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Cystic Fibrosis Transmembrane Regulator Correctors and Potentiators

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Cystic fibrosis (CF) is caused by loss-of-function mutations in the CF transmembrane conductance regulator (CFTR) protein, a cAMP-regulated anion channel expressed primarily at the apical plasma membrane of secretory epithelia. Nearly 2000 mutations in the CFTR gene have been identified that cause disease by impairing its translation, cellular processing, and/or chloride channel gating. The fundamental premise of CFTR corrector and potentiator therapy for CF is that addressing the underlying defects in the cellular processing and chloride channel function of CF-causing mutant CFTR alleles will result in clinical benefit by addressing the basic defect underlying CF. Correctors are principally targeted at F508del cellular misprocessing, whereas potentiators are intended to restore cAMP-dependent chloride channel activity to mutant CFTRs at the cell surface. This article reviews the discovery of CFTR potentiators and correctors, what is known regarding their mechanistic basis, and encouraging results achieved in clinical testing.

Cystic fibrosis (CF) is caused by loss-of-function mutations in the CF transmembrane conductance regulator (CFTR) protein, a cAMP-regulated chloride channel expressed primarily at the apical plasma membrane of secretory epithelia in the airways, pancreas, intestine, and other tissues. CFTR is a large, multidomain glycoprotein consisting of two membrane-spanning domains, two nucleotide-binding domains (NBD1 and NBD2) that bind and hydrolyze ATP, and a regulatory (R) domain that gates the channel by phosphorylation. There is limited crystal structure data on isolated cytoplasmic domains of CFTR, and low-resolution electron crystallographic data and homology modeling of full-length CFTR. Nearly 2000 mutations in the CFTR gene have been identified that produce the loss-of-function phenotype by impairing its translation, cellular processing, and/or chloride channel gating. The F508del mutation, which is present in at least one allele in ~90% of CF patients, impairs CFTR folding, stability at the endoplasmic reticulum and plasma membrane, and chloride channel gating (Dalemans et al. 1991; Denning et al. 1992; Lukacs et al. 1993; Du et al. 2005a). Other mutations primarily alter channel gating (e.g., G551D), conductance (e.g., R117H), or translation (e.g., G542X) (Welsh and Smith 1993). The fundamental premise of CFTR corrector and potentiator therapy for CF is that

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correction of the underlying defects in the cellular processing and chloride channel function of CF-causing mutant CFTR alleles will be of clinical benefit. Correctors are principally targeted at F508del cellular misprocessing, whereas potentiators are intended to restore cAMP-dependent chloride channel activity to mutant CFTRs at the cell surface. In contrast to current therapies, such as antibiotics, anti-inflammatory agents, mucolytics, nebulized hypertonic saline, and pancreatic enzyme replacement, which treat CF disease manifestations, correctors and potentiators correct the underlying CFTR anion channel defect.

This work is focused on corrector and potentiator therapy for CF. The goal is to normalize defective folding, plasma membrane targeting, surface stability, and channel function in cells expressing disease-causing CFTR mutants. An ideal therapy would be a single drug without off-target effects that normalizes mutant CFTR folding, processing, and function to resemble that of wild-type CFTR. Although remarkable progress has been made in the past decade in small-molecule correctors and potentiators in their discovery and rapid advancement to clinical trials, much work remains, in particular, for F508del correctors, in the identification of compounds with high efficacy and potency, understanding their mechanism of action, and establishing long-term clinical benefit.

STRATEGIES FOR CFTR DRUG DISCOVERY

Because of the paucity of structural information on full-length wild-type and F508del-CFTR, as well as the complexity of the defects caused by the F508del mutation, CFTR drug discovery has largely used phenotype assays based on CFTR chloride channel function. Genetically encoded, halide-sensing fluorescent proteins have been very useful in this regard. Yellow fluorescent protein (YFP) mutants have been identified whose fluorescence is strongly quenched (reduced) by iodide (Jayaraman et al. 2000), a halide that is efficiently transported by CFTR. The YFP mutant YFP-H148Q/I521L is brightly fluorescent and 50% quenched by 2–3 mM iodide (Galietta et al. 2001a). The general screening strategy for modulators of CFTR function is the generation of cells coexpressing CFTR (wild-type or mutant) together with the YFP iodide sensor. Test compounds can be added before assay of iodide influx, which involves measurement of the time course of cell fluorescence in response to iodide addition to the extracellular solution. Fisher rat thyroid (FRT) cells were found to be particularly useful for chloride channel drug discovery because of their epithelial origin and formation of tight junctions, rapid growth on uncoated plastic, strong adherence in multiwell plate format, ease of stable expression following transfection, and low basal halide permeability (Galietta et al. 2001b). An alternative screening approach has been the use of membrane potential-sensitive fluorescent dyes to measure CFTR-dependent membrane depolarization following chloride addition to the extracellular solution (Van Goor et al. 2006). An alternative assay approach for corrector testing is the appearance of mutant CFTR at the cell plasma membrane measured using an externally epitope-tagged CFTR with ELISA-based readout involving secondary antibodies (Carlile et al. 2007). A new generation of assays is under development that probes specific molecular interactions involved in F508del folding, such as domain–domain interactions or nucleotide-binding domain stability.

