

Personalized Medicine in Cystic Fibrosis

Dawning of a New Era

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Life expectancy in cystic fibrosis (CF) has improved substantially over the last 75 years, with a median predicted survival now approaching 40 years. This improvement has resulted largely from therapies treating end-organ manifestations. In an effort to develop drugs that would target the underlying defects in the CF transmembrane conductance regulator (CFTR), the Cystic Fibrosis Foundation embarked on a bold initiative in which it established collaborations with biopharmaceutical companies to support early-stage efforts to discover new medicines for CF. This has led to the development and clinical trial testing of several novel drugs targeting specific CFTR mutations. One drug, ivacaftor, was recently approved by the US Food and Drug Administration for the approximately 4% of patients with CF who have the G551D gating mutation. Drugs targeting F508del CFTR and premature termination codons, which would be applicable to 90% of patients with CF, are undergoing clinical trials. The impact of such drugs on CFTR biomarkers, such as sweat chloride and nasal potential difference, suggests that they may reset the clinical trajectory of CF, but their effect on long-term outcomes will remain unknown for many years. Nevertheless, development of CFTR-targeted drugs represents an important milestone in CF, perhaps revolutionizing the care of these patients in a fundamental way.

Keywords: CFTR; corrector; mutations; potentiator; suppressor

On January 31, 2012, the US Food and Drug Administration approved a novel compound, ivacaftor, for patients with cystic fibrosis (CF) with the G551D mutation. Ivacaftor's approval represents the first tangible product of an innovative collaboration between the Cystic Fibrosis Foundation (CFF), industry, and academia and is a milestone for a disease in which prognosis has improved steadily. Increased life expectancy until now has resulted from therapies derived from improved understanding of relationships between cystic fibrosis transmembrane conductance regulator (CFTR) gene defects and organ pathophysiology. Ivacaftor represents a therapy that targets the underlying cause of CF, defective proteins derived from mutations in the *CFTR* gene. Although ivacaftor provides compelling evidence for therapeutic efficacy based on genetic targets, much work remains to achieve the goal of a normal life expectancy for all patients with CF. In this perspective, we discuss the history of CF, the context in which

ivacaftor was developed, and the potential impact of this and other CFTR-active drugs on long-term outcomes.

CYSTIC FIBROSIS—HISTORICAL ASPECTS

Recognition of characteristic CF symptoms and their relationship to mortality has existed for centuries. References in medieval folklore spoke of premature death for an infant that tastes "salty" (1). Alonso y de los Ruyzes de Fonteca, professor of medicine at Henares in Spain, wrote that it was known that fingers tasted salty after rubbing the forehead of a bewitched child (2). Various other reports associated defective pancreatic function with steatorrhea and meconium ileus (3). Then, early in the 20th century, reports appeared linking lung disease with steatorrhea. In 1938, Dorothy Andersen coined the phrase "cystic fibrosis of the pancreas" in describing the autopsy of an infant (4). In 1953, di Sant'Agnes published that excessive salt loss in sweat was the explanation for heat prostration in several patients with CF (5). This observation led to the development of the sweat test, the most common and accurate test to make a CF diagnosis. Identification of the CFTR gene was reported simultaneously in 1989 by teams at the University of Michigan and Toronto (6–8). More than 1,900 mutations in CFTR have since been reported, although a handful of mutations account for the vast majority of patients with CF (9). The discovery that CFTR mutations cause CF created excitement among patients with CF and caregivers regarding an imminent "cure" by gene therapy. That promise remains unfulfilled, but recent advancements to improve mutant CFTR function offer the possibility of changing treatments from downstream, symptom-driven therapies to those that target the root cause of CF.

CFTR MUTATION NOMENCLATURE—WHAT'S IN A NAME?

