The Evolution of Cystic Fibrosis Care

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Cystic fibrosis (CF) is the most common life-limiting inherited illness of whites. Most of the morbidity and mortality in CF stems from impaired mucociliary clearance leading to chronic, progressive airways obstruction and damage. Significant progress has been made in the care of patients with CF, with advances focused on improving mucociliary clearance, minimizing inflammatory damage, and managing infections; these advances include new antimicrobial therapies, mucolytic and osmotic agents, and antiinflammatory treatments. More recently, researchers have targeted disease-causing mutations using therapies to promote gene transcription and improve channel function, which has led to impressive physiologic changes in some patients. As we develop more advanced, allele-directed therapies for the management of CF, it will become increasingly important to understand the specific genetic and environmental interactions that cause the significant heterogeneity of lung disease seen in the CF population. This understanding of CF endotypes will allow for more targeted, personalized therapies for future patients. This article reviews the genetic and molecular basis of CF lung disease, the treatments currently available, and novel therapies that are in development.

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ABBREVIATIONS: CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; ENaC = epithelial sodium channel

Cystic fibrosis (CF), which is an autosomal recessive defect occurring in approximately one in 3,500 live births based on data from neonatal screening,¹ is the most common, life-shortening inherited disease of whites. The life expectancy of a child born with CF has improved steadily, largely because of advances in disease surveillance and more aggressive treatment strategies. Nevertheless, patients with CF die too young, with much of the early morbidity and mortality from CF resulting from progressive airway involvement. Universal adoption of new-

born screening in the United States¹ has led to earlier diagnosis and treatment of CF, which has sparked hope that lung disease can be averted even before it begins, especially with the advent of newer agents that target the basic cellular defect and have the potential to radically change clinical outcomes. The use of defined endotypes, phenotypic subtypes that rely on a combination of genetics, biomarkers, environmental exposures, clinical outcome measures, and infectious and inflammatory factors to characterize disease, may better

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direct therapeutic interventions by targeting specific populations with CF. In this article, we review the pathophysiology of CF lung disease and describe how current and emerging therapies can both treat and possibly prevent this progressive lung disease.

Genetics and Pathophysiology of CF Airway Disease

To fully understand the current and newer therapies for CF, physicians must have a working knowledge of the basic pathophysiology of the disease. CF is clinically characterized by chronic sinopulmonary and GI manifestations, which are caused by abnormalities in the cystic fibrosis transmembrane conductance regulator (CFTR), a channel located at the surface of the cells lining the airway epithelium and in the submucosal glands that mediates cyclic adenosine monophosphate (cAMP)-regulated transport of chloride and other anions.²⁻⁶ The CFTR is functionally linked to the epithelial sodium channel (ENaC) and alternative apical chloride channels; thus, the CFTR defects lead to not only reduced chloride conductance but also dysregulation of ENaC activity. This failure of chloride secretion and sodium hyperabsorption leads to desiccation of the periciliary fluid layer and viscous mucus on the airway surface. The dehydrated secretions and excess solids in airway mucus impair mucociliary clearance, obstruct the airways, allow bacterial infection to become established, and subsequently incite an intense inflammatory response.⁷ Impaired activity of bactericidal proteins produced by airway epithelia related to altered bicarbonate secretion in the CF airway creates gaps in innate airway defenses and contributes to chronic infection (Fig 1).⁸

