Cystic Fibrosis and Its Management Through Established and Emerging Therapies

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Abstract

Cystic fibrosis (CF) is the most common life-shortening autosomal recessive disorder in the Caucasian population and occurs in many other ethnicities worldwide. The daily treatment burden is substantial for CF patients even when they are well, with numerous pharmacologic and physical therapies targeting lung disease requiring the greatest time commitment. CF treatments continue to advance with greater understanding of factors influencing long-term morbidity and mortality. In recent years, in-depth understanding of genetic and protein structure-function relationships has led to the introduction of targeted therapies for patients with specific CF genotypes. With these advances, CF has become a model of personalized or precision medicine. The near future will see greater access to targeted therapies for most patients carrying common mutations, which will mandate individualized bench-to-bedside methodologies for those with rare genotypes.

INTRODUCTION

Cystic fibrosis (CF) is the most common life-shortening autosomal recessive disorder in the Caucasian population (154) and is also well described, albeit at lower frequencies, in Hispanic, African American, and Middle Eastern ethnicities (158). The carrier frequency for CF is approximately 1 in 30 in the United States, with a birth rate of approximately 1 in 3,500. Globally, more than 70,000 people are currently living with CF, with the largest numbers of identified patients living in the United States (~30,000 in 2012) and Europe (~32,000 in 2010) (37, 54). The clinical features of CF were first described in 1938 by Dorothy Andersen (7), and the term cystic fibrosis was used to describe the pathology observed in the pancreas. Various other reports also described pancreatic dysfunction, steatorrhea, meconium ileus, and infant mortality (61, 151). Recognition of other characteristic CF symptoms and their relationship to mortality was also reported well before the twentieth century. Medieval folklore described the early demise of infants that tasted "salty" (23). Juan Alonso y de los Ruyzes de Fontecha, a professor of medicine at Henares in Spain, reported that fingers tasted salty after rubbing the forehead of a bewitched child (3). Efforts to isolate the causative gene localized it to chromosome 7 by the mid-1980s (117), and the cystic fibrosis transmembrane conductance regulator (CFTR) gene was fully sequenced in 1989 (71, 115, 116). Subsequently, the identification of both disease-causing and benign CFTR mutations has been extensive, with 2,006 known mutations described as of 2015 (66). Illustrating the efforts to centrally compile and assign functional and clinical data from CF patients with common and uncommon mutations, the Clinical and Functional Translation of CFTR (CFTR2) project had systematically collated mutation and phenotype data from 88,664 individuals as of August 2015 (35).

From the time of the first formal description of CF, advancements in symptom-based therapies have led to steady improvements in outcomes. These include development of CF care centers; advances in airway clearance techniques; and use of pancreatic replacement enzymes, systemic and topical antibiotics targeting CF-related lung infections, mucolytics, airway hydrators, and anti-inflammatories (119). During the 1990s, major efforts undertaken by numerous groups sought to develop gene replacement strategies to provide patients with normal, fully functional CFTR. Unfortunately, this approach proved to be difficult, with the researchers encountering significant barriers such as gene delivery, lack of persistent expression, and immune responses that limited repeat dosing (74, 77, 101, 120, 155, 162). In the early 2000s, a different tactic was undertaken: the development of small molecules that target specific protein defects associated with CFTR mutations. This approach was possible because of the large foundation of knowledge that assigned different CFTR protein defects to unique CFTR mutations (114). This class of medicines, known as CFTR modulators, was first reported in CF clinical trials in 2010 (1). The first medication to target a specific CF mutation received regulatory approval in 2012, with numerous ongoing efforts focused on genotype-specific therapies evolving in subsequent years. CF was also cited by US president Barack Obama in the 2015 State of the Union address as an example of success in "a new era of medicine—one that delivers the right treatment at the right time," a concept termed personalized or precision medicine (102). In this review, we summarize established CF therapies, describe various types of CFTR mutations, and discuss the development of CFTR modulators that target the underlying cause of CF rather than downstream symptoms. It is hoped that these drugs will be transformative in CF care and that the large investment in time, money, and patient participation will translate into novel therapies that help CF patients truly realize the hope and promise of personalized medicine.

CFTR PROTEIN FUNCTION AND ITS RELATIONSHIP TO DISEASE

CF is an autosomal recessive disorder caused by mutations in the CFTR gene. The CFTR protein is an ion-conducting channel that is a traffic ATPase, a protein family characterized by two transmembrane domains that secure the protein within the plasma membrane and two cytoplasmic nucleotide-binding domains. These nucleotide-binding domains form a heterodimer complex that binds and hydrolyzes ATP, a process that results in gating or regulation of the ion-channel function (114–116). CFTR also has a regulatory R domain that provides protein kinase A–dependent regulation (28). Functionally, CFTR conducts primarily chloride and bicarbonate but can also potentially conduct other small molecules, including glutathione and thiocyanate. It also provides regulation to the epithelial sodium channel (ENaC) and the outwardly rectified chloride channel (8, 67, 86, 87, 99, 119, 130, 138, 139, 156). CFTR is expressed in many tissues, reflected in the numerous disease manifestations associated with CFTR deficits. These include the airways, the sweat gland, the small and large intestine, the pancreas and biliary tree, and the vas deferens. In addition, recent studies have reported CFTR expression and defects in other organs and tissues, including the central nervous system, leukocytes, smooth muscle, and cartilage tissues of the large airway (12, 17, 113). In the lung, CFTR expression is highest in airway submucosal glands, but it is also present in pseudostratified epithelia of medium and large airways as well as cells lining the small airways (52, 53).

