Cystic Fibrosis: The Path to a Cure

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Clinical Interests: Care of patients with Cystic Fibrosis and non-CF related bronchiectasis

Research Interests: Understanding the role of estrogen and progesterone receptor modulation on gender disparities in airway disease

Purpose and Overview: The purpose of this discussion is to describe Cystic Fibrosis, the most common autosomal recessive genetic disease of Caucasians in the U.S. and demonstrate the huge advancements that have been made in the care for these patients. We will review the clinical features of CF and how they relate to abnormal gene function, discuss basic respiratory treatments used to treat these patients and outline new gene targeted therapies being developed.

Educational Objectives:

- Describe clinical features of cystic fibrosis
- Demonstrate role of CFTR in disease manifestations
- Explain the basic respiratory pathophysiology and treatments for CF
- Outline new CFTR modulating therapies for CF

INTRODUCTION:

Cystic fibrosis (CF) is the most common fatal autosomal recessive disease among Caucasian populations, with a frequency of 1 in 2000 to 3000 live births. It is a multisystem life shortening genetic disease first recognized in the 1930s in infants with malabsorption and failure to thrive. It is caused by mutations in cystic fibrosis transmembrane conductance regulator (CFTR), which codes for a c-AMP regulated chloride channel on chromosome 7.[1]

EPIDEMIOLOGY:

CF occurs in approximately 1:3000 Caucasians, 1:9200 Hispanics, 1:10,900 Native Americans, 1:15,000 African Americans and 1:30,000 Asian Americans and is becoming increasingly recognized in nonwhite populations not only in regions familiar with CF but also in South and East Asia, Africa, and Latin America. [2] Prevalence estimates are likely to rise with increasing use of newborn screening and increasing recognition of individuals with mild disease or disease limited to one organ system. There are 12 million people who carry at least one CF allelic mutation, the implications of this are not yet known.

CLINICAL FEATURES:

Mutations in CFTR, a complex chloride channel and regulatory protein found in epithelial cells of exocrine tissues, explains the majority of disease manifestations. Deranged transport of chloride or other CFTR-affected ions, such as sodium and bicarbonate, leads to thick, viscous secretions in the lungs, pancreas, liver, intestine, and reproductive tract, and to increased salt content in sweat gland secretions (Figure 1).[1, 3] The typical CF patient presents with multisystem disease involving several or all of these organs (Figure 2).

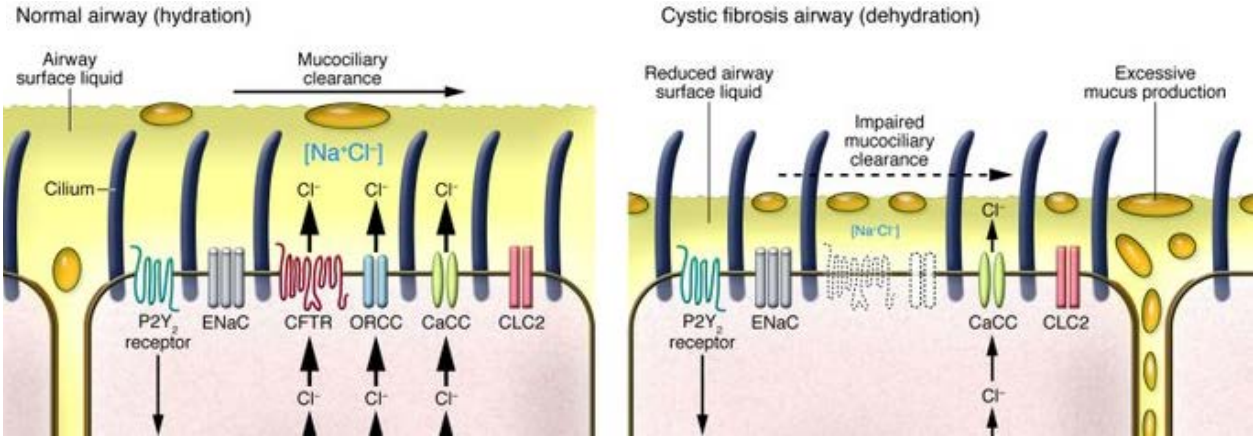

Figure 1. Regulation of air surface liquid composition by CFTR and other apical epithelial channel in normal airways and cystic fibrosis airways from Zeitlin PL. JCI 2008; 118: 3841-4.

Respiratory tract involvement — Typical respiratory manifestations of CF include a persistent cough with purulent sputum production, hyperinflation of the lung fields on chest radiograph, and pulmonary function tests consistent with obstructive airway disease. As the disease progresses, chronic bronchitis with or without bronchiectasis develops and is accompanied by acute exacerbations characterized by increased cough, dyspnea, increased sputum production, malaise, anorexia and weight loss. Colonization of the airways with pathogenic bacteria often occurs early in life. *Staphylococcus aureus* and *Haemophilus influenzae* are common pathogens during early childhood, but *Pseudomonas aeruginosa* is ultimately isolated from the respiratory secretions of most adult patients.[4] This predisposition to infection may be in part because of impaired clearance directly induced by a defect in CFTR. Management of CF respiratory disease is complex and includes bronchodilators, mucolytics, and antibiotics and will be discussed below.

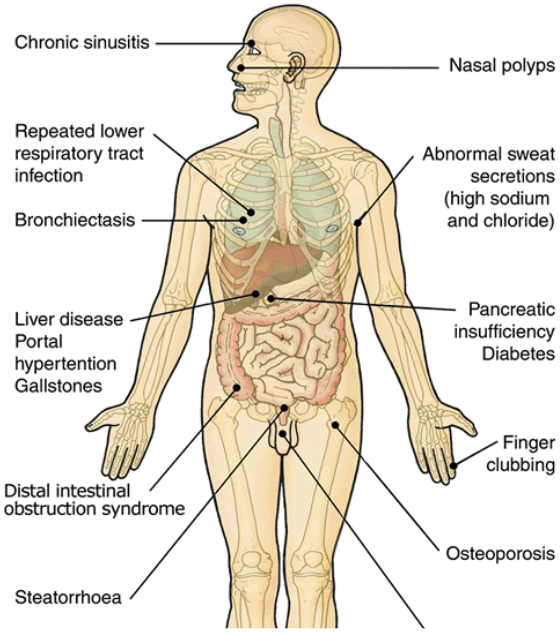

Figure 2. Clinical features of Cystic Fibrosis from Kliegman Nelson Essentials of Pediatrics, 2006.

Sinus disease — The majority of CF patients develop sinus disease. Radiographs reveal pan-opacification of the paranasal sinuses in 90 to 100 percent of patients older than eight months of age. Nasal polyposis is seen in 10 to 32 percent of patients.[5] Chronic sinusitis in CF is typically managed with frequent sinus rinses with salt solution and daily use of nasal steroids. Surgical intervention for polyp removal, dysimpaction of mucus and opening of sinus cavities for improved mucus draining is common.

