

Cystic fibrosis transmembrane conductance regulator modulators in cystic fibrosis: current perspectives

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Abstract: Mutations of the *CFTR* gene cause cystic fibrosis (CF), the most common recessive monogenic disease worldwide. These mutations alter the synthesis, processing, function, or half-life of CFTR, the main chloride channel expressed in the apical membrane of epithelial cells in the airway, intestine, pancreas, and reproductive tract. Lung disease is the most critical manifestation of CF. It is characterized by airway obstruction, infection, and inflammation that lead to fatal tissue destruction. In spite of great advances in early and multidisciplinary medical care, and in our understanding of the pathophysiology, CF is still considerably reducing the life expectancy of patients. This review highlights the current development in pharmacological modulators of CFTR, which aim at rescuing the expression and/or function of mutated CFTR. While only Kalydeco® and Orkambi® are currently available to patients, many other families of CFTR modulators are undergoing preclinical and clinical investigations. Drug repositioning and personalized medicine are particularly detailed in this review as they represent the most promising strategies for restoring CFTR function in CF.

Keywords: high-throughput screening, drug repositioning, personalized medicine, precision medicine, potentiators, correctors

Introduction

Cystic fibrosis and the CFTR gene

Cystic fibrosis (CF) is an inherited (recessive autosomal) chronic disease that affects the respiratory, digestive, and reproductive systems. Although intestinal symptoms are usually the first to occur during the life of the patient, it is the progressive lung damage, due to cycles of infection/inflammation, that finally leads to irreversible lung disease and death. With ~90,000 people diagnosed, a prevalence of 1/2,500 and about one carrier among 25 individuals, CF is the most common life-threatening Mendelian disorder worldwide. Advances in research and medical treatments have raised the life expectancy of CF newborns beyond 50 years; however, the current median age of survival for CF patients is still in the late 20s.

CF is caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene, which was cloned and identified as the gene affected in CF in 1989.¹ *CFTR* gene encodes the main anion channel expressed in the epithelium. Additionally, CFTR is also expressed in many other cells types (eg, fibroblasts,² neurons,³ cardiomyocytes,⁴

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and immune cells⁵⁻⁷), where its function is not always well known. Among the 2,000+ CFTR mutations identified so far (<http://genet.sickkids.on.ca>), only a fraction of them causes CF. These CF-causing mutations induce a decrease or a loss of function of CFTR at the plasma membrane. In the lung, the lack of CFTR leads to dehydration of the airway surface liquid and drives the cascade of pathological events characteristic of CF (Figure 1).

Structure of CFTR

The *CFTR* gene contains 27 exons spanning 250 kb on the long arm of chromosome 7 (7q31.2).^{8,9} The encoded mRNA is ~6.5 kb long and is translated into a protein of 1,480 amino acids. The CFTR protein belongs to the adenosine triphosphate (ATP)-binding cassette (ABC) transporters and functions as an adenosine 3',5'-cyclic monophosphate (cAMP)-regulated chloride channel in a variety of polarized epithelial cells.¹⁰ The predicted protein structure is shown in Figure 2.

The R domain is a unique structural feature of CFTR as it is not found in other ABC transporters.¹¹ The R domain is highly charged and contains multiple consensus sequences for protein kinase A phosphorylation^{12,13} as well as target sites for other kinases.¹⁴⁻¹⁶ Phosphorylation of the R domain of CFTR is necessary for channel activity: when unphosphorylated, the R domain inhibits CFTR.^{17,18} Although the

phosphorylation of the R region is required, it is not sufficient for opening the CFTR channel^{13,19-21} nor for the interaction with multiple binding partners.^{22,23} Moreover, phosphorylation of the R domain also regulates the membrane stability of CFTR by modulating the balance between endocytosis and exocytosis.²⁴

ATP binding and hydrolysis by the nucleotide-binding domains (NBDs) is a prerequisite to anion transport through CFTR channels.^{25,26} The two NBDs form a head-to-tail dimer

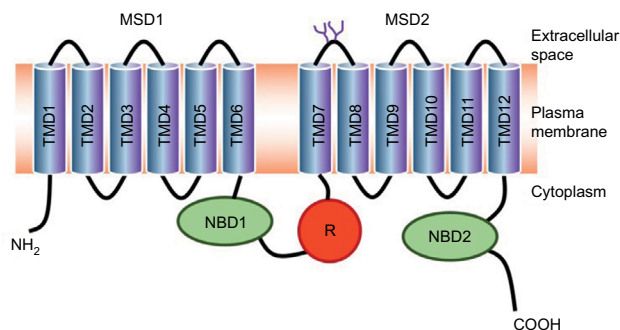


Figure 2 Predicted topology of CFTR protein.

Notes: It is composed of two repeated units made of a MSD followed by a NBD. The two repeated units are linked by a R domain. The MSDs consist of six hydrophobic transmembrane helices (or TMD). Several transmembrane helices contain one or more charged amino acids that control anion permeability. Extracellular loop 4 (between TMD7 and TMD8) contains two *N*-glycosylation sites.

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; MSD, membrane-spanning domain; NBD, nucleotide-binding domain; TMD: transmembrane domain; R, regulatory domain.