After the discovery of the gene, CFTR was identified as a member of the traffic ATPase family and a chloride channel (6, 8, 10). Subsequently it was shown to transport other anions (e.g., thiocyanate and bicarbonate) and to regulate ion transporters such as the epithelial sodium channel (ENaC) and alternative chloride channels (11–14). This work provided a new perspective for relationships between CFTR mutations and the molecular mechanism of their associated dysfunction. The CFTR Mutation Class system (Table 1 and Figure E1 in the online supplement) (14) helped to categorize the myriad CFTR mutations into groupings with similar manifestations and to define strategies to restore CFTR function based on mutation-specific defects. Members of classes I to III typically have minimal function, whereas members of classes IV and V retain partial function and are usually associated with lower sweat chloride and pancreatic sufficiency. Class I mutations produce biosynthetic defects, including premature termination codons (PTCs) and frameshift

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TABLE 1. CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR GENE MUTATION CLASS SYSTEM

Mutation Class	Nature of Defect	Examples	Pancreatic Function*	Modulator Strategy	Agents in Development
I	Biosynthesis	G542X	Insufficient	Suppressor Suppressor + potentiator?†	Ataluren
II	Trafficking	F508del	Insufficient	Corrector Corrector + potentiator	VX-809 + ivacaftor‡ VX-661 + ivacaftor‡
III	Channel gating	G551D	Insufficient	Potentiator	Ivacaftor (FDA approved, G551D) Other gating mutations?†
IV	Channel conductance	R117H	Sufficient	Potentiator?†	No clinical data available
V	Low transcript levels	3849+10kb C→T	Sufficient	Potentiator?†	No clinical data available

Definition of abbreviations: FDA = U.S. Food and Drug Administration.

*When *in trans* with a nonfunctional CFTR mutation.

† Theoretical approach based on nature of defect and known drug mechanism.

‡ Currently in phase II trials.

mutations that result in low levels of truncated and/or dysfunctional CFTR proteins. Class II mutations have folding or maturation defects, including the common F508del CFTR. They result in little detectable CFTR at the plasma membrane. Class III mutations reach the plasma membrane but have gating defects that limit channel opening (such as G551D CFTR). Class IV mutations produce CFTR channels that reach the plasma membrane but have low chloride conductance. Class V mutations typically result from splicing defects that reduce levels of wild-type CFTR at the cell membrane. It should be noted that among the more than 1,900 CF-causing mutations, detailed understanding of their effect on protein function is known for relatively few. It has also become apparent that the behavior of mutations is not restricted within one mutation class. For example, F508del CFTR has trafficking defects, reduced open channel time, and a shortened membrane half-life (15, 16). These observations support the concept of combining modulators to maximize CFTR level and function.

IMPROVED SYMPTOMATIC TREATMENTS TRANSLATE INTO IMPROVED LIFE EXPECTANCY

Over the last 75 years, CF life expectancy has increased from months to nearly 38 years (17, 18). A newborn with CF today may expect to live into the fifth or sixth decade (17). The reasons for improved life expectancy are varied and are summarized concisely in a recent review (19) (Figure 1). Pancreatic replacement enzymes (20) and mucus clearance techniques (i.e., chest physiotherapy) were important early treatments to extend longevity in childhood. Therapies targeting malnutrition, infection, inflammation, and mucus rheology have contributed significantly to improved outcomes. Furthermore, use of systemic antibiotics targeting *Staphylococcus aureus* and *Pseudomonas aeruginosa* have been associated with substantial improvement in survival (21). Additionally, advancements in airway clearance techniques/devices (22) and availability of inhaled tobramycin (23), recombinant human DNase (24), azithromycin (25), and hypertonic saline (26) have been key contributors. Hand-in-hand with these advancements, development of specialized CF care centers with dedicated team members has driven life expectancy up (27). More recent developments such as inhaled aztreonam (28) and emphasis on quality improvement (29) should push life expectancy higher.

TARGETING THE BASIC DEFECT

Notable by its absence in therapies contributing to improved survival in CF is gene therapy. Despite initial hope after discovery of CFTR, harnessing gene therapy for CF has proven so far to be

intractable. Initial trials based on viral or nonviral vectors did not translate into clinical benefits due to factors such as low expression of CFTR transgene and/or induction of immune responses (30, 31). Nevertheless, ongoing trials using cationic liposomes and plasmid DNA vectors potentially offer a genotype-independent therapy to all patients with CF (32).

Due in part to the initial failure of gene therapy, the CFF embarked on a bold initiative in 1998 in which it established collaborations with biopharmaceutical companies to support early-stage efforts to discover new medicines for CF. This program was likely the first and most successful example of venture philanthropy, whereby a nonprofit organization and a public company work together to address a common goal. To date, the CFF has invested more than \$300 million in its venture to develop novel drugs targeting mutant CFTR and continues to leverage early-phase CF-related research through this paradigm.

