

Review

Targeting F508del-CFTR to develop rational new therapies for cystic fibrosis

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The mutation F508del is the commonest cause of the genetic disease cystic fibrosis (CF). CF disrupts the function of many organs in the body, most notably the lungs, by perturbing salt and water transport across epithelial surfaces. F508del causes harm in two principal ways. First, the mutation prevents delivery of the cystic fibrosis transmembrane conductance regulator (CFTR) to its correct cellular location, the apical (lumen-facing) membrane of epithelial cells. Second, F508del perturbs the Cl⁻ channel function of CFTR by disrupting channel gating. Here, we discuss the development of rational new therapies for CF that target F508del-CFTR. We highlight how structural studies provide new insight into the role of F508 in the regulation of channel gating by cycles of ATP binding and hydrolysis. We emphasize the use of high-throughput screening to identify lead compounds for therapy development. These compounds include CFTR correctors that restore the expression of F508del-CFTR at the apical membrane of epithelial cells and CFTR potentiators that rescue the F508del-CFTR gating defect. Initial results from clinical trials of CFTR correctors and potentiators augur well for the development of small molecule therapies that target the root cause of CF: mutations in CFTR.

Keywords: ATP-binding cassette transporter; epithelial ion transport; cystic fibrosis; CFTR; chloride ion channel; F508del; CFTR corrector; CFTR potentiator

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Introduction

Salty sweat is diagnostic of cystic fibrosis (CF), an autosomal recessive genetic disease common in Caucasians^[1, 2]. The elevated concentration of salt in sweat is indicative of the underlying molecular defect in CF, the loss of chloride ion (Cl⁻) channel function in the apical (lumen-facing) membrane of epithelia lining ducts and tubes throughout the body^[1]. The impermeability of the apical membrane to Cl⁻ in CF disrupts fluid and electrolyte transport across epithelia and, hence, the function of a variety of organs. This leads to the wide-ranging manifestations of the disease, which include chronic lung disease, exocrine pancreatic insufficiency, meconium ileus (blockage of the terminal ileum), male infertility and salty sweat^[1, 2]. The median survival of CF patients in North America and Western Europe is around 40 years^[2].

There are two principal causes of debilitation and death in CF patients^[1, 3, 4]. First, chronic obstructive lung disease caused by thick tenacious mucus that prevents normal mucociliary clearance. Second, persistent bacterial infections, typically

with *Pseudomonas aeruginosa*, which result in bronchiectasis, respiratory failure and eventually death. Current therapies for CF include physiotherapy, mucolytic drugs and antibiotics to treat lung disease, and pancreatic enzyme replacement and supplementary nutrition to overcome gastrointestinal dysfunction^[1, 2]. These therapies treat the symptoms of CF; they do not target the root cause of the disease.

In 1989, the defective gene responsible for CF was identified and predicted to encode a protein with five domains: two membrane-spanning domains (MSDs), two nucleotide-binding domains (NBDs) and a unique regulatory domain (RD)^[5]. Shortly thereafter, the protein product of this gene, the cystic fibrosis transmembrane conductance regulator (CFTR), was demonstrated to be a unique member of the ATP-binding cassette (ABC) transporter superfamily^[6]. Instead of forming an ATP-driven pump like most family members, CFTR was demonstrated to function as an ATP-gated pathway for anion movement driven by the transmembrane electrochemical gradient^[7–10]. Subsequent research has aimed to understand the physiological role of CFTR, learn how CF mutations cause CFTR dysfunction and develop rational new therapies for CF patients. Here, we selectively review progress in the development of drug therapies for CF, focusing on small molecules

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that target the most common CF mutation, F508del, deletion of the three base pairs that result in the loss of the phenylalanine residue at position 508 of the CFTR protein sequence; 90% of CF patients carry at least one copy of the F508del mutation.