Nearly 2,000 disease-causing mutations in *CFTR*, a 27-exon gene on chromosome 7,⁹⁻¹¹ have been identified. Although racial and ethnic differences exist, the most common mutation is c.1521_1523delCTT, or delF508, which accounts for >70% of mutant alleles in the CF population.^{11,12} Mutations are usually grouped into six classes based on the protein product. Class 1 mutations consist of frameshift or nonsense variants that cause altered or impaired *CFTR* transcription, producing either messenger RNA that decays before nuclear export or truncated, nonfunctional protein. Class 2 mutations, such as delF508, result in misfolded protein that is trafficked to degradation pathways, has abnormal function, and is more rapidly cleared from the cell membrane. Mutations from both of these classes typically lead to more severe lung disease and pancreatic insufficiency. Class 3 mutations, including G551D, commonly called gating mutations, lead to a CFTR that is poorly responsive or nonresponsive to ATP activation of the nucleotide-binding domains, resulting in defective chloride conductance across the apical cell membrane. The clinical phenotypes and severity associated with class 3 mutations vary. Class 4, or conducting, mutations have impaired chloride conductance or transport caused by alterations in size and ion selectivity of the channel pore. Class 5 mutations are frequently splice mutants that reduce the rate of synthesis of functional CFTR and include intron 8 polythymidine variants. Class 6 mutations involve genetic defects that are near the N- or C-terminus and lead to aberrant membrane insertion, stability, or trafficking (Fig 2).^{2,6,13-18} Some mutations defy classification. For instance, delF508 acts as a class 2 (altered processing), class 3 (impaired gating), and class 6 (rapidly degraded) mutation, which potentially

Figure 1 – Epithelial pathophysiology in cystic fibrosis, characterized by altered anion secretion, sodium hyperabsorption, and dehydration of the apical surface fluid that leads to reduced periciliary fluid volume and pH, which interferes with mucociliary clearance and innate defenses, resulting in chronic infection. The decreased periciliary fluid volume also concentrates inflammatory mediators at the immediate epithelial surface. CFTR = cystic fibrosis transmembrane conductance regulator; Cl⁻ = chloride; ClCa = alternative chloride channel; ENaC = epithelial sodium channel; HCO₃⁻ = bicarbonate; H₂O = water; Na⁺ = sodium.

Figure 2 – Classes of CFTR mutations. ER = endoplasmic reticulum; mRNA = messenger RNA. See Figure 1 legend for expansion of other abbreviations.

has implications for molecular therapy. Other mutations are yet to be classified, either because of rarity of incidence or unclear effect on CFTR function.

Pulmonary disease is the primary cause of mortality in patients with CF,^{19,20} with considerable variability in presentation, severity, and progression of pulmonary involvement, even among patients with the same CFTR mutation.¹¹ Patient-level differences have been attributed to a variety of factors including genetic modifiers, sex, race, ethnicity, socioeconomic status, nutritional status, and exposure to airborne pollutants, including tobacco smoke.^{21–25} There is mounting evidence suggesting that pulmonary disease begins early in infancy. The lungs of neonates with CF have long been considered histologically normal, but animal models now indicate that structural abnormalities in the CF lung may occur in utero.^{26,27} Infants with CF are typically born at lower birth weight than their non-CF counterparts, and poor nutrition has an impact throughout life, particularly in early childhood when somatic growth is linked to lung and alveolar growth.^{28,29} Although it is unusual for neonates to have respiratory symptoms, older infants can develop tachypnea, wheezing, and cough that is frequently associated with respiratory viral infections. Studies of infants and preschool children with CF have found abnormal pulmonary function, structural changes, and increased inflammatory markers in BAL fluid occurring as early as 6 months of age.^{30–32} Moreover, there is significant disease heterogeneity within the lungs of a single patient, particularly in the early stages of disease.

The CF lungs are not infected at birth, but over time, various bacteria are intermittently found in airway

secretions.³¹ As bronchial obstruction evolves, the airway becomes persistently infected.³³ Acquisition of *Burkholderia cepacia*, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, and *Mycobacterium abscessus* has been associated with deteriorating lung function and poorer prognosis.^{33–40} Newly emerging data on the CF respiratory and gut microbiome suggest a complex interplay of microbes that contribute to, and are altered by, progressive CF lung disease.^{41,42}

