CFTR modulators: from mechanism to targeted therapeutics

Han-I Yeh[1,2,3], Katy Sutcliffe[4], David Sheppard[4], and Tzyh-Chang Hwang[1,2,3]

1. Department of Pharmacology, National Yang Ming Chiao Tung University, Taiwan
2. Department of Medical Pharmacology and Physiology, University of Missouri, USA
3. Dalton Cardiovascular Research Center, University of Missouri, USA
4. School of Physiology, Pharmacology and Neuroscience, University of Bristol, UK

Running title: Mechanisms of CF drugs

Key words: cystic fibrosis, chloride channels, ABC transporters, pharmacology

Correspondence:
Tzyh-Chang Hwang
134 Research Park Drive
University of Missouri, Columbia MO 65211
Email: hwangt@health.missouri.edu
Tel: +1 573-882-2271

Or

David Sheppard
Biomedical Sciences Building
University Walk
Bristol, BS8 1TD
Email: D.N.Sheppard@bristol.ac.uk
Tel: +44 117-331-2290

Section 1. Introduction

Three decades have passed since the cloning of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes an epithelial anion channel also named CFTR. CFTR maintains the homeostasis of water and electrolytes in epithelium-lining tissues such as the airways, pancreas, intestines, sweat duct, etc. [1, 2]. Loss-of-function mutations in the CFTR gene cause cystic fibrosis (CF), a multi-organ, chronic disorder with symptoms secondary to airway infection/inflammation, pancreatic insufficiency and anatomic abnormalities in the male reproductive system [3, 4]. Patients with CF also bear a higher risk of other CF-related conditions such as diabetes and gastrointestinal cancer [5, 6]. The past thirty years witness remarkable progress in both basic sciences and clinical medicine in the field: a thorough understanding of CFTR's function, detailed categorization of the molecular defects caused by pathogenic mutations, the solution of multiple atomic structures of CFTR, and above all, successful development of drug treatments for the majority of patients with CF.

Since the discovery of the first small-molecule CFTR modulator ivacaftor in 2009 (approved by the US Food and Drug Administration, FDA in 2012) [7], therapeutics targeting the fundamental cause of CF, the dysfunctional CFTR proteins, have substantially increased CF patients' life expectancy and dramatically improved their life quality. To date, ~90% of CF patients in the US benefit from CFTR modulator therapies developed by Vertex Pharmaceuticals [8, 9]. Although the remaining ~10% CF patients carrying rare mutations are not eligible for these medicines and still rely on symptomatic treatments, the CF community is galvanized towards developing novel therapeutics aimed at any left-behind mutations with the goal of delivering personalized cure to every individual with CF.

In this review, we start with the structural and functional studies of CFTR that are directly relevant to the actions of CFTR modulators, followed by a summary of the classification of CF-causing mutations. We then put emphasis on recent breakthroughs in the development of CFTR modulators and highlight the mechanistic studies that address how CFTR correctors and potentiators restore the function of CFTR mutants. For more comprehensive reviews of CFTR biosynthesis, structure/function, and regulation, we refer the readers to Csanády et al. [10], Hwang et al. [11], Pranke, Sermet-Gaudelus [12], and Al Salmani et al. [13].

Section 2. Atomic structures of CFTR and its modulators

In 2016, the long-awaited high-resolution structure of CFTR was solved by Prof. Jue Chen's lab using the cryogenic electron microscopy (cryo-EM) technology [14]. In the following years, a series of CFTR structures in different conformations and with CFTR modulators

bound was published by the same group [15-19]. An immediate gain from the cryo-EM structures of CFTR is to confirm the evolutionary relationship between CFTR and the exporters of the ATP binding cassette (ABC) transporter superfamily, as they all share a conserved architecture: an assembly of two transmembrane domains (TMDs, TMD1 and TMD2) and two cytosolic nucleotide binding domains (NBDs, NBD1 and NBD2). The TMDs in the ABC transport proteins form the substrate translocation pathway across the cell membrane, and the NBDs work as an engine harnessing the energy of ATP binding and hydrolysis to power the substrate transport cycle. CFTR inherits the canonical motifs of TMDs and NBDs but refashions its TMDs into an ion-conducting pore. Different from other ABC exporters is an additional regulatory domain (R domain) inserted between CFTR's two NBD/TMD complexes (**Figure 1A**).

Comparisons between the solved CFTR structures in different states reveal the distinct features of this channel: First, part of the R domain is wedged in between the two NBDs and the cytosolic extension of the TMDs in the unphosphorylated CFTR [14, 16]. Although the structure of the entire R domain was not resolved due to its lack of well-defined secondary structures, its position suggests an inhibitory effect on channel gating by steric hindrance. Consistent with this latter idea, in phosphorylated, ATP-bound CFTR structure, the density corresponding to the R domain was on the peripheries of the protein [15, 17]. Second, in phosphorylated ATP-bound CFTR structures, the two NBDs form a canonical head-to-tail heterodimer. The dimerization-induced conformational changes in the TMDs allow the permeation pathway to penetrate toward the extracellular side until reaching a constricted region surrounded by L102, F337, T338, and N1138 (**Figure 1B**). At this level, the pore is too small to accommodate a dehydrated chloride ion [15, 17], and hence this ATP-bound structure represents a closed state with dimerized NBDs. Internal to this constricted region is a large internal vestibule with a lateral portal flanked by 4^{th} transmembrane segment (TM4) and TM6 that are adorned with a number of positively-charged residues to attract anions towards the pore. Third and most unexpectedly, the TM8 is unwound by a helical break between residue 921 and 930, which was proposed to explain the asymmetric contribution of TMD1 and TMD2 to CFTR's pore formation.

Besides the determination of CFTR structures, the Chen's lab sets an important milestone in the journey of CF therapeutics—identifying two hotspots on CFTR for, respectively, potentiators [18] and type I correctors [19] (**Figure 2**). Individual amino acid residues interacting with these modulators were verified by elegant biochemical and biophysical studies [18-20]. The locations and exact amino-acid compositions of the binding sites establish a framework to understand how these small molecules affect CFTR gating or folding. Armed with these structural and functional mechanisms of CFTR modulators, the

field is at the cusp of materializing rational drug-design for CF.

Section 3. CFTR biochemistry and biophysics

CFTR biosynthesis, folding, trafficking, and degradation

Encoded by the *CFTR* gene on the seventh chromosome (7q31.2) [1], the mRNA transcript of CFTR is translated in the endoplasmic reticulum (ER) and further processed by the Golgi apparatus to make a multi-domain glycoprotein of 1480 amino acids with a molecular weight of ~170 kDa. As the synthesis of the nascent polypeptide of CFTR starts from the N- to the C-terminus, domain folding and insertion of the transmembrane segments into the ER membrane proceed co-translationally [21-23]. The newly synthesized TMD1 is first positioned in the ER membrane [24], followed by simultaneous growing and folding of the NBD1 polypeptide, an event accompanied by molecular chaperones that stabilize the cytosolic NBD1 during successive R domain synthesis [25-30]. Interactions between these three domains further stabilize the TMD1-NBD1-R domain complex and integration of TMD2 into the membrane ensues. Folding of NBD2 and assembly of the individual domains into a compact tertiary structure are completed post-translationally [31]. In the ER, CFTR is core-glycosylated at asparagine 894 and 900 in the fourth extracellular loop, creating the so-called immature form of CFTR (band B on Western blot) that is ready to enter the Golgi secretory pathway. On the other hand, misfolded CFTR resulting from a wide spectrum of mutations, including the most prevalent CF-causing mutation F508del, is targeted by ubiquitination and degraded via Endoplasmic Reticulum Associated Degradation (ERAD). Trafficked to the Golgi complex, the core-glycosylated CFTR is processed by multiple glycosyltransferases to become the fully-glycosylated or mature form of CFTR (band C on Western blot). Secretory vesicles then transport the mature CFTR to the apical plasma membrane, where it can finally exert its function as an ion channel. With a half-life of 12-24 hours [32, 33], a wild-type CFTR is internalized by endocytosis that sends the channel either to lysosomal degradation or to a recycling pathway back to the membrane.

Ion conduction and permeation

The pore of CFTR has a high anion-versus-cation selectivity but shows less degree of discrimination among small anions such as chloride, bromide, or nitrate [34-36]. For the two most prevalent, small, physiological anions, chloride and bicarbonate, CFTR has a permeability ratio ($P_{Cl^-}/P_{HCO_3^-}$) and a conductance ratio ($g_{Cl^-}/g_{HCO_3^-}$) both in the range of 3.5–4 [37, 38], enabling CFTR to play an essential role in transepithelial chloride and bicarbonate movement in the G-I tract and the airway. The overall architecture of CFTR's ion permeation pathway in the cryo-EM structures is largely consistent with a picture based on functional studies employing substituted cysteine accessibility method (SCAM) [39-46, reviewed in 47]: The pore consists of a narrow segment sandwiched by positively-charged

internal and external vestibules that attract anions to their respective entryway. Given that in the cryo-EM structure, the constricted area, which was proposed to be the selectivity filter and the gate, is too narrow for chloride movement [15, 17], how chloride ions outcompete other anions may await the solution of the open channel conformation.

Phosphorylation-dependent activation and ATP-dependent gating

Activation of CFTR requires cAMP-dependent PKA phosphorylation of the R domain, and the balance between phosphorylation and dephosphorylation processes in the cell determines the activity of CFTR [36, 48]. At least eight major PKA phosphorylation sites in the R domain have been identified [49-51]; phosphorylation of most of these serine residues stimulates CFTR channel activity whereas two of them (S737 and S768), once phosphorylated, are paradoxically inhibitory [52-54]. Nonetheless, even fully-phosphorylated CFTR prefers a closed conformation until ATP binds to its two ATP-binding sites to initiate dimerization of the two NBDs and gate opening (details in below). This ATP-dependent gating of phosphorylated CFTR is augmented incrementally with the degree of phosphorylation and that channels lacking the R domain are constitutively active with an open probability (P_o) that is compatible to that of a fully-phosphorylated wildtype CFTR [55, 56]. Since it seems unlikely that protein kinases can target an R domain wedged between the two TMD-NBD complexes, it was proposed that spontaneous dislodgement of the R domain from that inhibitory position renders the R domain accessible to protein kinases, which through simple binding and/or phosphorylation prevent R domain's return to the inhibitory position [57].

Once the R domain is released from the occluded location, binding of ATP to the two nucleotide binding sites (site 1 and site 2) induces dimerization of the NBDs [58, 59]. Since each NBD is connected to the TMDs through the intracellular loops, the conformational changes can be transmitted toward the TMDs to open the gate. Next, ATP hydrolysis in the catalytic site 2 disrupts the NBD dimer, leading to separation of the NBDs and gate closure. CFTR's site 1, on the other hand, is catalytically incompetent. Rather than terminating the opening burst by hydrolysis, ATP in site 1 likely plays a distinct role in stabilizing CFTR's structure during the gating motion [60-62]. In each gating cycle, CFTR's pore opening/closure is coupled to ATP-dependent formation/separation of the NBD dimer; the gating rate is thus determined by the catalytic turnover rate of site 2's ATPase activity (0.5–1 s^{-1}).