Utilizing cell-based high-throughput screening with YFP fluorescence readout, potent thiazolidinone (e.g., $CFTR_{inh}$-172) (Ma et al. 2002a) and glycine hydrazide (e.g., GlyH-101) (Muanprasat et al. 2004) inhibitors of wild-type CFTR have been identified (Fig. 1A), which are used widely as CF research tools. The glycine hydrazides target the external CFTR pore, which has allowed the development of nonabsorbable macromolecular conjugates that inhibit CFTR-dependent fluid secretion in the intestine (Sonawane et al. 2008). Recently, PPQ and related BPO classes of CFTR inhibitors, with IC_{50} down to ~5 nM, have been discovered (Tradtrantip et al. 2009a; Snyder et al. 2011). Small-molecule CFTR inhibitors are in preclinical development for therapy of enterotoxin-mediated secretory diarrheas and polycystic kidney disease. High-throughput screening has also yielded small-molecule activators of wild-type CFTR that tar-

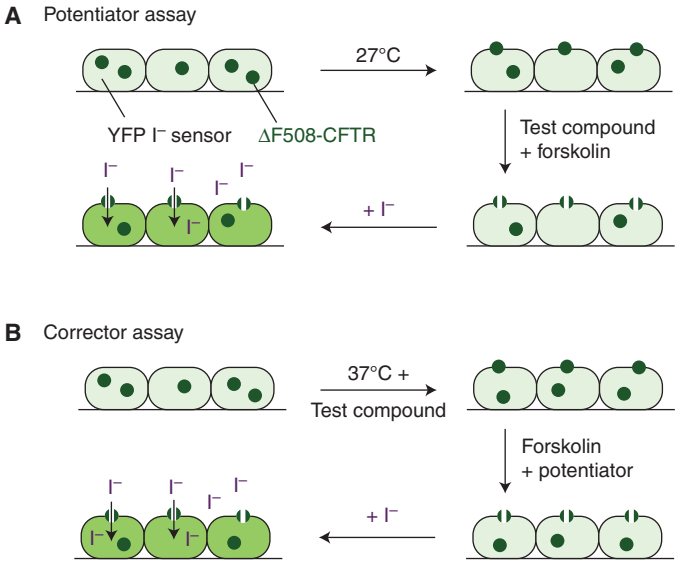

Figure 1. Cell-based screening assays for high-throughput identification of F508del-CFTR potentiators and correctors. (*A*) Potentiator assay: Fisher rat thyroid (FRT) cells coexpressing human F508del-CFTR and a halide-sensing yellow fluorescent protein (YFP) are incubated at reduced temperature (27°C) for 18–24 h before assay to target F508del-CFTR to the plasma membrane. Test compounds are added for 10 min in the presence of a cAMP agonist (forskolin) before iodide addition. F508del-CFTR function is assayed in a plate reader from the kinetics of YFP fluorescence quenching following iodide addition. (*B*) Corrector assay: Cells are incubated with test compounds at 37°C for 24 h. F508del-CFTR function is assayed by iodide addition in the presence of forskolin and a potentiator such as genistein.

get the channel directly (Ma et al. 2002b), as well as modulators of phosphodiesterase activity (Tradtrantip et al. 2009b).

Figure 2 diagrams high-throughput screening assays that have been used to identify small-molecule potentiators and correctors of F508del-CFTR. In these screens, potentiator activity is the ability of a compound to normalize mutant CFTR chloride channel gating when added just before assay of transport and generally in the presence of a cAMP agonist. Potentiator activity is assayed in F508del-CFTR-transfected epithelial cells following low-temperature incubation in which F508del-CFTR is targeted to the plasma membrane, with test compound (together with cAMP agonist) added just before assay (Yang et al. 2003). Potentiator-dependent restoration of F508del-CFTR channel function is assayed from the kinetics of decreasing YFP fluorescence following iodide addition. Corrector activity is the ability of a compound to promote cell-surface expression of F508del-CFTR generally following prolonged incubation. Corrector activity is assayed in F508del-CFTR-expressing cells by >12 h incubation with test compound at 37°C, followed by washout and addition of a potentiator such as the flavone genistein (together with cAMP agonist) just before measurement of iodide influx (Pedemonte et al. 2005a). Similar assays have been used to identify inhibitors and activators of calcium-activated chloride channels (Namkung et al. 2011a,b). The latter have potential therapeutic efficacy in CF by restoring chloride transport independent of CFTR rescue.