Two pharmaceutical companies have undertaken separate high-throughput screening efforts that addressed three different CFTR mutation targets, including (1) suppression of premature termination codons (PTC Therapeutics, South Plainfield, NJ), (2) “potentiation” of mutant CFTR at the plasma membrane (e.g., G551D CFTR, Vertex Pharmaceuticals, Cambridge, MA), and (3) “correction” of CFTR trafficking defects (F508del CFTR, Vertex Pharmaceuticals). Before and in parallel to these efforts, proof-of-concept studies in independent laboratories demonstrated that mutant CFTR function could be improved by a variety of small molecules in preclinical model systems and *in vivo* (16, 33–36).

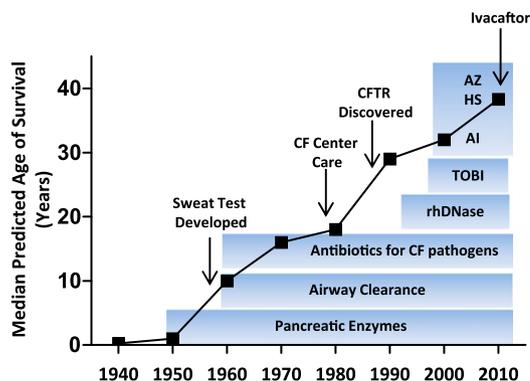


Figure 1. Survival in cystic fibrosis (CF). CF survival over time (line), associated CF therapies (bars), and milestones (arrows). Since CF was first described, steady increases in median survival have been associated with the introduction of new therapies and changes in the delivery of care. AI = inhaled aztreonam; AZ = azithromycin; CFTR = CF transmembrane conductance regulator; HS = hypertonic saline.

CFTR POTENTIATORS—OPENING MUTANT CFTR CHANNELS

Potentiators are CFTR modulators that increase the time CFTR spends in the open channel configuration, with increased chloride transport. Ivacaftor, the first potentiator to gain US Food and Drug Administration approval, is used for patients with CF with the G551D mutation (33). Although it is the second most common cause of CF, G551D is present in only about 4% of patients with CF (9). Ivacaftor has been studied in one phase II (37) and two pivotal phase III trials (38) (NCT00909727). In all three studies, ivacaftor treatment led to rapid, dramatic, and sustained improvements in FEV₁ and biomarkers of CFTR function (e.g., sweat chloride values below the 60 mmol CF diagnostic threshold, and moving nasal ion transport characteristics out of the CF range). In the larger phase III trial, ivacaftor-treated patients demonstrated highly significant improvements in (1) absolute FEV₁ percent predicted (increase > 10%), (2) weight (increase > 2.5 kg), (3) the respiratory domain of the CF Questionnaire Revised (CFQ-R) instrument, and (4) disease stability (reduced pulmonary exacerbations by >50%). Similar findings were seen in the phase III trial of patients aged 6 to 11 years, including absolute FEV₁ percent predicted improvement of greater than 12% and weight gain of 1.9 kg (NCT00909727). The responses in younger patients with CF were particularly striking, confirming the suspicion of “silent” disease in seemingly normal patients with CF and providing support to the notion that early disease is reversible (and potentially preventable). It remains to be seen if patients with other gating mutations can benefit from ivacaftor, but preclinical studies indicate chloride transport of other CFTR gating mutations can be increased by ivacaftor (39).

PTC SUPPRESSION AND CORRECTORS OF CFTR TRAFFICKING—GETTING MORE CFTR TO THE PLASMA MEMBRANE

Approximately 10% of patients with CF possess a PTC mutation, and nearly 90% of patients with CF possess F508del CFTR. Thus, modulators that target PTCs and F508del CFTR could benefit the majority of patients with CF. Unfortunately, mutations caused by PTCs and F508del are both tough nuts to crack, encompassing several unique and shared challenges at the cellular level, including inherent variability across numerous mutations (PTCs), the requirement that drugs restore translation (PTC suppressors) and trafficking (PTC suppressors and F508del correctors), and the need for functional protein that is delivered to the plasma membrane (suppressors and correctors).