The inflammatory response in CF airways is both protective and destructive, driven by local stimuli, mediators, and chemoattractants. The intense neutrophilic inflammation is remarkably compartmentalized, with disease limited to the airway.^{43–45} Several lines of evidence suggest that airway inflammation in CF may be exaggerated, with higher epithelial production of proinflammatory cytokines, but not all CF cell models and clinical studies have revealed a dysregulated response.⁴³ Indeed, several non-CFTR genetic modifiers, some of which are associated with inflammation, have been identified that can influence the severity of lung disease.^{11,46–48} Inflammatory mediators, such as neutrophil elastase, have been used as biomarkers for CF lung disease.⁴⁹ Neutrophil-derived proteases are released during phagocytosis and cell death and are central to the pathogenesis of CF lung disease by interference with nonspecific airway defenses, stimulation of epithelial chemokine secretion, and degradation of structural proteins, all of which lead to progressive bronchiectasis and bronchomalacia.⁵⁰

Assessment of CF Lung Disease

CF care relies on a multidisciplinary, center-based team approach that involves physicians, nurses, nutritionists, respiratory therapists, and other ancillary staff. Comprehensive care has contributed to improvements in morbidity and mortality in the CF population.⁵¹ Despite recommendations for standardized care based on randomized controlled trials and the availability of clinical guidelines from the Cystic Fibrosis Foundation that outline “best” practices for diagnosis, routine monitoring, and interventions to slow progression of lung disease,^{1,52–54} debate continues regarding optimal care, with the goal being early detection and aggressive treatment of disease. Routine surveillance studies are recommended, including regular spirometry to monitor the lung function of children ≥ 6 years of age, annual chest radiographs, and respiratory bacterial cultures to assess for pathogens in the lower respiratory tract. Studies have shown that respiratory culture results, particularly oropharyngeal

swab and BAL fluid, may differ by source.^{55,56} Even cultures of lavage fluid from different lung segments can have different results.⁵⁶ However, a 2011 randomized controlled study showed no apparent benefit in lavage-guided management of infection over standard oropharyngeal cultures in young children with CF.⁵⁷

Pulmonary function testing is an important tool in the clinical evaluation of the patient with CF, and FEV₁ has been linked directly to morbidity and mortality.⁵⁸ However, as the clinical outcomes in CF have improved, the rate of pulmonary function decline has slowed, thus making spirometry a less sensitive tool for recognizing and monitoring disease in patients with mild disease,⁵⁹ which has implications for both clinical care and trials. Children have considerable lung disease well before they are symptomatic or demonstrate either abnormal or deteriorating lung function.^{31,60} A growing number of centers reliably perform spirometry in preschool children. Infant pulmonary function testing using the raised volume rapid thoracoabdominal compression technique and plethysmography are considered useful adjuncts to standard care in centers with the necessary staffing, equipment, and expertise.^{61,62} Multiple breath washout testing, which assesses ventilation inhomogeneity, has been shown to be more sensitive than spirometry for the identification of early or mild CF lung disease.^{63,64} Despite a lack of normative data, some centers are beginning to use this approach clinically.

Sensitivity of the chest radiograph, particularly for early CF lung disease or minor changes, is relatively low. Findings can be inconsistent and evaluator dependent, even when using scoring systems.⁶⁵ Although CT scanning has been shown to be a more sensitive test for the identification of structural abnormalities, including air trapping and bronchiectasis, concern about repeated radiation exposure has limited its routine use. MRI of the lung has shown promise for the evaluation of both structural and ventilation abnormalities without radiation risk, although further development of testing protocols is still needed.⁶⁶

Current and Future Management of CF Lung Disease

Antimicrobial Therapies

Antibiotics are mainstays for the treatment of acute exacerbations and maintenance therapy for patients with chronic infection. At times, patients with CF will develop increasing respiratory signs and symptoms related to increasing bacterial burden, airway inflammation, and endobronchial obstruction, a phenomenon

termed a pulmonary exacerbation. Although a precise definition of pulmonary exacerbation remains elusive,⁶⁷ it is clinically manifested by a worsening cough, dyspnea, and sputum production, as well as systemic symptoms such as fatigue, anorexia, and weight loss.^{67,68} Frequent pulmonary exacerbations negatively affect prognosis.⁶⁹ Pulmonary function measures typically fall during exacerbations, and the treatment goal is a return to best baseline measures, irrespective of the patient's symptoms. However, many patients fail to reach this goal.⁷⁰ Although a few studies have attempted to define the optimal length of treatment,^{71,72} pulmonary exacerbations are generally treated with antibiotic therapy for 10 to 21 days, guided by bacterial isolates from respiratory secretions and aggressive airway clearance techniques.^{73,74}