POTENTIATORS

The practical drug discovery strategy used to date involves separate functional assays to screen for F508del-CFTR potentiators and correctors. We point out, however, that because channel gating and cellular processing are interrelated processes that each depend on F508del-CFTR

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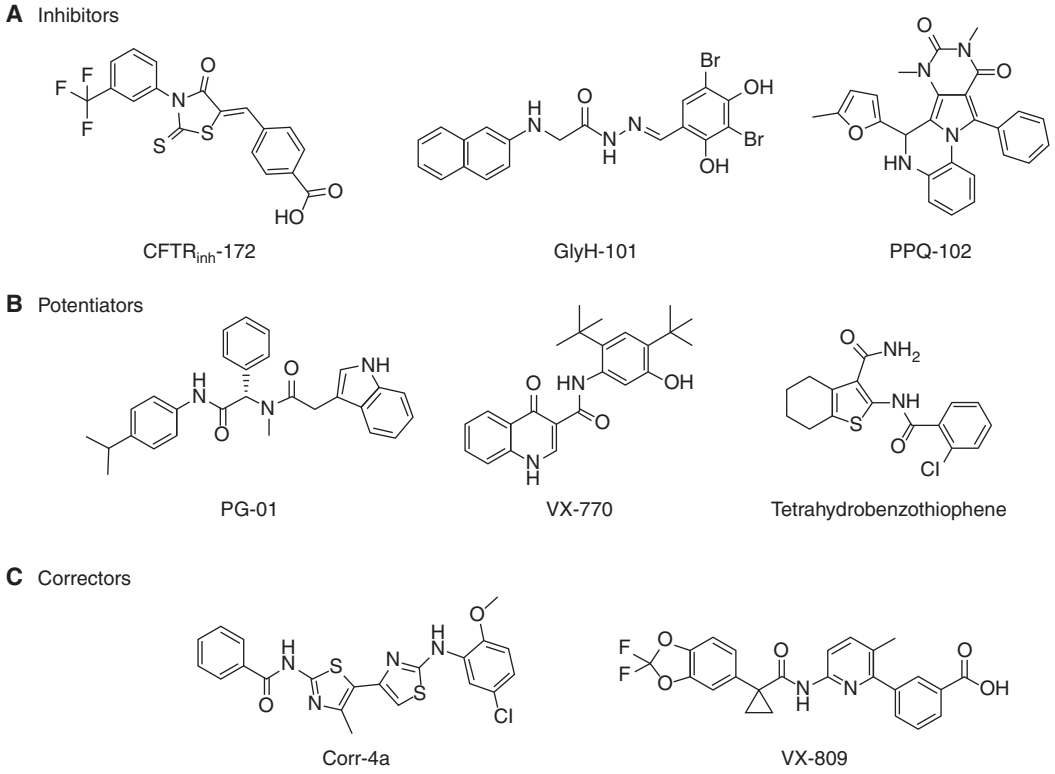

Figure 2. Chemical structures of small-molecule inhibitors of wild-type CFTR (*A*), potentiators (*B*), and correctors (*C*) of F508del-CFTR.

folding, the conventional, sharp distinction between potentiators and correctors is somewhat artificial and could hamper CF drug discovery, for example, in the identification of efficacious compounds for single-drug therapy of CF caused by the F508del mutation. Nevertheless, screening efforts have identified multiple chemical classes of potentiators and correctors, and, recently, a class of compounds with dual potentiator and corrector activities.

Before HTS, several chemical classes of efficacious F508del-CFTR potentiators were known, including flavones, xanthines, and benzimidazoles (Fig. 1B). A small screen of approved drugs and follow-on medicinal chemistry have identified dihydropyridine blockers of slow calcium channels as potentiators (Budriesi et al. 2011), although their development potential is unclear because of off-target cardiac and other effects. High-throughput screening efforts by the Verkman laboratory (Yang et al. 2003; Pedemonte et al. 2005b) and by Vertex Pharmaceuticals (Van Goor et al. 2006) yielded several additional classes of small-molecule potentiators. Interestingly, a relatively high "hit rate" was found for identification of potentiators, with many potential development candidates; however, these potentiators do not have significant corrector activity. The phenylglycine PG-01 (Fig. 1B), when added together with a cAMP agonist, activates F508del-CFTR chloride conductance with low nanomolar potency, with maximum efficacy comparable to that of the flavone genistein, restoring F508del-CFTR open probability to approximately that of wild-type CFTR. Ivacaftor (VX-770), an investigational agent identified by Vertex Pharmaceuticals, has similar efficacy. PG-01 and ivacaftor also correct defective channel gating in the CF-causing mutant G551D-CFTR, a CFTR "gating" mutant

whose primary defect is reduced channel open probability after maximal cAMP stimulation. Ivacaftor has undergone development for human use, as described further below. Although potentiators may be clinically useful for CF patients having G551D and possibly other gating/conductance mutations, a potentiator alone is unlikely to have clinical benefit in CF caused by the F508del mutation because of the small amount of F508del-CFTR targeted to the cell plasma membrane, a finding substantiated by the clinical evaluation of ivacaftor in F508del homozygotes (see below). Another concern with some available potentiators is that superphysiological concentrations of cAMP agonists are needed for potentiator efficacy following low-temperature or corrector rescue, probably because the rescued F508del-CFTR is in a nonnative conformation. Wild-type CFTR, in contrast, is activated strongly at low concentrations of cAMP without the need for a potentiator.