The F508del-CFTR mutant retains some residual channel function

When Rich *et al*^[11] found that expression of F508del-CFTR in CF airway epithelial cells failed to correct the defective Cl⁻ permeability of these cells and Cheng *et al*^[12] subsequently demonstrated that the F508del mutation disrupts CFTR biosynthesis and membrane trafficking in COS-7 cells, it was widely assumed that F508del-CFTR had no Cl⁻ channel function. Surprisingly however, Drumm *et al*^[13] demonstrated that F508del-CFTR generates a cAMP-activated Cl⁻ conductance when expressed in *Xenopus* oocytes. Because F508del-CFTR Cl⁻ currents had similar conduction and permeation properties, but reduced magnitude compared with those of wild-type CFTR, Drumm *et al*^[13] speculated that F508del-CFTR forms a channel with attenuated sensitivity to cAMP agonists, a conclusion that was to prove prescient. Concurrently, Dalemans *et al*^[14] used the patch-clamp technique to demonstrate that F508del-CFTR forms a Cl⁻ channel regulated by cAMP-dependent phosphorylation in Vero cells. The authors demonstrated that F508del-CFTR had many properties in common with those of wild-type human CFTR^[14]. However, there was one notable exception, the pattern of channel gating of F508del-CFTR differed dramatically from that of wild-type CFTR^[14].

Biophysical properties of the F508del-CFTR Cl⁻ channel

Like other mutations that affect specific residues within the NBDs^[15], F508del has no discernable effect on the conduction and permeation properties of the CFTR Cl⁻ channel. First, the F508del-CFTR Cl⁻ channel has a small single-channel conductance, which does not differ from that of wild-type CFTR (6–10 pS; *eg* Li *et al*^[16]). Second, like wild-type CFTR (but see^[17]), the current-voltage (I–V) relationship of F508del-CFTR is linear (*eg* Dalemans *et al*^[14]). Third, both wild-type and F508del-CFTR are highly selective for anions over cations ($P_{Na}/P_{Cl}=0.08$; *eg* Li *et al*^[16]). Fourth, wild-type and F508del-CFTR share the identical anion permeability sequence of Br⁻>Cl⁻>I⁻ (*eg* Dalemans *et al*^[14]). Finally, wild-type and F508del-CFTR both exhibit time- and voltage-independent gating behavior (*eg* Denning *et al*^[18]). Consistent with these data, using excised membrane patches from gallbladder epithelial cells of wild-type and F508del-CFTR mice French *et al*^[19] demonstrated that the F508del mutation is without effect on the biophysical properties of murine CFTR. Thus, the data suggest that the F508del mutation does not affect the pore properties of CFTR.

The gating defect of the F508del-CFTR Cl⁻ channel

Figure 1 illustrates the gating pattern of wild-type and F508del-CFTR Cl⁻ channels following phosphorylation by protein kinase A (PKA). The gating behavior of wild-type CFTR is characterized by frequent bursts of channel activity that are interrupted by brief flickery closures and separated

by longer closures between bursts (Figure 1). By contrast, the gating pattern of F508del-CFTR is characterized by infrequent bursts of channel activity that are interrupted by brief flickery closures, but separated by long closures of prolonged duration (Figure 1). Work by a number of investigators using a variety of cells and experimental conditions demonstrate that the open probability (P_o ; a measure of the average fraction of time that a channel is open) of F508del-CFTR is about one third that of wild-type CFTR^[14, 18, 20–23], although Miki *et al*^[24] argue that the P_o of F508del-CFTR is likely to be substantially lower (~fifteen-fold less than that of wild-type CFTR). Surprisingly, and in marked contrast to these data, other authors found that the P_o of F508del-CFTR did not differ from that of wild-type CFTR^[16, 19]. A likely explanation for these differences is that the rate of activation of F508del-CFTR is more than seven-fold slower than that of wild-type CFTR^[25].