Pancreatic disease — Insufficiency of the exocrine pancreas is present from birth in most patients with CF and effects 85% of patient at some time in their life.[6] Insufficient secretion of digestive enzyme, lipase, leads to malabsorption of fat and protein. Failure to thrive is a presenting sign in many infants and children. These problems can often be reversed with oral supplementation of pancreatic enzymes. In severe cases, the fat malabsorption can lead to deficiencies of the fat-soluble vitamins A, D, E, and K. Vitamin supplements are routinely given

to patients with CF, and vitamin deficiency is now less uncommon. Most patients with CF develop progressive pancreatic damage as a result of defective ductular and acinar pancreatic secretion. The vast majority of patients with classic CF have exocrine pancreatic insufficiency. Acute or recurrent pancreatitis can be seen, particularly in patients who are pancreatic sufficient, although they may subsequently progress to pancreatic insufficiency. Patients with exocrine pancreatic insufficiency often develop dysfunction of the endocrine pancreas, leading to glucose intolerance and CF-related diabetes.

CF-related diabetes - Cystic Fibrosis-related diabetes (CFRD) occurs in approximately 20% of adolescents and 40-50% of adults.[7] While it shares features of type I and type II diabetes, it is a distinct clinical entity. It is primarily caused by insulin insufficiency, but also has features of insulin resistance. Some have normal glucose tolerance because of the unusual combination of increased hepatic glucose production and increased peripheral glucose utilization; the latter adaptation is initially able to counteract the effects of insulin deficiency.[8] When impaired glucose tolerance or overt diabetes develops, it is characterized by reductions in both peripheral glucose utilization and hepatic insulin sensitivity. The standard medical therapy for CFRD is subcutaneous insulin, whether or not fasting hyperglycemia is present. [9]

Meconium ileus and distal ileal obstruction — Meconium ileus is the presenting problem in 10 to 20 percent of newborns with CF, and is virtually pathognomonic of the disease. Meconium ileus can occur in patients with a variety of CFTR mutations. A familial recurrence rate of nearly 30 percent suggests that other genetic modifiers predispose to the development of meconium ileus.[10] Small bowel obstruction may also occur in older children and adults, and is known as distal intestinal obstructive syndrome (DIOS). DIOS occurs in about 15 percent of adult patients with CF and is more common in patients with severe CFTR genotypes and advanced lung disease.[10] DIOS is caused by inspissated intestinal contents that completely or partially block the small intestinal lumen, most commonly at the ileocecal junction. The pathogenesis of DIOS is not fully understood, but several mechanisms have been suggested. The vast majority of patients with DIOS have pancreatic insufficiency. There have been only a few case reports of DIOS in patients with adequate pancreatic function. Intestinal dysmotility, either intrinsic or iatrogenic, has also been suggested as a factor in the development of this disorder. Intrinsic dysmotility has been implicated to explain the high rate of recurrence in some patients. Other factors that may have a role in the development of DIOS are abnormal mucins and water and electrolyte composition of the intestinal contents. When identified early, DIOS can usually be

controlled medically with laxatives and hypaque enemas.[11] Surgical intervention is sometimes required to alleviate severe obstruction but may be complicated by recurrent symptoms caused by adhesions and should be avoided if at all possible.

Biliary disease — Cholestatic liver disease caused by inspissated bile is present in many patients. In a minority of patients, the liver disease is progressive, with periportal fibrosis, cirrhosis, symptomatic portal hypertension, and variceal bleeding consistent with focal biliary cirrhosis. CF is the third leading cause for liver transplantation in late childhood.[12] Several observational studies have suggested that administration of ursodeoxycholic acid may slow the progression of liver disease related to CF, but the efficacy of this therapy has not been confirmed in randomized trials.[13] Cholelithiasis has been reported in up to 12 percent of patients, which is thought to be due to excessive loss of bile acids in the stool with consequent production of lithogenic bile.[12]

Infertility — More than 95 percent of men with CF are infertile because of defects in sperm transport, although spermatogenesis is not affected.[14] Most of these men have incompletely developed or absent vas deferens. These anomalies probably reflect a critical role for CFTR in the organogenesis of these structures, but are not fully understood. Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection can permit affected men to become biological fathers.[15]

The incidence of female infertility may be as high as 20 percent.[16] It is related to secondary amenorrhea (induced primarily by malnutrition) and the production of abnormally tenacious cervical mucus. When patients with CF become pregnant, maternal and fetal outcomes are generally favorable if the pre-pregnancy Percent predicted forced expiratory volume in 1 second (FEV1%) exceeds 50 to 60 percent of the predicted value.[17]

Musculoskeletal disorders — Patients with CF frequently have osteopenia or osteoporosis, and is seen in up to 30 percent of patients. [18-19] This appears to be caused by reduced rates of bone growth and accelerated rates of bone loss in addition to chronic vitamin D deficiency and frequent systemic steroid use. CF-associated arthropathy occurs in 2 to 9 percent of patients, and is characterized by brief episodes of pain and swelling of joints.[20] These features are occasionally accompanied by painful nodular skin lesions and purpura and are not yet fully understood.

Nephrolithiasis and nephrocalcinosis — Nephrolithiasis and nephrocalcinosis are common in patients with CF. The reported prevalence of microscopic nephrocalcinosis ranges from 27 to 92 percent and 3 and 6 percent of individuals with CF develop nephrolithiasis, compared to 1 to 2 percent of age-matched controls without CF.[21-22] Enteric hyperoxaluria (due to fat malabsorption resulting from decreased secretion of pancreatic enzymes) and hypocitraturia (due to chronic metabolic acidosis) are thought to be risk factors.

Mood disorders – Anxiety and depression are common as with many other young patients with chronic diseases. The lifetime prevalence of depression has been reported to be up to 11.5% in cystic fibrosis.[23] When treating these disorders, the effects of benzodiazapines on lung disease and selective serotonin re-uptake inhibitors (SSRI) on interactions with antibiotics including zyvox should be considered.

The incidence of many CF related co-morbidities rise particularly in adulthood (Figure 3).

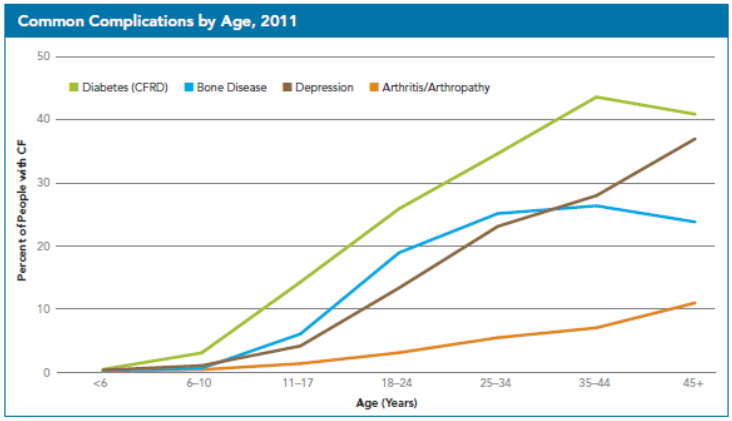

Figure 3. Prevalence of less common complications of cystic fibrosis from the Cystic Fibrosis Foundation Patient Registry 2011 Annual Data Report.