Understanding how loss of CFTR function contributes to pulmonary disease is a critical question in the field, as the vast majority of disease morbidity and mortality results from CFTR deficits in the lung. The impact of CFTR dysfunction in the lungs includes impaired mucus clearance, chronic infection with unique pathogens, and an exaggerated and chronic inflammatory response. Mechanistically, several models have been developed to explain how CFTR dysfunction at the cell and tissue levels translates into clinical disease manifestations. These can be broadly segregated into defects of (*a*) mucus hydration, (*b*) mucus formation and structure, and (*c*) cellular signaling defects that drive inflammation.

Several studies have suggested that abnormalities of the lung airway surface liquid (ASL) volume and mucus hydration are fundamental to disease pathology. A subcomponent of the ASL is the periciliary liquid layer (PCL), which normally approximates ciliary height and facilitates normal mucociliary clearance (20, 92, 93). ENaC is the primary regulator of sodium flow in the airways, with regulated chloride secretion provided via CFTR to increase ASL fluid volume (84, 140), leading to passive flow of water in response to ion flux (20, 46, 47). The specific CFTR and ion transport abnormalities that produce disease pathology are still under intense investigation. Defective CFTR function leads to reduced chloride transport, increased ENaC activity, and sodium resorption in human airway cells ex vivo (20), decreasing the PCL volume and producing a relatively greater proportion of solids in the ASL. The result is mucus stasis and airway obstruction, creating an environment conducive to inflammation and infection.

An alternative hypothesis has recently been described in CFTR-knockout piglets, in which ENaC activity is retained and ASL/PCL volume is not decreased. In this model, bicarbonate flux through CFTR is viewed as central to disease pathogenesis. Normal bicarbonate transport appears to be important for normal mucin formation in tissues, including in the airways (6, 63), and altered pH from reduced bicarbonate transport may limit the bactericidal activity of antimicrobial proteins within the ASL innate immune system (105). More recent studies have suggested that release of large mucus strands from submucosal glands may be defective, providing a nidus to establish infection (65). Finally, studies indicate that the inflammation in CF airways is out of proportion to that which is expected, even in the absence of infection, with high levels of neutrophils and cytokines present (13, 75, 122). This may be due to defects inherent to the epithelium or to

leukocytes that result from a loss of CFTR function (62, 106, 121). Alternatively, overexpression of ENaC in a mouse model produces mucus dehydration, airway plugging, and spontaneous airway influx of neutrophils (163). These results suggest that mucus abnormalities and dehydration secondary to CF ion transport defects may be sufficient to impart a proinflammatory phenotype. Upregulation of mucus production in the airways is also sufficient to impair clearance of pathogens (26, 27).

Whether CF lung disease results primarily from mucus dehydration, inherent mucus composition, excessive mucus production, cellular defects that promote inflammation, or a combination of these contributors remains an area of intense debate and research. Answers to these questions will help guide future interventions that target the root causes of CF organ disease manifestations.

CYSTIC FIBROSIS CLINICAL MANIFESTATIONS AND TREATMENTS

CF is a systemic disease involving multiple organ systems that largely parallels the distribution of CFTR expression throughout the body. The concept of increased mucus viscosity and inspissation of secretions encompasses many of the manifestations of CFTR dysfunction in end organs, including the sinuses, lung, pancreas, small and large intestines, liver, and vas deferens. Pulmonary complications resulting from CF currently account for up to 85% of CF mortality (57). CF pulmonary disease begins early in life and is characterized by progressive airway obstruction, as measured by the expiratory volume over the first second of a forced exhalation (FEV_1). Que et al. (108) reported that CF patients had a 2–3% absolute decline in FEV_1 on average per year, although recently rates of decline appear to have slowed considerably with the advent of improved therapies.

Bronchiectasis, an irreversible dilation and scarring of the airways, is a hallmark of CF lung disease. It can occur in the absence of clear symptoms, and as monitored by computerized tomography (CT), it has been reported in up to one-third of CF patients by the age of 3 years (134). This highlights the importance of early diagnosis, disease monitoring, and intervention in young children with CF to prevent irreversible lung damage.

The standard management of CF lung disease includes daily airway clearance of retained mucus, treatment of lung infections (through either acute interventions targeting new infections or cycled inhaled antibiotics to manage chronic airway infections), use of mucolytics such as dornase alfa to thin DNA-laden mucus secretions, inhalation of mucus hydrators such as hypertonic saline to thin secretions and enhance clearance, and oral dosing with anti-inflammatories to suppress the chronic neutrophilic airway inflammation characteristic of the CF airway (46, 50, 59, 76, 110, 123, 124, 142). These treatments are effective but cumbersome, and Sawicki et al. (127) have estimated that CF adults spend nearly two hours daily on routine treatments (focused largely on lung disease management) when well. In addition, CF lung disease is punctuated with acute pulmonary exacerbations, in which increased pulmonary symptoms require extra interventions (such as oral or intravenous antibiotics, increased airway clearance, and hospitalization) to restore lung function. Pulmonary exacerbations are now appreciated as sentinel events in CF, as up to 25% of patients fail to restore lung function to the pre-event baseline (125, 126).

Approximately 85% of CF patients have exocrine pancreas insufficiency, with diminished or absent delivery of digestive enzymes to the small intestine. The endocrine pancreas is also frequently involved as CF patients age, which is manifest initially as glucose intolerance and eventually as CF-related diabetes (37). The earliest presentation of CF can be meconium ileus (occurring in approximately 10% of CF patients), with intestinal blockage by inspissated luminal secretions in the fetus or newborn that can be life threatening and require surgical management. In older patients, distal intestinal obstructive syndrome can also occur, producing partial or full bowel obstruction.

