Cystic Fibrosis: Translating Molecular Mechanisms into Effective Therapies

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Abstract

Cystic fibrosis is a genetic disease that affects approximately 75,000 individuals around the world. Long regarded as a lethal and life-limiting disease, with the most severe manifestations expressed in the progressive decline of lung function, treatment advances focusing on airway clearance and management of chronic lung infection have resulted in improved outcomes for individuals with cystic fibrosis. These advances have been realized in conjunction with an improved understanding of the genetic basis of this disease, dating back to the discovery of the cystic fibrosis gene in 1989. The identification of the cystic fibrosis gene and the advancement of our understanding of the resultant cystic fibrosis transmembrane

conductance regulator protein have led to the development of a new class of cystic fibrosis therapies designed to directly impact the function of this protein. These therapeutic developments have progressed, targeting the various mutations that can cause cystic fibrosis. These new medications, known as cystic fibrosis transmembrane conductance regulator modulators, have changed the landscape of cystic fibrosis care and cystic fibrosis research. Their demonstrated effect in patients with specific cystic fibrosis mutations has ignited the hope that such therapies will soon be available to more individuals with this disease, moving the cystic fibrosis community significantly closer to the ultimate goal of curing this disease.

Keywords: cystic fibrosis; CFTR; drug development

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In his seminal 1962 work, “The Structure of Scientific Revolutions,” Thomas Kuhn introduced the concept of the “paradigm shift” into the lexicon of the scientific community (1). The changing landscape of treatment options in cystic fibrosis (CF) stands as a contemporary example of a paradigm shift. The therapeutic advances introduced in care of the patient with CF over the last few decades have primarily targeted the clinical consequences of the genetic mutation, such as inhaled antibiotics for chronic airways infection, airway clearance techniques for the excessive inflammatory secretions obstructing the airways, and pancreatic enzyme replacement as well as nutritional support for impaired nutritional status.

Modest improvements in treatment and management were achieved, but dramatic improvement in disease outcomes could only occur by treating the basic underlying problem in the hopes of preventing those downstream consequences.

In 1989, the discovery of the CF gene provided hope that a more powerful and fundamental understanding of the disease would inevitably lead to new therapies that could revolutionize the approach to disease management. The promise of gene therapy, in which a normal copy of the CF gene could replace the function of the mutated gene, has not been realized (2). Yet study of the gene, its resultant protein, and the biologic processes mediated by this protein ultimately laid the foundation for the work

that has led to a paradigm shift in the treatment of patients with CF. Novel therapies are now available that target the basic pathophysiology of the disease, heralded in 2012 by the U.S. Food and Drug Administration’s (FDA) approval of ivacaftor for use in individuals with CF who carry the *G551D-CFTR* mutation (3). This approval marked a milestone in the advancement of CF treatment, wherein a therapy designed to improve cystic fibrosis transmembrane conductance regulator (CFTR) protein function and address the underlying cause of the clinical manifestations of CF might now fulfill the promise first inspired with the discovery of the CF gene. Ongoing research efforts have led to advances that aim to provide an even

greater therapeutic effect as well as reach a greater proportion of individuals with CF (4). The novel and emerging treatment options in CF are the direct result of the convergence of molecular genetics and advances in clinical investigation. The work that has propelled the introduction of these new therapies has simultaneously given renewed hope that CF can be transformed from a life-limiting disease to a lifelong chronic disease, possibly paving the way to an eventual cure for individuals with CF.

Pathophysiology of CFTR Mutations

There are approximately 2,000 distinct genetic mutations that cause CF (5). They all lead to the phenotypic manifestations of the disease through their impact on the CFTR protein—an epithelial chloride ion channel found in multiple organ systems and tissues. A classification system has been developed that categorizes these mutations into six groups on the basis of their impact on CFTR production, processing, or function

(Figure 1). Although the molecular mechanisms differ among the groups, the end result is a reduction in the quantity of CFTR protein, a reduction in the function of the CFTR protein, or both (6). It is the integrated result of quantity and functionality of the CFTR protein at the cell surface that accounts for the aggregate activity of CFTR, and the degree of reduction in this activity corresponds with disease phenotype and severity (7). In addition, there are other factors (e.g., genetic, environmental) that also contribute to the clinical phenotype (8).

