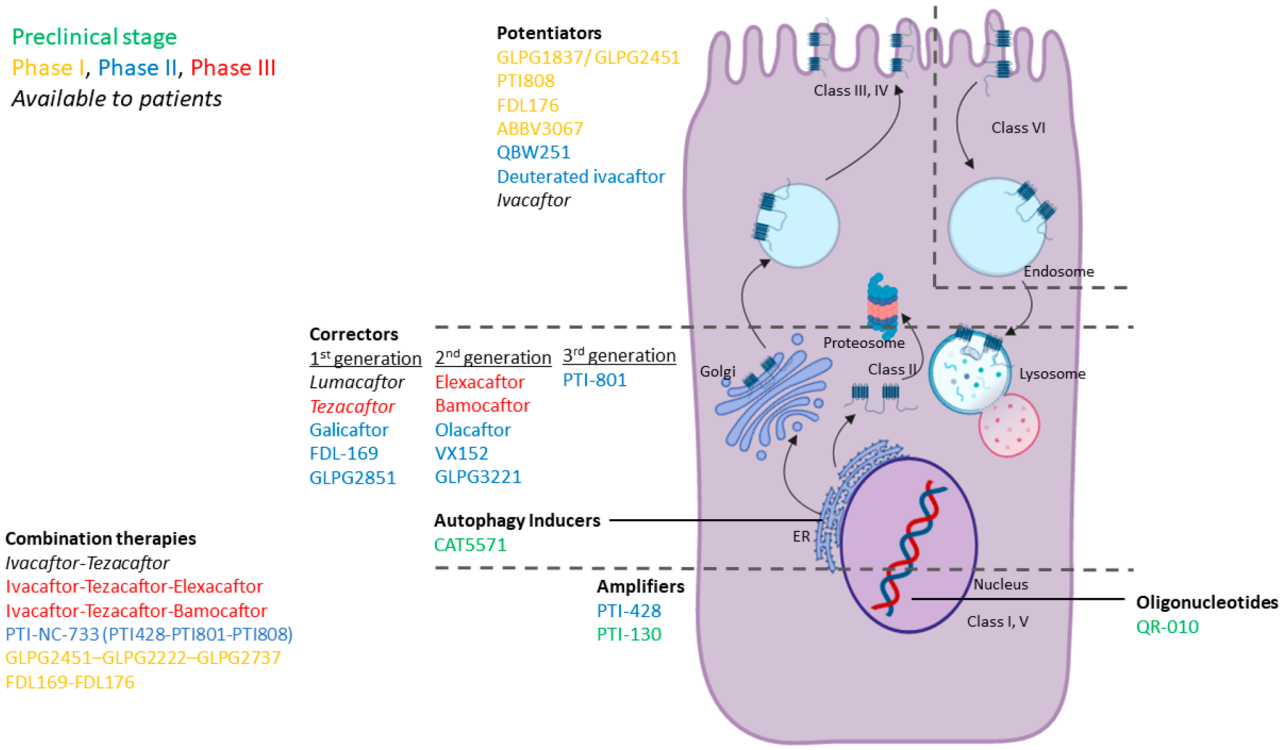

Figure 2. A schematic of the cystic fibrosis airway epithelial cell with CFTR mutations divided into six categories based on pathogenic mechanism and color-coded treatment options classically categorized based on their molecular action as well as their availability to patients. Green represents preclinical stage, yellow = phase 1 clinical trial, blue = phase 2 clinical trial, red = phase 3 clinical stage, and black represents approval by the FDA and EMEA, respectively.

investigated the addition of galicaftor (orally once daily for 29 days) to standard ivacaftor therapy.[23,24] The authors report that dose-dependent decreases in sweat chloride concentrations were seen in galicaftor-treated subjects (maximum decrease in FLAMINGO: −17.6 mmol/L [galicaftor 200 mg], $p < 0.0001$. ALBATROSS: −7.4 mmol/L [galicaftor 300 mg], $p < 0.05$).[23] The triple combination of C2 corrector GLPG-2737, C1 corrector galicaftor, and potentiator GLPG-2451 are intended to collectively increase the activity of the mutated copies of CFTR. In the phase 1b FALCON trial the combination of galicaftor with GLPG-2737 with or without GLPG-2451 is being investigated for efficacy, safety, and tolerability in 10 patients that are either homozygous or heterozygous (with a potentiator nonresponsive) for the F508del mutation (ClinicalTrials.gov Identifier: NCT03540524). Results are to be expected in mid-2020.