This is generally managed via cathartic therapies but can become dominant and challenging for daily care. The vast majority of males with CF are infertile owing to interruption of formation of the vas deferens, although spermatogenesis itself remains intact (45). Chronic sinusitis is also common in CF and, although not a leading cause of mortality, may be burdensome and require numerous surgical interventions (111). Reduced bone mineral density affects more than half of CF patients and can result from vitamin D deficiencies, malnutrition, chronic inflammation, and possibly direct effects on osteoblast/osteoclast balance, which increases in prevalence with age (9, 36). Other deficiencies of fat-soluble vitamins (A, E, and K) can occasionally be clinically manifest in CF and are managed with chronic daily vitamin replacement therapy. Severe liver involvement is relatively uncommon, but 1–2% of patients develop severe cirrhosis, portal hypertension, and ultimately liver failure that can necessitate liver transplantation (136).

Despite the variety and seriousness of the consequences associated with CF, life expectancy has increased dramatically over the past several decades. In 1938, 70% of CF patients died by 1 year of age (7). With the systematic application of evidence-based therapies, the US median predicted survival rose from ~31 years in 2002 to ~41 years as of 2013 (37). Therapies throughout this period targeted the pathologic downstream disease manifestations that develop as a consequence of CFTR dysfunction. Pancreatic insufficiency is treated with enzyme replacement therapy along with close monitoring of growth and nutritional parameters, with caloric and nutrient supplementation as needed (18, 137). Even with enzyme replacement, the required caloric intake can be up to 200% of that required for a comparable non-CF individual (137), and tube feedings, via either a nasogastric or surgically placed gastrostomy tube, are necessary in a significant number of CF individuals. Importantly, with the advent of universal newborn screening for CF in the United States, severe malnutrition is often averted. The growth and cognitive outcomes of children identified by newborn screening are better than those of patients diagnosed by clinical manifestations, and improved nutrition in childhood is directly associated with better growth and future adult lung function (19, 81).

CYSTIC FIBROSIS PULMONARY DISEASE THERAPIES

Treatments directed at improving CF pulmonary disease are the most numerous and varied and can be broadly categorized as (*a*) airway clearance treatments (both mechanical and pharmacologic), (*b*) antibiotics, and (*c*) anti-inflammatories. Airway clearance, performed several times per day, involves both inhaled therapies that thin airway secretions and various modalities to facilitate the movement of secretions into the larger airways, where they are coughed up and then either expectorated or swallowed. Numerous modalities of mechanical airway clearance exist; common approaches include manual chest percussion (performed systematically, targeting all lung zones), various handheld devices that assist in performing positive expiratory pressure and oscillatory positive expiratory pressure, and use of a high-frequency chest compression vest. The literature on airway clearance is reviewed in the US Cystic Fibrosis Foundation airway clearance guidelines (58). Studies have shown benefits from all methods of airway clearance, and no one modality is obviously superior to any other. Positive expiratory pressure and oscillatory positive expiratory pressure require active performance and training in proper technique, whereas vest therapy can be more time consuming and requires a much more expensive device. The current recommendation is that personal preference should drive decision making in order to facilitate adherence (58). Importantly, aerobic exercise is currently regarded as inadequate clearance by itself, but it does offer other health benefits associated with physical activity.

Two inhaled medications have been carefully studied and shown to improve mucus clearance, and are now central to airway clearance regimens: nebulized recombinant human DNase

(dornase alfa) and nebulized hypertonic saline. Dornase alfa helps to break down excess DNA present in cellular debris and the mucus of chronically inflamed airways and has shown benefit in CF patients with both mild and moderate-to-severe disease (57, 97), improving lung function and reducing the incidence of pulmonary exacerbations. Dornase alfa has also been studied in younger CF patients and in patients with advanced CF lung disease, with some evidence of benefits (78, 107). Hypertonic saline increases airway hydration and thus increases mucociliary clearance (46). Although less extensively studied, 7% hypertonic saline has been shown to modestly improve lung function and provide strong beneficial effects on pulmonary exacerbations, reducing the incidence by nearly 50% compared with saline-treated controls (50). Both dornase alfa and hypertonic saline are currently recommended for CF patients beginning in childhood (46, 57, 97).

The *Pseudomonas aeruginosa* bacterium frequently infects CF patients; the prevalence of *Pseudomonas* infection increases with age and becomes the most frequently identified pathogen in adulthood (36). Eradication strategies have emerged over the past several years that target nonmucoid strains and can delay the development of chronic infection (94, 112, 141, 142). Colonization with *Pseudomonas* (particularly mucoid strains) is associated with a more rapid decline in pulmonary status (51). Therefore, antibiotic therapy directed toward *Pseudomonas* has been extensively studied for chronic outpatient use. Inhaled tobramycin has been available for more than 15 years and is now widely used, with a strong recommendation for its use to manage chronic *Pseudomonas* infection (57). Several tobramycin-based formulations have been subsequently developed with variable use and/or regulatory approval, including concentrated tobramycin solutions for nebulization (Bethkis, T100, and Vantobra) and a dry powder formulation (TOBI Podhaler). Inhaled aztreonam lysinate used in a cycled fashion also improves lung function and reduces the incidence of pulmonary exacerbations, prompting regulatory approval for use to manage chronic *Pseudomonas* infections (97). One head-to-head comparison of aztreonam lysinate and tobramycin inhalation solution has been conducted in patients chronically receiving tobramycin inhalation solution therapy, demonstrating minor lung function benefits for subjects treated with aztreonam lysinate as opposed to tobramycin inhalation solution over three treatment cycles for six months (11). A dry powder form of colistin (colistimethate sodium) has recently been studied and approved in Europe, with similar effects on lung function compared with tobramycin inhalation solution over three cycles lasting six months (128).