There is limited information about the mechanism of action of potentiators, but they are likely to involve a diverse range of cellular mechanisms. The Rowe laboratory has investigated potentiator mechanisms with a focus on R-domain phosphorylation (Pyle et al. 2011). Results suggest that potentiation of cAMP-dependent CFTR activation occurs independent of R-domain phosphorylation, whereas agents that activate CFTR itself without altering the response to cAMP resulted in robust R-domain phosphorylation. Other results have suggested that VRT-532, a CFTR potentiator that enhances the cAMP responsiveness of CFTR, potentiates CFTR gating by directly interacting with G551D or F508del CFTR to restore its defective ATPase activity (Pasyk et al. 2009; Wellhauser et al. 2009). Agents that potentiate CFTR by altering its cAMP dependence likely interact with CFTR itself, perhaps facilitating the conformation change involved in CFTR gating, although disruption of protein–protein interactions may also be involved in the action of some potentiators (Eckford and Bear 2011). Other indirect mechanisms of CFTR activation, including activation of cAMP through activation of phosphodiesterases or inhibition of phosphatases, or even alteration of electrochemical gradients for chloride transport by action on basolateral K^+ channels, the NKCC cotransporter or the Na^+/K^+ ATPase are also possible, and may underlie the effects of some putative "potentiators" that may be better classified as nonspecific activators (Pyle et al. 2011).

CORRECTORS

The identification and development of correctors of F508del-CFTR cellular misprocessing presents a much greater challenge than that for potentiators because of the involvement of multiple components of the cellular quality control machinery that are expressed in a cell-specific manner, and because of the multiplicity of defects conferred by the F508del mutation (Du et al. 2005b; He et al. 2010; Thibodeau et al. 2010). Correctors could act as "pharmacological chaperones" by interacting with F508del-CFTR itself, facilitating its folding and cellular processing, or as "proteostasis regulators" by modulating the cellular quality-control machinery to alter F508del-CFTR recognition and processing (Mu et al. 2008; Powers et al. 2009). Although pharmacological chaperones have the potential for greater target selectivity and there is a precedent for their utility in other protein folding diseases, proteostasis regulators may produce greater efficacy, particularly in combination with agents that directly enhance CFTR folding.

Neutraceuticals and drugs approved for other indications have been reported to have F508del-CFTR corrector activity, including curcumin (Egan et al. 2004), miglustat (Noel et al. 2008; Norez et al. 2009), and sildenafil (Lubamba et al. 2008; Robert et al. 2008). However, follow-up studies, and in the case of curcumin, a small clinical trial (see below), have failed to confirm bona fide or robust corrector action of these compounds. The compound 4-phenylbutryate (Buphenyl), which is approved for an inherited disorder of urea metabolism, increases F508del-CFTR plasma membrane expression in cell culture models, probably acting as a proteostatis regulator. However, in a clinical trial involving nasal potential difference measurements, F508del CF patients showed minimal benefit (Zeitlin et al. 2002). Down-regulation of Aha1,

an Hsp90 cochaperone (Wang et al. 2006), and inhibition of HDAC7 (Hutt et al. 2010), increase plasma membrane F508del-CFTR in cell culture models by facilitating F508del-CFTR folding and stability, acting by a proteostasis regulator mechanism.

Several classes of small-molecule F508del-CFTR correctors have been identified by high-throughput screening (Fig. 1C). The original study identified four classes of compounds, including the bithiazole corr-4a, which increased F508del-CFTR cell-surface expression after 12–24 h incubation at 37°C, resulting in increased chloride conductance in transfected cell models and human F508del-CFTR bronchial cell cultures (with addition of a potentiator and cAMP agonist) (Pedemonte et al. 2005b). Follow-up medicinal chemistry yielded bithiazole analogs with improved potency and pharmacological properties (Yu et al. 2008), with EC_{50} ~300 nM for the most potent bithiazole. However, in F508del bronchial cell culture models, available correctors normalize chloride conductance to only 10%–15% of that in wild-type bronchial cell cultures. Screening by Vertex Pharmaceuticals has yielded other classes of correctors (Van Goor et al. 2006, 2010), with the most promising compound, VX-809, in phase II clinical trials as discussed below. Other small-scale screening efforts have yielded additional candidate correctors, including the approved drug glafanine (Robert et al. 2010), the phenylhydrazone RDR1 (Sampson et al. 2011), and a few compounds from computational screening (Kalid et al. 2010), although these candidate correctors have quite low efficacy.

The molecular mechanisms of corrector action are poorly understood. Initial studies on corr-4a supported a mechanism of direct interaction with F508del-CFTR, as corr-4a improved F508del-CFTR folding at the endoplasmic reticulum and did not correct other misfolded proteins, including some CFTR mutants (Pedemonte et al. 2005b). Cys-cross-linking data supported the idea that corr-4a interacts with F508del-CFTR in the endoplasmic reticulum to promote its folding (Loo et al. 2008), although indirect effects involving proteostasis regulation cannot be excluded. One study suggested that corr-4a targets and stabilizes NBD2 (Grove et al. 2009), whereas another study suggested that corr-4a action may also involve interference with ubiquitination (Jurkuvenaite et al. 2010). There is also evidence for direct interaction of corrector VRT-325 with F508del-CFTR, as it alters CFTR ATPase activity (Kim Chiaw et al. 2010) and protease susceptibility (Yu et al. 2011). Notwithstanding these and other lines of suggestive evidence, compelling data remains lacking about the mechanism of action of available correctors.