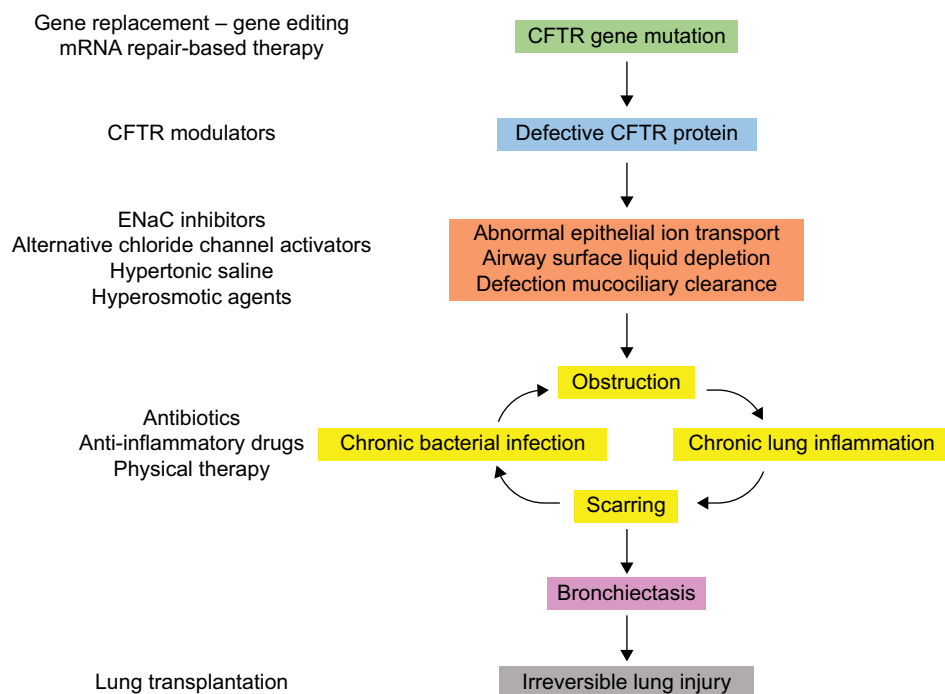


Figure 1 Pathophysiology of CF lung disease and potential therapies targeting the basic defect or the symptoms.

Note: In the absence of conclusive data on gene therapy, CFTR modulators are the most proximal therapy for CF currently in development.

Abbreviations: CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; ENaC, epithelial sodium channel; mRNA, messenger RNA.

with two ATP-binding sites located at the dimer interface.²⁷ Within this dimer, ATP binds to NBD1 but is hydrolyzed at the NBD2 ATP-binding site.^{27–29} ATP interaction with NBDs facilitates their dimerization and induces conformational changes in the membrane-spanning domains required for the gating of CFTR channel.²⁶

CFTR function(s): not only a chloride channel

CFTR is the only ABC transporter functioning as an ion channel. The characteristic properties of CFTR-associated conductance are a linear current–voltage relationship and a single conductance of 6–11 pS.^{30,31} Although CFTR may also transport negatively charged organic molecules such as gluconate³² and glutathione,^{33,34} it is mostly selective for monovalent anion. In vivo, it mainly transports Cl^- and HCO_3^- .^{31,35} Lack of apical Cl^- secretion in CF epithelial cells had already been characterized several years before the discovery of the *CFTR* gene.³⁶ Over the past few years, it has become apparent that CFTR-dependent bicarbonate secretion, required for normal expansion of mucins (the main component of mucus), is also defective in patients with CF.³⁷ Therefore, the role of CFTR in CF pathogenesis is both due to lack of Cl^- , resulting in low hydration of the airway surface liquid, and decrease of HCO_3^- transport, which maintain mucins in an aggregated and poorly soluble form.

In addition to the defective apical Cl^- and HCO_3^- secretion (due to the absence or dysfunction of CFTR), the hyperabsorption of Na^+ through hyperactive epithelial sodium channel (ENaC) is another hallmark of CF epithelia.³⁶ The failure of mutated CFTR proteins to regulate ENaC activity is proposed to play a major role in the pathophysiology of CF lung disease.^{38–40} How does CFTR regulate ENaC and how much CFTR is needed to do so is still debated.^{41,42} It has been reported that CFTR and ENaC physically interact in several cell types.^{43–45} CFTR could also decrease the open probability of ENaC,^{46,47} possibly by protecting ENaC against endogenous proteolytic cleavage.⁴⁸ Finally, CFTR could modulate ENaC stability at the plasma membrane⁴⁹ or regulate the electric coupling between the two channels.^{50,51}

CFTR controls many other ion channels and transporters.⁵² Besides its own ability to transport Cl^- and HCO_3^- , CFTR indirectly modulates the transports of these ions by regulating, for instance, several members of the solute carrier 26 (SLC26) family.⁵³ While some of these proteins function as $\text{Cl}^-/\text{HCO}_3^-$ exchange proteins and participate in pH regulation, SLC26A9 is a chloride channel expressed in the apical

membrane of epithelial cells and is constitutively active in human bronchial epithelial cells (HBECs).^{54–56} It contributes to cAMP-dependent chloride secretion and its activity is maximal when coexpressed with wild-type (WT) CFTR.⁵⁵

Classes of CFTR mutations

Six classes of CFTR mutations have been described (Table 1). Mutations of classes I, II, III, and VI are considered as severe as they are associated with little to no CFTR protein at the plasma membrane, while mutations of classes IV and V generate milder phenotypes as they lead to only partial loss of CFTR activity.^{57,58}