In addition to *Pseudomonas*, inherently antibiotic-resistant pathogens such as *Burkholderia cepacia*, *Achromobacter*, *Stenotrophomonas*, and nontuberculous mycobacteria as well as fungal infections with *Aspergillus* can be problematic in a minority of patients, requiring specialized treatment regimens that can be highly burdensome and associated with drug toxicities. Jennings et al. (69) recently demonstrated that chronic infection with methicillin-resistant *Staphylococcus aureus* is associated with increased mortality, and effective approaches to manage infection are currently under investigation.

Anti-inflammatory medications have also been investigated for use in CF, including macrolide antibiotics and nonsteroidal anti-inflammatory drugs; the medications in these classes that have been studied in large controlled trials in CF include azithromycin (a macrolide antibiotic, although its chronic use in CF is for its ability to reduce neutrophilic inflammation) and ibuprofen (76, 83, 123, 124). Azithromycin administered three times a week reduces pulmonary exacerbations and improves weight and lung function in patients chronically infected with *Pseudomonas*, and has similar benefits for pulmonary exacerbations and weight in younger patients not infected with *Pseudomonas*. It has also been examined in patients with chronic obstructive pulmonary disease and non-CF bronchiectasis, demonstrating benefits for pulmonary exacerbation frequency in both of these populations (2, 4, 143). Ibuprofen taken twice daily at doses that achieve appropriate blood levels reduces the decline in lung function and is most effective in pediatric patients.

Although outcome measures have varied between studies, both therapies are generally well tolerated. Azithromycin is recommended for use in patients over 6 years of age irrespective of *Pseudomonas* colonization (57, 97), but regular screening for infection with nontuberculous mycobacteria is recommended. As data for the use of ibuprofen are somewhat more limited, and the most sizable study focused on pediatric patients with an FEV_1 of >60% predicted (83), the Cystic Fibrosis Foundation pulmonary care guidelines currently recommend ibuprofen only in patients 6–17 years of age (97). Potential renal toxicity can be a concern with high-dose ibuprofen use in combination with other nephrotoxic medications, and this can become problematic in the setting of systemic aminoglycoside antibiotics in particular (80). In addition, ibuprofen therapy requires monitoring of serum levels, which limits ease of use (97).

In the context of these pulmonary-directed therapies, optimal nutritional status remains critical, because body mass index is an independent predictor for FEV_1 over time (36, 137). Site of care itself is also important, with improved respiratory outcomes in patients who are followed at comprehensive CF care centers (85).

Hospitalization is a significant burden in the care of CF patients, both financially and as it relates to quality of life (22, 56). Patients are frequently admitted for pulmonary exacerbations that can involve some combination of worsening of cough, sputum production, decline in FEV_1, weight loss, and other systemic symptoms. Intravenous antibiotic therapy is a mainstay of inpatient therapy, with numerous studies examining optimal regimens to maximize antibiotic efficacy while reducing side effects [reviewed extensively in the Cystic Fibrosis Foundation guidelines (56)]. Particular focus has been given to dosing regimens for aminoglycosides (14, 82, 135, 150, 157). Another key component to hospital-based care is an increase in patients' airway clearance therapies. Although studies examining outpatient management of exacerbations are limited, current guidelines recommend that even though hospitalization is itself burdensome, exacerbations should be managed through inpatient care unless comparable treatment with intravenous antibiotics and an increased airway clearance regimen can be accomplished outside of the hospital (56).

CYSTIC FIBROSIS GENETICS

As noted above, there are more than 2,000 known mutations in the CFTR gene. However, a select few mutations define the vast majority of cases. The most common mutation is F508del, a three-base-pair (in-frame) deletion at position 508 in the coding region of the gene, leading to loss of a phenylalanine residue. F508del occurs in 86.4% of US patients (46.5% are homozygous for the mutation, with ~40% carrying at least one allele) and 82.7% of European patients (37, 54). Rates are lower in certain global populations (158). The F508del mutation was identified in the process of initially sequencing the CFTR gene (68, 71). Knowledge of CF patients' CFTR genotypes varies worldwide. The Cystic Fibrosis Foundation offers a Mutation Analysis Program that provides free mutation identification for patients whose genotype is unknown (36). In Europe, the percentage of patients whose genotypes are fully known varies by country and is dependent largely on whether full sequencing is performed at diagnosis or only an assessment for common mutations is performed (54).

Disease-causing CFTR mutations are commonly classified by their effect on the CFTR protein itself (119). Knowledge of the different defect classes and how they result in CFTR dysfunction has informed the development of new therapies. Class I mutations are biosynthetic defects, including frameshift mutations and those resulting in a premature termination codon (PTC); regardless of whether a given mutation affects mRNA transcription, protein translation, or both, the result is an absence of working CFTR at the cell membrane. Class I mutations are the most abundant in number of those cataloged. Class II mutations (which include F508del) affect intracellular

trafficking of the CFTR protein, leading to little if any mature protein at the plasma membrane. Class III mutations result in abnormal gating, such that there is a normal amount of CFTR at the plasma membrane but its channel gating is abnormal, limiting ion transport and protein function. Class IV mutations produce mutant CFTR that has abnormal conductance of chloride, but with retained trafficking to the plasma membrane. Class V mutations are often splicing mutations that lead to a reduced level of CFTR gene transcription and subsequent protein that reaches the plasma membrane, and class VI mutations cause reduced stability of the protein at the cell surface.