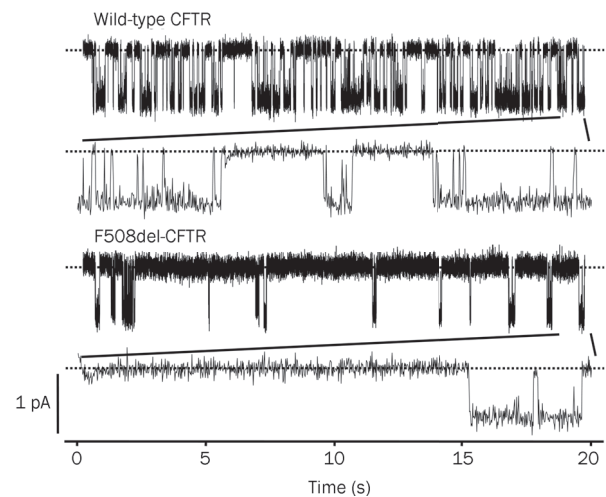


Figure 1. Single-channel activity of wild-type and F508del-CFTR. Representative recordings of wild-type and F508del-CFTR Cl⁻ channels in excised inside-out membrane patches from C127 cells expressing recombinant CFTR. ATP (1 mmol/L) and PKA (75 nmol/L) were continuously present in the intracellular solution, voltage was clamped at -50 mV, and a large Cl⁻ concentration gradient was imposed across the membrane patch ($[Cl^-]_{Ext}=10$ mmol/L; $[Cl^-]_{Int}=147$ mmol/L). Dashed lines indicate where the channels are closed and downward deflections correspond to channel openings. Beneath each of the prolonged 20 s recordings, the last 1 s of the record is shown on an expanded scale. Other details are as described in Cai and Sheppard^[23]. Modified, with permission, from Cai and Sheppard^[23].

To understand how the F508del mutation disrupts CFTR channel gating, several investigators have examined the gating kinetics of the F508del-CFTR Cl⁻ channel. Dalemans *et al*^[14] first demonstrated that in cell-attached membrane patches on Vero cells voltage-clamped at -60 mV, the F508del mutation was without effect on open times, but decreased mean closed times five-fold compared with those of wild-type CFTR. Haws *et al*^[20] and our group^[23, 26] examined the gating kinetics of F508del-CFTR in membrane patches from BHK

and C127 cells. Both groups found that the F508del mutation was without effect on open and closed times within bursts. Instead, F508del caused a large decrease in P_o by (i) markedly prolonging the closed time interval between bursts and (ii) reducing mean burst duration^[20, 23, 26]. These data suggest that the F508del mutation disrupts CFTR channel gating in two ways: first, F508del dramatically slows the rate of entry into a burst of channel activity. Second, F508del accelerates the rate of channel closure.

F508del is located at a critical interface in the CFTR gating pathway

The F508del mutation profoundly disrupts CFTR channel gating by slowing dramatically the rate of channel opening and by accelerating the rate of channel closure. An explanation for the gating behavior of F508del-CFTR is provided by the ATP-driven NBD dimerization model of CFTR channel gating^[27, 28]. This model integrates the results of functional studies of CFTR channel gating with biochemical and structural data. Structural studies of ABC transporters suggest that the NBDs are organized as a head-to-tail dimer with two ATP-binding sites located at the NBD1:NBD2 interface^[29–32]. The data suggest that one ATP-binding site is formed by the Walker A and B motifs of NBD1 and the LSGGQ motif of NBD2 (termed site 1), while the other is formed by the Walker A and B motifs of NBD2 and the LSGGQ motif of NBD1 (termed site 2) (Figure 2). However, photolabeling studies argue that the ATP-binding sites of CFTR are not equivalent in function; site 1 stably binds nucleotides, whereas site 2 rapidly hydrolyses them^[33, 34]. Because CFTR Cl^- channels transit between the closed and open configurations in seconds, Vergani *et al*^[27, 28] interpreted the photolabeling data to suggest that CFTR channel gating

is controlled by ATP binding and hydrolysis at site 2, driving cycles of NBD dimer assembly and disassembly. To test this model, Vergani *et al*^[28] applied mutant cycle analysis to residues predicted to lie on opposite sides of the NBD1:NBD2 dimer interface. Of note, the authors demonstrated that R555 (NBD1) and T1246 (NBD2) are energetically coupled only in open channels, arguing convincingly that the NBDs undergo dynamic reorganization during channel gating^[28]. For further discussion of how ATP gates the CFTR Cl^- channel, see^[35–37].

Using the ATP-driven NBD dimerization model of CFTR channel gating^[27, 28], Roxo-Rosa *et al*^[26] speculated that the exceptionally slow rate of channel opening of F508del-CFTR might be explained by F508del inducing misfolding and/or structural instability of NBD1, which would hamper ATP binding. Moreover, the reduced open time of F508del-CFTR might reflect weakening of the binding energy for stable NBD1:NBD2 dimer formation by the mutation^[26]. In support of this idea, Pissarra *et al*^[38] demonstrated that the solubilizing mutations used to promote crystallization of human NBD1^[32] traffic F508del-CFTR to the surface and abrogate, albeit incompletely, the channel's gating defect. Thus, deletion of F508 might cause intrinsic misfolding and/or structural instability of NBD1^[26].