Plenty of subtleties in how the NBDs control the gate are still under debate [10, 11]. Based on the prevailing mechanism of ABC exporters, which depicts a rigid-body motion of the TMD/NBD complexes, CFTR's gate in the TMD is open if and only if the NBDs are

dimerized (**Figure 3A**) [63]. An alternative gating model featuring an energetic coupling between TMDs and NBDs was also proposed [64] (**Figure 3B**). The essence of this latter model, in contrast to the strict coupling model, is that NBD dimerization facilitates, but not obligates, gate opening and vice versa. A consequent distinction between the strict coupling and the energetic coupling mechanisms is the stoichiometry between the gating cycle and ATP consumption: the former follows a one-to-one stoichiometry and the latter is nonintegral. Both models agree that the dimer interface at site 2 opens up upon ATP hydrolysis to release the hydrolytic products, but they differ in the subsequent events. Strict coupling thesis holds that as soon as the dimer interface breaks open, the gate is closed, and the gating cycle completes at the full separation of the two NBDs. In contrast, the energetic coupling model incorporates a post-hydrolytic state with an open gate and emptied site 2, and a closed state with dimerized NBDs. In other words, the coupling between NBDs and the gate in TMDs is probabilistic: NBD dimerization promotes but not obligates gate opening, and gate opening promotes NBD dimerization, resulting in a nonintegral stoichiometry between the ATP hydrolysis cycle and the gating cycle [65].

Section 4. CF-causing mutations (classification) and mechanisms of CFTR dysfunction

To function as a phosphorylation-activated and ATP-gated anion channel, CFTR requires a delicate balance between protein stability and relatively fast conformational dynamics which are responsive to environmental cues. CFTR mutations which disrupt this balance, as well as those that inhibit proper biogenesis of CFTR proteins, could lead to CFTR dysfunction and CF.

Currently, over 2100 variants have been reported for the CFTR gene (http://www.genet.sickkids.on.ca/), although not all of these cause CF. By far the most prevalent variant is the deletion of the phenylalanine at position 508 in the NBD1, termed F508del, with an estimated 90% of individuals with CF possessing at least one copy of this variant. The remaining 10% of individuals with CF bear comparatively rare mutations in CFTR. The less most prevalent variants are G542X, G551D, N1303K and W1282X accounting for just 2.6 %, 2.0 %, 1.8 % and 1.5 % of patients in the CFTR2 database [66]. The mutation sites span the entire CFTR protein (**Figure 4**). Comparatively fewer variants are found in the R domain, consistent with the much greater sequence diversity and a lack of secondary structure in this region [67]. These disparate locations of pathogenic mutations explain why these variants result in differing mechanisms for CFTR dysfunction—from loss of protein expression and stability to altered gating and conductance properties. Thus, this highly heterogeneous patient population makes drug development challenging, as no single compound is likely to be effective across the entire spectrum of CFTR variants

and disease phenotypes.

CFTR variants have been classified into six major classes according to their underlying functional defects [68-71] (**Table 1**):

Class I variants result in defective protein synthesis by mutations that cause frameshifts, alternative splicing or the introduction of stop codons. As less, or prematurely truncated, protein is synthesized, CFTR expression is often severely reduced or indeed absent. For example, both the G542X and W1282X variants introduce stop codons resulting in abrogated protein production (with a remaining 5 to 10 % of wild-type level [72]) and a severe CF phenotype [73-75].

Class II variants affect protein folding and transport to the cell surface by causing CFTR to misfold and be targeted for degradation in the endoplasmic reticulum (ER). This severely reduces the number of functional CFTR molecules reaching the plasma membrane. The common F508del variant is a typical Class II variant. F508del cells express mostly immature CFTR, whilst the mature, fully glycosylated form is absent [76], indicating a folding and subsequent maturation defect. It has been proposed that loss of F508 results in increased instability of the NBD1 domain [77], which is crucial for proper folding.

Class III variants alter channel regulation and gating. This is classically characterised by a reduction in open probability in electrophysiological recordings, without alteration in current amplitude [78]. G551D is an example of a Class III variant. Due to the electrostatic repulsion between the negatively charged aspartate side chain and the phosphate groups of ATP, ATP-dependent gating is abolished in G551D-CFTR [78-80]. Interestingly, the Class II mutation F508del also results in dysfunctional channel gating, an effect that could be attributed to the loss of the F508-mediated NBD1-ICL4 interaction which communicates conformational changes in the NBDs to the CFTR pore [14, 15, 81]. Moreover, class III variants are not confined to the NBDs. F312del on TM5 and R117H on ECL1, both exhibit moderate effects on channel gating [82-84], despite residing some 70 Å and 90 Å away from the ATP binding sites, respectively. The loss of an R117-mediated hydrogen bond has been recently proposed as the mechanism by which R117 mutations alter gating [85], whilst the structural mechanism for defective gating in F312del-CFTR is unknown but could be related to the location of this residue at the TM8 hinge [17]. These mutations, which cause gating defects via different mechanisms, highlight the complex allosteric network which regulates CFTR function.

Class IV variants lead to a reduced chloride conductance by impeding chloride ion flow

through the pore [86]. Often, these are residue substitutions in the pore region resulting in the loss of a positively charged side chain [87], for example R347P and R334W. These mutations are likely to reduce anion conductance by the loss of favourable electrostatic interactions between the chloride ions and the pore-lining side chains [14].

Class V variants exhibit reduced expression at the membrane. Unlike Class I and II variants, these variants are synthesized and folded correctly, but display mutations in promotor or splicing regions which reduce the overall abundance of the protein. For example, the A455E variant alters CFTR processing, resulting in less protein at the cell surface [88]. Patients with the A455E variant exhibit a relatively mild disease phenotype [89], possibly attributed to the functional A455E-CFTR which does reach the plasma membrane [88, 90].

Class VI variants result in reduced plasma membrane expression and increased protein turnover, caused by conformational instability at the plasma membrane and/or in the post-ER compartments, or the introduction of internalization signals to remove CFTR from the cell membrane. Several CFTR-interacting proteins, including keratin, PDZ domain containing proteins and SNARES, regulate the expression and stability of CFTR on the cell surface [91]. Mutations at the regions of CFTR which interact with these regulatory proteins can therefore lead to Class VI dysfunction. For example, C-terminal truncation mutations, such as Q1412X, confer increased sensitivity to degradation [69], possibly due to the loss of a C-terminal hydrophobic residue patch which in wildtype CFTR may interact with the cytoskeleton to strengthen membrane stability [92, 93].

Although this classification system is useful in broadly understanding the impacts of different CF-causing variants, most variants have defects in multiple classes. For instance, as described above, F508del-CFTR exhibits both a Class II folding defect and a Class III gating defect [76, 94]. In addition, membrane-expressed F508del-CFTR is subject to increased turnover, characteristic of a Class VI defect [32, 95]. Similarly, P67L, classically described as a Class II variant, is also sensitive to modulators designed to act on gating and conductance defects (termed potentiators, see Section 5.1) [96]. Not surprisingly, given its location on ECL1, the R117H mutant also exhibits reduced chloride conductance [86], a Class IV defect, alongside dysfunctional gating. Finally, it was recently reported that the Class VI variant Q1412X also confers a gating defect, characterised by a reduced P_o, which may be due to the C-terminal truncation impairing the structural integrity of the NBD2 ATP binding site [97]. Even within classes, there is considerable intraclass variability in the precise mechanism of CFTR dysfunction. For instance, whilst both variants are Class III defects, G551D exhibits a much more severe gating defect than F312del [79, 84]. In

contrast to G551D and F508del [79, 94], which primarily reduce the opening rate, the R117H variant decreases P_o by shortening the burst durations and prolonging intraburst and interburst closures [82, 83, 85]. This is by no means an exhaustive list of cross-class variants and intraclass variability, but hopefully presents the challenge of assigning binary classes to these multi-mechanism defects.

Correctly assigning variants to their respective classes is essential for drug development. Correcting either the folding or gating defect of F508del in isolation is insufficient to fully restore wildtype function [7, 98], whereas using a combination of modulators which act on different mechanisms of CFTR dysfunction improves treatment efficacy [99]. Therefore, an updated classification system allowing for combinations of the previously defined six classes has been proposed [100]. This updated system allows for mechanistic subdivisions for certain variants, and this increased detail in classifying CFTR variants paves the way for personalized therapies for CF based on an individual's specific variant class fingerprint, termed "theratyping" [100-102].

Whilst the structural mechanism of dysfunction caused by a particular CFTR variant can largely explain the behaviour of the protein *in vitro*, it is important to remember that disease severity in CF patients can also be influenced by other external factors, including modifier genes, the environment, and socioeconomic status [103-105]. Moreover, where lung damage has already occurred, correcting the underlying defects in CFTR does not reverse the existing lung damage [106].

Section 5. Rectification of CFTR dysfunction with CFTR modulators

CFTR Potentiators

Potentiators are a group of small molecules that enhance chloride flux through CFTR by increasing its P_o. Patients with Class III (e.g. G551D) or Class IV (e.g. R117H) mutations benefit from CFTR potentiator monotherapy (ivacaftor) [7, 107], and combinational therapy of ivacaftor plus correctors is now a gold standard for patients carrying at least one allele of F508del [8].

Long before the discovery of ivacaftor, pioneering studies using small molecules to modulate CFTR gating demonstrated that multiple steps in CFTR gating may be targeted to enhance CFTR activity. The most straightforward strategy is to increase intracellular cAMP, which activates PKA-dependent phosphorylation of CFTR. This idea was exemplified by IBMX (3-isobutyl-1-methylxanthine), a compound that indirectly elevates cAMP level by inhibiting phosphodiesterase [108, 109]. Since opening of CFTR is mainly governed by ATP-induced NBD dimerization, another apparent strategy to increase P_o is to stabilize the

dimeric NBDs with high-affinity hydrolysable ATP analogs. Three such chemically modified compounds were developed, including N^6-(2-phenylethyl)-ATP (P-ATP), 2′-deoxy-ATP (2′-dATP), and N^6-(2-phenylethyl)-2′-deoxy-ATP (P-dATP). As expected, all of them potentiate CFTR activity [110-112]. The horizontal stacking between the additional benzene ring of P-ATP and the aromatic side chains of residues in the ATP-binding site (e.g., Y1219) accounts for its high-affinity, whereas the underlying structural mechanism for potentiation by 2′-dATP was unclear. Despite these encouraging proof-of-concept studies, clinical utility of IBMX and ATP analogs is limited, and they are now mostly used as tools to study CFTR function in the lab.

Meanwhile, numerous small molecules were reported to potentiate CFTR via distinct mechanisms. Genistein, a plant-derived isoflavone, was the first potentiator shown to directly act on CFTR gating [113-116]. Curcumin, another natural compound that increases the P_o of both wildtype CFTR and mutant channels with defective ATP-dependent gating (e.g., G551D- and NBD2del-CFTR), exhibits additive effects with genistein [117, 118]. Interestingly, a chloride channel blocker NPPB (5-nitro-2-(3-phenylpropyl-amino) benzoic acid) was found to work as a dual-acting modulator, which blocks the chloride current but potentiates CFTR activity simultaneously [119, 120]. Along this line, 9-anthracene carboxylic acid (9-AC) also acts as a voltage-dependent pore blocker and voltage-independent potentiator presumably through two different binding sites [121].