Further mechanistic studies remain a high priority, as they may offer clues to understand the limited efficacy of available correctors. One commonality among F508del-CFTR correctors has been their limited efficacy of CFTR rescue following treatment by a single compound (or pathway, in the case of RNA interference). It has been suggested that single agents may have limited efficacy (therapeutic "ceiling") because of the complex, multiple defects in F508del-CFTR folding (Sloane and Rowe 2010). It remains to be determined whether the limited efficacy of available correctors can be overcome with improved understanding of the folding pathway and molecular partners of F508del-CFTR. As a more immediate approach to enhance efficacy, it has been shown that combinations of F508del correctors can have additive or even synergistic effects (Bridges 2010; Lin et al. 2010), supporting the idea that more than one cellular mechanism is relevant to the cellular recognition and degradation of F508del-CFTR. The use of CFTR potentiators in combination with CFTR correctors represents another option to overcome deficits in efficient functional rescue for the protein. For example, VX-770 approximately doubles the effect of VX-809 in F508del HBE cells (Van Goor et al. 2010).

There has been interest in the identification of single compounds with dual corrector and potentiator activities, with the rationale being the practicality of single versus double drug therapy. Of the available correctors, the bithiazoles and aminoarylthiazoles appear to at least partially normalize F508del-CFTR folding, as their prolonged incubation results in greater chloride conductance in response to a cAMP agonist (Pedemonte et al. 2011). In a proof-

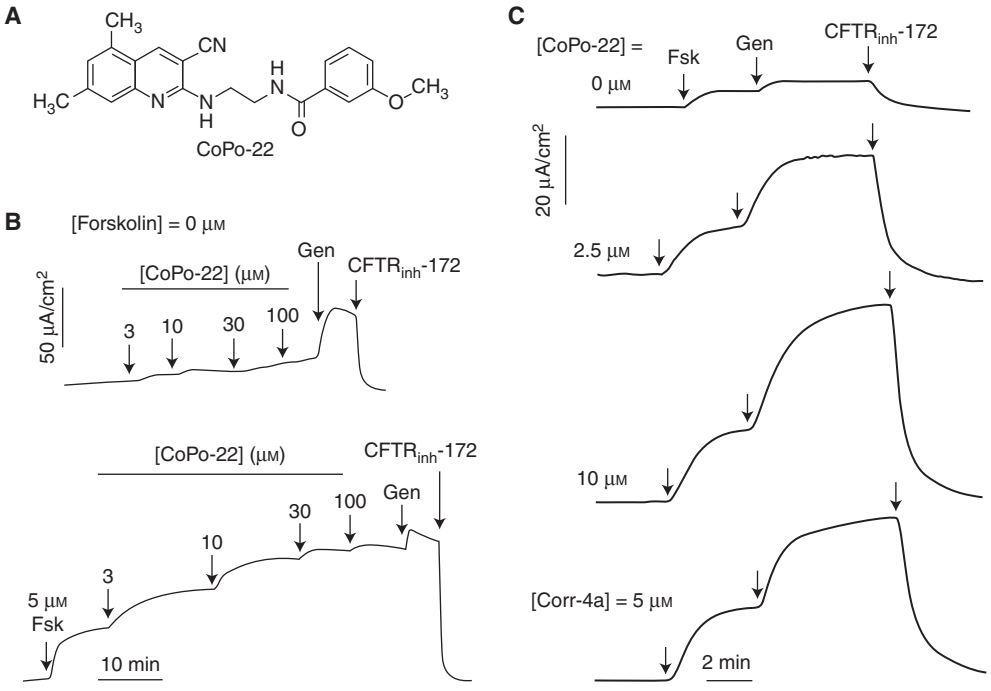

Figure 3. Dual potentiator and corrector activities of cyanoquinoline CoPo-22. (*A*) CoPo-22 structure. (*B*) Potentiator activity assayed by measurement of short-circuit current in FRT cells expressing F508del-CFTR. Assays performed at 0 and 5 μM forskolin as indicated. Effects of reference potentiator genistein shown, and CFTR inhibitor $CFTR_{inh}$-172. (*C*) Corrector activity measured following 18 h incubation with indicated concentrations of CoPo-22. Data for bithiazole corr-4a shown for comparison. Chloride conductance measured following addition of high concentrations of forskolin and genistein. (From Phuan et al. 2011; adapted, with permission, from the author.)

of-concept study, a hybrid bithiazole-phenylglycine corrector potentiator was synthesized, which, when cleaved by intestinal enzymes, yields an active bithiazole corrector and phenylglycine potentiator (Mills et al. 2010). Recently, the Verkman laboratory identified a cyanoquinoline class of F508del-CFTR correctors with independent potentiator activity (Phuan et al. 2011). Corrector-potentiator-22 (CoPo-22, Fig. 3A) rapidly increased chloride current when added to low-temperature rescued cells (potentiator activity, Fig. 3B), as well as increased cell-surface expression and chloride current after prolonged incubation (corrector activity, Fig. 3C). Although the corrector and potentiator efficacies of CoPo-22 are comparable to those of corr-4a and genistein, respectively, their EC_{50} are in the low micromolar rather than nanomolar range. Whether improved cyanoquinoline analogs can be development candidates for CF therapy is unclear, although these studies offer proof of concept for the possibility of dual-action corrector-potentiator compounds.