The eradication of *P aeruginosa* from the lower respiratory tract has become a cornerstone of CF treatment in the United States during the past 2 decades. Several different eradication strategies, which typically consist of single agents or combinations of systemic and inhaled antibiotics, have been tested.⁷⁵ Although strategies to clear initial *P aeruginosa* infection have been shown to delay chronic colonization and slow lung function decline, *Pseudomonas* eventually becomes established in the airways in most older children with CF.⁷⁶ Chronic suppressive therapy with inhaled antibiotics such as tobramycin and aztreonam has been shown to decrease the frequency of pulmonary exacerbations; the impact of these therapies on mortality remains unknown.^{77,78} Other antibiotics currently being developed for inhaled administration include vancomycin, ciprofloxacin, and levofloxacin.^{79,80}

Mucolytic and Osmotic Agents

Because the primary physiologic manifestation of CF in the lungs is defective mucociliary clearance, it is only logical that several therapies have been developed to target this defect. To maintain lung health, airway secretions need to be mobilized to not only relieve airway obstruction but also reduce infection and airway inflammation. Effective airway clearance techniques are essential components of CF therapy, although few data have clearly demonstrated the long-term effect of airway clearance on lung function and virtually no studies have compared the efficacy of different methods.^{81,82} Aerosolized mucolytic agents have been incorporated into CF care to clear airway secretions. Dornase alfa (recombinant human deoxyribonuclease I) cleaves the DNA released in high concentrations by degraded neutrophils present in CF mucus, thus reducing sputum viscosity and leading to slower lung function decline and fewer pulmonary exacerbations.^{68,83,84} N-acetylcysteine, which acts through

hydrolysis of disulfide bonds in mucin, has a long history of use as a mucolytic agent for patients with CF, although few studies support its effectiveness.⁸⁵

Other inhaled therapies have targeted fluid and ion balance in the airways as a means of improving mucociliary clearance. Inhaled hypertonic saline, which leads to a temporary increase in mucociliary clearance by increasing the depth of the periciliary fluid space and lowering mucus osmolality, has been widely adopted in children and adults with CF.^{86,87} Its efficacy in younger subjects has yet to be established.⁸⁸ More recently, inhaled mannitol has been proposed as an osmotic agent designed to rehydrate the airway surface layer, and several studies suggest inhalation of mannitol may improve FEV₁.^{89,90} However, there are concerns about possible adverse effects. While this therapy is available for adults in Europe, Australia, and New Zealand, it has not been approved by the US Food and Drug Administration.

Another therapeutic strategy to increase periciliary fluid depth is to alter the function of other non-CFTR ion channels at the apical surface. A uridine triphosphate nucleotide analog (denufosol) activates non-CFTR chloride channels and inhibits sodium absorption by stimulating P2Y₂ receptors on epithelial cells,⁹¹ but a large phase 3, placebo-controlled study in patients with CF failed to show an effect.^{92,93} Several studies have examined agents that block ENaC, but have shown little benefit to date. Studies examining newer ENaC inhibitors are in the early stages.⁹⁴

Antiinflammatory Therapies

Chronic neutrophilic inflammation is central to the pathogenesis of CF lung disease. Neutrophilic inflammation is present from early infancy,⁹⁵ even before the child is symptomatic. Inflammation in the CF lung is highly compartmentalized and confined to the airway lumen, whereas the alveolar space is relatively spared. Various antiinflammatory agents have been applied to CF lung disease.^{96,97} High-dose ibuprofen has been shown to significantly decrease the rate of lung function decline in younger children with CF,⁹⁸ but its use is somewhat limited because of compliance and potential renal and gastric toxicities. Alternate-day treatment regimens with systemic corticosteroids have also been shown to significantly slow lung function decline, although there is even greater concern for adverse effects.⁹⁹ Inhaled corticosteroids have shown some benefit in terms of preservation of lung function, but long-term use has been associated with increased risk of glucose intolerance and reduced growth velocity.¹⁰⁰