Mutations that affect the normal transcription of the gene (class I) result in complete absence of CFTR protein. Examples include mutations (e.g., G542X) involving premature stop codons that prevent complete transcription of the gene, resulting in a truncated (nonsense) protein. Once mRNA is transcribed, it must be further edited to remove the unnecessary introns and splice the exons to form a mature mRNA ready for translation of protein. Some CF mutations (e.g., 3849 + 10 kb C → T) cause inefficient splicing of these exons (class V), resulting in little to no CFTR protein. Translation of the mRNA

creates a protein that must be subsequently processed (e.g., folded) and trafficked to its working site in the apical membrane of the cell. Mutations that result in impairment to these processes (class II; e.g., F508del, the most common mutation) also lead to little to no CFTR protein. Finally, there are mutations that result in sufficient protein trafficked to the apical membrane, but they do not function properly, such as when the gating mechanism is impaired (class III; e.g., G551D) or conductance through the channel is impeded (class IV; e.g., R117H).

To increase functional CFTR at the apical cell membrane, treatment approaches to these aberrant processes must address these mutation-specific issues. For example, mutations that result in little to no CFTR at the cell surface require a different approach from those mutations for which CFTR is present at the cell surface but does not function properly. The principal example of this latter approach is the development of ivacaftor, which was approved for the treatment of patients with the *G551D-CFTR* mutation. G551D is a gating mutation in which the protein is present in the cell

Figure 1. Classes of cystic fibrosis transmembrane conductance regulator (CFTR) mutations. Reprinted by permission from Reference 6.

membrane but the channel is essentially closed much of the time, resulting in decreased CFTR activity (9). Ivacaftor increases the probability of the channel being open, thereby increasing its activity (9). However, ivacaftor alone will not be effective in patients who have no protein at the cell surface, such as occurs with class I and II mutations. A key example is the most common mutation, F508del, in which misfolded CFTR protein is targeted for destruction and never reaches the cell surface (6). Similarly, those mutations that result in a premature stop codon, with truncation of mRNA and production of nonsense protein, lead to complete absence of CFTR at the cell surface (10). Such mutations will require different approaches that allow for the transcription of mRNA to “read-through” false stop codons and provide a complete template for translation into protein or that stabilize the processing and trafficking of CFTR to the cell membrane (6, 11, 12).

CFTR Modulator Development

The expanded knowledge of CFTR biology coincided with the technological development of high-throughput screening, which permitted the rapid assessment of large numbers of compounds as candidates to increase CFTR activity (referred to as CFTR modulators) (13). Using convenient assays for CFTR activity, large libraries of compounds on the order of thousands per day could be studied to see if they enhanced CFTR activity. Compounds that were “hits” could be further studied to understand their mechanism of action and their potential for future human trials. This approach led to the discovery of compounds that could bind to CFTR and act as 1) potentiators, increasing the function of CFTR; or 2) correctors, enabling more CFTR to reach the cell surface. In truth, the latter does not “correct” the CFTR protein, but the nomenclature has persisted. “Correctors” are better described as compounds that improve the processing and trafficking of CFTR. There is also a new class of compounds discovered through screening that lead to increased activity but do not function as either a potentiator or corrector. Rather they “amplify” CFTR production through increases in both mRNA and protein and could perhaps be used in conjunction with correctors (14–16).

Ivacaftor was identified as a compound that could increase CFTR activity and was eventually introduced into clinical development, leading to the pivotal phase III clinical trials investigating the safety and efficacy of ivacaftor in patients with at least one G551D mutation. The medication was approved based on demonstration of a sustained increase in forced expiratory volume in 1 second (FEV₁) percent predicted of 10.6% (absolute change) and a marked (55%) reduction in the rate of pulmonary exacerbations compared with subjects who received placebo (9, 17). A decrease in sweat chloride concentration was also noted, documenting the systemic activity of the drug on CFTR function (9). Ivacaftor has been shown to be efficacious in real-world settings for longer periods of follow-up. There was also improvement in nutritional parameters, since shown to be associated with normalization of small bowel pH, likely from improved bicarbonate secretion (18). Subsequent benefits have been shown in sinus disease (resolution of symptoms and radiographic improvement) and CF-related diabetes (normalization of hemoglobin A1C without insulin therapy) (19, 20). The drug has since had its labeled indications broadened to include 33 mutations within classes III, IV, and V on the basis of clinical and *in vitro* data.

The success of ivacaftor not only offered great benefit to those patients with the G551D mutation but also demonstrated that the strategy of targeting CFTR can lead to direct, measurable clinical benefits, offering hope to patients with other mutations. Individuals with at least one copy of the G551D mutation represent only 4.4% of the CF population, and the expansion of ivacaftor’s indication increases the number of eligible patients to close to 15% of the entire CF population (21, 22).