CLINICAL DEVELOPMENT OF CFTR POTENTIATORS

Progress in the clinical testing of potentiators and correctors of CFTR function in CF patients is among the most exciting developments in CF therapeutics. Although considerable challenges remain to bring therapeutic options to CF patients with different CFTR mutations, recent data have established proof of concept that rescue of CFTR-mediated anion transport can result in clinical benefit, and have opened a new

era of CF treatment options that address the fundamental defect in the disease (Sloane and Rowe 2010). Table 1 summarizes clinical trial data.

Just as the discovery of CFTR correctors has been more challenging than that for potentiators, clinical progress toward developing a safe and efficacious CFTR modulator has moved more swiftly for CFTR potentiators, led by studies of ivacaftor. Following phase 1 testing in normal subjects and CF volunteers, ivacaftor was evaluated in a phase 2 randomized, double-blind, ascending dose trial that used both a crossover component and a confirmatory parallel group design. The trial focused on CF subjects with the G551D-CFTR allele, a gating mutation with low open probability at baseline, but relatively sensitive to ivacaftor in vitro (Van Goor et al. 2009). Ivacaftor was safe and well tolerated following administration for 2 and 4 weeks. Clinically significant and dose-dependent improvements in CFTR activity were observed in three ivacaftor dose groups (75 mg, 150 mg, and 250 mg, each bid) as measured by nasal potential difference and sweat chloride (Accurso et al. 2010). Improved pulmonary function was also observed, including an ~8.7% increase in lung function as measured by FEV1% in the 150-mg bid group. The median reduction in sweat chloride of ~59 mEq at the maximally effective dose resulted in a mean sweat chloride of ~55 mEq, a value below the traditional diagnostic threshold of CF. CFTR-dependent anion transport measured by nasal potential difference (NPD) also improved a degree postulated to confer clinical benefit as predicted by genotype–phenotype correlations (Wilschanski et al. 2006). A summary of the ivacaftor data following 14-day administration is shown in Figure 4, which provided the first example of a systemic drug that restored CFTR activity and conferred meaningful clinical improvement in lung function (Accurso et al. 2010).

Phase 3 trials in G551D CF patients include two long-term randomized placebo-controlled clinical trials. In a trial in older children and adults (age 12 and above) (Ramsey et al. 2011), the primary end point was achieved, establishing an ~10.5% improvement in FEV1% at 24 weeks, which was sustained at 48 weeks. In addition, secondary clinical end points showed significant improvement, including a 55% reduction in the probability of experiencing a pulmonary exacerbation during the course of the study, a 3.1-kg weight gain (compared with 0.9 kg in the placebo group), and an improvement in respiratory symptoms as assessed by the CFQ-R, a patient reported quality-of-life index. Similar to the phase 2 study, sweat chloride improved to mean level below the diagnostic threshold of CF (60 mEq/L). Similar results were reported in a smaller study that enrolled pediatric G551D CF patients age 6–12. The mean improvement in FEV1 was 12.5% following 24 weeks of treatment, with similar improvement in sweat chloride. In both clinical studies ivacaftor was safe and well tolerated, and potential mechanistic-based toxicities such as secretory diarrhea were not observed.

These results have established the proof-of-concept that targeting the restoration of CFTR activity using a small-molecule potentiator is safe and feasible, and could confer clinical benefit. The degree of improvement in spirometry among participants of the phase 3 trial of ivacaftor compares favorably to that of commonly used therapies for chronic CF care, including inhaled recombinant human DNase (Fuchs et al. 1994), inhaled tobramycin (Ramsey et al. 1999), azithromycin (Saiman et al. 2003), and hypertonic saline (Elkins et al. 2006). These results provide a rationale for continued evaluation of CFTR potentiators and correctors, and for their application to CF caused by other CFTR mutations including F508del.

Although principally designed as a safety study, ivacaftor has also been tested in CF patients homozygous for F508del-CFTR. Commensurate with predictions regarding its in vitro efficacy, a 4-month placebo-controlled trial revealed no statistically significant change in FEV1 following ivacaftor treatment (Flume et al. 2011). Interestingly, sweat chloride did improve by a small amount in the ivacaftor treatment group (3 mEq reduction compared with placebo), suggesting that low levels of F508del-CFTR at the cell surface can respond to a CFTR

Table 1. Summary of selected clinical trials evaluating CFTR potentiators and correctors in CF subjects