At the turn of the century, adoption of high throughput drug screening further accelerated the discovery of potential chemical scaffolds for CFTR potentiators, including phenylglycine (e.g., PG-01) [122], sulfonamide (e.g., SF-01) [122], and tetrahydrobenzothiophene (e.g., CFTR potentiator P5) [123]. Moreover, dual-acting compound such as VRT-532 with both potentiator and corrector activities was discovered [124], creating the possibility for one drug to target different functional defects in CFTR mutants. Although the above compounds were not used clinically, they contribute to an increasingly thorough understanding of how CFTR's function can be modulated.

The first landmark discovery of small molecules of clinical applicability is with ivacaftor (VX-770) [7]. In patients with G551D mutation, ivacaftor improved predicted FEV_1 (Forced Expiratory Volume in One second) by 10.5% compared to the placebo, markedly decreased sweat chloride concentration by ~50 $mmolL^{-1}$, and reduced the frequency of pulmonary exacerbations [125]. These robust beneficial clinical outcomes easily won the FDA's approval for ivacaftor monotherapy for people carrying Class III or Class IV (R117H) mutations. Ivacaftor was also used later in combination with correctors for patients with F508del mutation.

In vitro mechanistic studies suggest that unlike IBMX, phosphorylation is not required for ivacaftor's potentiation, as channels lacking the entire R domain are still responsive to ivacaftor [126]. In addition to potentiation on ATP-dependent gating on wildtype CFTR, ivacaftor also prolongs non-hydrolytic closure of hydrolysis-deficient mutants and promotes CFTR activity in the absence of ATP [126]. Moreover, G551D- and NBD2del-CFTR respond to ivacaftor, indicating that NBD dimerization is not essential for its action [80, 127]. Given the hydrophobic nature of ivacaftor and its effectiveness from both extracellular and intracellular applications, it was postulated that the target site for ivacaftor resides in the TMDs. Since ivacaftor works additively with nitrate, which potentiates CFTR activity in a manner identical to ivacaftor but most likely binds to the TMD-water interface as a hydrophilic anion, the binding site for the hydrophobic ivacaftor was proposed to lie in the TMD-lipid interface [127]. Indeed, a binding pocket for ivacaftor mainly comprised of hydrophobic amino acids at the TMD-lipid interface was identified by cryo-EM (**Figure 2**, **A-C**) [18]. The interactions between each amino acid residue and ivacaftor were also verified by examining the mutational effect on the sensitivity of the channel to ivacaftor and GLPG-1837, which despite significant structural differences binds to the same spot as ivacaftor [20, 128]. For instance, the cryo-EM structure shows that residue Y304 stabilizes the overall structure of the binding site by forming a hydrogen bond with the neighboring TM8. Mouse CFTR, which instead has a phenylalanine at the 304th residue, is irresponsive to ivacaftor. Replacing mouse F304 with tyrosine restores the effect of ivacaftor, and substitution of the human Y304 with phenylalanine significantly reduces the affinity of ivacaftor. Meanwhile, the possibility of multiple binding sites for ivacaftor continues to be explored. For instance, Csanady and Torocsik (2019) argued that the kinetics of activation/deactivation time course of ivacaftor are most compatible with two drug binding sites in the TMDs [129]. Using photoactive probe and molecular dynamic simulation, Laselva et al., (2021) determined an additional binding pocket at the intracellular loop 4 (ICL4) for ivacaftor [130].

Undoubtedly, the identification of ivacaftor's binding site makes a monumental stride toward the goal of structure-based drug design. However, thermodynamic analysis on gating modulation suggests that the efficacy of a potentiator is determined by its different binding affinities between the closed and open channels [128]. The implication is that to optimize the effect of a CFTR potentiator, one needs to compare the binding modes of the drug on both closed and open channel conformation, the latter of which remains unsolved. Moreover, as GLPG1837, which is more efficacious but less potent than ivacaftor, shares the same binding site with ivacaftor and most residues contributing to ivacaftor's binding also interact with GLPG1837, detailed comparison between multiple potentiators binding modes on the same site will provide insights into how manipulation on their chemical structures can achieve better potency and efficacy.

The triple combination therapy introduced a novel CFTR corrector elexacaftor (VX-445), which is later discovered to be a dual-acting compound with potentiator activity. In human nasal and bronchial epithelial cell cultures, acute addition of elexacaftor increases the activity of wildtype, F508del and gating mutants of CFTR including G551D and G1244E [131-133]. The EC_{50} of elexacaftor's potentiation is ~1 nM [132, 133], a hundred-fold lower than its EC_{50} for the corrector activity [134], suggesting a distinct binding site for its potentiating effect. More importantly, elexacaftor exhibits synergy with ivacaftor on G551D-CFTR, as the effectiveness of the individual potentiator is promoted by the presence of the other potentiator [132, 133]. Of note, elexacaftor also synergistically potentiates R117H-CFTR with ivacaftor, implying an even broader application in CF therapies [132].

The pharmacological synergism of elexacaftor and ivacaftor indicates a distinct mechanism of action and binding site for elexacaftor as a potentiator. Elexacaftor does not alter the intracellular cAMP level [132], but the potentiator activity of elexacaftor seems to be correlated with the phosphorylation level of CFTR. Laselva et al. (2021) reported in a pilot study that acute addition of elexacaftor enhances the response of F508del-CFTR to forskolin, but the potentiating effect of elexacaftor is diminished if the channels were already activated by 10 μM forskolin [131], contradicting other two reports [132, 133]. Exactly how elexacaftor works remains unclear. Nevertheless, since ivacaftor only partially restores the function of several gating mutants, including G551D, the combination of ivacaftor and elexacaftor presents a promising opportunity for new CF therapies in patients carrying Class III mutations.

CFTR Correctors

CFTR correctors aid protein folding, processing, delivery to and stability on the plasma membrane. Correctors fall into two main classes: those acting as molecular chaperones to directly engage the CFTR protein [135, 136], and those which indirectly aid CFTR folding by modulating proteostasis [137-139]. We will focus on the former as direct modulators of CFTR.

Although CFTR correctors have so far garnered more success than potentiators, with three corrector compounds licensed in the clinic at the time of writing (apps.cff.org/trials/pipeline/), the molecular mechanism of corrector action is not as well understood compared to that of potentiators. Moreover, correctors alone are unable to fully rescue F508del-CFTR [98] and their efficacy in improving the clinical phenotype is limited [140, 141]. A more thorough understanding of the mechanism of action of CFTR correctors may aid the development of more efficacious compounds.

Most studies support a direct interaction between the corrector and CFTR. A series of correctors including corr-4a were shown to inhibit cysteine cross-linking between CFTR TMDs [136], whilst NMR and thermal stability data on isolated NBD1 indicated conformational shifts upon binding of the corrector CFFT-001 [142]. Indeed, putative binding sites for lumacaftor, the first corrector to be licensed for treatment of CF, have been defined by both *in silico* computational methods [143] and structural biology [19, 144].

Based on their putative binding sites and their interaction fingerprint with other corrector compounds, correctors have been classified into three categories [134, 145]. Type I correctors were proposed to bind to and stabilise the NBD1-TMD1 or NBD1-TMD2 interface, but cryo-EM structures of CFTR in complex with type I correctors demonstrated a common binding pocket within the TMD1 [19, 134, 146]. Type II correctors are thought to act upon NBD2 and its interfaces with other parts of CFTR, and include experimental compounds such as corr-4a [147], but no type II correctors are yet licensed for clinical use. Finally, Type III correctors may directly stabilise NBD1 [134]. Elexacaftor, part of the recently approved combination therapy elexacaftor-tezacaftor-ivacaftor is designated a type III corrector [134].

A definitive mechanism of action for CFTR correctors remains elusive. This is partly due to a lack of consensus on the location of a binding site on CFTR. The most intensively studied compound in this regard is the type I corrector lumacaftor. Consistent with experiments on domain fragments showing an interaction with TMD1 [148-150] and NMR data showing a stabilisation of the NBD1-TMD1 interface [144], molecular docking studies identified potential binding pockets for lumacaftor at different sites in this region. *He et. al.* used a homology model of CFTR to dock lumacaftor to the hydrophobic groove between the helical loop of ICL4 and NBD1, close to F508 [151]. Indeed, this pocket was hypothesised as a hotspot for interdomain stability and a series of novel corrector compounds were identified by an *in silico* screen against this site [152]. However, more recently, a study combining molecular modelling and site-directed mutagenesis uncovered two alternative binding sites for lumacaftor proximal to the lasso motif; the first comprised residues from the “elbow” region of the lasso motif, TM1 and TM3, whereas the second site was further towards the extracellular side of CFTR and included TM3 and TM4 [143]. These binding site disparities were ultimately settled by the cryo-EM structure of the CFTR/type I corrector complexes, showing that lumacaftor and tezacaftor bind to the TM1 elbow pocket to stabilize the TMD1 in protein folding (**Figure 2, D-F**) [19].

Due to their ability to synergise with type I correctors [134], type II and III correctors are

expected to bind to alternative sites on CFTR. Corr-4a, a type II corrector, is proposed to bind to TMD2 [148], whilst elexacaftor (type III) is able to bind isolated NBD1 [134]. Baatallah et al (2021) proposed that elexacaftor, as a corrector, has two potential binding sites in the TMD1 and NBD1, the latter being shared with lumacaftor [143]. A definitive answer to the binding site(s) for elexacaftor still awaits structural evidence. As corrector monotherapy has limited efficacy in fully rescuing CFTR dysfunction [140, 141], nowadays combining multiple correctors, which potentially bind at different sites and hence utilise different modes of action, appears to be the gold standard to correct variants exhibiting multiple defects in biogenesis [153-155].

Beyond structural and functional studies of CFTR correctors, clinical trials are invaluable in providing real-world data on the efficacy of correctors and corrector combinations in CF patients. The first clinical trial of lumacaftor in F508del homozygous individuals showed minimal improvement in sweat chloride and lung function, indicating that lumacaftor monotherapy was insufficient to restore CFTR function to the point of clinical benefit [140]. However, when used as a combination therapy with the potentiator ivacaftor, a much greater clinical effect was seen, with patients experiencing an increased FEV_1 and reduced exacerbation frequency with long term therapy [156, 157]. Similarly, the corrector ABBV-2222 had limited efficacy when given alone [141], but further trials are underway to evaluate the efficacy of ABBV-2222 in combination with a CFTR potentiator (trial number NCT03969888). Recently, clinical trials of lumacaftor/ivacaftor combination therapy in children firstly aged 6 - 11 years old, and subsequently in children under 5, have shown good safety and efficacy profiles and suggest that early intervention with modulator therapies may be able to modify the course of disease into adulthood [158, 159]. The triple combination therapy has been proved efficacious in CF patients over the age of 12 [9] and children aged 6 to 11 (trial number NCT03691779), showing significant improvements in lung function, sweat chloride and quality of life. Trials in children aged two to five are currently underway (trial number NCT04537793).

Despite the success of the triple combination therapy, treatments for nonsense and many rare mutations that do not benefit from CFTR modulators are still inadequate. However, strategies that override the premature stop codons as well as replacing or repairing the messenger RNA or DNA template are under development, including RNA therapy, antisense oligonucleotide therapy, gene editing and gene therapy. As this review focuses on CFTR modulators, we recommend the following reviews to readers who are interested in other treatments for CF: Cooney et al. [160], Hodges, Conlon [161], Pranke et al. [162], Fajac, Sermet [163].