F508del is by far the most common mutation, affecting patients as either homozygotes (47%) or heterozygotes (39%) (22). As noted earlier, ivacaftor would not be expected to be effective for these patients, because they lack CFTR protein at the cell surface, a prediction confirmed in clinical trials (23). Rather, correctors are needed to address the consequences of the F508del mutation by stabilizing the folding process and allowing more CFTR protein to traffic to the cell membrane. The first of these correctors to advance through clinical trials was lumacaftor, which in preclinical studies resulted in an increase in CFTR quantity

(24). As the quantity of CFTR protein was only modestly increased, the question in clinical trials was whether that was sufficient to result in a clinically significant benefit. Because the final CFTR product transported to the apical cell membrane in response to lumacaftor is not fully functional, it was believed that ivacaftor would also be necessary to potentiate its function. The clinical trials thus examined a combination of the two drugs (25). The phase III clinical trials, TRAFFIC and TRANSPORT, demonstrated effects that were not as robust as those seen in studies of ivacaftor in subjects with G551D; the absolute increase in lung function compared with placebo was only 2.6% for lumacaftor/ivacaftor compared with 10.6% in the ivacaftor trial, and the reduction in pulmonary exacerbations was 34% compared with 55% in the previous trial. However, the improvement in lung function and reduction in the frequency of pulmonary exacerbations was sustained in the lumacaftor/ivacaftor–treated subjects when compared with placebo (26). These benefits were seen in patients homozygous for F508del, but studies of heterozygotes did not demonstrate clinical benefit (27). Subsequent to the approval of lumacaftor/ivacaftor by the FDA, real-world experience has demonstrated a longer-term sustained reduction in the rate of decline of lung function (28). Offsetting the modest benefits of this combination are the potential adverse effects of chest tightness and acute drops in lung function experienced by some patients. Although typically transient and manageable for many patients, some find these symptoms intolerable (29). Also, there are significant drug–drug interactions with lumacaftor, including antibiotics such as rifampin, antifungals such as voriconazole, antiseizure medications such as phenytoin, and hormonal contraceptives (30).

The approval of ivacaftor and lumacaftor/ivacaftor provided a CFTR modulator to close to 60% of individuals with CF. Despite the success represented by these accomplishments, significant challenges remained. Approximately 40% of patients did not have an available therapy, and the modest effectiveness of the combined corrector and potentiator approach in individuals with the F508del mutation suggested the need for more effective options.

The introduction of tezacaftor, a new corrector, addresses some of the problems

with lumacaftor. Although similar to lumacaftor in its mechanism of action, tezacaftor has a longer half-life, less potential for drug–drug interactions, and fewer side effects. A phase III study of tezacaftor/ivacaftor in individuals homozygous for F508del showed similar clinical benefits as seen with lumacaftor/ivacaftor: a 4% absolute increase in percent predicted FEV₁ and a 35% lower rate of pulmonary exacerbations (31). On the basis of these results, tezacaftor/ivacaftor was

recently approved by the FDA for use in individuals homozygous for F508del and for individuals with one copy of F08del and one of 26 residual function mutations that are known to be responsive to ivacaftor (32) (Table 1). However, it was clear that either a more potent corrector or a more effective combination of correctors was needed. Because they have similar targets in the processing of CFTR, combining lumacaftor and tezacaftor did not represent a strategy likely to succeed. However, the development

of next-generation CFTR correctors (e.g., VX-659 and VX-445), which target different parts of the F508del-CFTR protein pathway, provided additional stabilization and increased trafficking of CFTR to the cell surface. *In vitro* studies demonstrated that when these next-generation correctors were combined with tezacaftor and ivacaftor in F508del homozygous cell lines, CFTR activity was increased to greater than 50% of normal, surpassing the effect seen in ivacaftor and the G551D mutation (33). Moreover, *in vitro* studies of these compounds in cell lines with only one F508del allele demonstrated restoration of close to 50% of CFTR activity, offering hope that a triple-combination approach could provide benefit to those patients heterozygous for F508del (33). Unlike lumacaftor, tezacaftor does not cause symptoms of chest tightness or acute drops in lung function, nor does it have the same potential drug–drug interactions (29, 31). For these reasons, it is currently being studied as part of a triple-combination regimen, in conjunction with a “next-generation” CFTR corrector and ivacaftor (34–36).

There are other potential strategies to achieve the goal of increasing the quantity of F508del CFTR protein at the cell surface. Amplifiers increase both CFTR transcript and immature protein levels, potentially creating more substrate available for interaction with correctors (37, 38). Medications to block the turnover of CFTR in the cell membrane, prolonging its presence, thus far have been unsuccessful but remain a tactic to consider. Ultimately, it is likely that at least a three-drug regimen will be necessary to optimize the clinical benefit for patients with the F508del mutation.