Study	Compound	Mutation	Enrollment age	*N*	Duration	Δ FEV1% predicted	Δ In CF exacerbation
CFTR potentiator trials							
Accurso et al. 2010	Ivacaftor (VX-770)	G551D	18 yr and older	39	14–28 d	10.8% Relative improvement[a]	N/A
Ramsey et al. 2011	Ivacaftor (VX-770)	G551D	6 yr and older	161	48 wk	10.6% Absolute improvement; 16.7% relative improvement	55% Reduction
Flume et al. 2012	Ivacaftor (VX-770)	F508del homozygous	18 yr and older	140	16 wk	No significant improvement	
CFTR corrector trials							
McCarty et al. 2002	CPX	F508del homozygous	18–38 yr	37	24 h	No significant change	N/A
Rubenstein and Zeitlin 1998	4-Phenyl butyrate	F508del homozygous	14 yr and older	18	1 wk	N/A	N/A
Zeitlin et al. 2002	4-Phenyl butyrate	F508del homozygous	18 yr and older	19	1 wk	No significant change	N/A
Goss et al. 2006	Curcuminoids	F508del homozygous	18 yr and older	9	14 d	No significant change	N/A
Clancy et al. 2011	VX-809	F508del homozygous	18 yr and older	89	28 d	No significant change	N/A

[a]Pooled data at 14 days from 14-d (part 1) and 28-d (part 2) study; all changes with the 150-mg dose group.
[b]20-g Dose group.
[c]−1.8 mV Change at day 4, but not sustained at day 7; 20-g dose group.

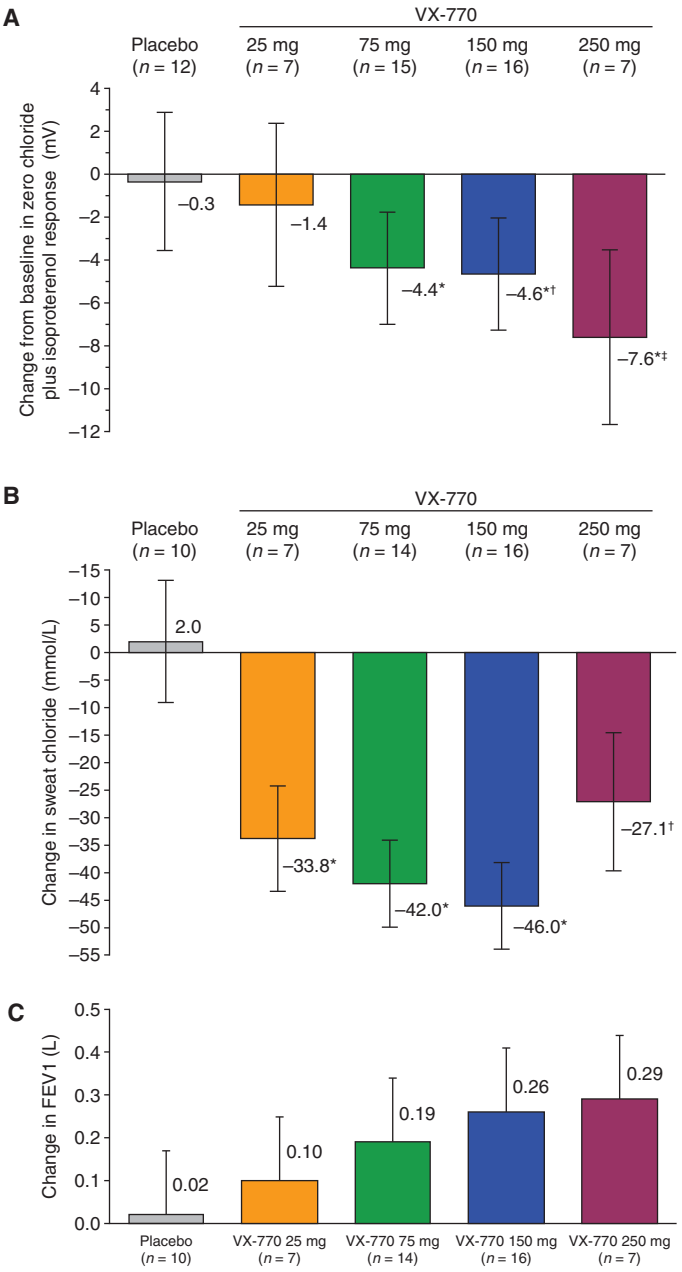

Figure 4. Results of a phase 2 clinical trial evaluating ivacaftor in G551D CF patients. Results show the relationship between CFTR activity measured by NPD: (*A*) *, $P < 0.01$ within subject; †, $P < 0.05$ vs. placebo; ‡, $P < 0.01$ vs. placebo, and sweat chloride; (*B*) *, $P < 0.001$ within subject and vs. placebo; †, $P < 0.001$ within subject and $P < 0.01$ vs. placebo with pulmonary function assessed by change in FEV1% predicted; (*C*) *, $P < 0.01$ within subject; †, $P < 0.05$ vs. placebo; ‡, $P < 0.01$ vs. placebo. (Data used in creation of figures from Accurso et al. 2010.)

potentiator, but at levels insufficient to confer clinical benefit. CFTR correctors will thus likely be necessary to restore clinically significant CFTR activity in vivo.