However, two lines of evidence argue against the idea that deletion of F508 causes misfolding of NBD1. First, F508del perturbs the local topography of NBD1, without affecting domain folding^[32], but see Pissarra *et al*^[38]. Second, F508 is located at the surface of NBD1, where it might interact with the MSDs^[31, 32]. The residue is remote from the NBD1:NBD2 interface, the location of the ATP-binding sites (Figure 2).

Following the elucidation of the atomic structure of the ABC transporter Sav1866, the multidrug transporter of *S aureus*^[39],

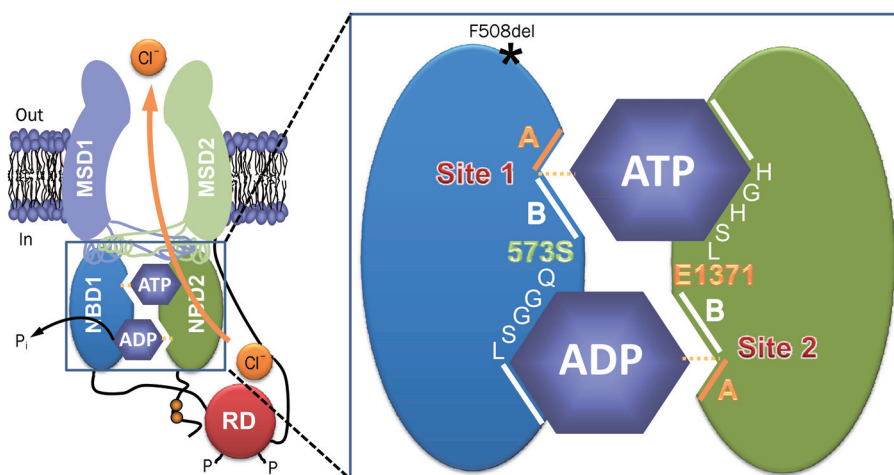


Figure 2. The organization of the ATP-binding sites in CFTR. The simplified model shows the molecular architecture of ATP-binding site 1 (site 1) and ATP-binding site 2 (site 2) in an open CFTR Cl^- channel. Each ATP-binding site is formed by the Walker A and B motifs (labeled A and B, respectively) of one NBD and the LSGGQ motif of the other NBD. Site 2 contains a canonical LSGGQ motif, whereas site 1 contains a non-canonical LSGGH motif. Site 2 also contains a catalytic base (E1371) at the distal end of the Walker B motif, but this residue is absent in site 1 (S573). The location of the CF mutation F508del on the surface of NBD1 opposite intracellular loop 4 (ICL4) is shown by an asterisk. Abbreviations: MSD, membrane-spanning domain; NBD, nucleotide-binding domain; P, phosphorylation of the RD; P_i , inorganic phosphate; RD, regulatory domain. In and out denote the intra- and extracellular sides of the membrane, respectively. See text for further information. Modified, with permission, from Hwang and Sheppard^[36].

structural models of the entire CFTR protein have been developed^[40–42] and used to understand better CFTR function (eg Alexander *et al*^[43]). Of note, these structural models have provided important new insight into how the F508del mutation disrupts CFTR channel gating. They also reveal the function of the four intracellular loops (ICLs), which connect transmembrane segments within the MSDs. Each ICL consists of two long α -helical extensions of transmembrane segments with an intervening short α -helix at its most cytoplasmic location orientated parallel to the plane of the membrane. Because this short α -helix interacts with the NBDs, it is termed the coupling helix^[40, 41].

For two reasons, the positions of the ICLs in structural models of CFTR are notable. First, the ICLs communicate both with the same and the opposite NBD (eg ICL1 (MSD1) with NBD1 and ICL2 (MSD1) with NBD2^[40, 41]). Prior to these structural models, communication between the NBDs and MSDs was presumed to be only vertical (eg NBD1:MSD1). However, the structural models of Serohijos *et al*^[40] and Mornon *et al*^[41] argue that communication between the NBDs and MSDs is both vertical and orthogonal (eg NBD1:MSD1 and NBD1:MSD2). Second, the coupling helix of ICL4 interacts with the surface of NBD1 in the region of F508^[40, 41]. This observation provides an explanation for why the F508del mutation profoundly disrupts CFTR channel gating. The mutation affects a residue located at a critical interface in the CFTR gating pathway, the sequence of conformation changes initiated by ATP-driven NBD dimerization, which leads to opening of the CFTR pore and Cl⁻ flow through the channel. Thus, understanding the interface between the F508 region of NBD1 and the coupling helix of ICL4 is central to the development of drug therapies that target the root cause of CF.