PATHOGENESIS OF LUNG DISEASE:

Despite the multi-system nature of CF, progressive obstructive lung disease remains the leading cause of morbidity and mortality. Decline in forced expiratory volume in one second (FEV1) tracks along with mortality (Figure 4).[4] The main reason for this lies in defective mucociliary clearance of the airways due to CFTR dysfunction (Figure 5). CFTR mutations result in defective chloride secretion to the surface of the airway epithelium.[24] This leads to a decreased

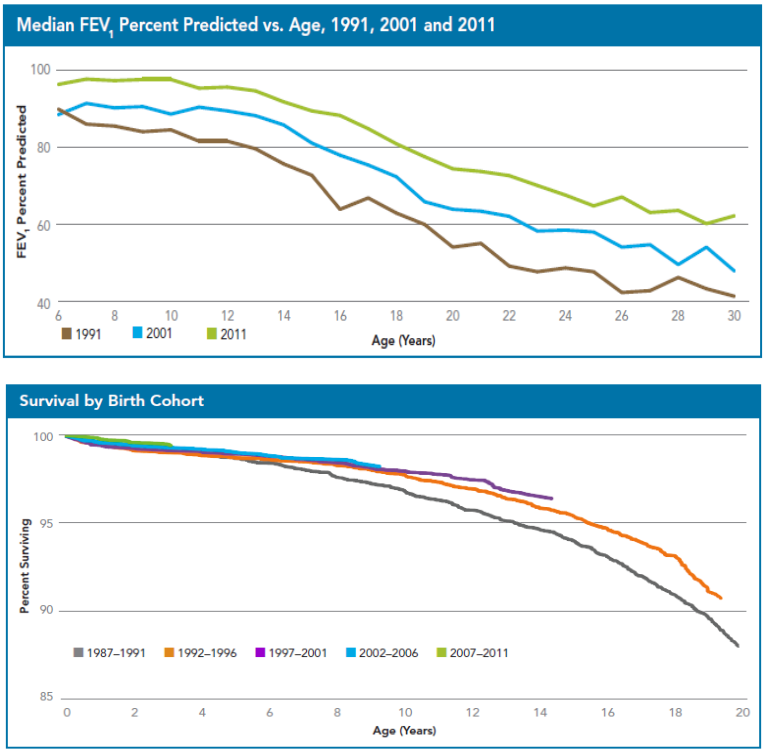

Figure 4. (Top) Lung function decline observed over time/age in CF patients with notable improvement in the past 20 years. (Bottom) Survival curves in all age and gender groups with CF over time with notable improvement in the past 20 years. Data from the Cystic Fibrosis Foundation Patient Registry 2011 Annual Data Report.

air surface liquid layer which leaves mucus thick, sticky and viscous. This cycle leads to poor cilia function, lack of clearance of mucus and pathogens from the lungs and resulting inflammation and infection that cause recurrent pulmonary exacerbations. Respiratory features of the disease include chronic cough and purulent sputum production, airway obstruction with wheezing and chest tightness, microbial colonization with frequent infections and progressive irreversible thickened inflamed airways known as bronchiectasis.

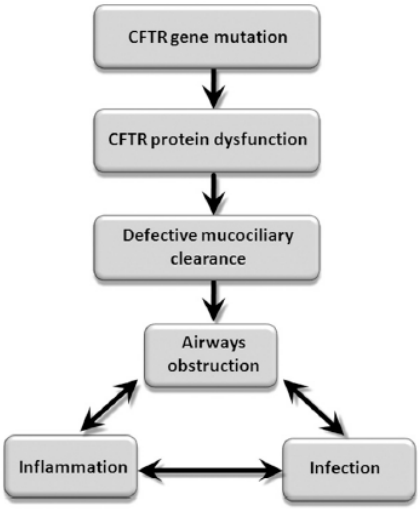

Figure 5. Pathogenesis of lung disease in CF from Hoffman LR, Ramsey BW. Chest 143; 2013: 207-213.

HISTORY OF PROGRESS: Despite the grim picture depicted of the array of clinical features found in CF patients, huge advancements have been made in treating the disease such that life expectancy has gone from 5 years of age in the 1950s to 38 years of age in 2012 (Figure 6).[4] CF was once known as a Pediatric disease, but with the advent of many important therapies, the adult and pediatric CF populations are near equal.[4] This is largely due to the efforts of the Cystic Fibrosis Foundation in pairing with pharmaceutical companies to organize multi-center clinical trials focused on treatments to improve the lives of patients with CF and to the discovery of the abnormal gene in CF, Cystic Fibrosis Transmembrane conductance Regulator, CFTR, which was found simultaneously by three separate groups in 1989.[25] Since that time, an array of therapies have been developed geared toward improving the underlying pathophysiology of the disease and most recently to correcting the underlying protein defect.

Figure 6. (Left) Median Predicted Survival of CF patients from 1987-2011 in 5 year increments. (Right) Prevalence of adults and children with CF over time. Data from the Cystic Fibrosis Foundation Patient Registry 2011 Annual Data Report.

CFF DRUG DEVELOPMENT PIPELINE – The CF Foundation in conjunction with multiple pharmaceutical companies have been studying new treatments geared towards targeting many different aspects of the underlying cause of the disease (Figure 7).[24]

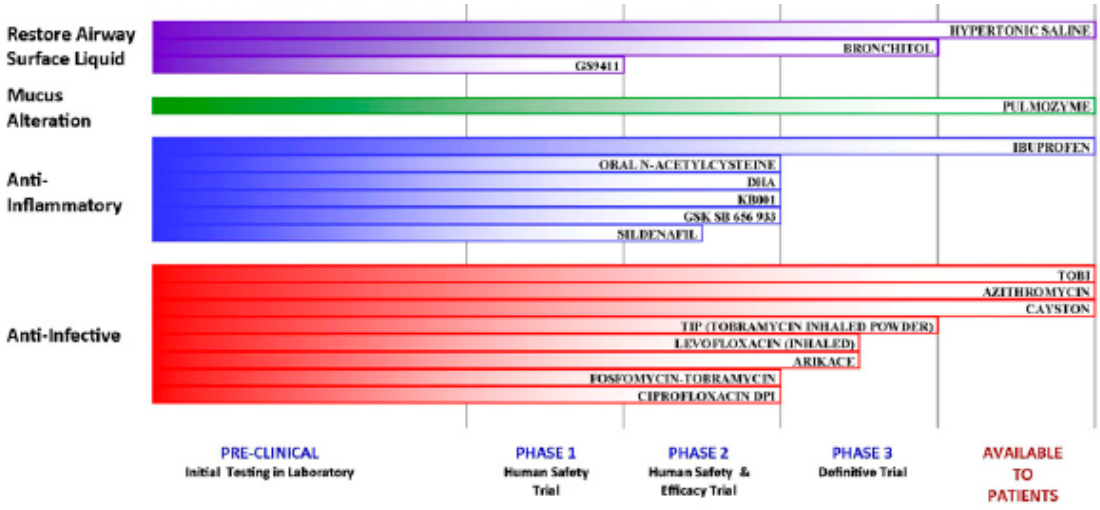

Figure 7. Drugs in development for CF focused on underlying respiratory pathology from Hoffman LR, Ramsey BW. Chest 143; 2013: 207-213.