Section 6. Conclusion and future perspective

Decades of rigorous research on CF and CFTR have paved the way for recent successes in developing personalized medicine for patients with CF. Moreover, researchers are just beginning to materialize the potential of structure-based drug design using CFTR as the target. When the interaction between the drug and its binding partners is revealed down to an atomic level, optimization of efficacy and affinity by modifying the chemical structure of the drug becomes feasible. It is anticipated that precision drug design, in addition to high-throughput drug screening, would play a major role in the development of next generation CFTR modulators. The discovery of dual-acting molecules also raises the possibility of designing a single drug that rectifies multiple function defects, which will simplify the therapeutic regimen.

Although CFTR modulators have been used in patients for over a decade and their effectiveness on pulmonary functions are well-documented, the long-term benefits on patients, especially the extrapulmonary effects, are harder to evaluate [164]. On the other hand, evidence for CFTR's role in other airway diseases suggests a therapeutic potential of CFTR modulators for diseases such as chronic obstructive pulmonary disease and chronic bronchitis [165]. More research integrating structural, functional, and clinical studies on CF and CFTR is still much needed to accelerate the progress toward a cure for those afflicted by CF.

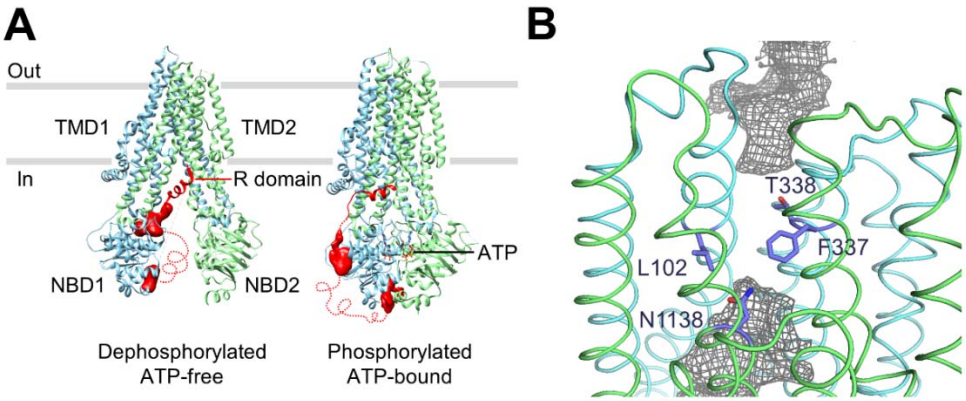

Figure 1. Cryo-EM structures of human CFTR. (A) Atomic structures of the dephosphorylated, ATP-free (PDB: 5UAK) and phosphorylated ATP-bound (PDB: 6MSM) human CFTR. **(B)** Constriction region of the pore. The gray mesh is the chloride-accessible space defined by a probe with the size of a dehydrated chloride (1.8Å). Transmembrane helices in TMD1 and TMD2 are colored in cyan and green respectively.

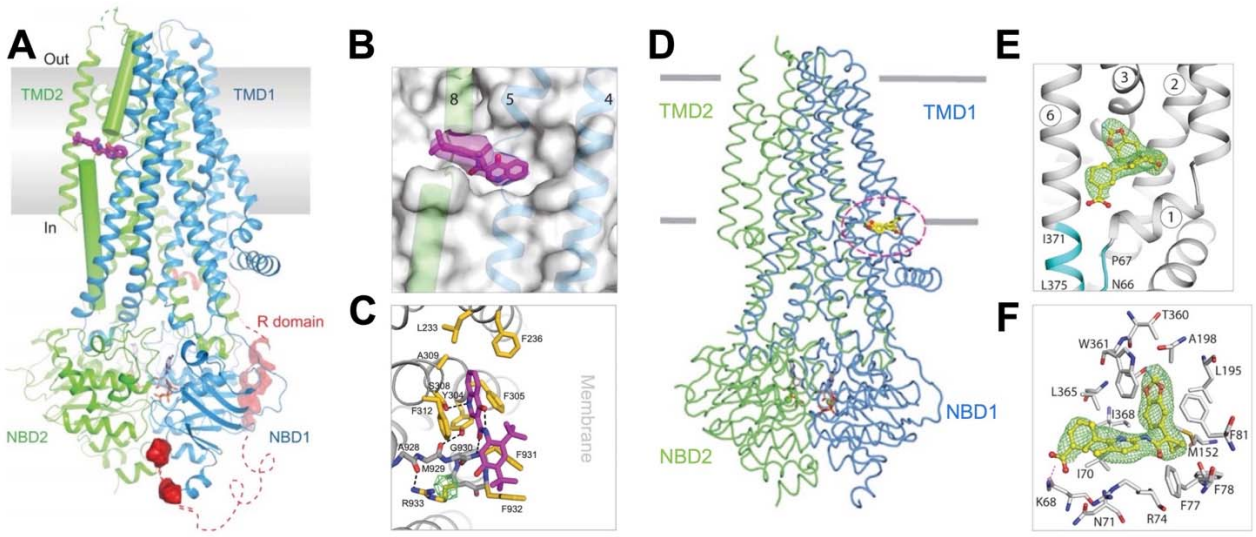

Figure 2. Cryo-EM structure of CFTR modulators ivacaftor and lumacaftor bound to CFTR. (**A**) The structure of phosphorylated, ATP-bound CFTR with ivacaftor (magenta). (**B**) An expanded view of ivacaftor binding site. Transmembrane segment (TM) 4, 5, and 8 are indicated. (**C**) Amino acid residues of the ivacaftor binding site. Residues within van der Waals distances are shown in yellow, and hydrogen bonds are indicated with dashed lines. An unknown density between R933 and ivacaftor is shown as green mesh. (**D**) The structure of phosphorylated, ATP-bound CFTR in complex with lumacaftor (yellow). (**E**) Lumacaftor binding site formed by TM1, 2, 3, and 6. (**F**) Detailed architecture of the lumacaftor binding site. Residues within 4.5 Å of lumacaftor are shown as gray sticks. A salt bridge between K68 and lumacaftor is indicated by a magenta dashed line. (Adopted from Liu et al., 2019 and Fiedorczuk and Chen 2021 with permission).

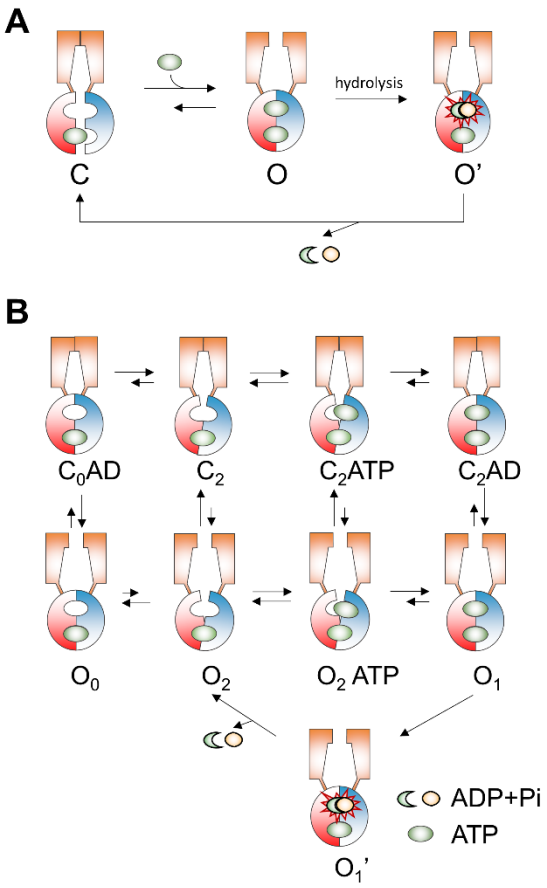

Figure 3. Strict-coupling and energetic-coupling models for CFTR gating. (**A and B**) Cartoon representation of the strict-coupling (A) and energetic-coupling (B) gating scheme. Orange trapezoids: TMDs. Red and blue hemispheres: NBD1 and NBD2, respectively. Green ovals: ATP. C: closed states. O: open states.

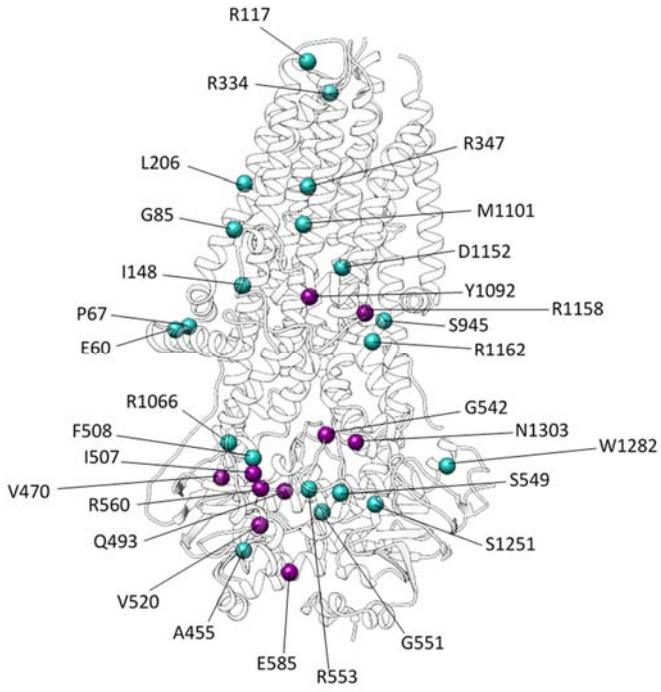

Figure 4. Location of the most prevalent single point CFTR variants mapped onto the CFTR structure. The structure of the phosphorylated and ATP-bound human CFTR (PBD: 6MSM) is shown in light grey, with the positions of the 30 most common single amino acid variants depicted as spheres. The variants approved for treatment with the triple therapy ivacaftor-tezacaftor-elexacaftor (Trikafta) are shown in cyan, and variants not yet approved for modulator treatment in purple.

	Description	Examples	Locations	Treatments
Class I	Defective protein synthesis	G542X R553X W1282X		Read-through agents, NMD inhibitors; ELX-02[§]
Class II	Defective folding and transport	P67L R117H A455E F508del R1070W N1303K		Correctors; lumacaftor*, tezacaftor*, elexacaftor*, VX-121[§], ABBV-2222[§]
Class III	Defective regulation and gating	P67L R117H F312del F508del G551D Q1412X		Potentiators; ivacaftor*, ABBV-3067[§], ABBV-191[§]
Class IV	Reduced anion conductance	R117H R334W R347P		
Class V	Reduced protein abundance	A445E P574H		Amplifiers; nesolicaftor[§]
Class VI	Increased turnover	F508del A561E N1303K Q1412X		Stabilizers; cavosonstat (discontinued)
*approved; [§]in clinical trials				

Table 1. Classes of CF-causing CFTR variants. The CFTR mutation classification system, including example variants, their location on the CFTR structure and treatment options.

References

1. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science. 1989;245(4922):1066-73. doi: 10.1126/science.2475911.