Class I mutations are nonsense mutations causing defects in mRNA splicing or premature insertion of a stop codon in the polypeptidic chain synthesis. They account for ~10% of the *CFTR* mutations worldwide and are particularly prevalent in the Ashkenazi Jewish population where they reach 50% of *CFTR* alleles, with W1282X being the most frequent mutation of this population.⁵⁹

Class II mutations cause defective protein processing and trafficking to the plasma membrane. Among these, the most common CF allele F508del-*CFTR* is found in ~70% of the patients (The Clinical and Functional Translation of CFTR [CFTR2]; <http://cfr2.org>). The deletion of the phenylalanine at position 508 of the CFTR protein causes CFTR misfolding and prolonged retention of the protein in the endoplasmic reticulum, followed by rapid degradation by the ubiquitin-proteasome pathway.^{60,61}

Class III mutations are relatively rare mutations characterized by altered gating and reduced open probability of the channel. The G551D mutation, also known as the Celtic mutation, is the prototype of class III mutation and represent ~2%–3% of CF alleles in north west and central Europe but is less common in other parts of Europe.⁶²

Class IV mutant proteins are correctly inserted at the plasma membrane but the channel single conductance is altered. The most frequent class IV mutations encountered in patients are R117H (1.3%) and R347P (0.37%).

Class V (eg, A455E and 2789+5G→T) and VI (eg, 4326delTC and 4279insA) mutations lead to reduced amount of CFTR protein at the plasma membrane, by affecting CFTR mRNA (stability, alternative splicing, etc) or increasing the turnover of the CFTR protein, respectively.

Some CFTR mutations display more than one type of dysfunctions. For example, in addition to trafficking defect, F508del-*CFTR* also presents with characteristic defects of classes III and IV, with a reduced open probability⁶³ and decreased membrane stability,⁶⁴ respectively.

Table I Classes of CFTR mutations

Class	Class I	Class II	Class III	Class IV	Class V	Class VI
Type of defect	No functional protein	Trafficking defect	Defective channel regulation	Decreased channel conductance	Reduced synthesis	Decreased stability
Examples of mutations	G542X W1282X R553X 621+1G→T	F508del N1303K I507del R560T	G551D G178R G551S S549N	R117H R347P R117C R334W	3849+10kbC→T 2789+5G→A 3120+1G→A 5T	4326delTC Q1412X 4279insA

Abbreviation: CFTR, cystic fibrosis transmembrane conductance regulator.

First modulators and natural compounds

The expression and activity of CFTR channels are regulated by many intracellular signaling pathways. The most known modulator of the CFTR chloride channel is intracellular cAMP, and the activity of CFTR is mainly regulated via phosphorylation by various protein kinases and dephosphorylation by protein phosphatases.

Naturally occurring compounds inducing phosphorylation of the channel were among the first CFTR modulators identified.⁶⁵ Alkylxanthines, such as caffeine, theophylline, and theobromine, are found in plants such as coffee or chocolate beans or tea leaves. Among them, 3-isobutyl-1-methylxanthine inhibits phosphodiesterases (PDEs) to enhance CFTR phosphorylation by preventing its dephosphorylation.^{66–68} Patch clamp single-channel recordings also suggested that some xanthine derivatives can directly activate CFTR channel to increase open probability of the channel independently of cAMP levels,⁶⁹ possibly through direct binding to NBD1.⁷⁰

Soybeans and soy food (eg, tofu, soy flour, and soy milk) contain large amount of isoflavones,⁷¹ such as genistein (5,7-dihydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one). Genistein is a protein tyrosine kinase inhibitor that was found to activate CFTR, independently from protein kinase A or PDE activity.⁷² In contrast with 3-isobutyl-1-methylxanthine,⁷³ it does not inhibit PDE activity but it requires CFTR phosphorylation to increase open probability of the channel.⁷² This is particularly true to mutated protein F508del-^{74,75} and G551D-CFTR⁷⁵ channels for which genistein restores phosphorylation-dependent activation of the channel by direct binding to NBD1.⁷⁶

Curcumin exhibits structural similarities to isoflavones and might bind directly to CFTR protein⁷⁷ to rescue F508del-CFTR trafficking in vitro.^{78,79} In vivo, curcumin increased survival rate of F508del-CFTR mice by preventing gastrointestinal obstruction in treated animals as compared with controls.⁸⁰ Moreover, curcumin corrects CFTR-dependent Cl⁻ transport across nasal and rectal epithelium of F508del-CFTR

mice.⁸⁰ These effects are still controversial as many other studies failed to reproduce them.^{81,82}

Resveratrol is a natural polyphenol compound with antioxidant and anti-inflammatory properties that has been shown to activate CFTR-mediated chloride transport in epithelial cells in vitro^{83,84} and in vivo^{85–87} independently from [cAMP]_i or R domain phosphorylation.⁸⁷ Two independent studies also demonstrated that resveratrol corrects F508del-CFTR trafficking in CF cell lines⁸⁸ and in CF mouse models.⁸⁶ However, doses required for such effects might be difficult to achieve in vivo.⁸⁹

Very low cytotoxicity and high abundance of natural compounds in regular aliment make them an appealing therapeutic option. It seems difficult to achieve sufficiently high concentration of these compounds from food intake only; therefore, administering purified compounds at higher doses could be considered. They may not be selective enough, however, as they often regulate various cellular and biochemical functions.