MAJOR ADVANCEMENTS IN CF CARE: (cff.org)

Since the discovery of the CF gene in 1989, major steps in understanding the pathophysiology of the disease have occurred with development of a number of new treatments.

- **1989 –** Discovery of CF gene
- **1993** - FDA approves nebulized dornase alpha (Pulmozyme)
- **1997** - FDA approves tobramycin inhalation solution (TOBI)
- **2002** - Study shows oral azithromycin improves CF lung health in people with Pseudomonas
- **2004** – Study shows nebulized 7% hypertonic saline helps improves lung function and reduces hospital stays
- **2010** - FDA approves aztreonam inhalation solution (Cayston)
- **2012** - FDA approves VX-770 (Kalydeco) for patients with G551D mutation

THERAPIES TO TREAT LUNG DISEASE:

Mucolytics

Dornase alfa: The CF airway is characterized by abundant neutrophils that release DNA when they undergo necrosis. Extracellular DNA and actin are responsible for increased sputum viscosity and result in mucus obstruction, impaired mucociliary clearance, infection and airway inflammation. Dornase alfa cleaves extracellular DNA, resulting in reduced DNA concentration and length, as well as reduced sputum viscosity. This change facilitates airway clearance, relieves obstruction and improves pulmonary function. In addition, inhaled dornase alfa may alter bacterial infection by cleaving DNA in the biofilm of bacteria and making these bacteria more sensitive to antibiotic killing. This is supported by evidence that shows lower rates of *Staphyloccocus aureus* acquisition with the use of dornase alfa in patients with CF and early lung disease.

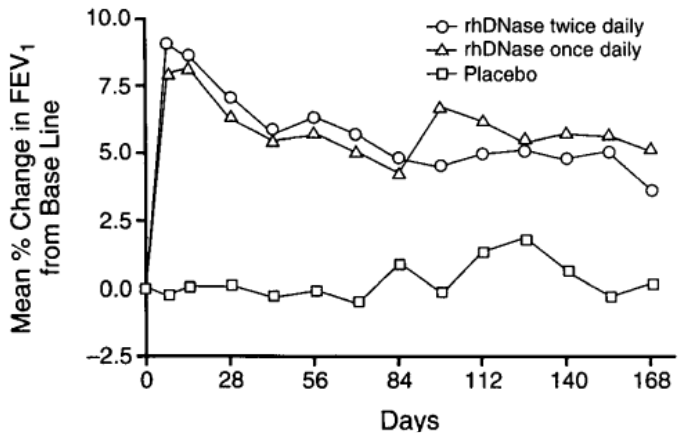

Figure 8. Phase III multi-center clinical trial results showing change in FEV1% of rhDNase once versus twice daily versus placebo from Fuchs et al. NEJM 1994; 331:631-642.

A randomized, blinded, placebo-controlled trial of 968 stable CF patients with an FVC% >40 showed that the daily inhalation of 2.5 mg of nebulized DNase for six months resulted in a statistically significant improvement in FEV1% of approximately 6 percent (Figure 8).[26] A small but statistically significant reduction in the number of hospital days for exacerbations of respiratory disease was also seen in patients receiving the drug. Since its FDA approval in 1993, dornase alpha has been a mainstay of respiratory treatment for CF lung disease. Of note, it was not found to be beneficial in patients with non-CF related bronchiectasis.[27]

N-acetylcysteine: N-acetylcysteine, a free sulfhydryl reagent that cleaves disulfide bonds within mucus glycoproteins, can liquify CF sputum *in vitro*, and has antioxidant properties. Although originally developed as an inhaled mucolytic agent, there are no well-designed studies that demonstrate its clinical utility. Furthermore, its potential to induce airway inflammation and/or

bronchospasm in a subgroup of patients and to inhibit ciliary function has led to reduction in its use.

Restore Air Surface Liquid

The current model invoking airway dehydration in the pathophysiology of CF lung disease provides a strong rationale for treatment to restore airway surface liquid (ASL) soon after diagnosis.

Inhaled hypertonic saline: Hypertonic saline has been administered by inhalation to hydrate inspissated mucus that is present in the airways of patients with CF. It is presumed that the high osmolality of the solution draws water from the airway epithelium to re-establish the aqueous surface layer that is deficient in CF. The effectiveness of this strategy was shown by a study of 24 patients (≥14 years of age) with stable CF who were treated with 5 mL of 7 percent saline four times per day for 14 days. The mucus clearance rate was improved after 1 and 14 days of hypertonic saline treatment, and there were modest improvements in lung function and symptom scores.[28]

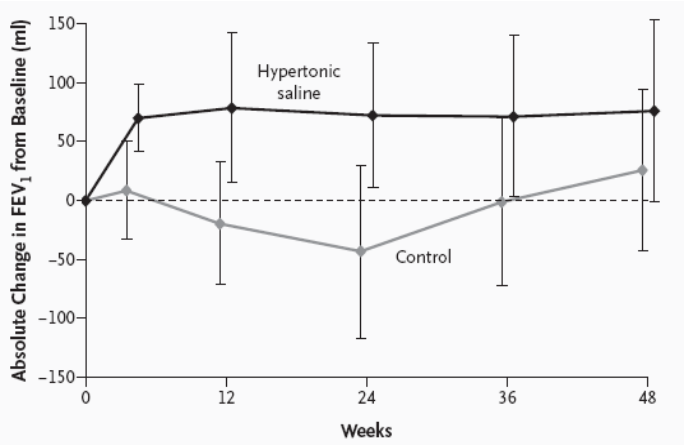

Figure 9. Phase III multi-center clinical trial results showing absolute change in FEV1 of patients treated with 7% hypertonic saline versus 0.9% saline from Elkins et al. NEJM 2006; 354: 229-240.