The most prevalent CFTR mutations reflect the range of mutation classes. The most common mutation, F508del, is primarily a class II mutation (discussed further below); the second most common, G542X, is a class I mutation with a PTC; and the third most common, G551D, is a class III mutation. Regarding overall protein function, class I–III mutations typically lead to absent CFTR function, whereas class IV–VI mutations can have residual function. Regarding localization, CFTR is generally absent from the plasma membrane in class I–II mutations but is present (albeit in varying levels) in class III–VI mutations. Finally, these classifications are helpful for understanding how CFTR defects result in CF disease, but many mutations have defects across the spectrum of mutation classes. For example, F508del-CFTR is primarily a trafficking mutation (class II), but F508del-CFTR at the cell membrane has reduced gating (class III) and reduced plasma membrane stability (class VI) (70, 145).

THERAPIES FOR GATING AND CONDUCTANCE MUTATIONS: IVACAFTOR

An understanding of the CFTR mutation classes has facilitated the development of medications to restore CFTR function. Investigations undertaken at both academic centers and pharmaceutical companies, often in collaboration with the Cystic Fibrosis Foundation, have led to numerous promising compounds (24, 60, 103, 144, 145, 153). One such drug, ivacaftor (VX-770, trade name Kalydeco, developed by Vertex Pharmaceuticals), is approved internationally for use in patients with an expanding number of specific CFTR mutations. Ivacaftor was identified by high-throughput screening as an agent capable of restoring CFTR function to G551D-CFTR, a class III (gating) mutation; agents such as ivacaftor that address gating mutations are referred to as potentiators of CFTR. In vitro studies demonstrated that ivacaftor increases the probability of the channel being open; chloride secretion increased tenfold in G551D-CFTR airway epithelia, and excess sodium and fluid resorption (functions of ENaC) were reduced (144). Notably, ivacaftor apparently acted only on CFTR activated by protein kinase A—i.e., by normal physiologic regulatory pathways (144). Additional investigation revealed that ivacaftor potentiates other CFTR channels as well, including wild-type CFTR, other CFTR gating mutations, and F508del-CFTR (79, 144, 145).

Only one gating mutation is necessary for patients to benefit from ivacaftor therapy, and clinical studies have demonstrated significant improvements irrespective of the second allele. The initial clinical trials of ivacaftor investigated patients with at least one G551D mutation and demonstrated both clinically and statistically significant improvements in FEV_1 and weight in patients as young as age 6 (39, 109). Older patients (over age 12) also demonstrated improvements in pulmonary exacerbation frequency (reduced by ~60%) and respiratory symptoms. Biomarkers of CFTR function—sweat chloride levels and nasal potential difference—improved as well (1, 39, 109). The improvement in sweat chloride was such that it normalized in some individuals (i.e., was below the diagnostic threshold for CF). Observational data have suggested improvement in CF sinus symptoms, mucociliary clearance, and intestinal pH, although these have not been endpoints examined in controlled trials (133). A pilot study looking at insulin secretion (reflective

of endocrine rather than exocrine pancreas function) in response to intravenous and oral glucose administration demonstrated improvements in four of five patients who had started ivacaftor (16). Similarly, a case report described resolution of CF-related diabetes following initiation of ivacaftor therapy (64). Together, these findings demonstrate the effect of ivacaftor on CFTR in numerous tissues throughout the body. Benefits have been shown to persist, with a long-term follow-up trial extending to 144 weeks showing continued beneficial effects (95). Ivacaftor also improves markers of airway disease in those with milder phenotypes (patients with a normal FEV_1), suggesting benefit even in those with less overt disease pathology (38). An additional study (the KONNECTION trial) demonstrated that ivacaftor is effective in a variety of other class III (gating) CFTR mutations, leading to FDA approval of ivacaftor for eight additional gating mutations (40). Similarly, a study of ivacaftor in patients with gating mutations was completed in children 2–5 years of age (ClinicalTrials.gov ID NCT01705145); the results led to FDA approval for use of ivacaftor in this age group (149).

Ivacaftor has also been investigated for use in a class IV mutation, specifically the fairly common R117H-CFTR mutation. This mutation is unusual in that phenotypic expression differs depending on the number of thymidine repeats within one of the introns of the CFTR gene. The 5T genotype (i.e., five thymidine repeats) leads to aberrant gene splicing and absence of exon 9, which correlates with R117H individuals who have a more severe disease phenotype, whereas 7T and 9T genotypes demonstrate normal splicing and typically a milder phenotype (30, 31). R117H patients with the 5T modifier who received ivacaftor showed improvements in sweat chloride levels (25), and a subsequent clinical trial demonstrated improvement in FEV_1 and respiratory symptoms that reached statistical significance in adult patients, although not in pediatric patients (100). Based on these results, ivacaftor has received FDA approval for use in patients with the R117H mutation independent of T status.