Drug repositioning

The goal of drug repositioning is to identify new indications of marketed drugs in particular for rare and neglected diseases.⁹⁰ They have multiple advantages over innovative treatments: they are considered safer, as they have already undergone extensive toxicology and safety assessment, they are often less expensive, and shortage is less likely to occur.

Iminosugars that interfere with *N*-glycosylation are approved for the treatment of Gaucher disease.⁹¹ Although strong in vitro^{92,93} and preclinical evidence^{92,94} demonstrated that *N*-butyldeoxynojirimycin (miglustat, Zavesca®) corrects both Cl⁻ and Na⁺ transport by restoring the trafficking defect of F508del-CFTR, a Phase II clinical trial failed to demonstrate significant changes in chloride transport measured by nasal potential difference (NPD), sweat chloride, or force expiratory volume in 1 second (FEV₁) in CF patients.⁹⁵

PDE5 inhibitors (iPDE5) and soluble guanylyl cyclase activators are currently approved for the treatment of erectile dysfunction⁹⁶ and pulmonary hypertension.⁹⁷ They both lead

to increased intracellular cGMP content, although the final mechanism of action on CFTR is still unknown. Some data suggested two distinct effects: a cGMP-dependent increase in CFTR activity and a cGMP-independent effect on CFTR trafficking.⁹⁸ Some *in vitro* studies required 1,000-fold greater concentration than what is used in the clinic to observe an effect on CFTR trafficking.^{98,99} *In vivo* preclinical studies have yet showed that improvement in chloride transport could be achieved with clinical doses of iPDE5, such as sildenafil and vardenafil, in CF mice.^{100,101} Outcomes of a Phase IIa open-label study aiming at investigating safety and efficacy of sildenafil in CF lung disease were recently published.¹⁰² No change in sputum IL-8 was noted, but sputum neutrophil elastase content was significantly reduced after treatment. However, pharmacokinetic profiles of sildenafil suggested that CF patients may eliminate sildenafil at a faster rate than non-CF patients.

Similar to iPDE5, riociguat (BAY 63-2521) increases intracellular cGMP levels in a concentration-dependent manner and in synergy with nitric oxide (NO).^{103,104} It is a soluble guanylate cyclase activator developed by Bayer, already approved for pulmonary arterial hypertension. A Phase II trial is currently ongoing for adult CF patients homozygous for F508del mutation (NCT02170025).

Ibuprofen has long been known for its anti-inflammatory properties, and has been showed to significantly slow the decline in FEV₁ in CF patients.¹⁰⁵ This effect was solely attributed to its anti-inflammatory effect. However, a recent *in vitro* study demonstrated that ibuprofen is also an efficient F508del-CFTR corrector via inhibition of cyclooxygenase-1.¹⁰⁶

Approved for the treatment of cystinosis,¹⁰⁷ cysteamine is a proteostasis regulator that restores autophagy, which is defective in CF.^{108,109} This is associated with a rescue and stabilization of F508del-CFTR at the plasma membrane.^{110,111} Given orally together with epigallocatechin gallate (EGCG, a flavonoid derived from green tea, contained in dietary supplements), cysteamine significantly reduced sweat chloride levels and levels of pro-inflammatory markers TNF- α and IL-8 during a small pilot study in homozygous F508del-CFTR patients.¹¹¹ An open-label Phase II trial involving 34 patients met the primary end point of efficacy, with a significant reduction in sweat chloride concentration of -18.0 mmol/L, but no significant difference in FEV₁ was observed.¹¹²

Escin, extracted from horse chestnut tree, possesses anti-inflammatory effects and is already used in patients with chronic venous insufficiency, hemorrhoids, and post-traumatic edema.¹¹³ Escin significantly enhanced CFTR

function in Fisher rat thyroid cells transfected with different CFTR class I mutants (G542X, W1282X) and in primary HBECs isolated from G542X/F508del and W1282/F508del patients.¹¹⁴ By contrast, escin failed to improve CFTR function in HBECs from a patient homozygous for F508del, demonstrating that Escin acts as a readthrough agent for nonsense mutations.

All these compounds are excellent illustrations that, as for many other rare diseases, CF therapy may benefit from drug repositioning as a strategy to speed up drug development.

Genotype-specific therapies

With the development of high-throughput screening (HTS) assays allowing rapid screening of thousands of small molecules, many families of chemical structures have been identified. Thanks to expanding knowledge of the structure and function of CFTR, and to increased understanding of the different functional consequences of CFTR mutations, structure–activity relationship and optimization of the most promising lead compounds have led to a series of potential pharmacological therapies for CF to correct CFTR defects at different levels.^{114–117} CFTR modulators can be categorized according to the class of mutation or dysfunction that they aim at targeting (Figure 3 and Table 2).