Suppressors of Premature Termination Codons

PTCs result when single base-pair substitutions create an erroneous stop codon within the open reading frame of a gene. Suppressors of PTCs, such as aminoglycoside antibiotics, are able to bind eukaryotic ribosomes and cause the insertion of a near cognate amino-acyl tRNA into the ribosomal A site (40). This process can allow the ribosome to “readthrough” the PTC and produce some full-length protein that is derived from PTC-containing mRNA. This approach has been extensively tested in proof-of-concept studies using aminoglycosides to suppress PTCs (gentamicin, amikacin, geneticin) (40) and has had demonstrated efficacy *in vitro*, in animal models of CF and muscular dystrophy, as well as in small numbers of patients with CF. Currently, there is one oral compound (ataluren; PTC Therapeutics) in clinical trials to treat CF caused by PTCs. It possesses no antibacterial properties but does bind eukaryotic ribosomes to suppress PTCs in cell- and animal-based disease

models. Ataluren was studied in three phase II, randomized, dose-ascending, open-label trials in CF. Each study demonstrated short-term tolerability of ataluren, and two demonstrated improvements in CFTR function (across a number of PTC mutations) as measured by the nasal potential difference (NPD). One study also demonstrated improvements in CFTR localization to the nasal cell membrane, whereas another demonstrated improvement in cough over 3 months (34, 41). The third study failed to demonstrate improvements in NPD, and all three studies were limited by small numbers and absence of placebo groups (42).

Ataluren is currently being assessed in a large phase III randomized, double-blind, placebo-controlled trial. The study is for 48 weeks and evaluates safety, efficacy, and tolerability of a single dose group compared with placebo (NCT00803205). The results of this exciting trial are anticipated soon.

Correctors of F508del CFTR

The F508del mutation disrupts folding of nascent CFTR, causing retention in the endoplasmic reticulum and subsequent proteasomal degradation (14). The result is minimal protein escaping intracellular degradation. CFTR correctors increase F508del CFTR protein at the plasma membrane, and Vertex Pharmaceuticals has developed two F508del correctors that have advanced to clinical trials. One F508del CFTR corrector (VX-809, lumicaftor) has been assessed in a multicenter, randomized, double-blind, placebo-controlled trial in adults homozygous for F508del CFTR. Eighty-nine subjects were enrolled to evaluate safety and efficacy of 28 days of VX-809 treatment compared with placebo across four doses (43). The safety profile of VX-809 was similar to placebo, and the VX-809 group had a modest, dose-dependent reduction in sweat chloride. Although no other biomarkers or outcome measures improved, the results demonstrated that F508del CFTR was a viable drug target, laying the foundation for combination trials with a potentiator. Indeed, *in vitro* results of VX-809 combined with ivacaftor have reported increased F508del CFTR activity relative to VX-809 alone (as assessed by chloride currents in primary human bronchial epithelial cells) (44). Ivacaftor has also been assessed as a monotherapy in F508del CFTR homozygous subjects, building off of the premise that some of these patients may have low levels of F508del CFTR at the plasma membrane. In a 3-month, double-blind, placebo-controlled study, ivacaftor did modestly reduce sweat chloride values relative to baseline and placebo values (<3 mmol, $P < 0.05$), but did not improve clinical efficacy measures (45). In addition, a second F508del CFTR corrector (VX-661) has also entered clinical trials in F508del CFTR homozygous adults, which will assess treatment with VX-661 alone and in combination with ivacaftor in a dual dose ascending study format (NCT01531673). Together, it is hoped that these studies will provide sufficient data regarding safety, tolerability, pharmacokinetics, and bioactivity to support phase III studies that target the most common cause of CF.

Although the effect of rare CFTR mutations on protein function is often unknown, it is possible that additional patients could benefit from ivacaftor, VX-809, or other CFTR-targeted drugs. Thus, it will be important to identify additional endpoints that quantitatively assess CFTR function. Ongoing studies are assessing emerging CF biomarkers such as lung clearance index, hyperpolarized helium ventilation by magnetic resonance imaging, sputum biomarkers, mucus rheology and clearance, and intestinal pH (NCT01521338, NCT01161537, NCT01262352). Many of these endpoints may eventually be useful in future studies testing CFTR modulators, particularly in young patients or patients with milder disease status.