Patients treated with hypertonic saline had considerably fewer pulmonary exacerbations requiring antibiotic therapy (mean number of exacerbations per participant 0.39 versus 0.89) and fewer days absent from school or work or unable to participate in usual activities (7 versus 24 days). Treatment with hypertonic saline was not associated with worsening bacterial infection or inflammation. The treatment was well tolerated. In a larger randomized double blinded placebo controlled trial of 164 CF patients treated with 7% hypertonic saline twice daily versus 0.9% saline, the absolute difference in lung function between groups was significant ($p = 0.03$) when averaged across all post-randomization visits in the 48-week treatment period (Figure 9).[29] As compared with the control group, the hypertonic-saline group had significantly higher FEV1 values (68 ml). The hypertonic

saline group also had significantly fewer pulmonary exacerbations (relative reduction, 56 percent; p = 0.02) and a significantly higher percentage of patients without exacerbations (76 percent, as compared with 62 percent in the control group; p = 0.03).

Mannitol: Treatment with mannitol by inhalation is associated with modest improvement in some clinical outcomes. This was shown in two similarly-designed phase 3 trials studying the efficacy and safety of inhaled dry powdered mannitol.[30-31] In each trial, more than 300 subjects were randomized to receive twice daily mannitol, 400 mg by inhalation, compared to a control group receiving a low dose of mannitol (40 mg). Subjects were not permitted to use hypertonic saline during the study. The primary endpoint for both trials was absolute change in FEV1 from baseline, averaged over the duration of the trial. The first study met this endpoint, reporting a relative improvement in FEV1 of 93 mL in the treatment group compared to that of the control group (p <0.001). Exacerbation rates were also reduced (p = 0.045). The second trial just missed the endpoint, reporting a relative improvement in FEV1 of 54.1 mL in the treatment group compared to control (p = 0.059). Exacerbation rates were reduced, but did not reach statistical significance (p = 0.31). However, secondary endpoints and post-hoc analyses using other spirometry-based measures were statistically better in the treatment group. The improvement with mannitol treatment was sustained during a 26-week study extension period.

Denufosol: Activation of alternative chloride channels, calcium-activated chloride channels, can promote chloride secretion in airway cells lacking CFTR function. One approach to activate this channel is via the stimulation of surface P2Y2 purinergic receptors. Denufosol, a P2Y2 agonist, was studied in a short term (24 week) double blind phase III trial.[32] Study drug by inhalation three times daily produced a 48 mL improvement in FEV1 (p<0.047) versus a 3 mL improvement in the control group. However, a longer (48 week) and larger phase 3 double blind placebo controlled trial of 466 patients failed to show a statistically significant effect on lung function.[33]

ANTIBIOTICS AND ANTI-INFECTIVES

PATHOGENS — Chronic bacterial infection within the airways occurs in most patients with CF; the prevalence of each bacterial type varies with the age of the patient (Figure 10).

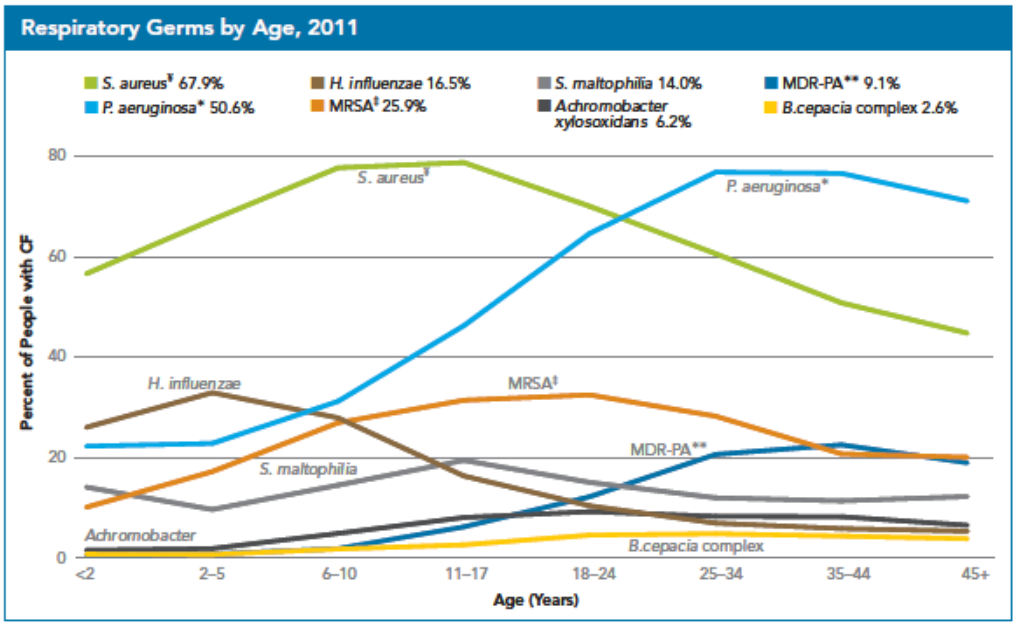

Figure 10. Percentile of CF patients with different bacteria found in the lungs by age of acquisition from the Cystic Fibrosis Foundation Patient Registry 2011 Annual Data Report.

Pseudomonas aeruginosa — For reasons that are poorly understood, the CF airway is particularly susceptible to *Pseudomonas aeruginosa*, with infection occurring as early as the first year of life. The prevalence of P. *aeruginosa* increases as patients' age, such that more than 73% of adults are chronically infected.[34] With prolonged infection, P. *aeruginosa* converts to a mucoid phenotype by the production of alginate. This mucoid phenotype is seen infrequently in populations without CF but is manifested by over 66 percent of the P. *aeruginosa* isolated from patients with CF.[4] Chronic infection with P. *aeruginosa* is an independent risk factor for accelerated loss of pulmonary function and decreased survival. Conversion of P. *aeruginosa* to the mucoid phenotype worsens prognosis.

Staphylococcus aureus — *Staphylococcus aureus* is the bacterial pathogen most frequently identified in respiratory secretions of CF infants and children.[35] It remains a significant pathogen throughout adulthood. It's impact on long term lung health is not yet well described.

Methicillin-resistant *Staphylococcus aureus* (MRSA) — MRSA has become more prevalent in the CF population, increasing from 2.1 percent in 1996 to 26 percent in 2009.[4] A study of nearly 20,000 CF patients in the United States found that MRSA was associated

with 1.3 times the risk of death compared with individuals never infected with MRSA.[36] Multivariate analysis showed that MRSA was an independent risk factor whose effect could not be explained by its association with other known risk factors including age, sex, diabetes, pancreatic status, FEV1 at baseline, and socioeconomic status, or co-infection with *Burkholderia cepacia* (B. *cepacia*) complex or P. *aeruginosa*.

Burkholderia cepacia complex — Advances in bacterial genetics have revealed that *Burkholderia cepacia*, which was originally thought to be a single species, is now known to constitute multiple separate species, each of which is a member of the *Burkholderia cepacia* complex. The species most commonly isolated from the sputum of CF patients are *Burkholderia multivorans* and *Burkholderia cenocepacia*.