Therapies targeting class I

Development of premature termination codon (PTC) “read-through” agents allow ribosomes to continue translation through class I nonsense mutations to produce full-length CFTR protein. Almost 20 years ago, aminoglycosides, such as gentamicin, were first described as a potential pharmacological approach for class I mutations.^{118–120} In addition to its potent bactericidal activity, gentamicin displayed beneficial effects on electrophysiological parameters assessed by NPD *in vivo* after topical nasal application^{121,122} or intravenous administration^{118,123} in CF patients with at least one class I mutation. However, high inter-individual variability in clinical benefits was observed, in particular between patients carrying only one and those carrying two nonsense mutations.¹²⁴ In addition, high nephron- and oto-toxicity render *per os* or systemically administered aminoglycosides not well suited for long-term use. To tackle this, new series of synthetic aminoglycoside derivatives were developed through a systematic structure-based approach.^{125,126} NB30, NB54, and NB124 had significantly reduced toxicity^{125,127} and demonstrated superior *in vitro* readthrough activity in HBE cell lines or primary cells expressing at least one nonsense CFTR mutation.^{125,128} Moreover, when systemically administrated to *cftr*^{-/-} mice expressing human

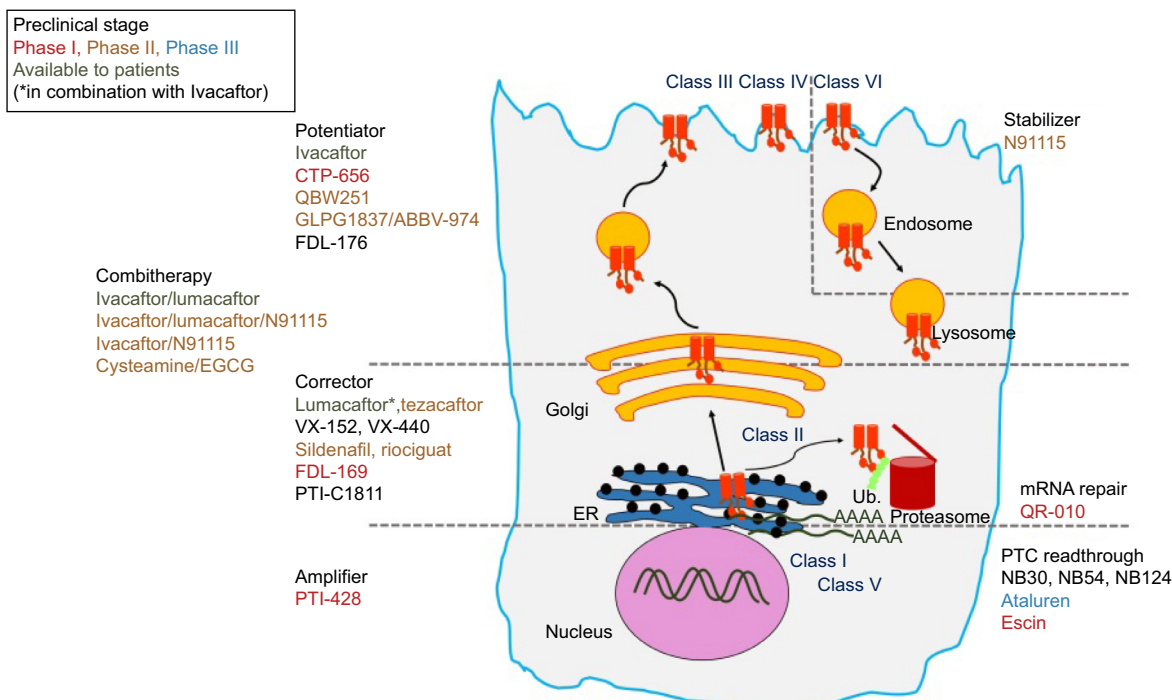


Figure 3 Overview of the most advance CFTR modulators in preclinical and clinical studies, with regard to the class of CFTR mutations and the primary defect of the corresponding mutant protein.

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; PTC, premature termination codon; EGCG, epigallocatechin gallate; ER, endoplasmic reticulum; Ub, ubiquitin; mRNA, messenger RNA.

CFTR-G542X,¹²⁹ NB54 and NB124 restored CFTR activity measured ex vivo across intestinal epithelium to at least 5% of the current observed in WT animals.^{127,128}

Through HTS, PTC Therapeutics™ (Dublin, Ireland) identified PTC-124 (3-[5-(2-fluorophenyl)-1,2,4-oxadiazol-3-yl]-benzoic acid), ataluren. It is an orally bioavailable small molecule inducing complete translation of proteins containing premature nonsense mutations without affecting the normal stop codons.¹³⁰ In the initial Phase II trial, CF adults with at least one *CFTR* nonsense mutation received oral treatment with ataluren for 14 days followed by a washout period of 14 days.¹³¹ CFTR function measured by NPD was restored and a small decrease in ENaC activity was also recorded. Moreover, patients presented with slight increase in FEV₁ and bodyweight, and some of them reported an improvement in pulmonary symptoms such as cough. A pediatric trial was conducted with children of age 6 and older, and demonstrated similar improvements in CFTR function although it did not correlate with FEV₁.¹³² Despite these encouraging data, ataluren did not provide a significant improvement in FEV₁ of a Phase III placebo-controlled trial.¹³³ Interaction with chronically inhaled tobramycin could be a cause, as the subgroup of patients not receiving inhaled aminoglycosides showed a more robust improvement in FEV₁ (+5.7% predicted) together with fewer pulmonary exacerbations (−40%)

in the ataluren group as compared to the placebo. Moreover, variable responses were found among patients with different genotypes suggesting that readthrough agents may not work for all class I mutations.