Harbeson *et. al* (2017) evaluated the metabolic and pharmacological effects of two novel deuterated ivacaftor analogues, CTP-656 (VX-561, d_9-ivacaftor) and d_{18}-ivacaftor in six healthy volunteers.[25] Importantly, the *in vitro* pharmacological data confirmed that CFTR activity of ivacaftor and its major metabolites remained unaffected by ivacaftor deuteration. The data suggest that deuterated ivacaftor has a greater exposure and longer half-life than traditional ivacaftor; this may warrant a once-a-day dosing schedule. In the first half of 2019, a phase 2 randomized, double-blind study was initiated to explore the efficacy and safety of d_9-ivacaftor versus ivacaftor monotherapy or placebo in CF patients bearing 1 of the following 9 CFTR mutations on at least 1 allele: G551D, G178R, S549N, S549R, G551S, G1244E, S1251N, S1255P, or G1349D (ClinicalTrials.gov Identifier: NCT03911713). Results are to be expected in mid-2020.

Using a high-throughput screen Giuliano *et al.* identified novel small molecules displaying synergistic activity when combined with ivacaftor and lumacaftor.[26] By definition these novel CFTR modulators do not fit the traditional potentiator/corrector category. These novel first-in-class compounds have been named *amplifiers*. The authors report that the activity of a corrector (lumacaftor) and potentiator (ivacaftor) in combination with the amplifier PTI-CH nearly doubles when each compound is used at a concentration greater than its EC_{90}.[26] Unlike proteostasis regulators PTI-801 and PTI-808 that indirectly improve CFTR folding, amplifiers such as PTI-428 and PTI-130 are compounds that increase the protein load by boosting CFTR expression. To correct CFTR function, amplifiers are to be combined with correctors and potentiators. In 2018, a randomized, double-blind, placebo-controlled phase 1 study was conducted enrolling 48 patients for 14-days of dosing ivacaftor + lumacaftor with PTI-801 at three escalating dose levels or placebo. PTI-801 demonstrated a statistically significant improvement in sweat chloride and BMI in the 14-day treatment period; however, a trend in improvement in $ppFEV_1$ was observed, albeit this was not statistically significant (press release proteostasis 2018). Currently, the Phase 2 study of the once-a-day triplet regimen, which includes PTI-428, a novel CFTR amplifier, PTI-808, a potentiator, and PTI-801are being initiated.

The hit compound QBW251 identified and optimized by a membrane potential-based screen and subsequent triage by high throughput and Ussing chamber assays employs native and recombinant cell-based systems expressing F508del.[27] In mutations associated with gating defects such as the class II

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F508del mutation, QBW25l proved to be a potent and effective CFTR potentiator. In primary human bronchial epithelial (hBE) cells homozygous for the F508del mutation, QBW251 displayed increased currents to levels −20% of those observed in healthy individuals. Furthermore, QBW251 displayed consistent high selectivity for CFTR and good solubility, high cellular permeability *in vitro*, and moderate-to-high plasma protein binding (89−95%). In *in vivo* pharmacokinetic studies QBW25l displayed good oral bioavailability with a half-life (e.g., in rats, bioavailability of 90% when given 3 mg/kg, half-life 6.7 h ± 2.5 h).[27] In a randomized, double-blind placebo-controlled study (ClinicalTrials.gov Identifier: NCT02190604) pharmacokinetics/pharmacodynamic parameters, tolerability, and safety of single and multiple ascending doses of QBW251 were assessed in healthy subjects and multiple doses in CF patients. In people with CF, the change in lung clearance index (LCI) was measured between baseline and Day 15. LCI improved slightly in patients on QBW251 450 mg but this was not clinically significant. Since then QBW251 has been tested in a 4-week study for patients with chronic obstructive pulmonary disease (COPD). No improvements in LCI were observed; however, QBW251 demonstrated improvement over placebo in sweat chloride (−5.2 mmol/L, 90% probability) suggesting target engagement, and fibrinogen (−40 mg/dL, 99% probability) suggesting an indirect anti-inflammatory effect.[28]