Chronic infection with B. cepacia complex bacteria is associated with an accelerated decline in pulmonary function and shortened survival in CF.[37] Lung transplantation in patients infected with B. cepacia complex is associated with recurrent and often severe infection, with poor outcomes, particularly for those carrying B. cenocepacia and is has become a relative contraindication.

Other pathogens — Other pathogens have been identified in respiratory secretions of CF patients, with varying prevalence including *Haemophilus influenza*, *Stenotrophomonas maltophilia*, *Achromobacter* (formerly *Alcaligenes*) *xylosoxidans*, Nontuberculous mycobacteria, including Mycobacterium *avium* (MAI) complex, Mycobacterium *abscessus*, and *Aspergillus* species.[4]

Investigations are ongoing to determine which members of this complex microbiome are important for driving pulmonary exacerbations and disease progression.

Inhaled antibiotics — Aerosolized Tobramycin and aztreonam have been approved by the FDA for CF patients colonized with *Pseudomonas*. These are the only 2 approved inhaled antibiotics for chronic maintenance suppression of *Pseudomonas* or other bacteria in CF.

Inhaled tobramycin — Treatment with nebulized tobramycin (TOBI) in patients chronically infected with P. *aeruginosa* improves lung function and reduces acute pulmonary exacerbations. The pivotal study of inhaled tobramycin was a 24-week randomized, double blind, multicenter trial in 520 patients with stable CF.[38] Twice daily treatment with 300 mg of inhalational tobramycin solution in cycles of 28 days on the medication followed by 28 days

off was compared with a control group (Figure 11). Subjects receiving tobramycin had a 10% higher FEV1 at 20 weeks, a decrease in the sputum density of P*. aeruginosa*, and a 26% decrease in the likelihood of hospitalization during the trial. In a two-year, open-label follow-up of patients participating in the above study, ongoing use of inhaled tobramycin was associated with greater improvement in FEV1 and with an increase in body mass index. Peak serum tobramycin concentrations averaged <1 mcg/mL, and no significant nephrotoxicity or ototoxicity was detected.

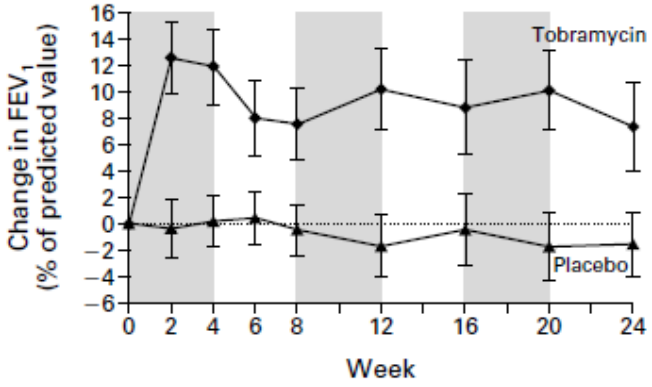

Figure 11. Phase III multi-center clinical trial results showing improved FEV1% in TOBI versus placebo group from Ramsey et al. NEJM 1999; 340: 23-30.

Inhaled aztreonam lysine — Aztreonam, a monobactam antibiotic with anti-Pseudomonal activity has also undergone large scale clinical trials. In a randomized trial, 211 subjects with chronic pseudomonal lung infection were given either inhaled aztreonam lysine (75 mg) or placebo for 28 days.[39] The group treated with inhaled aztreonam had a longer time before needing additional anti-Pseudomonal antibiotics (92 days) as compared with those given placebo (71 days) (Figure 12). Furthermore, patient-reported respiratory symptom scores, FEV1, and *Pseudomonas* density in sputum samples also improved in the group given aztreonam.

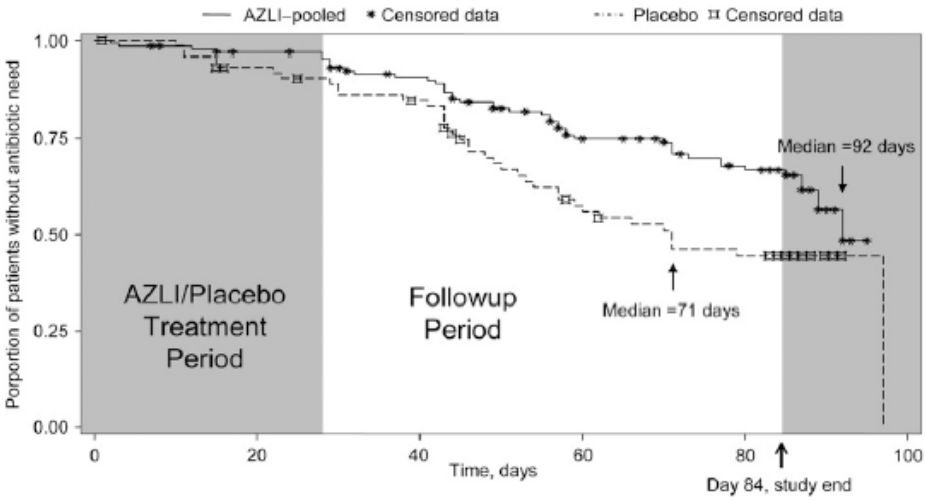

Figure 12. Phase III multi-center clinical trial results showing longer duration to next course of antibiotics in Aztreonam treated group versus placebo from McCoy et al. AJRCCM 2008; 178: 921-928.

Inhaled colistin — The polypeptide antibiotic colistin has anti-Pseudomonal activity, which is preserved against many multidrug-resistant strains. To achieve benefit without the systemic toxicity, the inhalation route also has been used for colistin. The renal and neurotoxicity of inhaled colistin appears minimal when 150 mg of colistimethate sodium is diluted in 2 mL of sterile water and administered by nebulizer twice per day. However, bronchospasm may be induced, particularly among patients with a history of wheezing, atopy, or asthma. In a small clinical trial, 40 subjects with CF were randomized to receive twice a day colistin or isotonic saline by inhalation.[40] A high dropout rate compromised the value of the study; only 11 saline subjects and 18 colistin subjects completed the three-month treatment period. Comparison of the groups showed that those receiving colistin had better clinical symptom scores and preservation of pulmonary function. In a randomized trial, one month treatment with either colistin or tobramycin resulted in a decrease in bacterial load; however, only tobramycin therapy was associated with improvement in lung function.[41] Thus, inhaled colistin appears to be potentially effective but may be inferior to inhaled tobramycin. No studies have compared colistin with inhaled aztreonam. Aerosolized colistin is not approved by the FDA for CF.

The success of these antibiotics has induced a number of pharmaceutical companies to develop other inhaled antibiotics for CF including aerosolized Ciprofloxacin, Fosfomycin and Tobramycin combined, Levofloxacin, liposomal Amikacin, and tobramycin inhalation as a dry powder. Studies of these agents are ongoing.