Therapies targeting class II

The aim of class II targeting compounds is to rescue the trafficking defect of mutant CFTR and therefore increase the quantity of mutated CFTR protein inserted in the plasma membrane. Soon after the identification of the CFTR gene, Denning et al⁶¹ demonstrated that low-temperature incubation (eg, 27°C) restores F508del-CFTR expression at the plasma membrane. This was the first evidence that the trafficking defect of F508del-CFTR could be modified to allow partial escape from the endoplasmic reticulum quality control and functional expression on the cell surface. Additional in vitro proofs of mutant CFTR druggability were obtained with chemical chaperones^{134,135} or the transcriptional regulator butyrate.¹³⁶ In vitro, 4-phenylbutyrate (4-PBA), an analog of butyrate, corrects the trafficking defect of F508del-CFTR¹³⁷ by modulating the interaction with 70 kDa Heat shock protein (Hsp) family Hsc70.¹³⁸ 4-PBA was one of the first corrector to be tested in a pilot clinical trial for CF, where it slightly improved CFTR activity in nasal epithelium but did not reduce sweat chloride concentration.¹³⁹

Table 2 Mechanisms of action of pharmacological modulators of CFTR available to CF patients or under preclinical development as mono- and/or combitherapies for CF

Classes of compounds		Target	Mechanism of action	Pharmacological compounds
Monotherapies	PTC read-through	Class I (nonsense mutations)	Generate a full-length CFTR by complete translation of CFTR transcript	Ataluren Escin NB30, NB54, NB124
	mRNA repair therapy	All classes	Repair the CFTR mRNA to generate a WT-CFTR transcript	QR-010 (specific for F508del-CFTR)
	Correctors	Class II	Rescue F508del-CFTR to the plasma membrane	Lumacaftor, tezacaftor, VX-152, VX-440 Sildenafil, riociguat FDL-169 PTI-C1811
	Potentiators	Class III Class IV	Bind to CFTR to increase open probability	Ivacaftor CTP-656 QBW251 GLPG1837/ABBV-974 FDL-176
Combitherapies	Corrector/potentiator	Class II/III	Rescue F508del-CFTR to the plasma membrane, increase CFTR open probability	Lumacaftor/ivacaftor Tezacaftor/ivacaftor Lumacaftor/QBW251
	Corrector 1/corrector 2/ potentiator	Class II/III	Rescue F508del-CFTR to the plasma membrane via two distinct mechanisms and increase CFTR open probability	Tezacaftor/VX-152/ivacaftor Tezacaftor/VX-440/ivacaftor
	Corrector/stabilizer	Class II	Rescue F508del-CFTR to the plasma membrane and enhance rescued F508del-CFTR stability	Cysteamine/EGCG
	Corrector/potentiator/stabilizer	Class II/III/VI	Rescue F508del-CFTR to the plasma membrane, increase CFTR open probability, and enhance rescued F508del-CFTR stability	Lumacaftor/ivacaftor/N91115
	Potentiator/stabilizer	Class VI	Increase CFTR open probability and enhance rescued F508del-CFTR stability	Ivacaftor/N91115
	Amplifier/other modulator(s)	All classes	Increase the amount of immature CFTR to provide more substrate for other modulators to act upon	PTI-428/other(s)

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; CF, cystic fibrosis; PTC, premature termination codon; mRNA, messenger RNA; WT, wild type.

With the development of HTS assays and medicinal chemistry, many families of new chemical structures with corrector properties have emerged. Their corrector activities are exerted through direct modulation of protein folding^{140,141} or cellular proteostasis,¹⁴² or may act as pharmacological chaperons.¹⁴³ While many of the compounds available so far, such as corr-4a¹¹⁶ or VRT-325,¹⁴¹ will never progress beyond the status of “bench tools”, some hits have been identified and optimized in view of clinical assessments. The most advanced corrector for F508del-CFTR is VX-809 (lumacaftor), developed by Vertex Pharmaceuticals (Boston, MA, USA). VX-809 restores F508del-CFTR trafficking by improving its folding and stabilizing membrane-spanning domain 1.^{144,145} Four weeks of oral lumacaftor as monotherapy in homozygous F508del-CFTR patients was demonstrated safe and well tolerated.¹⁴⁶ Sweat chloride contents were significantly decreased with treatment in a dose-dependent manner. However, lumacaftor failed to demonstrate any therapeutic benefit for lung function as it did not change

FEV₁ nor modulate NPD parameters. This lack of clinical effect suggested that corrector-based monotherapies are not efficient enough to improve lung function because they do not target the other biological defects of F508del-CFTR, ie, decreased membrane stability and open probability.

Because in vitro studies showed that CFTR potentiator VX-770 (see ivacaftor, classes III and IV) improved the open probability of VX-809-rescued F508del-CFTR,¹⁴⁵ a new Phase II¹⁴⁷ and a Phase III¹⁴⁸ studies with combination of lumacaftor and ivacaftor were conducted. Overall, the absolute increase in FEV₁ was modest (+3%).¹⁴⁸ Currently marketed as Orkambi®, the ivacaftor–lumacaftor combination has been heavily challenged because it seems no more efficient than conventional multitherapies¹⁴⁹ for a price outrageously tenfold higher.¹⁵⁰ More importantly, two in vitro studies evidenced negative interference between ivacaftor and several correctors including lumacaftor, as prolonged exposure of HBECs with ivacaftor decreases the stability of lumacaftor-corrected F508del-CFTR.^{151,152} This could explain

in part the modest improvement of lung function observed in patients taking lumacaftor/ivacaftor.