2. Bear CE, Li CH, Kartner N, Bridges RJ, Jensen TJ, Ramjeesingh M, et al. Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). Cell. 1992;68(4):809-18. doi: 10.1016/0092-8674(92)90155-6.

3. Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. N Engl J Med. 2005;352(19):1992-2001. doi: 10.1056/NEJMra043184.

4. O'Sullivan BP, Freedman SD. Cystic fibrosis. Lancet. 2009;373(9678):1891-904. doi: 10.1016/s0140-6736(09)60327-5.

5. Moran A, Dunitz J, Nathan B, Saeed A, Holme B, Thomas W. Cystic fibrosis-related diabetes: current trends in prevalence, incidence, and mortality. Diabetes Care. 2009;32(9):1626-31. doi: 10.2337/dc09-0586.

6. Neglia JP, FitzSimmons SC, Maisonneuve P, Schöni MH, Schöni-Affolter F, Corey M, et al. The Risk of Cancer among Patients with Cystic Fibrosis. New England Journal of Medicine. 1995;332(8):494-9. doi: 10.1056/nejm199502233320803.

7. Van Goor F, Hadida S, Grootenhuis PD, Burton B, Cao D, Neuberger T, et al. Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. Proc Natl Acad Sci U S A. 2009;106(44):18825-30. doi: 10.1073/pnas.0904709106.

8. Keating D, Marigowda G, Burr L, Daines C, Mall MA, McKone EF, et al. VX-445–Tezacaftor–Ivacaftor in Patients with Cystic Fibrosis and One or Two Phe508del Alleles. New England Journal of Medicine. 2018;379(17):1612-20. doi: 10.1056/NEJMoa1807120.

9. Barry PJ, Mall MA, Alvarez A, Colombo C, de Winter-de Groot KM, Fajac I, et al. Triple Therapy for Cystic Fibrosis Phe508del-Gating and -Residual Function Genotypes. The New England journal of medicine. 2021;385(9):815-25. doi: 10.1056/NEJMoa2100665.

10. Csanády L, Vergani P, Gadsby DC. Structure, Gating, and Regulation of the CFTR Anion Channel. Physiological Reviews. 2018;99(1):707-38. doi: 10.1152/physrev.00007.2018.

11. Hwang TC, Yeh JT, Zhang J, Yu YC, Yeh HI, Destefano S. Structural mechanisms of CFTR function and dysfunction. J Gen Physiol. 2018;150(4):539-70. doi: 10.1085/jgp.201711946.

12. Pranke IM, Sermet-Gaudelus I. Biosynthesis of cystic fibrosis transmembrane conductance regulator. Int J Biochem Cell Biol. 2014;52:26-38. doi: 10.1016/j.biocel.2014.03.020.

13. Al Salmani MK, Sondo E, Balut C, Sheppard DN, Singh AK, Pedemonte N. Molecular Physiology and Pharmacology of the Cystic Fibrosis Transmembrane Conductance Regulator. In: Hamilton KL, Devor DC (eds) Studies of Epithelial Transporters and Ion Channels Physiology in Health and Disease Springer, Cham. 2020. doi: https://doi.org/10.1007/978-3-030-55454-5_16.

14. Zhang Z, Chen J. Atomic Structure of the Cystic Fibrosis Transmembrane Conductance Regulator. Cell. 2016;167(6):1586-97.e9. doi: 10.1016/j.cell.2016.11.014.

15. Zhang Z, Liu F, Chen J. Conformational Changes of CFTR upon Phosphorylation and ATP Binding. Cell. 2017;170(3):483-91.e8. doi: 10.1016/j.cell.2017.06.041.
16. Liu F, Zhang Z, Csanady L, Gadsby DC, Chen J. Molecular Structure of the Human CFTR Ion Channel. Cell. 2017;169(1):85-95.e8. doi: 10.1016/j.cell.2017.02.024.
17. Zhang Z, Liu F, Chen J. Molecular structure of the ATP-bound, phosphorylated human CFTR. Proceedings of the National Academy of Sciences. 2018;115(50):12757. doi: 10.1073/pnas.1815287115.
18. Liu F, Zhang Z, Levit A, Levring J, Touhara KK, Shoichet BK, et al. Structural identification of a hotspot on CFTR for potentiation. Science. 2019;364(6446):1184-8. doi: 10.1126/science.aaw7611.
19. Fiedorczuk K, Chen J. Mechanism of CFTR correction by type I folding correctors. Cell. 2022;185(1):158-68.e11. doi: 10.1016/j.cell.2021.12.009.
20. Yeh H-I, Qiu L, Sohma Y, Conrath K, Zou X, Hwang T-C. Identifying the molecular target sites for CFTR potentiators GLPG1837 and VX-770. The Journal of General Physiology. 2019;151(7):912. doi: 10.1085/jgp.201912360.
21. Kleizen B, van Vlijmen T, de Jonge HR, Braakman I. Folding of CFTR is predominantly cotranslational. Mol Cell. 2005;20(2):277-87. doi: 10.1016/j.molcel.2005.09.007.
22. Du K, Sharma M, Lukacs GL. The DeltaF508 cystic fibrosis mutation impairs domain-domain interactions and arrests post-translational folding of CFTR. Nat Struct Mol Biol. 2005;12(1):17-25. doi: 10.1038/nsmb882.
23. Kim SJ, Skach WR. Mechanisms of CFTR Folding at the Endoplasmic Reticulum. Front Pharmacol. 2012;3:201. doi: 10.3389/fphar.2012.00201.
24. Lu Y, Xiong X, Helm A, Kimani K, Bragin A, Skach WR. Co- and posttranslational translocation mechanisms direct cystic fibrosis transmembrane conductance regulator N terminus transmembrane assembly. J Biol Chem. 1998;273(1):568-76. doi: 10.1074/jbc.273.1.568.
25. Rosser MF, Grove DE, Chen L, Cyr DM. Assembly and misassembly of cystic fibrosis transmembrane conductance regulator: folding defects caused by deletion of F508 occur before and after the calnexin-dependent association of membrane spanning domain (MSD) 1 and MSD2. Mol Biol Cell. 2008;19(11):4570-9. doi: 10.1091/mbc.E08-04-0357.
26. Loo MA, Jensen TJ, Cui L, Hou Y, Chang XB, Riordan JR. Perturbation of Hsp90 interaction with nascent CFTR prevents its maturation and accelerates its degradation by the proteasome. EMBO J. 1998;17(23):6879-87. doi: 10.1093/emboj/17.23.6879.
27. Meacham GC, Lu Z, King S, Sorscher E, Tousson A, Cyr DM. The Hdj-2/Hsc70 chaperone pair facilitates early steps in CFTR biogenesis. EMBO J. 1999;18(6):1492-505. doi: 10.1093/emboj/18.6.1492.
28. Wang X, Matteson J, An Y, Moyer B, Yoo JS, Bannykh S, et al. COPII-dependent export of cystic fibrosis transmembrane conductance regulator from the ER uses a di-acidic exit code. J Cell Biol. 2004;167(1):65-74. doi: 10.1083/jcb.200401035.
29. Pind S, Riordan JR, Williams DB. Participation of the endoplasmic reticulum chaperone calnexin

(p88, IP90) in the biogenesis of the cystic fibrosis transmembrane conductance regulator. J Biol Chem. 1994;269(17):12784-8.
30. Yang Y, Janich S, Cohn JA, Wilson JM. The common variant of cystic fibrosis transmembrane conductance regulator is recognized by hsp70 and degraded in a pre-Golgi nonlysosomal compartment. Proc Natl Acad Sci U S A. 1993;90(20):9480-4. doi: 10.1073/pnas.90.20.9480.
31. Du K, Lukacs GL. Cooperative assembly and misfolding of CFTR domains in vivo. Mol Biol Cell. 2009;20(7):1903-15. doi: 10.1091/mbc.E08-09-0950.
32. Lukacs GL, Chang XB, Bear C, Kartner N, Mohamed A, Riordan JR, et al. The delta F508 mutation decreases the stability of cystic fibrosis transmembrane conductance regulator in the plasma membrane. Determination of functional half-lives on transfected cells. J Biol Chem. 1993;268(29):21592-8.
33. Ward CL, Kopito RR. Intracellular turnover of cystic fibrosis transmembrane conductance regulator. Inefficient processing and rapid degradation of wild-type and mutant proteins. J Biol Chem. 1994;269(41):25710-8.
34. Quinton PM. Chloride impermeability in cystic fibrosis. Nature. 1983;301(5899):421-2. doi: 10.1038/301421a0.
35. Anderson MP, Gregory RJ, Thompson S, Souza DW, Paul S, Mulligan RC, et al. Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. Science. 1991;253(5016):202-5. doi: 10.1126/science.1712984.
36. Berger HA, Anderson MP, Gregory RJ, Thompson S, Howard PW, Maurer RA, et al. Identification and regulation of the cystic fibrosis transmembrane conductance regulator-generated chloride channel. J Clin Invest. 1991;88(4):1422-31. doi: 10.1172/jci115450.
37. Poulsen JH, Fischer H, Illek B, Machen TE. Bicarbonate conductance and pH regulatory capability of cystic fibrosis transmembrane conductance regulator. Proc Natl Acad Sci U S A. 1994;91(12):5340-4. doi: 10.1073/pnas.91.12.5340.
38. Linsdell P, Tabcharani JA, Rommens JM, Hou Y-X, Chang X-B, Tsui L-C, et al. Permeability of Wild-Type and Mutant Cystic Fibrosis Transmembrane Conductance Regulator Chloride Channels to Polyatomic Anions. Journal of General Physiology. 1997;110(4):355-64. doi: 10.1085/jgp.110.4.355.
39. Bai Y, Li M, Hwang TC. Dual roles of the sixth transmembrane segment of the CFTR chloride channel in gating and permeation. J Gen Physiol. 2010;136(3):293-309. doi: 10.1085/jgp.201010480.
40. Gao X, Bai Y, Hwang TC. Cysteine scanning of CFTR's first transmembrane segment reveals its plausible roles in gating and permeation. Biophys J. 2013;104(4):786-97. doi: 10.1016/j.bpj.2012.12.048.
41. Zhang J, Hwang TC. The Fifth Transmembrane Segment of Cystic Fibrosis Transmembrane Conductance Regulator Contributes to Its Anion Permeation Pathway. Biochemistry. 2015;54(24):3839-50. doi: 10.1021/acs.biochem.5b00427.
42. Zhou J-J, Li M-S, Qi J, Linsdell P. Regulation of conductance by the number of fixed positive