CLINICAL DEVELOPMENT OF CFTR CORRECTORS

A number of initial studies to establish the efficacy of correctors of F508del processing in CF patients were unsuccessful, but have advanced the clinical evaluation of CFTR modulators (Table 1). Among the first to be tested was the adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX), a corrector identified by Eidelman et al. (1992) (Guay-Broder et al. 1995). Although no efficacy was observed in a randomized multicenter trial that tested sweat chloride and nasal potential difference following 14-d administration, the study indicated the importance of standardization of biomarkers of CFTR activity, which was in its early stages at the time (McCarty et al. 2002). Subsequent efforts to characterize the activity of CFTR modulators would benefit from improvements, including standardization of sweat chloride testing using pilocarpine iontophoresis and the Macroduct collection device (Rowe et al. 2007). Similarly, nasal potential difference testing has benefited from a more rigorously standardized protocol that incorporates methodological improvements that enhance signal-to-noise ratio and allow electronic data capture for centralized and blinded interpretation (Solomon et al. 2010).

Although significant enthusiasm surrounded the initial discovery of curcumin as a putative CFTR corrector, curcuminoid derivatives did not improve CFTR activity in a two-center study that evaluated changes in nasal potential difference (Goss et al. 2006). Minimal levels of free curcumin were detected by mass spectroscopy, suggesting poor absorption could have contributed to results in the clinic, in addition to questions regarding lack of efficacy as a F508del processing corrector suggested by in vitro studies (Song et al. 2004; Grubb et al. 2006). As curcumin also shows robust activity as a CFTR potentiator (Grubb et al. 2006; Wang et al. 2007), its activity as a direct CFTR activator may have confounded initial studies that relied heavily on a murine model that expresses detectable levels of F508del-CFTR at the cell surface (Egan et al. 2004; Ostedgaard et al. 2007).

The investigational agent VX-809 represents the first corrector molecule discovered by high-throughput screening to be tested in CF patients. Its efficacy as a corrector was validated in a panel of primary human bronchial epithelial cells derived from F508del homozygotes before human testing (Van Goor et al. 2010). Although VX-809 is less active in comparison to ivacaftor in G551D HBE cells (which restored CFTR-dependent short-circuit current to ~35%–50% of wild-type levels), activity was sufficient to increase short-circuit current to ~16% of wild-type levels. In F508del homozygous subjects, VX-809 induced a dose-dependent reduction in sweat chloride, with a maximal reduction of 8 mEq/L compared with placebo (Clancy et al. 2011). However, no significant change in CFTR-dependent PD or improvement in lung function were found. Thus, VX-809 was not sufficient to confer clinical improvement in a short-term (28-d) study when used as a single agent.

Because of limitations in the efficacy of VX-809 as a monotherapy in vitro and in vivo, and the effect of ivacaftor on VX-809-treated F508del HBE cells, the use of ivacaftor in combination with VX-809 represents a natural progression to enhance restoration of CFTR activity in the clinical setting, and is currently being tested in ongoing clinical studies.

RELATIONSHIP OF CLINICAL FINDINGS TO PRECLINICAL EFFICACY MODELS

Clinical studies testing potentiators and correctors provide insight on the use and relative performance of preclinical models. Although potentiators and correctors work through different cellular mechanisms, the degree of activity detected by changes in sweat chloride generally correlates across dose groups (and pharmacologic agents) to the degree of correction observed in primary human bronchial epithelial cells. For example, when the activity of various doses of ivacaftor was tested in G551D CF subjects, NPD

and sweat chloride improved to ~30% of the activity observed in normal (non-CF) patients (Rowe et al. 2010), a level compatible with less severe pulmonary dysfunction and a nonclassic CF phenotype based on known genotype–phenotype correlations in the disease (Wilschanski et al. 2006). This also matched the level of correction observed following in vitro treatment of primary human bronchial epithelial cells derived from individuals with at least one copy of the G551D mutation. In total, these data provide reasonable guidance on the efficacy limits necessary to observe a change in clinical outcome associated with "conversion" to a more mild CF phenotype (e.g., a change in lung function) (Rowe et al. 2010), and support the utility of electrophysiological measurements on primary human bronchial epithelial cell cultures as a useful preclinical surrogate of clinical efficacy. Whether this compares favorably with testing in new large animal models of CF that recapitulate many aspects of human disease remains to be determined (Rogers et al. 2008; Sun et al. 2010).

FUTURE CHALLENGES

The rapid progress over the past decade in small-molecule corrector and potentiator therapy for CF is perhaps the most exciting recent advance in the field, as it offers the possibility of a treatment that corrects the underlying CFTR defect in the appropriate target cells. However, the limited efficacy of first-generation correctors, and the lack of understanding of their mechanism of action, mandates the need for further basic and clinical research. Additional screening efforts are indicated to identify safe and potent correctors with high efficacy. However, because of the complex, multifaceted folding and thermodynamic defects imparted by the F508del mutation, the feasibility of identifying single correctors with high efficacy in normalizing F508del-CFTR cellular processing remains unproven. New screening paradigms, perhaps targeted to specific, well-characterized defects in F508del-CFTR folding and structure, may be needed, as will consideration of combination corrector therapy, such as compounds targeted to the F508del-CFTR protein combined with agents that alter the cellular proteostasis machinery. There also remains a need for better in vitro preclinical surrogate assays of compound action, and a need to better understand the determinants of clinical efficacy and the thresholds of CFTR activation required to produce sustained clinical benefit.

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