Azithromycin has gained acceptance for its antiinflammatory effects, although the precise mechanism of action in CF is unclear. In a large multicenter study in the United States, patients with CF who were > 6 years of age and infected with *P aeruginosa* who were treated with azithromycin three times weekly had better lung function and fewer pulmonary exacerbations when compared with those who were given a placebo. A subsequent clinical trial in subjects who were not chronically infected with *P aeruginosa* did not reveal a difference in lung function, though there was a benefit in terms of a reduction in pulmonary exacerbations and improved nutritional status.^{101,102} Few adverse effects have been reported, which may explain some of the popularity of this agent. There are contraindications to its use, specifically nontuberculous mycobacterial infections, where use of azithromycin has the potential to induce antibiotic resistance, and prolonged QT interval. Other oral antiinflammatory agents currently under study include N-acetylcysteine, docosahexaenoic acid, and sildenafil.¹⁰³ Their effects on lung function and airway inflammation have yet to be established.

CFTR Genotype-Specific Therapies

Historically, treatment of CF has focused on the downstream effects of CFTR dysfunction—impaired mucociliary clearance, chronic infection, and chronic inflammation.⁴⁵ The ideal therapy for CF lung disease would directly treat the disease proximate to the gene or protein defect, allowing for normal or near-normal CFTR function. Despite its early promise, gene therapy has yet to be successful in patients.¹⁰⁴ One of the most exciting advances in CF therapeutics is the discovery of small molecules that alter mutant CFTR function.¹⁰⁵

Through the Cystic Fibrosis Foundation Therapeutic Development Network, a nonprofit clinical trials network established to facilitate the development and conduct of all phases of clinical trials,¹⁰⁶ several novel therapies are being tested or introduced to CF care (Fig 3). Some agents have allele- or mutation-specific effects.

Class 1 mutations, which include nonsense mutations that result in premature termination of CFTR messenger RNA, account for roughly 10% of CFTR mutations. Several agents allow the ribosome to read through the premature stop codon but not through normal termination codons. Despite promising early trials, a large phase 3 clinical trial with ataluren (PTC124), an oral medication that promotes ribosomal read-through, showed no improvement in FEV₁ compared with placebo, though the results may have been confounded by aminoglycoside use.¹⁰⁷

Figure 3 – Recently approved and newer agents that are being tested for cystic fibrosis through the Therapeutics Development Network. FDA = US Food and Drug Administration. See Figure 1 legend for expansion of other abbreviation. (Modified with permission from the Cystic Fibrosis Foundation.¹⁰⁶)

In contrast, a Food and Drug Administration-approved oral medication, ivacaftor, acts as a CFTR potentiator, improving protein function in patients with class 3 mutations, and has been shown to dramatically improve lung function and quality of life and reduce sweat chloride levels and pulmonary exacerbations, in patients with the G551D mutation (Fig 4).¹⁰⁸ Its indication was recently expanded to several other gating mutations,¹⁰⁹ which

unfortunately still represents only a minority of patients with CF.