Table 1. SUMMARY OF MAINTENANCE RESPIRATORY THERAPIES

Type of airway clearance therapy	Agent	Maint Therapy
Bronchodilators	Albuterol/Xopenex	BID
Mucolytics	Pulmozyme/dornase alpha	QD
Restoration of airway surface liquid	7% hypertonic saline	BID
Chest physiotherapy	Chest physiotherapy	BID

Inhaled antibiotics (if Pseudomonas +)	TOBI (tobramycin) Cayston (aztreonam)	BID TID
Anti-inflammatories	Ibuprofen Azithromycin	QD TIW

Problems with current therapies:

Despite significant improvements in life expectancy of patients with CF, several major problems still exist in the therapies that have been developed this far. These first of these is the burden of the time it takes to adhere to the appropriate medical regimen. Between breathing treatments, exercise, sinus care, adequate nutrition and vitamins, as well as glucose control, patients are overwhelmed with the time it takes to stay well. Second, the majority of the therapies developed have been geared toward lung disease with very little developed for sinus disease, hepatobiliary disease and GI luminal disease. Therapies are also incredibly expensive and often unaffordable even with insurance. Finally, these therapies target the underlying pathophysiology, but do not cure the direct cause of the disease.

ALTERNATIVE APPROACHES

Gene Therapy

The prospects for developing gene therapy to treat CF improved markedly in 1989 with the sequencing and cloning of the CFTR gene. Because mutations in CFTR account for virtually all cases of CF, correcting abnormalities in this single gene should theoretically cure the disease, regardless of the genotype. Although *in vitro* methods have demonstrated feasibility in many systems, efficient gene transfer and persistent CFTR expression have not been achieved in clinical trials.[42] Multiple questions remain unanswered in regards to gene therapy including what cells need to express CFTR, what is the best method of delivery, and what level of CFTR expression is needed to reverse the phenotype. To overcome these barriers, research in this field is focusing on developing vectors for the safe delivery of a normal CFTR gene to the airways of patients with CF. Currently, only one group, the UK Cystic Fibrosis Gene Therapy Consortium, is actively involved in clinical trials of patients with CF. This group has been using liposomes to transfer plasmids containing a functional CFTR cDNA to the airway epithelium.[42] Results from their single-dose pilot study involving a few human subjects demonstrated the successful delivery of the CFTR gene to the airway

with the resultant expression of a normal CFTR protein. Unfortunately, the treatment also caused mild flu-like symptoms and a transient decrease in measures of pulmonary function. A planned multi-dose trial has been delayed until methods to circumvent these problems are found.

Gene Targeted Therapy

CFTR protein modulation, an alternative to gene transfer therapy represents the next wave of therapeutic development. These are small molecule oral systemically effective agents that are being developed by high through put screening to target underlying CFTR protein abnormalities.

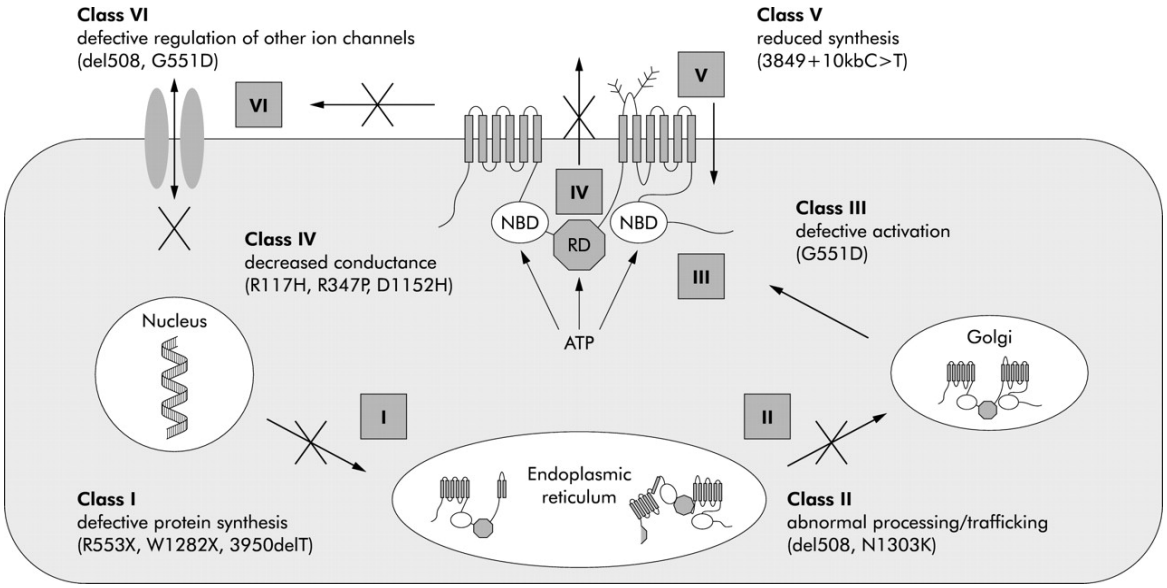

Figure 13. Schematic depiction of am epithelial cell with functional problem with the six type of CFTR mutations from 921-928.

To understand the benefit and effectiveness of these therapies, it is important to understand the different classes or categories of CFTR mutations. More than 1900 different CFTR mutations have been identified in the CFTR gene though less than 90 account for the vast majority of CF phenotypes. They have been grouped into different classes based upon their effects on CFTR structure and/or function (Figure 13).

Class I mutations: Defective protein production. Class I mutations include nonsense, frameshift, and splice junction mutations including G542X, W1282X, R553X, R1162X,

621+1G>T, 1717-1G>A. They account for approximately 10% of CF disease causing mutations and are considered severe. Nonsense mutations in CFTR introduce premature stop codons so that the messenger RNA is truncated and no mature CFTR protein is produced. This type of mutation accounts for 2 to 5 percent of cases of CF worldwide. Potential treatments for patients with this type of mutation include drugs that allow the ribosome to read through premature stop codons during translation, while still honoring true stop codons. Ataluren (PTC124) is a small molecule which binds to ribosomes and allows read-through of premature stop codons in CFTR mRNA (Figure 14). In a phase 2 study without a placebo control, 23 patients with one nonsense mutation received PTC124. The primary outcome consisted of measurements reflecting aspects of transepithelial potential difference. Significant improvement in total chloride transport was seen with modest improvements in lung function and body weight.[43] A phase 3 multicenter, controlled, efficacy and safety study of CF patients with one CFTR gene nonsense mutation (class I), and FEV1 of 40% to 90% predicted showed promising results. This trial was just completed and results are still unpublished but revealed at the last CF Foundation meeting, showing no significant difference in treatment group versus placebo.[24] However, subanalysis revealed that when patients on inhaled antibiotics (primarily TOBI) were excluded, significant improvements were seen in the ataluren treated group. This is thought to be due to the known feature of aminoglycosides in binding 16S rRNA and also suppressing premature stop codons. Thus a repeat trial excluding people on aminoglycoside therapy is likely to be repeated. This novel therapy may present some potential risks such as the suppression of native stop codons, however, molecular tests specifically conducted to monitor such cellular processes showed that PTC124 seems very selective for premature stop signals and not for native stop codons.