A slightly different approach is the development CFTR modulators classed into 4 categorical cohorts that function either synergistically or additively on primary F508del HBE: (1) PS1, potentiator set (e.g., ivacaftor, FD2033129, FDL-176) increasing the channel open probability; (2) CS1, early corrector set (e.g., lumacaftor, tezacaftor, FDL304) correcting nucleotide binding domain (NBD)1 portion of F508del CFTR; (3) CS2, CFTR Stabilizer (FD2052160) improving CFTR stability in membrane; (4) CS3, CFTR Amplifier (FD1881042, FDL-438) increasing the band B of CFTR.[29] The safety, pharmacokinetic and pharmacodynamic parameters of the clinical candidate FDL-169 are currently being investigated in a phase 1 study (ClinicalTrials.gov Identifier: NCT03093714). There are no clinical results available to date; however, based on the *in vitro* FDL-169 suggested to employ the same mode of action as lumacaftor but with better drug properties such as plasma protein binding and distribution in the lung.[30] Furthermore, in two *in vitro* studies the combination of FDL-169 + FDL-176 or a novel second site correct FD2052160 increased the CFTR protein expression and the chloride transport into primary human bronchial epithelial cells similar in efficacy to FDL-169, ivacaftor, or tezacaftor.[31,32] The increase of combination FD2052160 + FDL-169 in chloride transport was ∼2-fold higher than FDL-169 alone; which was comparable to the effect of FD2052160 combined with tezacaftor or lumacaftor. The novel site 2 corrector series have the potential to increase efficacy of corrector/potentiator combinations currently in clinical development.

The important proof-of-concept of targeting basic defects is both feasible and can have a tremendous clinical benefit to patients with CF. It has added to the success of the first CFTR modulators ivacaftor, lumacaftor, and tezacaftor. Currently, with novel CFTR modulators with enhanced efficacy and complementary modes of action emerging from the development pipeline, it has become realistic that in the near future next-generation combination therapies would be able to expand the patient cohort to include almost the entire CF patient population. Additionally, to new emerging other approaches to CFTR modulators have been investigated to restore CFTR function. Eluforsen (QR-010) is an antisense oligonucleotide constructed to restore the CFTR function by binding to the mRNA region of the F508 encoded deletion.[33] Drevinek *et al.* report that in patients homozygous for F508del CFTR up to 50 mg of inhaled eluforsen dosed 3 times per week for 4 weeks was safe and well tolerated. The patients displayed low systemic exposure, stable lung function, and demonstrated improvement in the CF Questionnaire Revised Respiratory Symptom Score.[33] Liu *et al.* reported that CAT-5571, a potent autophagy activator enhances trafficking of CFTR to the cell surface of hBE that is homozygous for the F508del CFTR.[34] CAT-5571 is a novel conjugate of a cysteine moiety covalently linked to docosahexaenoic acid which has been shown to activate autophagy resulting in improved clearance of lung infections caused by *Burkholderia cenocepacia* and *Pseudomonas aeruginosa*.[35] Interestingly, in addition to effects on bacterial clearance, CAT-5571 has been shown to enhance chloride transport when used in combination with ivacaftor + lumacaftor.[35] Albeit, CAT-5571 *per se* is not a CFTR modulator, it acts on the CFTR channel. Compounds with a novel mode of action such as CAT-5571 represent a potential new therapeutic in the CFTR modulating landscape.

The exciting developments in the CF pipeline in the past decade has been unprecedented, and optimizing clinical trial designs has to become a very high priority, in particular when testing new CFTR modulators in patients that already receiving CFTR modulators. Recent evidence from clinical studies suggests that the pathogenesis of chronic obstructive pulmonary disease (COPD) may be impacted by mucociliary dysfunction. Therefore, CFTR modulators may also prove beneficial to promote mucus clearance and enhance airway surface hydration in other lung diseases such as COPD, even though they were originally intended for CF.

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ABBREVIATIONS

BMI	l
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
COPD	chronic obstructive pulmonary disease
EC_{90}	drug concentration that induces 90% of the maximum response after a specified exposure time

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FDA	Food and Drug Administration
FEV_1	forced expiratory volume in 1 s
HBE	human bronchial epithelial
LCI	lung clearance index
MF	minimal function
$ppFEV_1$	percent predicted FEV1

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