PREMATURE TERMINATION CODON SUPPRESSION

A subgroup of class I mutations have been the target of ongoing investigation. PTCs are the result of single-base-pair substitutions creating an in-frame termination codon; upon reaching a PTC, a ribosome and associated mRNA-translating structures detach from the mRNA strand, yielding a truncated protein with little or no function (15, 104, 118). It is possible, however, to reduce the proofreading fidelity of the ribosomal unit and allow read-through of a PTC, resulting in a full-length protein, a phenomenon initially noted with aminoglycoside antibiotics. Studies have shown that aminoglycoside antibiotics do this both in vitro and in vivo in CF patients (32, 49, 89, 90, 96, 118, 132, 160). Based on this finding, the compound PTC124, or ataluren, was developed as a non-aminoglycoside molecule proposed to induce PTC read-through. Following several phase II studies (33, 72, 131, 159), Kerem et al. (73) recently reported the results of a phase III study of ataluren. They did not observe improvements in nasal potential difference or sweat chloride compared with placebo. A subgroup analysis noted improvements in FEV_1 compared with placebo among patients who were not using inhaled aminoglycoside antibiotics, which appear to interfere with ataluren activity in vitro; notably, only FEV_1 data, and not nasal potential difference or sweat chloride data, were reported for the subgroup. Investigation of ataluren is continuing in an ongoing phase III trial that specifically excludes individuals using inhaled aminoglycosides (ClinicalTrials.gov ID NCT02107859). Additionally, the development of new aminoglycosides that lack antimicrobial activity has been considered, specifically for PTC-suppressing indications. Preclinical data in models of human airway epithelia as well as mice expressing the G542X class I mutation have shown that synthetic aminoglycoside derivatives can suppress PTCs and that the resultant CFTR is amenable to augmentation with ivacaftor (161).

RESTORATION OF F508DEL-CFTR

As mentioned above, the F508del-CFTR mutation confers multiple abnormalities to the resultant CFTR protein. F508del is considered a class II mutation that causes protein misfolding; the vast majority of synthesized protein is degraded within the 26S proteasome, leading to very limited expression on the cell surface (43). However, the CFTR that does reach the cell surface demonstrates both abnormal gating and increased turnover, behaving like CFTR with both class III and class VI defects (42, 88). To date, a single medication alone has been unable to restore adequate function to F508del-CFTR to result in clinical improvement (21, 34, 55). However, combination therapy has proven promising.

Whereas agents such as ivacaftor that alter gating and conductance are termed potentiators, molecules that target the misfolding of F508del are termed correctors. Several such corrector compounds were discovered using high-throughput screening methods in cells stably expressing F508del-CFTR, similar to the techniques used to develop ivacaftor. One such agent, called lumacaftor (VX-809, developed by Vertex Pharmaceuticals), has been developed alongside ivacaftor. In vitro studies demonstrated that lumacaftor increased F508del-CFTR maturation and expression at the plasma membrane, increased half-life at the plasma membrane, enhanced F508del-CFTR-mediated chloride transport, and increased ASL height (145). The overall in vitro effect of VX-809 restored F508del-CFTR activity to ~15% that of wild-type CFTR. Addition of ivacaftor (which can potentiate F508del-CFTR) in these lumacaftor models increased activity to nearly 30% that of wild-type CFTR (145). Therefore, in moving lumacaftor development forward, efficacy studies combined the use of lumacaftor and ivacaftor. A multicohort phase II served as the basis for several different dosing regimens in subsequent studies (21).

Within the past year, two large phase III trials, enrolling more than 1,100 individuals, assessed lumacaftor/ivacaftor combination therapy in patients with two F508del mutations: the TRAFFIC (ClinicalTrials.gov ID NCT01807923) and TRANSPORT (Clinical Trials.gov ID NCT01807949) trials (152). Two dose cohorts and one placebo group were included in these 24-week randomized, placebo-controlled trials of patients over 12 years of age. Ivacaftor/lumacaftor treatment led to significant improvements in FEV_1 (2.8–3.3% predicted compared with placebo) and pulmonary exacerbations (30–40% reduction) and a statistically significant increase in body mass index. Side effects included a higher rate of liver function test abnormalities related to the drug and more frequent reports of chest tightness in treated patients compared with placebo (152). These results led to FDA approval of a single-pill combination (taken every 12 hours) of lumacaftor/ivacaftor (Orkambi) for patients age 12 and older with two copies of F508del-CFTR (147).

A second CFTR corrector, VX-661, has shown activity in phase II trials [reported in abstract form (48)] and more recently was explored in combination with ivacaftor (148). Preliminary data indicate improvement in FEV_1 and sweat chloride levels compared with controls, and phase III trials with VX-661 are currently enrolling patients (ClinicalTrials.gov IDs NCT02392234, NCT02516410, and NCT02412111). As reported in a press release from Vertex Pharmaceuticals (148), these studies will examine VX-661 in combination with ivacaftor for patients homozygous for the F508del mutation, and additional studies will examine F508del heterozygous patients with various second CF-causing alleles (nonfunctional, gating, or partially functional). One of the notable limitations of lumacaftor has been the inability to achieve clinical benefit in patients with only one F508del mutation (21). As opposed to potentiator therapies for patients with class III or class IV mutations, which can yield significant benefits in patients with one ivacaftor-responsive CFTR mutation, lumacaftor/ivacaftor thus far requires two F508del-CFTR alleles to produce clinical improvement. Although the current VX-661/ivacaftor program will test whether this

corrector/ivacaftor combination therapy produces benefits in patients with only one F508del-CFTR allele, our understanding that multiple folding defects contribute to F508del-CFTR dysfunction has prompted consideration of future studies combining multiple CFTR correctors to achieve greater efficacy than can be achieved with any single corrector agent.

A concern that has arisen in recent in vitro studies of ivacaftor is the potential for negative effects on the stability of corrected F508del-CFTR. Two separate groups demonstrated that chronic (several days) administration of ivacaftor led to destabilization of corrected F508del-CFTR protein in primary human airway epithelial cells (29, 146). These results were seen with both VX-809- and VX-661-corrected F508del-CFTR. Veit et al. (146) tested other potentiators not yet in clinical use and did demonstrate that one agent [P5 (ΔF508act-02)], which is a potentiator of F508del-CFTR but not of other mutations, did not have this same negative interaction (91). Notably, the findings were specific to F508del-CFTR and did not extend to G551D-CFTR. These results do not negate the utility of ivacaftor therapy, as the clinical data reported thus far do indicate clear clinical benefits of ivacaftor/lumacaftor in F508del-CFTR homozygous subjects. They do suggest, however, that superior results may be achievable with alternate potentiators in patients with an F508del-CFTR mutation. As with consideration of combining several correctors, a significant achievement would be a therapy yielding clinical improvement in patients with a single F508del-CFTR allele. If successful, this would help extend modulator therapy to nearly 90% of CF patients based on genotype.