Rational new therapies for CF that target defects in F508del-CFTR

To target the root cause of CF, future therapies should (i) overcome the F508del-CFTR processing defect and traffic the mutant protein to the apical membrane^[12]; (ii) extend the residence time of F508del-CFTR at the apical membrane^[44] and abrogate channel “rundown” (eg Schultz *et al*^[22]) and (iii) rescue the defective channel gating of F508del-CFTR^[14]. Thus, small molecules with two or possibly three types of activity are required to restore function to the F508del-CFTR Cl⁻ channel.

Small molecules that overcome the processing defect of F508del-CFTR and traffic the mutant protein to the apical membrane are termed CFTR correctors because they rescue the cell surface expression of F508del-CFTR^[45, 46]. CFTR correctors might interact with CFTR itself, by acting as either substrate mimics or active site inhibitors. Alternatively, they might target one or more of the many CFTR interacting proteins that orchestrate and control processing of CFTR, its delivery to, and expression at the apical membrane. This latter group of CFTR correctors is termed proteostasis regulators because they aim to treat CF by manipulating the concentration, conformation, quaternary structure and/or location of CFTR^[47].

Small molecules that repair the gating defect of the F508del-CFTR Cl⁻ channel are termed CFTR potentiators because they do not open silent channels, but instead enhance ATP-dependent channel gating following the phosphorylation of F508del-CFTR by PKA^[45]. Although some agents (eg bromotetramisole^[48]) enhance CFTR gating by modulating activity of the protein kinases and phosphatases that control the phosphorylation status of CFTR, CFTR potentiators interact directly with CFTR to enhance channel gating. Interestingly, a small number of compounds have been identified which possess both CFTR corrector and potentiator activity^[49, 50]. These small molecules are termed CFTR corrector-potentiators or dual-acting molecules.

Because there is insufficient information at the present time to design rationally CFTR correctors and potentiators, the strategy of choice to identify drug-like small molecule CFTR modulators is high-throughput screening (HTS)^[45]. HTS exploits a reliable, sensitive, cost-effective assay to screen libraries of chemically diverse small molecules (eg approved drugs^[51], drug-like chemicals^[52] and natural products^[53]) to identify lead compounds for medicinal chemistry optimization. For example, Alan Verkman (UCSF, San Francisco, USA) used Fischer rat thyroid cells, epithelial cells devoid of CFTR expression and cAMP-stimulated Cl⁻ currents^[54] engineered to co-express recombinant human CFTR and a green fluorescent protein (GFP) with ultra high halide sensitivity in a halide flux assay (eg Yang *et al*^[52]). By contrast, Vertex Pharmaceuticals (San Diego, USA) employed NIH-3T3 cells expressing recombinant human CFTR in a fluorescence resonance energy transfer (FRET)-based membrane voltage-sensing assay (eg Van Goor *et al*^[55]). Both of these HTS assays monitor the change in CFTR-mediated anion flux elicited by CFTR modulators in real time.

By screening 150 000 drug-like compounds using F508del-CFTR expressing FRT cells, Verkman's group were the first to identify CFTR correctors using HTS^[56]. Among the chemical scaffolds with CFTR corrector activity, the bisaminomethylthiazole corr-4a (Figure 3) deserves special attention. Pedemonte *et al*^[56] demonstrated that corr-4a is equipotent to low temperature correction at restoring function to F508del-CFTR-expressing human bronchial epithelia (CFBE), achieving levels of CFTR function approximately 8% of that of human bronchial epithelia expressing wild-type CFTR (HBE). Of special note, the aminoarylthiazole corr-2b identified by Pedemonte *et al*^[56] exhibits dual activity as both a CFTR corrector and a CFTR potentiator^[50]. When compared with small molecules that act as CFTR correctors, corr-2b generated double the amount of forskolin-activated CFTR Cl⁻ current (I_{FSK}) relative to the total CFTR Cl⁻ current measured in the presence of forskolin and the CFTR potentiator genistein (I_{TOT}) (CFTR correctors (eg corr-4a): $I_{\text{FSK}}/I_{\text{TOT}} \sim 40\%$; corr-2b: $I_{\text{FSK}}/I_{\text{TOT}} \sim 80\%$; see Figure 1 of Pedemonte *et al*^[50]). Moreover, the authors demonstrated that aminoarylthiazoles do not act as typical CFTR potentiators because they require protein synthesis to exert their effects (see below and^[50]). Elucidation of the mechanism of action of corr-2b and related dual-acting small molecules is