charges in the intracellular vestibule of the CFTR chloride channel pore. Journal of General Physiology. 2010;135(3):229-45. doi: 10.1085/jgp.200910327.
43. Wang W, El Hiani Y, Linsdell P. Alignment of transmembrane regions in the cystic fibrosis transmembrane conductance regulator chloride channel pore. Journal of General Physiology. 2011;138(2):165-78. doi: 10.1085/jgp.201110605.
44. El Hiani Y, Linsdell P. Changes in accessibility of cytoplasmic substances to the pore associated with activation of the cystic fibrosis transmembrane conductance regulator chloride channel. J Biol Chem. 2010;285(42):32126-40. doi: 10.1074/jbc.M110.113332.
45. Qian F, El Hiani Y, Linsdell P. Functional arrangement of the 12th transmembrane region in the CFTR chloride channel pore based on functional investigation of a cysteine-less CFTR variant. Pflugers Arch. 2011;462(4):559-71. doi: 10.1007/s00424-011-0998-2.
46. Beck EJ, Yang Y, Yaemsiri S, Raghuram V. Conformational Changes in a Pore-lining Helix Coupled to Cystic Fibrosis Transmembrane Conductance Regulator Channel Gating*. Journal of Biological Chemistry. 2008;283(8):4957-66. doi: https://doi.org/10.1074/jbc.M702235200.
47. El Hiani Y, Linsdell P. Conformational changes opening and closing the CFTR chloride channel: Insights from cysteine scanning mutagenesis. Biochemistry and Cell Biology. 2014;92(6):481-8. doi: 10.1139/bcb-2014-0038 %M 25367045.
48. Anderson MP, Berger HA, Rich DP, Gregory RJ, Smith AE, Welsh MJ. Nucleoside triphosphates are required to open the CFTR chloride channel. Cell. 1991;67(4):775-84. doi: 10.1016/0092-8674(91)90072-7.
49. Cheng SH, Rich DP, Marshall J, Gregory RJ, Welsh MJ, Smith AE. Phosphorylation of the R domain by cAMP-dependent protein kinase regulates the CFTR chloride channel. Cell. 1991;66(5):1027-36. doi: 10.1016/0092-8674(91)90446-6.
50. Picciotto MR, Cohn JA, Bertuzzi G, Greengard P, Nairn AC. Phosphorylation of the cystic fibrosis transmembrane conductance regulator. J Biol Chem. 1992;267(18):12742-52.
51. Seibert FS, Tabcharani JA, Chang XB, Dulhanty AM, Mathews C, Hanrahan JW, et al. cAMP-dependent protein kinase-mediated phosphorylation of cystic fibrosis transmembrane conductance regulator residue Ser-753 and its role in channel activation. J Biol Chem. 1995;270(5):2158-62. doi: 10.1074/jbc.270.5.2158.
52. Hegedus T, Aleksandrov A, Mengos A, Cui L, Jensen TJ, Riordan JR. Role of individual R domain phosphorylation sites in CFTR regulation by protein kinase A. Biochim Biophys Acta. 2009;1788(6):1341-9. doi: 10.1016/j.bbamem.2009.03.015.
53. Vais H, Zhang R, Reenstra WW. Dibasic phosphorylation sites in the R domain of CFTR have stimulatory and inhibitory effects on channel activation. American Journal of Physiology-Cell Physiology. 2004;287(3):C737-C45. doi: 10.1152/ajpcell.00504.2003.
54. Csanády L, Seto-Young D, Chan KW, Cenciarelli C, Angel BB, Qin J, et al. Preferential phosphorylation of R-domain Serine 768 dampens activation of CFTR channels by PKA. J Gen Physiol. 2005;125(2):171-86. doi: 10.1085/jgp.200409076.

55. Bompadre SG, Ai T, Cho JH, Wang X, Sohma Y, Li M, et al. CFTR gating I: Characterization of the ATP-dependent gating of a phosphorylation-independent CFTR channel (DeltaR-CFTR). J Gen Physiol. 2005;125(4):361-75. doi: 10.1085/jgp.200409227.
56. Csanády L, Chan KW, Seto-Young D, Kopsco DC, Nairn AC, Gadsby DC. Severed channels probe regulation of gating of cystic fibrosis transmembrane conductance regulator by its cytoplasmic domains. J Gen Physiol. 2000;116(3):477-500. doi: 10.1085/jgp.116.3.477.
57. Mihályi C, Iordanov I, Töröcsik B, Csanády L. Simple binding of protein kinase A prior to phosphorylation allows CFTR anion channels to be opened by nucleotides. Proc Natl Acad Sci U S A. 2020;117(35):21740-6. doi: 10.1073/pnas.2007910117.
58. Vergani P, Nairn AC, Gadsby DC. On the mechanism of MgATP-dependent gating of CFTR Cl- channels. J Gen Physiol. 2003;121(1):17-36.
59. Vergani P, Lockless SW, Nairn AC, Gadsby DC. CFTR channel opening by ATP-driven tight dimerization of its nucleotide-binding domains. Nature. 2005;433(7028):876-80. doi: 10.1038/nature03313.
60. Bompadre SG, Cho JH, Wang X, Zou X, Sohma Y, Li M, et al. CFTR gating II: Effects of nucleotide binding on the stability of open states. J Gen Physiol. 2005;125(4):377-94. doi: 10.1085/jgp.200409228.
61. Powe AC, Jr., Al-Nakkash L, Li M, Hwang TC. Mutation of Walker-A lysine 464 in cystic fibrosis transmembrane conductance regulator reveals functional interaction between its nucleotide-binding domains. J Physiol. 2002;539(Pt 2):333-46. doi: 10.1113/jphysiol.2001.013162.
62. Yeh HI, Yu YC, Kuo PL, Tsai CK, Huang HT, Hwang TC. Functional stability of CFTR depends on tight binding of ATP at its degenerate ATP-binding site. J Physiol. 2021;599(20):4625-42. doi: 10.1113/JP281933.
63. Csanady L, Vergani P, Gadsby DC. Strict coupling between CFTR's catalytic cycle and gating of its Cl- ion pore revealed by distributions of open channel burst durations. Proc Natl Acad Sci U S A. 2010;107(3):1241-6. doi: 10.1073/pnas.0911061107.
64. Jih KY, Hwang TC. Nonequilibrium gating of CFTR on an equilibrium theme. Physiology (Bethesda). 2012;27(6):351-61. doi: 10.1152/physiol.00026.2012.
65. Jih KY, Sohma Y, Hwang TC. Nonintegral stoichiometry in CFTR gating revealed by a pore-lining mutation. J Gen Physiol. 2012;140(4):347-59. doi: 10.1085/jgp.201210834.
66. Sosnay PR, Siklosi KR, Van Goor F, Kaniecki K, Yu H, Sharma N, et al. Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. Nat Genet. 2013;45(10):1160-7. doi: 10.1038/ng.2745.
67. Ostedgaard LS, Baldursson O, Vermeer DW, Welsh MJ, Robertson AD. A functional R domain from cystic fibrosis transmembrane conductance regulator is predominantly unstructured in solution. Proc Natl Acad Sci U S A. 2000;97(10):5657-62. doi: 10.1073/pnas.100588797.
68. De Boeck K, Amaral MD. Progress in therapies for cystic fibrosis. Lancet Respir Med. 2016;4(8):662-74. doi: 10.1016/s2213-2600(16)00023-0.

69. Haardt M, Benharouga M, Lechardeur D, Kartner N, Lukacs GL. C-terminal truncations destabilize the cystic fibrosis transmembrane conductance regulator without impairing its biogenesis. A novel class of mutation. J Biol Chem. 1999;274(31):21873-7. doi: 10.1074/jbc.274.31.21873.
70. Marson FAL, Bertuzzo CS, Ribeiro JD. Classification of CFTR mutation classes. Lancet Respir Med. 2016;4(8):e37-e8. doi: 10.1016/s2213-2600(16)30188-6.
71. Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell. 1993;73(7):1251-4. doi: 10.1016/0092-8674(93)90353-r.
72. Hamosh A, Rosenstein BJ, Cutting GR. CFTR nonsense mutations G542X and W1282X associated with severe reduction of CFTR mRNA in nasal epithelial cells. Hum Mol Genet. 1992;1(7):542-4. doi: 10.1093/hmg/1.7.542.
73. Sharma N, Evans TA, Pellicore MJ, Davis E, Aksit MA, McCague AF, et al. Capitalizing on the heterogeneous effects of CFTR nonsense and frameshift variants to inform therapeutic strategy for cystic fibrosis. PLoS Genet. 2018;14(11):e1007723. doi: 10.1371/journal.pgen.1007723.
74. Shoshani T, Augarten A, Gazit E, Bashan N, Yahav Y, Rivlin Y, et al. Association of a nonsense mutation (W1282X), the most common mutation in the Ashkenazi Jewish cystic fibrosis patients in Israel, with presentation of severe disease. Am J Hum Genet. 1992;50(1):222-8.
75. Yeh JT, Hwang TC. Positional effects of premature termination codons on the biochemical and biophysical properties of CFTR. J Physiol. 2020;598(3):517-41. doi: 10.1113/jp278418.
76. Cheng SH, Gregory RJ, Marshall J, Paul S, Souza DW, White GA, et al. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell. 1990;63(4):827-34. doi: 10.1016/0092-8674(90)90148-8.
77. Chong PA, Farber PJ, Vernon RM, Hudson RP, Mittermaier AK, Forman-Kay JD. Deletion of Phenylalanine 508 in the First Nucleotide-binding Domain of the Cystic Fibrosis Transmembrane Conductance Regulator Increases Conformational Exchange and Inhibits Dimerization. J Biol Chem. 2015;290(38):22862-78. doi: 10.1074/jbc.M115.641134.
78. Cai Z, Taddei A, Sheppard DN. Differential sensitivity of the cystic fibrosis (CF)-associated mutants G551D and G1349D to potentiators of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl- channel. J Biol Chem. 2006;281(4):1970-7. doi: 10.1074/jbc.M510576200.
79. Bompadre SG, Sohma Y, Li M, Hwang TC. G551D and G1349D, two CF-associated mutations in the signature sequences of CFTR, exhibit distinct gating defects. J Gen Physiol. 2007;129(4):285-98. doi: 10.1085/jgp.200609667.
80. Lin W-Y, Jih K-Y, Hwang T-C. A single amino acid substitution in CFTR converts ATP to an inhibitory ligand. The Journal of General Physiology. 2014;144(4):311-20. doi: 10.1085/jgp.201411247.
81. Lukacs GL, Verkman AS. CFTR: folding, misfolding and correcting the DeltaF508 conformational defect. Trends Mol Med. 2012;18(2):81-91. doi: 10.1016/j.molmed.2011.10.003.
82. Yu YC, Sohma Y, Hwang TC. On the mechanism of gating defects caused by the R117H mutation in cystic fibrosis transmembrane conductance regulator. J Physiol. 2016;594(12):3227-44. doi: 10.1113/jp271723.