GENE THERAPY

In contrast to restoring CFTR function through CFTR modulation, gene therapy aims to provide CF patients with wild-type CFTR. Such a treatment should be effective independent of patients' CFTR mutations (i.e., CFTR mutation agnostic). Armstrong et al. (10) have described historical approaches to gene therapy. Multiple challenges exist, most notably the efficient delivery of transgenes and host inflammatory response. Although early research explored a variety of viral vectors, more recent studies have examined repeat dosing of nonviral vectors. This approach was utilized in a recent trial published by the UK Cystic Fibrosis Gene Therapy Consortium (5). The phase II study dosed 62 placebo and 78 active drug patients (over 12 years of age) in a randomized, double-blind, placebo-controlled trial. Patients received pGM169/GL67A (plasmid CFTR cDNA/cationic liposome) or placebo (saline) once a month for 12 months. The results showed stabilization of FEV_1 over a one-year period in subjects receiving the lipid-based nonviral vector complexed with plasmid CFTR cDNA versus placebo; stratification by lung function indicated improvement in those beginning the study with more severe respiratory disease.

A notable limitation of current research in gene therapy is that gene delivery has historically been through the airway. As such, restoration of wild-type CFTR activity is currently limited to the CF lung. Ultimately, an ideal gene therapy strategy would be introduced early enough to prevent the development of disease and could be delivered systemically and targeted to CF-affected organs in order to address all manifestations of CF-associated disease. Despite these challenges, the capacity to develop genotype-agnostic gene therapy that addresses CF lung disease would be a major step to bringing CFTR-directed therapy to all CF patients.

FUTURE THERAPIES AND CONSIDERATIONS REGARDING PERSONALIZED CYSTIC FIBROSIS CARE

Therapies for CF have changed dramatically in the last several years. The approval of Kalydeco in 2012 marked the introduction of the first treatment specific to a particular genetic defect in CF

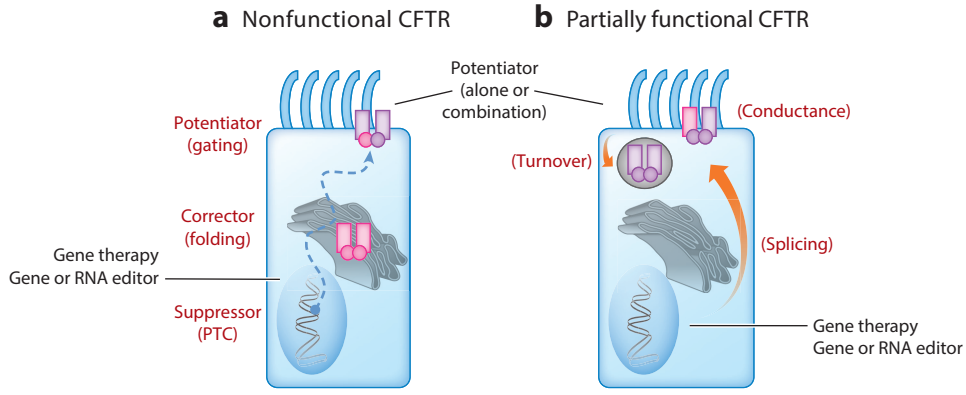

Figure 1

Strategies to restore cystic fibrosis transmembrane conductance regulator (CFTR) function. Various defect classes in CFTR production are shown in parentheses. Those shown in panel *a* have little, if any, endogenous function, whereas those shown in panel *b* frequently have some measurable baseline activity. Small molecules have been approved or are under development to potentiate CFTR that is responsive and available at the plasma membrane (potentiators), to correct folding (correctors), and to suppress premature termination codons (PTCs) (suppressors). Combining a potentiator with a corrector improves the function and clinical outcome of patients with two copies of F508del-CFTR, and potentiator monotherapy restores function to several CFTRs with gating defects and at least one mutation that has conductance and gating defects (R117H-CFTR). Combining modulators may improve the function of select CFTR mutations. Gene therapy can restore CFTR function independent of genotype, whereas gene editing and/or RNA editing can be designed to target numerous mutations in all CFTR mutation classes.

and has been championed as an example of personalized or precision medicine. With expanded genotype indications for Kalydeco and this year's approval of Orkambi for patients homozygous for F508del-CFTR, mutation-specific therapies are available for approximately half of the CF population. (In practice, this number is lower owing to age restrictions on the medications' approval, but childhood use is being further studied and expanded.) An obvious future target in drug development is achieving effective correction and potentiation in those with only one F508del-CFTR allele, accounting for approximately 40% of CF patients. With ongoing research in PTC mutations, this leaves a small minority of patients with rarer mutations without targeted therapies potentially on the horizon. Options for such individuals might include DNA-editing (CRISPR/Cas9) or RNA-editing therapies (which could be designed specifically for a patient's particular disease-causing mutation) or stem cell–based therapies to replace disease-affected epithelia with progenitor cells containing functional CFTR (98, 129). **Figure 1** summarizes currently available therapies and future options of molecular targets for given mutations. Testing of various CFTR modulators using laboratory models derived from patients' own cells may allow screening of a variety of existing compounds for evidence of activity for a particular mutation, or guiding selection of agents when several options are available (e.g., which corrector combinations are most effective for a given patient with the F508del-CFTR mutation) (41).