Even if a triple-combination regimen can offer benefit to all patients who have at least one copy of F508del, and the potentiators can be used for those with responsive protein, approximately 5% of patients will not benefit from these therapies. Specifically, these strategies will be ineffective for mutations that result in complete absence of CFTR expression; 3% of patients have two nonsense mutations, or premature stop codons, and another 2% of patients have two canonical splice or deletion mutations (22). Premature stop codons will require a strategy that will allow the transcription of mRNA to “read through” the false stop and provide a

Table 1. U.S. Food and Drug Administration–approved therapies for cystic fibrosis–causing mutations

	Ivacaftor	Lumacaftor/Ivacaftor	Tezacaftor/Ivacaftor
Class I			
Class II		F508del (homozygous)	F508del (homozygous) F508del (heterozygous*)
Class III	G178R G551D G551S G1244E G1349D G1069R [†] S549N S549R S1251N S1255P		
Class IV	R117H R117C D110E D110H D1152H F1052V R347H R352Q R74W		R117C D110E D110H D1152H F1052V R74W R347H R352Q
Class V	A455E A1067T D579G D1270N E56K E193K E831X F1074L K1060T L206W P67L R1070W R1070Q S977F 711 + 3A→G 2789 + 5G→A 3272-26A→G 3849 + 10kbC→T		A455E A1067T D579G D1270N E56K E193K E831X F1074L K1060T L206W P67L R1070W S977F 711 + 3A→G 2789 + 5G→A 3272-26A→G 3849 + 10kbC→T
Class VI			
Other	S945L [‡]		S945L [‡]

*Approved for patients with one copy of the F508del mutation in combination with a mutation known to be responsive to ivacaftor (responsive mutations are listed in the table).

[†]Also believed to have a trafficking defect resulting in less cystic fibrosis transmembrane conductance regulator.

[‡]Multiple effects on the cystic fibrosis transmembrane conductance regulator protein.

complete template for translation into protein. Ataluren, a drug that works by this mechanism, was tested in phase III clinical trials but did not ultimately achieve its aims (12). There are, however, ongoing efforts to develop drugs that read through nonsense mutations, and the Cystic Fibrosis Foundation continues to dedicate substantial resources to these drug discovery efforts.

Study Design and Endpoint Challenges

Designing clinical trials that can demonstrate safety and efficacy can be challenging, as clinical endpoints are needed that are meaningful, but traditional endpoints may no longer be relevant. For example, it may be difficult to demonstrate improvement in lung function in an otherwise healthy child with CF (39, 40). In addition, there are some patients whose mutations are so rare it would be impossible to run a clinical trial in the usual sense, because there are not sufficient numbers of patients to participate. The FDA, acknowledging the barriers to conducting traditional clinical trials for patients with relatively rare CF mutations, has endorsed “an alternative approach based on precision medicine” that permits use of *in vitro* assays as a surrogate to clinical trials (*see below*) (41, 42). Although not endorsing a particular

approach, the FDA’s openness to the need for alternative strategies for testing therapies in individuals with rare mutations has led to a number of cell-based models that have been used.

The search for a biomarker that could identify patients who would respond to the various combinations of CFTR modulators has been somewhat elusive. Sweat chloride concentration, long the gold standard in the diagnosis of CF, has been used as a surrogate marker of CFTR activity, both at baseline and in response to CFTR modulator therapies. However, the variability in sweat chloride measurements (both within and between subjects), a poor correlation between sweat chloride changes and clinical endpoints, and difficulty in establishing a clear threshold value that corresponds to clinical endpoints, make sweat chloride concentration a rather imperfect biomarker (43, 44). A novel approach has been to develop *in vitro* cell-based models that could be assayed for CFTR activity. This method was deemed acceptable by the FDA as they expanded the indication of ivacaftor to additional mutations on the basis of results from studies using Fischer rat thyroid cells, rather than clinical data derived from actual patients (45). In addition, stem-cell culture from patients can be used to create a reliable *in vitro* assay of drug response. Such cultures are being used to create intestinal organoids, which can show a real-time response to CFTR modulator therapy (46). These

responses have been correlated with *in vivo* indices of drug response, such as changes in lung function and sweat chloride concentration (47). The ongoing development of these *in vitro* biomarkers, derived directly from patients with CF and showing a clear relationship with clinical endpoints, may allow for the expansion of CFTR modulator therapy in keeping with the FDA’s recognition of alternative approaches on the basis of precision medicine.

Future Challenges and Opportunities

A number of challenges remain in the development of CFTR modulators: 1) more potent and effective modulators are needed; 2) patients will need to be treated early in the course of the disease, before the complications of CF are established; and 3) the agents need to be safe and affordable for what is likely a lifetime of therapy. Despite these challenges, the success realized in the development of these therapies has provided new optimism that the advancement of CF research will continue to push toward a new paradigm in which CF is no longer a life-limiting disease. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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