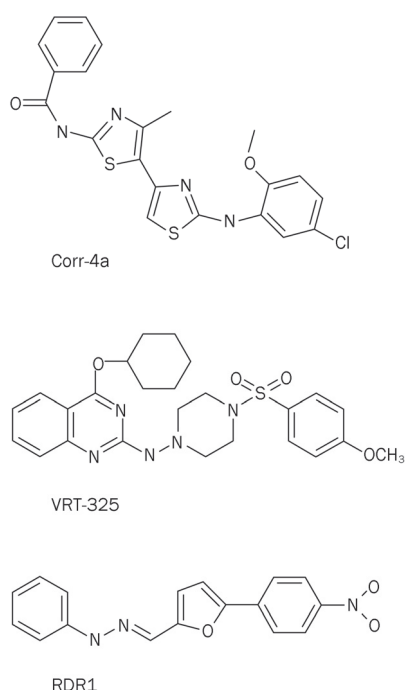


Figure 3. Chemical structures of some CFTR correctors identified by HTS. Abbreviations: Corr-4a, N-[2-(5-Chloro-2-methoxy-phenylamino)-4'-methyl-[4,5']bithiazolyl-2'-yl]-benzamide; VRT-325, 4-Cyclohexyloxy-2-[1-[4-(4-methoxy-benzenesulfonyl)-piperazin-1-yl]-ethyl]-quinazoline; RDR1, 5-(4-nitrophenyl)-2-furaldehyde 2-phenylhydrazone.

a priority for future research.

In a ground-breaking program funded by the Cystic Fibrosis Foundation (Bethesda, USA), a nonprofit, donor-supported organization, Vertex Pharmaceuticals identified 13 distinct chemical scaffolds with CFTR corrector activity after screening ~164 000 chemically diverse drug-like small molecules^[55]. Following medicinal chemistry optimization, Vertex Pharmaceuticals identified the quinazoline VRT-325 (Figure 3) as a potent and efficacious CFTR corrector that enhances the maturation of native F508del-CFTR protein and augments CFTR-mediated transepithelial Cl⁻ secretion in CFBE^[55]. Biochemical studies of VRT-325 suggest that it acts at the endoplasmic reticulum to promote CFTR folding^[55]. Because VRT-325 decreases the apparent ATP affinity of purified, reconstituted F508del-CFTR^[57], it might rescue the processing and trafficking of F508del-CFTR, at least in part, by interacting directly with the mutant protein.

To identify CFTR correctors that interact directly with F508del-CFTR, Sampson *et al*^[58] employed differential scanning fluorimetry, which identifies ligands of a target protein by monitoring their effects on the thermal unfolding of the protein. Among 224 hits identified in a previous HTS for CFTR correctors, just one chemical, the substituted phenylhydrazone RDR1 (Figure 3), was able to thermally stabilize murine F508del-CFTR^[58]. As with previous studies of CFTR correctors by the Hanrahan and Thomas groups^[59, 60], the authors deployed a battery of biochemical and functional

assays to investigate F508del-CFTR rescue by RDR1 in heterologous cells, polarized epithelia and genetically-modified mice. The authors' data demonstrate that RDR1 thermally stabilizes murine F508del-NBD1, increases the maturation of human F508del-CFTR protein and augments the function of human CFTR *in vitro* and murine CFTR *in vivo*^[58]. Taken together, these data and the additive effect of RDR1 treatment and low temperature incubation on human F508del-CFTR maturation argue convincingly that RDR1 is a CFTR corrector that targets directly F508del-NBD1 to exert its effects. Identification of the RDR1-binding site on CFTR should be an important goal of future research.