A notable caveat with current mutation-directed directed therapies is that CFTR restoration is incomplete, and responses to drugs vary. Kalydeco set the bar high for CFTR-directed therapies, producing significant improvements in numerous clinical outcome domains. By comparison, Orkambi clearly has clinical effects but a lesser effect size. **Table 1** compares the effects of the two medications across multiple clinical outcomes in their respective phase III trials. With long-term use of both medications, there may be different natural histories in the respective patient

Table 1 Comparison of the phase III trials that led to initial FDA approval of Kalydeco (ivacaftor) and Orkambi (lumacaftor/ivacaftor)

	VX-770/ivacaftor trials (Kalydeco)		TRAFFIC/TRANSPORT trials (Orkambi)		
Summary	VX-770 in patients ≥12 years old with at least one G551D mutation		Lumacaftor plus ivacaftor in patients ≥12 years old who are homozygous for F508del (included two study arms)		
Duration	48 weeks		24 weeks		
Number of subjects	78 placebo	83 ivacaftor	368 receiving 600 mg daily lumacaftor	369 receiving 400 mg q12h lumacaftor	371 placebo
Mean age (years)	24.7	26.2	24.5	25.3	25.4
Proportion of subjects less than 18 years of age	22%	23%	26.1%	26.6%	25.9%
Sex (% female)	51%	53%	49.5%	49.3%	48.8%
Mean BMI (kg/m^2)	21.9	21.7	21.0	21.5	21.0
Range of BMI (kg/m^2)	15.2–38.6	14.8–38.9	14.2–35.1	14.6–31.4	14.1–32.2
Mean baseline FEV_1 (% predicted)	63.7%	63.5%	60.8%	60.5%	60.4%
Proportion of subjects with FEV_1 <70% predicted	58%	59%	72.0%	72.0%	71.7%
Mean absolute change in FEV_1 predicted from baseline versus placebo, at 24 weeks	+10.6% ($p < 0.0001$)		+3.3% ($p < 0.001$)	+2.8% ($p < 0.001$)	NA
Hazard ratio for pulmonary exacerbation versus placebo	0.455 ($p = 0.001$)		0.70 ($p = 0.001$)	0.61 ($p < 0.001$)	NA
CFQ-R score versus placebo (points)	+8.6 ($p < 0.0001$)		+3.1 ($p = 0.007$)	+2.2 ($p = 0.05$)	NA
Mean weight difference from baseline versus placebo (kg)	+2.7 ($p < 0.0001$)		NA		
Mean BMI difference from baseline versus placebo (kg/m^2)	NA		+0.28 ($p < 0.001$)	+0.24 ($p < 0.001$)	NA

Data are from Ramsey et al. (109) and Wainwright et al. (152) for the VX-770/ivacaftor trials and TRAFFIC/TRANSPORT trials, respectively. Abbreviations: BMI, body mass index; CFQ-R, Cystic Fibrosis Questionnaire Revised; FEV_1, expiratory volume over the first second of a forced exhalation; NA, not applicable.

populations as differences in drug effects change disease trajectory over time. This might be expected to play out with other new therapies as well, depending on the degree of CFTR restoration and the relative ability of drugs to act on CFTR in a range of tissues. These existing differences should also motivate ongoing research with newer correctors and potentiators to maximize the clinical benefits that can be achieved.

In considering personalized therapies, one of the major limitations of recent therapeutic breakthroughs such as ivacaftor is that they do not replace existing therapies. All of these studies have been conducted as add-on therapies, and thus the relative benefits of established treatments in the context of CFTR modulation are unknown. Patients continue to require pancreatic enzyme replacement, to perform airway clearance, and to have exacerbations requiring hospital admission, albeit with a lower frequency of exacerbations (95). Restoration of CFTR function has not been demonstrated to reverse existing damage to exocrine pancreatic function and appears unlikely to

repair severely bronchiectatic airways. Additionally, the potential for long-term negative sequelae of these therapies will require monitoring. Of great interest will be the introduction of ivacaftor, lumacaftor/ivacaftor or other combination therapies, and other future medications in progressively younger patients. If CFTR function is restored early enough, perhaps the onset of chronic respiratory symptoms or permanent pancreatic insufficiency can be delayed or even prevented, and likewise, CF-related diabetes or liver disease incidence may change. In addition, recent data from the CF pig point to congenital abnormalities in airway cartilage formation and raise the question of whether other prenatal CFTR-dependent defects will be identified (12, 44).

Ultimately, the hope would be to prevent the disease phenotype from developing, obviating the need for treatments that address the pathologic consequences of CFTR dysfunction. Expanding CFTR modulators to younger CF patients and/or advancing the capabilities in gene therapy to systemic delivery could conceivably hold the same promise if instituted early. Whether this can be achieved with any treatment modality is unknown but prompts the question of whether therapies could be sought that would be efficacious in utero to prevent the earliest of complications, such as meconium peritonitis, pancreatic injury, and impaired airway development. Hand in hand with this line of consideration is the need for sensitive monitoring tools in the youngest patients and for older patients in the context of studies or decisions to remove therapies. Indeed, withholding established but cumbersome therapies has the greatest potential positive impact on patient quality of life but must be considered in the context of the long-term impact of these decisions. The field of CF therapeutics will continue to change rapidly in the coming years, and as this occurs, the natural history of the disease may also change dramatically. The ultimate goal is to bring CFTR-targeted therapies to all patients at an early enough age to delay or even prevent many manifestations of the disease, and to personalize the addition of traditional therapies based on patient need.

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