Correction of the class 2 mutation delF508 is the holy grail of CF therapeutics, because it could potentially change the clinical course of 90% of all affected individuals in the United States. Numerous small molecule correctors are in the therapeutic pipeline,¹⁰⁶ including

Figure 4 – Correcting the molecular defect in cystic fibrosis. Newer agents have mutation-specific effects on defective CFTR proteins. Class 3 or gating mutations, such as G551D, result in a defective CFTR that is unable to regulate chloride flow because of impaired ATP activation of the nucleotide-binding domains, which is corrected by the potentiator, ivacaftor. Class 2 mutations, such as delF508, result in misfolded protein that is trafficked to degradation pathways, but the corrector, lumacaftor, acts as a chaperone and traffics the defective protein to an apical surface. Nevertheless, the delF508 protein still has impaired channel function and will require addition of a potentiator to correct the chloride conductance. See Figure 1 and 2 legends for expansion of abbreviations.

lumacaftor, which has been shown to act as a chaperone for the defective CFTR protein and partially restored cyclic adenosine monophosphate-mediated chloride secretion in delF508 human bronchial epithelial cells in vitro. Early experience with lumacaftor alone showed a modest drug response only,¹¹⁰ which is not surprising because the rescued delF508 protein is still a defective channel (Fig 3). Recent clinical trials combining lumacaftor with ivacaftor to improve mutant channel gating reportedly have yielded promising results. However, studies using airway epithelial cell models have shown that ivacaftor interfered with the effect of lumacaftor on delF508 CFTR, indicating that this combination of agents may have limitations.^{111,112}

Shift Toward Primary Prevention/Treatment of Infants and Young Children

Although advances over the past 20 years have led to tremendous progress in the treatment of CF lung disease, these therapies are currently aimed at treating existing lung disease and slowing disease progression, which is often described as secondary or tertiary disease prevention. It is clear that lung disease begins very early in life, and CF may provide a striking opportunity for the primary prevention-initiation of therapies before measurable disease is present.¹¹³ Universal implementation of prenatal and neonatal screening programs in the United States means that infants are usually diagnosed before manifesting respiratory symptoms. The emergence of novel genotype-, mutation-, or class-specific therapeutics that target the basic defects allows for personalized treatments. These discoveries have raised the hope that CF lung disease can be prevented before it starts, well before children develop respiratory symptoms.

For these primary prevention strategies to succeed, more sensitive outcome measures that can identify the earliest changes in lung disease are needed for use in infants and young children. Very few interventional studies have examined treatments in children < 6 years of age, largely because of the difficulty of measuring a treatment effect. Early physiologic and structural changes in the CF lung are relatively subtle, which makes demonstration of a significant treatment response challenging. In addition, there are few adequately sensitive, low-risk techniques to measure disease in this population. Further development of methods that can detect the earliest changes of CF lung disease while being minimally invasive, and ideally imparting no risk from radiation or sedation, is critical for the

identification and longitudinal following of disease in young children.

As we move forward toward an era of personalized medicine, the primary prevention of CF lung disease will likely be accomplished by comprehensively defining endotypes of patients with CD, using genotype (including *CFTR* and modifier genes) and phenotype, combined with knowledge of environmental exposures and social factors, to stratify patients by risk and likely disease manifestations. Endotyping may also improve outcomes in therapeutic trials by better classifying those subjects most likely to benefit from interventions and adding the power to identify specific responder groups. This approach has the potential to improve clinical care by identifying those patients at highest risk, in whom a particular therapy (or combination of therapies) will likely have the greatest impact while minimizing risk.

Conclusions

Several novel therapies are being tested or have recently been introduced to CF care, with even more treatments in the pipeline. These new therapies have brought the exciting possibility of prevention of CF lung disease, an unprecedented opportunity to profoundly change the morbidities and mortality associated with CF, forever changing the patients' lives. The goal of managing patients with CF without measurable lung disease is potentially within our grasp.

The adoption of many of these therapeutic approaches has and will continue to improve the health of patients with CF, but the growing number of treatments is beginning to present clinical dilemmas. What therapies should be given to which patients? When do we begin therapies in young children? How do we minimize risk as well as the physical, financial, emotional, and social burdens to the patients while providing the most aggressive care possible? As therapeutic advances continue, particularly with the move toward genotype-directed treatments, better classification of patients with CF from infancy onwards will play an important role in characterizing disease progression and response to therapies. Endotyping of patients will need to become the norm and will likely have a significant impact in both future research studies and clinical care.

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