To identify CFTR potentiators that rescue the gating defect of F508del-CFTR, Yang *et al*^[52] studied FRT cells expressing low temperature-corrected F508del-CFTR. A screen of 100 000 compounds identified six novel classes of high-affinity F508del-CFTR potentiators^[52]. However, by screening additional structural analogues, Yang *et al*^[52] discovered tetrahydrobenzothiophenes (*eg* ΔF508_{act}-02; Figure 4), which potentiate F508del-CFTR with $K_d < 100$ nmol/L. Subsequently, Pedemonte *et al*^[61] screened 50 000 compounds searching for further ligands that rescue the gating defect of F508del-CFTR. After secondary analyses, Pedemonte *et al*^[61] identified phenylglycines and sulfonamides that potentiate F508del-CFTR with nanomolar potency. Interestingly, by screening a library of 2000 compounds, including drugs approved for clinical use, Pedemonte *et al*^[51] demonstrated that the antihypertensive drugs 1,4-dihydropyridines (DHPs) act as F508del-CFTR potentiators by a mechanism independent of their effects on voltage-gated Ca²⁺ channels. To identify DHPs that potentiate F508del-CFTR without inhibiting voltage-gated Ca²⁺ channels, Pedemonte *et al*^[62] investigated structure-activity relationships

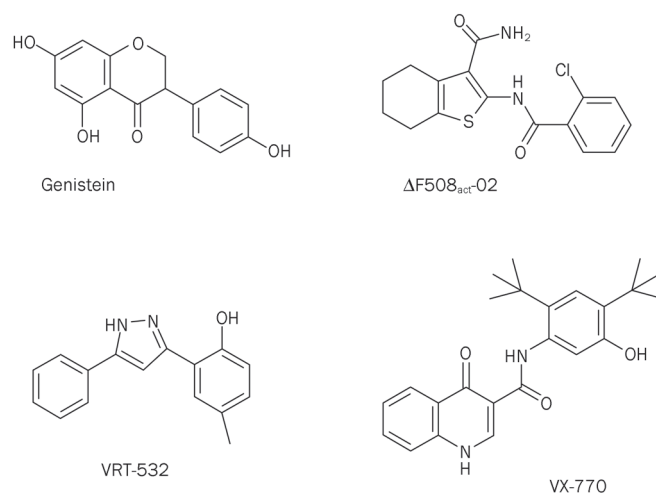


Figure 4. Chemical structures of some CFTR potentiators identified by HTS. Abbreviations: ΔF508_{act}-02, 2-(2-chlorobenzamido)-4,5,6,7-tetrahydro-3H-indene-1-carboxamide; VRT-532, 4-methyl-2-(5-phenyl-1H-pyrazol-3-yl)phenol; VX-770, N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide. For comparison, the chemical structure of genistein is shown.

using a panel of 333 analogues of felodipine, the most potent CFTR potentiator identified in their original screen. The authors' data demonstrate that substituents with hydrophobic groups enhance the potency of DHPs as CFTR potentiators^[62]. They also reveal that some DHPs are excellent lead compounds for the development of therapeutically active CFTR potentiators.

To identify therapeutically active potentiators of F508del-CFTR, Vertex Pharmaceuticals screened 122000 synthetic compounds from their compound collection using NIH-3T3 cells expressing temperature-corrected F508del-CFTR^[55]. After careful scrutiny, Van Goor *et al*^[55] selected for further study 53 compounds consisting of 10 distinct chemical scaffolds. One compound, the pyrazole VRT-532 (Figure 4), rescued the gating defect of F508del-CFTR by accelerating the rate of channel opening and slowing the rate of channel closure^[55]. Critically, VRT-532 augmented robustly CFTR-mediated transepithelial Cl⁻ secretion in CFBE (EC₅₀, 2.7±0.2 μmol/L^[55]). Importantly, the effects of VRT-532 on CFBE were synergistic with the CFTR corrector VRT-325. CFBE incubated with VRT-325 and then treated with cAMP agonists and VRT-532 generated levels of CFTR-mediated transepithelial Cl⁻ secretion in CFBE >20% of those observed in HBE^[55].