83. Sorum B, Torocsik B, Csanady L. Asymmetry of movements in CFTR's two ATP sites during pore opening serves their distinct functions. Elife. 2017;6. doi: 10.7554/eLife.29013.
84. Raraigh KS, Paul KC, Goralski JL, Worthington EN, Faino AV, Sciortino S, et al. CFTR bearing variant p.Phe312del exhibits function inconsistent with phenotype and negligible response to ivacaftor. JCI Insight. 2022;7(6). doi: 10.1172/jci.insight.148841.
85. Simon MA, Csanady L. Molecular pathology of the R117H cystic fibrosis mutation is explained by loss of a hydrogen bond. Elife. 2021;10. doi: 10.7554/eLife.74693.
86. Sheppard DN, Rich DP, Ostedgaard LS, Gregory RJ, Smith AE, Welsh MJ. Mutations in CFTR associated with mild-disease-form Cl- channels with altered pore properties. Nature. 1993;362(6416):160-4. doi: 10.1038/362160a0.
87. Gong X, Linsdell P. Maximization of the rate of chloride conduction in the CFTR channel pore by ion-ion interactions. Arch Biochem Biophys. 2004;426(1):78-82. doi: 10.1016/j.abb.2004.03.033.
88. Sheppard DN, Ostedgaard LS, Winter MC, Welsh MJ. Mechanism of dysfunction of two nucleotide binding domain mutations in cystic fibrosis transmembrane conductance regulator that are associated with pancreatic sufficiency. EMBO J. 1995;14(5):876-83.
89. Gan KH, Veeze HJ, van den Ouweland AM, Halley DJ, Scheffer H, van der Hout A, et al. A cystic fibrosis mutation associated with mild lung disease. N Engl J Med. 1995;333(2):95-9. doi: 10.1056/nejm199507133330204.
90. Fulmer SB, Schwiebert EM, Morales MM, Guggino WB, Cutting GR. Two cystic fibrosis transmembrane conductance regulator mutations have different effects on both pulmonary phenotype and regulation of outwardly rectified chloride currents. Proc Natl Acad Sci U S A. 1995;92(15):6832-6. doi: 10.1073/pnas.92.15.6832.
91. Guggino WB, Stanton BA. New insights into cystic fibrosis: molecular switches that regulate CFTR. Nat Rev Mol Cell Biol. 2006;7(6):426-36. doi: 10.1038/nrm1949.
92. Duan Y, Sun Y, Zhang F, Zhang WK, Wang D, Wang Y, et al. Keratin K18 increases cystic fibrosis transmembrane conductance regulator (CFTR) surface expression by binding to its C-terminal hydrophobic patch. J Biol Chem. 2012;287(48):40547-59. doi: 10.1074/jbc.M112.403584.
93. Haggie PM, Stanton BA, Verkman AS. Increased diffusional mobility of CFTR at the plasma membrane after deletion of its C-terminal PDZ binding motif. J Biol Chem. 2004;279(7):5494-500. doi: 10.1074/jbc.M312445200.
94. Dalemans W, Barbry P, Champigny G, Jallat S, Dott K, Dreyer D, et al. Altered chloride ion channel kinetics associated with the delta F508 cystic fibrosis mutation. Nature. 1991;354(6354):526-8. doi: 10.1038/354526a0.
95. Heda GD, Tanwani M, Marino CR. The Delta F508 mutation shortens the biochemical half-life of plasma membrane CFTR in polarized epithelial cells. Am J Physiol Cell Physiol. 2001;280(1):C166-74. doi: 10.1152/ajpcell.2001.280.1.C166.
96. Sabusap CM, Wang W, McNicholas CM, Chung WJ, Fu L, Wen H, et al. Analysis of cystic fibrosis-associated P67L CFTR illustrates barriers to personalized therapeutics for orphan diseases. JCI

Insight. 2016;1(14). doi: 10.1172/jci.insight.86581.
97. Yeh JT, Yu YC, Hwang TC. Structural mechanisms for defective CFTR gating caused by the Q1412X mutation, a severe Class VI pathogenic mutation in cystic fibrosis. J Physiol. 2019;597(2):543-60. doi: 10.1113/jp277042.
98. Van Goor F, Hadida S, Grootenhuis PD, Burton B, Stack JH, Straley KS, et al. Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. Proc Natl Acad Sci U S A. 2011;108(46):18843-8. doi: 10.1073/pnas.1105787108.
99. Boyle MP, Bell SC, Konstan MW, McColley SA, Rowe SM, Rietschel E, et al. A CFTR corrector (lumacaftor) and a CFTR potentiator (ivacaftor) for treatment of patients with cystic fibrosis who have a phe508del CFTR mutation: a phase 2 randomised controlled trial. Lancet Respir Med. 2014;2(7):527-38. doi: 10.1016/S2213-2600(14)70132-8.
100. Veit G, Avramescu RG, Chiang AN, Houck SA, Cai Z, Peters KW, et al. From CFTR biology toward combinatorial pharmacotherapy: expanded classification of cystic fibrosis mutations. Mol Biol Cell. 2016;27(3):424-33. doi: 10.1091/mbc.E14-04-0935.
101. Cutting GR. Cystic fibrosis genetics: from molecular understanding to clinical application. Nat Rev Genet. 2015;16(1):45-56. doi: 10.1038/nrg3849.
102. Han ST, Rab A, Pellicore MJ, Davis EF, McCague AF, Evans TA, et al. Residual function of cystic fibrosis mutants predicts response to small molecule CFTR modulators. JCI Insight. 2018;3(14). doi: 10.1172/jci.insight.121159.
103. Szczesniak R, Rice JL, Brokamp C, Ryan P, Pestian T, Ni Y, et al. Influences of environmental exposures on individuals living with cystic fibrosis. Expert Rev Respir Med. 2020;14(7):737-48. doi: 10.1080/17476348.2020.1753507.
104. O'Neal WK, Knowles MR. Cystic Fibrosis Disease Modifiers: Complex Genetics Defines the Phenotypic Diversity in a Monogenic Disease. Annu Rev Genomics Hum Genet. 2018;19:201-22. doi: 10.1146/annurev-genom-083117-021329.
105. Paranjapye A, Ruffin M, Harris A, Corvol H. Genetic variation in CFTR and modifier loci may modulate cystic fibrosis disease severity. J Cyst Fibros. 2020;19 Suppl 1:S10-S4. doi: 10.1016/j.jcf.2019.11.001.
106. Duckers J, Lesher B, Thorat T, Lucas E, McGarry LJ, Chandarana K, et al. Real-World Outcomes of Ivacaftor Treatment in People with Cystic Fibrosis: A Systematic Review. J Clin Med. 2021;10(7). doi: 10.3390/jcm10071527.
107. Yu H, Burton B, Huang CJ, Worley J, Cao D, Johnson JP, Jr., et al. Ivacaftor potentiation of multiple CFTR channels with gating mutations. J Cyst Fibros. 2012;11(3):237-45. doi: 10.1016/j.jcf.2011.12.005.
108. Al-Nakkash L, Hwang TC. Activation of wild-type and deltaF508-CFTR by phosphodiesterase inhibitors through cAMP-dependent and -independent mechanisms. Pflugers Arch. 1999;437(4):553-61.
109. Schultz BD, Frizzell RA, Bridges RJ. Rescue of dysfunctional deltaF508-CFTR chloride channel

activity by IBMX. J Membr Biol. 1999;170(1):51-66. doi: 10.1007/s002329900537.
110. Bompadre SG, Li M, Hwang TC. Mechanism of G551D-CFTR (cystic fibrosis transmembrane conductance regulator) potentiation by a high affinity ATP analog. J Biol Chem. 2008;283(9):5364-9. doi: 10.1074/jbc.M709417200.
111. Miki H, Zhou Z, Li M, Hwang TC, Bompadre SG. Potentiation of disease-associated cystic fibrosis transmembrane conductance regulator mutants by hydrolyzable ATP analogs. J Biol Chem. 2010;285(26):19967-75. doi: 10.1074/jbc.M109.092684.
112. Tsai MF, Li M, Hwang TC. Stable ATP binding mediated by a partial NBD dimer of the CFTR chloride channel. J Gen Physiol. 2010;135(5):399-414. doi: 10.1085/jgp.201010399.
113. Hwang TC, Wang F, Yang IC, Reenstra WW. Genistein potentiates wild-type and delta F508-CFTR channel activity. American Journal of Physiology-Cell Physiology. 1997;273(3):C988-C98. doi: 10.1152/ajpcell.1997.273.3.C988.
114. Weinreich F, Wood PG, Riordan JR, Nagel G. Direct action of genistein on CFTR. Pflügers Archiv. 1997;434(4):484-91. doi: 10.1007/s004240050424.
115. French PJ, Bijman J, Bot AG, Boomaars WE, Scholte BJ, de Jonge HR. Genistein activates CFTR Cl- channels via a tyrosine kinase- and protein phosphatase-independent mechanism. The American journal of physiology. 1997;273(2 Pt 1):C747-53. doi: 10.1152/ajpcell.1997.273.2.C747.
116. Wang F, Zeltwanger S, Yang IC, Nairn AC, Hwang TC. Actions of genistein on cystic fibrosis transmembrane conductance regulator channel gating. Evidence for two binding sites with opposite effects. J Gen Physiol. 1998;111(3):477-90.
117. Yu YC, Miki H, Nakamura Y, Hanyuda A, Matsuzaki Y, Abe Y, et al. Curcumin and genistein additively potentiate G551D-CFTR. J Cyst Fibros. 2011;10(4):243-52. doi: 10.1016/j.jcf.2011.03.001.
118. Sohma Y, Yu YC, Hwang TC. Curcumin and genistein: the combined effects on disease-associated CFTR mutants and their clinical implications. Curr Pharm Des. 2013;19(19):3521-8.
119. Csanady L, Torocsik B. Structure-activity analysis of a CFTR channel potentiator: Distinct molecular parts underlie dual gating effects. J Gen Physiol. 2014;144(4):321-36. doi: 10.1085/jgp.201411246.
120. Lin WY, Sohma Y, Hwang TC. Synergistic Potentiation of Cystic Fibrosis Transmembrane Conductance Regulator Gating by Two Chemically Distinct Potentiators, Ivacaftor (VX-770) and 5-Nitro-2-(3-Phenylpropylamino) Benzoate. Mol Pharmacol. 2016;90(3):275-85. doi: 10.1124/mol.116.104570.
121. Ai T, Bompadre SG, Sohma Y, Wang X, Li M, Hwang TC. Direct effects of 9-anthracene compounds on cystic fibrosis transmembrane conductance regulator gating. Pflugers Arch. 2004;449(1):88-95. doi: 10.1007/s00424-004-1317-y.
122. Pedemonte N, Sonawane ND, Taddei A, Hu J, Zegarra-Moran O, Suen YF, et al. Phenylglycine and sulfonamide correctors of defective delta F508 and G551D cystic fibrosis transmembrane conductance regulator chloride-channel gating. Mol Pharmacol. 2005;67(5):1797-807. doi: 10.1124/mol.105.010959.