CFTR-TARGETED DRUGS, LONG-TERM OUTCOMES AND COST

A critical unanswered question in the CF care community is the impact of CFTR-targeted drugs on long-term outcomes. One can, however, attempt to gauge the impact of CFTR-targeted drugs by examining relationships between residual CFTR function and associated clinical phenotypes. Obligate heterozygotes (i.e., parents of patients with CF) have half the amount of CFTR relative to noncarriers and essentially an indistinguishable phenotype and similar *in vivo* CFTR function (sweat chloride, NPd). At the other end of the spectrum, patients with CF with two severe CFTR mutations have little if any detectable CFTR function, with sweat chloride values near 100 mmol/L. Increasing levels of CFTR function are found in patients with pancreatic-sufficient CF (PS-CF), in patients with congenital bilateral absence of the vas deferens and partial function CFTR mutations, and possibly other disorders (e.g., chronic idiopathic pancreatitis) (46). If sweat chloride values can be used as a general guide to relate CFTR restoration to phenotype across these patient groups, then biomarker benchmarks that might translate into outcomes can be defined. Using these benchmarks, one might predict that moving sweat chloride values into the mid-70s would approximate the PS-CF phenotype, and mid-50s the congenital bilateral absence of the vas deferens phenotype (47). The outcomes of these patient groups are drastically different than pancreas-insufficient CF, with age of death a decade later for PS-CF (48) and survival that is indistinguishable from the general population for patients with congenital bilateral absence of the vas deferens. Unfortunately, sweat chloride changes in response to ivacaftor treatment have not been directly predictive of improvements in FEV₁, and the long-term effects of ivacaftor treatment will likely be highly dependent on lung function before beginning therapy and rate of FEV₁ decline. Furthermore, patients with CF with partial CFTR function are not disease free and can develop bronchiectasis with chronic sinopulmonary infections, diabetes, and other end-organ manifestations. Thus, treatment of residual disease will likely remain a mainstay of CF care when CFTR modulators are available, and biomarker benchmarks that approximate those observed in obligate heterozygote carriers of CFTR mutations may be more appropriate long-term therapeutic goals.

The annual cost of ivacaftor treatment is approximately \$300,000 in the United States. Because roughly 1,000 G551D patients with CF will be immediate candidates for the drug, the immediate public health impact of its cost is likely to be limited. The impact on other public health systems outside of the United States could be much more significant, however, where healthcare budgets are fixed in the public sector. Indeed, a recent editorial discussed this concern in European countries, where Vertex is currently seeking European Union approval for ivacaftor (49). Furthermore, if a drug targeting F508del CFTR is successful and approved, up to 90% of patients with CF could be candidates, with potentially much larger effects on healthcare economics. Speculating the long-term societal costs of these agents is highly complex, as a number of variables require consideration, including pricing in a larger CF market, the reduction in healthcare costs of treated patients (e.g., reduced pulmonary exacerbations and potential reduction in standard expensive treatments), and increased economic contributions of working CF individuals to the workforce. At the patient level, ivacaftor improves how patients feel, which is arguably the most immediately meaningful clinical efficacy outcome and is impossible to quantify in economic terms. Evaluation of ivacaftor's effects on these parameters should help elucidate societal and individual cost/benefit ratios of CFTR modulators.

CONCLUSIONS

New modulators of mutant CFTR target proximal events in CF disease pathogenesis, potentially revolutionizing CF care. Although exciting results have been reported, a number of critical questions remain unanswered, including whether restoring CFTR function offers long-term benefits, what aspects of CF disease can be reversed or prevented by modulator treatment, and whether strategies that target PTCs and F508del CFTR have sufficient potency to impact established clinical efficacy measures. Indeed, the results of the ivacaftor studies in patients with G551D CFTR CF have set high expectations for future modulator strategies. CF researchers and care providers are likely to be challenged by unprecedented questions, such as prioritization of established therapies, management of patient and family expectations, and development of a knowledge base to select modulators for patients with CF harboring mutations with limited pathophysiologic information. In any event, with the advancement of CFTR modulator therapies, the next several years could be transformative for CF care, heralding an era in which therapeutic choices are driven by personalized genetic information.

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

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