Vertex Pharmaceuticals is currently expanding its drug portfolio by developing more correctors such as VX-661 (tezacaftor), for which they claimed slightly better clinical efficacy (+4.8% FEV₁) than VX-809 when combined with ivacaftor in patients with two copies of F508del-CFTR.¹⁵³ Moreover, VX-661 showed additional benefit (+4.6% FEV₁) in patients carrying both F508del- and G551D-CFTR mutations and who were already taking ivacaftor.¹⁵³

More next-generation correctors such as VX-152 and VX-440 will be evaluated in combination with VX-661/ivacaftor as triple combinations (VX-152/VX-661/ivacaftor and VX-440/VX-661/ivacaftor) in homozygous F508del patients and patients with one F508del associated with a second mutation that results in minimal CFTR function. In vitro, these triple combinations resulted in an increase in chloride transport in HBECs approximately threefold higher than with lumacaftor/ivacaftor.

Other drug discovery companies have undertaken development of correctors. Among them, PTI-C1811 (Proteostasis Therapeutics, Cambridge, MA, USA) and FDL-169 (Flatley Discovery Lab, Charlestown, MA, USA) act through different mechanisms than VX-809 and are both claimed to have similar or superior in vitro activity when combined with potentiators.

Unlike CFTR correctors that act at the protein level, ProQR Therapeutics NV (Leiden, the Netherlands) developed QR-010, a single-strand modified RNA specifically designed to repair the F508del mutation at the mRNA level to generate a WT-CFTR transcript. In vivo in a preclinical mouse model, QR-010 demonstrated a robust increase in CFTR activity measured by NPD¹⁵⁴ and a restoration of CFTR-dependent salivary secretion rates.¹⁵⁵ QR-010 is now being tested in two clinical trials. In a Phase Ib study (NCT02532764), single and multiple ascending doses will assess QR-010 safety and tolerability in F508del homozygous patients. The second study (NCT02564354) is exploratory proof-of-concept study in CF patients with at least one copy of F508del. It will explore whether intranasal administration of QR-010 can restore function of the CFTR protein as measured by NPD.

Therapies targeting class III and IV

Class III and IV mutations are considered mild because they produce full-length CFTR that inserts into the plasma membrane where it can correctly interact with other proteins. However, chloride transport is reduced because the open

probability (class III) or the single conductance (class IV) of the channel is altered. Pharmacological compounds that enhance CFTR function at the cell membrane are called potentiators.

VX-770 (ivacaftor) was identified by HTS in epithelial cells expressing G551D-CFTR.¹⁵⁶ Early clinical investigations enrolling patients with at least one G551D mutations provided encouraging efficacy data (as measured by NPD and sweat chloride concentration) in patients receiving 150 mg ivacaftor twice a day, together with a safety profile comparable to the placebo group.¹⁵⁷ Longer studies (STRIVE and ENVISION), up to 48 weeks, also demonstrated that patients aged 6 years and older treated with ivacaftor gained significantly more weight and their frequency of pulmonary exacerbation was reduced by 55% with ivacaftor as compared to placebo.^{158,159} During the KONNECTION study, ivacaftor resulted in significant improvement in FEV₁ (+10.7% at 8 weeks), sweat chloride, and body mass index in patients carrying one of the following non-G551D alleles: G178R, S549N, S549R, G551S, G970R, G1244E, S1251N, S1255P, or G1349D.¹⁶⁰ Results were comparable to those observed during the STRIVE and ENVISION studies (+10.6% FEV₁) in patients with G551D mutation.^{158,159}

Because CF lung disease is progressive, treating patients as early as possible was the aim of the KIWI study which enrolled preschoolers (2.5–5 years old) with one G551D mutation. Ivacaftor seemed to be safe in that cohort, although extended results are awaited.¹⁶¹

During the initial screening, ivacaftor was shown to also potentiate activity of rescued F508del-CFTR.¹⁵⁶ As expected with a potentiator, a clinical trial with ivacaftor for F508del/F508del patients failed to support its use as a monotherapy for this class of patients.¹⁶² As of now, ivacaftor (Kalydeco®; Vertex Pharmaceuticals) is the only potentiator approved for CF patients aged 2 years and older who carry at least one of the following mutations: G551D, G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N, S549R or R117H. Ivacaftor is seen by the CF community as a proof of principle of clinical benefit from a CFTR modulator, and its approval was a very significant milestone in CF treatment.

Concert Pharmaceuticals Inc. (Lexington, MA, USA) is applying deuterium chemistry to enhance the pharmacokinetic properties of ivacaftor. This approach was tested in CF patients with class III mutations in a Phase I crossover comparison of deuterated ivacaftor (CTP-656) vs ivacaftor. CTP-656 demonstrated a longer half-life of the compound in plasma compared to ivacaftor, supporting the possibility to reduce the dosing to once a day.¹⁶³

QBW251 is a potentiator developed by Novartis Pharmaceuticals. In vitro data showed superior efficacy of QBW251 as compared to ivacaftor when both are combined with lumacaftor. Phase II trial (NCT02190604) has been conducted and some outcomes have been recently presented.¹⁶⁴ CF heterozygous patients with at least one class III to VI mutation were enrolled (including patients with one F508del mutation as it can be considered either as class II, III, or VI). A separate arm of the study enrolled only F508del-CFTR homozygous patients. Orally administered QBW251 (150 mg or 450 mg, twice a day) for 2 weeks was safe and well tolerated in the 40 CF patients.¹⁶⁴ In patients with a residual function, QBW251 (450 mg) statistically increased FEV₁ over placebo by 7.3%, an increase that is considered as clinically relevant for lung function and very similar to that observed with ivacaftor. As for ivacaftor, QBW251 monotherapy did not demonstrate any efficacy in patients with two copies of F508del.