123. Yang H, Shelat AA, Guy RK, Gopinath VS, Ma T, Du K, et al. Nanomolar affinity small molecule correctors of defective Delta F508-CFTR chloride channel gating. J Biol Chem. 2003;278(37):35079-85. doi: 10.1074/jbc.M303098200.
124. Van Goor F, Straley KS, Cao D, González J, Hadida S, Hazlewood A, et al. Rescue of DeltaF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. Am J Physiol Lung Cell Mol Physiol. 2006;290(6):L1117-30. doi: 10.1152/ajplung.00169.2005.
125. Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Dřevínek P, et al. A CFTR Potentiator in Patients with Cystic Fibrosis and the G551D Mutation. New England Journal of Medicine. 2011;365(18):1663-72. doi: 10.1056/NEJMoa1105185.
126. Jih KY, Hwang TC. Vx-770 potentiates CFTR function by promoting decoupling between the gating cycle and ATP hydrolysis cycle. Proc Natl Acad Sci U S A. 2013;110(11):4404-9. doi: 10.1073/pnas.1215982110.
127. Yeh HI, Yeh JT, Hwang TC. Modulation of CFTR gating by permeant ions. J Gen Physiol. 2015;145(1):47-60. doi: 10.1085/jgp.201411272.
128. Yeh HI, Sohma Y, Conrath K, Hwang TC. A common mechanism for CFTR potentiators. J Gen Physiol. 2017;149(12):1105-18. doi: 10.1085/jgp.201711886.
129. Csanády L, Töröcsik B. Cystic fibrosis drug ivacaftor stimulates CFTR channels at picomolar concentrations. Elife. 2019;8. doi: 10.7554/eLife.46450.
130. Laselva O, Qureshi Z, Zeng Z-W, Petrotchenko EV, Ramjeesingh M, Hamilton CM, et al. Identification of binding sites for ivacaftor on the cystic fibrosis transmembrane conductance regulator. iScience. 2021;24(6):102542. doi: https://doi.org/10.1016/j.isci.2021.102542.
131. Laselva O, Bartlett C, Gunawardena TNA, Ouyang H, Eckford PDW, Moraes TJ, et al. Rescue of multiple class II CFTR mutations by elexacaftor+tezacaftor+ivacaftor mediated in part by the dual activities of elexacaftor as both corrector and potentiator. European Respiratory Journal. 2021;57(6):2002774. doi: 10.1183/13993003.02774-2020.
132. Shaughnessy CA, Zeitlin PL, Bratcher PE. Elexacaftor is a CFTR potentiator and acts synergistically with ivacaftor during acute and chronic treatment. Scientific Reports. 2021;11(1):19810. doi: 10.1038/s41598-021-99184-1.
133. Veit G, Vaccarin C, Lukacs GL. Elexacaftor co-potentiates the activity of F508del and gating mutants of CFTR. J Cyst Fibros. 2021;20(5):895-8. doi: 10.1016/j.jcf.2021.03.011.
134. Veit G, Roldan A, Hancock MA, Da Fonte DF, Xu H, Hussein M, et al. Allosteric folding correction of F508del and rare CFTR mutants by elexacaftor-tezacaftor-ivacaftor (Trikafta) combination. JCI Insight. 2020;5(18). doi: 10.1172/jci.insight.139983.
135. Pedemonte N, Lukacs GL, Du K, Caci E, Zegarra-Moran O, Galietta LJ, et al. Small-molecule correctors of defective DeltaF508-CFTR cellular processing identified by high-throughput screening. J Clin Invest. 2005;115(9):2564-71. doi: 10.1172/jci24898.
136. Wang Y, Loo TW, Bartlett MC, Clarke DM. Correctors promote maturation of cystic fibrosis transmembrane conductance regulator (CFTR)-processing mutants by binding to the protein. J Biol

Chem. 2007;282(46):33247-51. doi: 10.1074/jbc.C700175200.
137. Pankow S, Bamberger C, Calzolari D, Martinez-Bartolome S, Lavallee-Adam M, Balch WE, et al. F508 CFTR interactome remodelling promotes rescue of cystic fibrosis. Nature. 2015;528(7583):510-6. doi: 10.1038/nature15729.
138. Hegde RN, Parashuraman S, Iorio F, Ciciriello F, Capuani F, Carissimo A, et al. Unravelling druggable signalling networks that control F508del-CFTR proteostasis. Elife. 2015;4. doi: 10.7554/eLife.10365.
139. Hanrahan JW, Sato Y, Carlile GW, Jansen G, Young JC, Thomas DY. Cystic Fibrosis: Proteostatic correctors of CFTR trafficking and alternative therapeutic targets. Expert Opin Ther Targets. 2019;23(8):711-24. doi: 10.1080/14728222.2019.1628948.
140. Clancy JP, Rowe SM, Accurso FJ, Aitken ML, Amin RS, Ashlock MA, et al. Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation. Thorax. 2012;67(1):12-8. doi: 10.1136/thoraxjnl-2011-200393.
141. Bell SC, Barry PJ, De Boeck K, Drevinek P, Elborn JS, Plant BJ, et al. CFTR activity is enhanced by the novel corrector GLPG2222, given with and without ivacaftor in two randomized trials. J Cyst Fibros. 2019;18(5):700-7. doi: 10.1016/j.jcf.2019.04.014.
142. Hudson RP, Chong PA, Protasevich, II, Vernon R, Noy E, Bihler H, et al. Conformational changes relevant to channel activity and folding within the first nucleotide binding domain of the cystic fibrosis transmembrane conductance regulator. J Biol Chem. 2012;287(34):28480-94. doi: 10.1074/jbc.M112.371138.
143. Baatallah N, Elbahnsi A, Mornon JP, Chevalier B, Pranke I, Servel N, et al. Pharmacological chaperones improve intra-domain stability and inter-domain assembly via distinct binding sites to rescue misfolded CFTR. Cell Mol Life Sci. 2021;78(23):7813-29. doi: 10.1007/s00018-021-03994-5.
144. Hudson RP, Dawson JE, Chong PA, Yang Z, Millen L, Thomas PJ, et al. Direct Binding of the Corrector VX-809 to Human CFTR NBD1: Evidence of an Allosteric Coupling between the Binding Site and the NBD1:CL4 Interface. Mol Pharmacol. 2017;92(2):124-35. doi: 10.1124/mol.117.108373.
145. Okiyoneda T, Veit G, Dekkers JF, Bagdany M, Soya N, Xu H, et al. Mechanism-based corrector combination restores DeltaF508-CFTR folding and function. Nature chemical biology. 2013;9(7):444-54. doi: 10.1038/nchembio.1253.
146. Singh AK, Fan Y, Balut C, Alani S, Manelli AM, Swensen AM, et al. Biological Characterization of F508delCFTR Protein Processing by the CFTR Corrector ABBV-2222/GLPG2222. J Pharmacol Exp Ther. 2020;372(1):107-18. doi: 10.1124/jpet.119.261800.
147. Grove DE, Rosser MF, Ren HY, Naren AP, Cyr DM. Mechanisms for rescue of correctable folding defects in CFTRDelta F508. Mol Biol Cell. 2009;20(18):4059-69. doi: 10.1091/mbc.E08-09-0929.
148. Laselva O, Molinski S, Casavola V, Bear CE. Correctors of the Major Cystic Fibrosis Mutant Interact through Membrane-Spanning Domains. Mol Pharmacol. 2018;93(6):612-8. doi: 10.1124/mol.118.111799.

149. Ren HY, Grove DE, De La Rosa O, Houck SA, Sopha P, Van Goor F, et al. VX-809 corrects folding defects in cystic fibrosis transmembrane conductance regulator protein through action on membrane-spanning domain 1. Mol Biol Cell. 2013;24(19):3016-24. doi: 10.1091/mbc.E13-05-0240.
150. Loo TW, Bartlett MC, Clarke DM. Corrector VX-809 stabilizes the first transmembrane domain of CFTR. Biochem Pharmacol. 2013;86(5):612-9. doi: 10.1016/j.bcp.2013.06.028.
151. He L, Kota P, Aleksandrov AA, Cui L, Jensen T, Dokholyan NV, et al. Correctors of DeltaF508 CFTR restore global conformational maturation without thermally stabilizing the mutant protein. FASEB J. 2013;27(2):536-45. doi: 10.1096/fj.12-216119.
152. Kalid O, Mense M, Fischman S, Shitrit A, Bihler H, Ben-Zeev E, et al. Small molecule correctors of F508del-CFTR discovered by structure-based virtual screening. J Comput Aided Mol Des. 2010;24(12):971-91. doi: 10.1007/s10822-010-9390-0.
153. Carlile GW, Yang Q, Matthes E, Liao J, Radinovic S, Miyamoto C, et al. A novel triple combination of pharmacological chaperones improves F508del-CFTR correction. Sci Rep. 2018;8(1):11404. doi: 10.1038/s41598-018-29276-y.
154. Lin S, Sui J, Cotard S, Fung B, Andersen J, Zhu P, et al. Identification of synergistic combinations of F508del cystic fibrosis transmembrane conductance regulator (CFTR) modulators. Assay and drug development technologies. 2010;8(6):669-84. doi: 10.1089/adt.2010.0313.
155. Veit G, Xu H, Dreano E, Avramescu RG, Bagdany M, Beitel LK, et al. Structure-guided combination therapy to potently improve the function of mutant CFTRs. Nat Med. 2018;24(11):1732-42. doi: 10.1038/s41591-018-0200-x.
156. Wainwright CE, Elborn JS, Ramsey BW, Marigowda G, Huang X, Cipolli M, et al. Lumacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del CFTR. N Engl J Med. 2015;373(3):220-31. doi: 10.1056/NEJMoa1409547.
157. Konstan MW, McKone EF, Moss RB, Marigowda G, Tian S, Waltz D, et al. Assessment of safety and efficacy of long-term treatment with combination lumacaftor and ivacaftor therapy in patients with cystic fibrosis homozygous for the F508del-CFTR mutation (PROGRESS): a phase 3, extension study. Lancet Respir Med. 2017;5(2):107-18. doi: 10.1016/s2213-2600(16)30427-1.
158. McNamara JJ, McColley SA, Marigowda G, Liu F, Tian S, Owen CA, et al. Safety, pharmacokinetics, and pharmacodynamics of lumacaftor and ivacaftor combination therapy in children aged 2-5 years with cystic fibrosis homozygous for F508del-CFTR: an open-label phase 3 study. Lancet Respir Med. 2019;7(4):325-35. doi: 10.1016/s2213-2600(18)30460-0.
159. Ratjen F, Hug C, Marigowda G, Tian S, Huang X, Stanojevic S, et al. Efficacy and safety of lumacaftor and ivacaftor in patients aged 6-11 years with cystic fibrosis homozygous for F508del-CFTR: a randomised, placebo-controlled phase 3 trial. Lancet Respir Med. 2017;5(7):557-67. doi: 10.1016/S2213-2600(17)30215-1.
160. Cooney AL, McCray PB, Jr., Sinn PL. Cystic Fibrosis Gene Therapy: Looking Back, Looking Forward. Genes (Basel). 2018;9(11). doi: 10.3390/genes9110538.
161. Hodges CA, Conlon RA. Delivering on the promise of gene editing for cystic fibrosis. Genes Dis.

2019;6(2):97-108. doi: 10.1016/j.gendis.2018.11.005.
162. Pranke I, Golec A, Hinzpeter A, Edelman A, Sermet-Gaudelus I. Emerging Therapeutic Approaches for Cystic Fibrosis. From Gene Editing to Personalized Medicine. Front Pharmacol. 2019;10:121. doi: 10.3389/fphar.2019.00121.
163. Fajac I, Sermet I. Therapeutic Approaches for Patients with Cystic Fibrosis Not Eligible for Current CFTR Modulators. Cells. 2021;10(10). doi: 10.3390/cells10102793.
164. Sergeev V, Chou FY, Lam GY, Hamilton CM, Wilcox PG, Quon BS. The Extrapulmonary Effects of Cystic Fibrosis Transmembrane Conductance Regulator Modulators in Cystic Fibrosis. Ann Am Thorac Soc. 2020;17(2):147-54. doi: 10.1513/AnnalsATS.201909-671CME.
165. Solomon GM, Fu L, Rowe SM, Collawn JF. The therapeutic potential of CFTR modulators for COPD and other airway diseases. Curr Opin Pharmacol. 2017;34:132-9. doi: 10.1016/j.coph.2017.09.013.