Subsequently, Vertex Pharmaceuticals screened 228 000 chemically diverse drug-like compounds to identify chemical scaffolds for development into therapeutically active CFTR potentiators. Following medicinal chemistry optimization, Vertex Pharmaceuticals identified VX-770 (Figure 4), a potent, selective and orally bioavailable CFTR potentiator^[63]. Interestingly, by increasing the frequency and duration of channel openings, VX-770 (1 μmol/L) restored the channel activity (measured by P_o) of F508del-CFTR to wild-type CFTR levels^[63]. Moreover, treatment of CFBE (genotype F508del/G551D) with VX-770 (10 μmol/L) increased airway surface liquid volume and ciliary beat frequency to levels about half those of HBE^[63].

Based on its performance in preclinical studies, VX-770 became the first CFTR potentiator to be tested in the clinic. The drug was first tested in 39 adult CF patients carrying the CFTR mutation G551D, which has no effect on the processing and trafficking of CFTR, but profoundly disrupts channel gating^[64, 65]. The CF patients in this study took VX-770 orally in a randomized, double-blind, placebo-controlled trial^[66]. VX-770 was well tolerated by CF patients, and at high concentration (150 mg), VX-770 decreased the sweat Cl⁻ concentration to a level approaching the normal range (<60 mmol/L) and improved lung function (measured by forced expiratory volume in one second, FEV₁) by 9%^[66]. Further clinical studies of VX-770 are ongoing. Of special note, initial results from the phase III clinical trial of VX-770 on 83 CF patients with the G551D mutation demonstrated a sustained improvement in lung function at 48 weeks with drug-treated CF patients 55% less likely to experience a pulmonary exacerbation (<http://investors.vrtx.com/releasedetail.cfm?ReleaseID=551869>). Vertex Pharmaceuticals indicate that they plan to apply for US and European drug approval later in 2011 (<http://investors.vrtx.com/releasedetail.cfm?ReleaseID=551869>).

Knowledge of how ATP gates the CFTR Cl⁻ channel, particularly the ATP-driven NBD dimerization model^[27, 28], provides explanations for the mechanism(s) of action of CFTR potentiators. Ai *et al*^[67] first proposed that genistein and other CFTR potentiators might enhance CFTR channel gating by affecting NBD dimerization. The authors speculated first that the binding of genistein at the interface of the NBD dimer might lower the free energy of the transition state and, hence, accelerate channel opening^[67]. Second, the authors proposed that genistein might slow the rate of channel closure by stabilizing the NBD dimer conformation^[67]. Finally, the authors argued that the binding site for genistein might be located at the dimer interface^[67]. Consistent with this idea, Moran *et al*^[68] used a molecular model of the NBD1:NBD2 dimer to show that genistein, apigenin and a series of novel CFTR potentiators identified by HTS bind to CFTR at the dimer interface. As predicted^[69] and verified^[70] by functional data, this drug-binding site is distinct from the two ATP-binding sites of CFTR. Moreover, sequences from both NBD1 (Walker A, Walker B and LSGGQ) and NBD2 (LSGGQ) contribute to the drug-binding site, with those from NBD1 forming a cavity in which CFTR potentiators dock^[68]. Following the development of structural models of the entire CFTR protein^[40-42], *in silico* structure-based screening is likely to become a powerful tool to identify small molecules that interact directly with F508del-CFTR and other CF mutants. Of note, using this approach Kalid *et al*^[49] identified a ligand-binding site in the vicinity of F508 at the interface of the NBDs and MSDs. Finally, differences in the molecular pharmacology of CFTR homologs from different species (eg human and murine CFTR^[63, 71, 72]) argue that chimeric CFTR proteins may be valuable tools to identify where CFTR potentiators dock with CFTR^[73].

Conclusions

Two decades after the identification of the defective gene responsible for CF, therapies based on a molecular understanding of the disease are beginning to be tested in the clinic. Early results from these trials are encouraging. They raise the prospect of personalized medicine, whereby specific therapies are designed to target precisely the genetic defects harbored by individuals afflicted by CF. However, the development of efficacious and safe drug therapies for CF patients will require much more work. For example, it is currently unknown how much CFTR function is required to rescue CF mutants, whether drug therapy for CF is mutation specific and if long-term treatment with CFTR correctors and potentiators causes adverse effects. Answers to these pressing questions will play an important role in shaping future therapeutic strategies for CF.

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Author contribution

The authors researched the literature and wrote the review.

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