The potentiator GLPG1837/ABBV-974 is codeveloped by Galapagos NV (Mechelen, Belgium) and AbbVie Pharmaceuticals (North Chicago, IL, USA). Phase I has demonstrated that single (up to 2 g) and multiple doses (up to 800 mg twice a day for 14 days) of GLPG1837/ABBV-974 were safe and well tolerated in healthy volunteers.¹⁶⁵ Two Phase II open-label studies are ongoing and will explore GLPG1837/ABBV-974 safety, tolerability, and efficacy in CF patients with G551D (SAPHIRA1) and S1251N (SAPHIRA2).

Therapies targeting class V and VI

Currently, there is no clinical data available for class V-specific therapies. For class VI, a new class of compounds increasing the half-life of CFTR protein at the plasma membrane has recently attracted interest. VRT-325 and Corr-4a were prototypes for this type of compounds, so-called “stabilizers”, which are meant to be complementary to existing and future CFTR modulators.

N91115, developed by Nivalis Therapeutics (Boulder, CO, USA), is an inhibitor of *S*-nitroglutathione (GSNO) reductase and aimed at increasing intracellular levels of GSNO. GSNO induces the *S*-nitrosylation of the cellular chaperone Hsp70/Hsp90 organizing protein which prevents the association of CFTR with Hsp70/Hsp90 organizing protein.^{166–168} N91115 was proven safe and well tolerated in CF patients with two F508del alleles.¹⁶⁹ N91115 has recently received the status of Orphan Drug designation by the FDA and two Phase II clinical studies are ongoing (see “Combitherapies and personalized medicine” section). In the near future, potentiators may also prove useful to provide maximal activation of class VI mutants.

Combitherapies and personalized medicine

Many pharmacological agents are currently in development to correct mutant CFTR activity in CF. These agents are becoming increasingly specific, and aim at targeting patients with particular genotype. The most advanced treatment for CF currently available for patients is ivacaftor. This is a typical example of personalized medicine where only individuals with specific mutations can be treated with this drug. Although ivacaftor provides a significant improvement in lung function, this may not be achievable in every CF patient with a single compound. More specifically, in patients carrying CFTR mutations displaying multiple dysfunctions, such as F508del-CFTR, combination of several molecules will likely lead to better clinical results. Here, the biological defects of F508del-CFTR could be ideally addressed by a triple combination of a corrector to increase the amount of F508del-CFTR protein expressed in the plasma membrane, a potentiator to enhance its open probability and a stabilizer to increase its half-life at the plasma membrane.

To tackle the membrane instability of rescued F508del-CFTR, Nivalis is currently evaluating N91115 safety and efficacy in combination with lumacaftor/ivacaftor in homozygous F508del patients (NCT02589236) and with ivacaftor in patients with one F508del and a gating mutation (NCT02724527) in two Phase II clinical trials.

Another new class of compounds is currently investigated by Proteostasis Therapeutics. They are developing PTI-428, a CFTR amplifier, which aims to selectively increase the amount of immature form of CFTR protein to provide other CFTR modulators with more substrate to act upon.¹⁷⁰ PTI-428 received Fast Track designation from the FDA and a Phase I is ongoing to assess its safety, tolerability and pharmacokinetics in CF patients (NCT02718495).

In the near future, one can also envisage combitherapies with activators of alternative chloride channels or with inhibitors of the ENaC.¹⁷¹

One of the biggest challenges to implement personalized medicine for CF will be to develop new in vitro models to better predict the individual response of patient to different combinations of treatments. Development and use of experimental materials based on patient tissues (such as airway and intestinal organoids or induced pluripotent stem cells) will hopefully provide new powerful assays to better anticipate the individual clinical benefit of CFTR modulators.

Conclusion

Many classes of compounds restoring the function of CFTR mutants have been identified; however, most of them, such as natural compound curcumin, were never translated into therapy mainly because of lack of benefit to patients as well as off-target effects or low bioavailability. Drug repositioning, through the exciting examples of the cystamine/EGCG combination or sildenafil, may speed up the development of novel therapies for CF. Currently, ivacaftor alone or in combination with lumacaftor are the only pharmacological modulators of CFTR approved for the treatment of CF. The combination lumacaftor/ivacaftor has been highly challenged as they do not seem to provide significant improvement in lung function as compared to conventional therapies. Ivacaftor targets only a specific CFTR mutant (G551D-CFTR) which is found in <2% of the patients. Finally, these two marketed therapies cost over USD 250,000/year (a tenfold increase as compared to usual multitherapies) for a modest improvement in the quality of life of patients. Thus, there is still a major and urgent need for new molecules and therapeutic approaches to be developed for treating CF.

Acknowledgment

TL and SN received financial support from the European Commission under the H2020-PHC-13-2014 funding program (PRO-CF-MED, ref. 633545) granted to ProQR Therapeutics NV.

Disclosure

The authors report no conflicts of interest in this work.

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