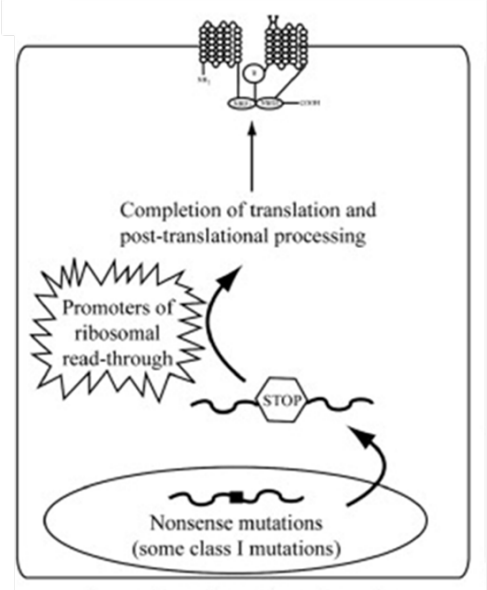

Figure 14. Schematic depiction of promoters that could read through premature stop codons, CFTR from Kreindler JL. Pharmacology &Therapeutics 2010; 125: 219-29.

Class III mutations: Defective regulation — Class III CFTR mutations are commonly missense mutations, single base pair mutations that lead to changes in a single amino acid residue. One of these mutations, G551D exchanges an aspartate residue for a glycine residue at position 551. This leads to abnormal CFTR regulation where CFTR protein reaches the apical epithelial cell surface, but has poor conductance or gating. High-throughput screening of combinatorial libraries has identified candidate molecules (called "potentiators") that improve activation of these abnormal channels and enhance chloride flux in cells expressing at least some of these class III mutations (Figure 15).[45] The G551D mutation is present in about 5 percent of patients with cystic fibrosis, making it one of the more common disease-causing mutations in this class. Ivacaftor (VX-770, Kalydeco) is a potentiator drug that has substantial benefits in patients with at least one G551D allele. A phase III multi-center randomized double blind placebo controlled trial was completed and published in 2010, showing VX-770 increased FEV1% and partially restored chloride transport as measure by sweat test and nasal epithelial electrical potential difference (Figure 16).[44]

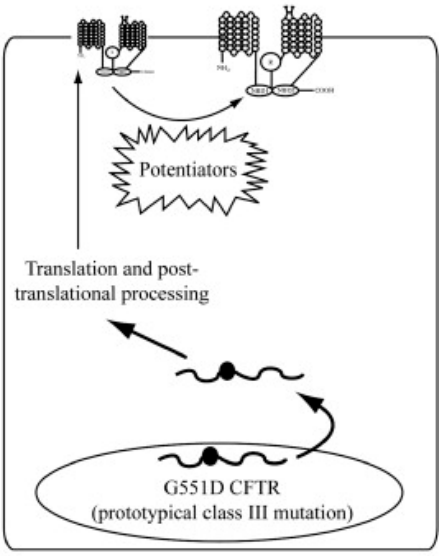

Figure 15. Schematic depiction of CFTR potentiators that improve chloride secretion in patients with the gating mutation, G551D from Kreindler JL. Pharmacology &Therapeutics 2010; 125: 219-29.

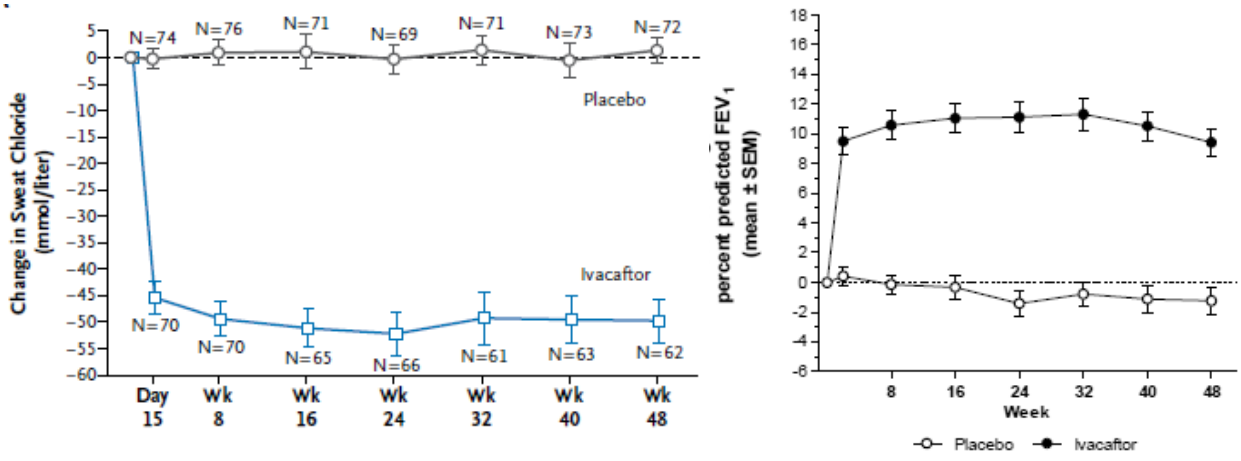

Figure 16. Sweat chloride results and FEV1 results from phase III clinical trial of VX-770 for G551D from Accurso et al. New England Journal of Medicine 2010; 363:1991-2003.

This drug was subsequently FDA approved in Jan 2012, which hit headlines around the world and was named Forbes most important new drug of 2012 because this was the first drug to treat the underlying cause of CF!

Class II mutations: Defective protein processing

Mutations causing defective protein processing are the most common cause of CF worldwide. These include the F508del mutation, which causes improper folding of the CFTR protein during its synthesis. F508del is a deletion of three nucleotides that remove the codon for phenylalanine at position 508. This results in an incorrectly folded CFTR protein that is unable to escape the endoplasmic reticulum for further processing and the majority of it is degraded in the proteosome. As a result, very little mature F508del CFTR protein is trafficked to its targeted location on the apical surface of cells. Because of the high frequency of F508del mutations in CF, major efforts have been put forth to identify drugs that can assist with F508del folding and permit its transfer to the apical surface of expressing cells. Candidate molecules called "correctors" allow F508del to fold properly to be transported to the apical surface. One such drug, VX-809, has reached clinical trials. In a phase 2 trial to test safety and tolerability, adult patients with homozygous F508del mutations were randomized to receive one of four doses of VX-809 or placebo for 28 days.[45] VX-809 treatment resulted in a small but statistically significant decrease in sweat chloride values in a dose-dependent manner. This small trial did not detect improvements in pulmonary function or nasal potential difference, and in the subset of patients that provided rectal biopsy tissue, no maturation of F508del-CFTR was observed. An additional molecule VX-661 is being evaluated as well.

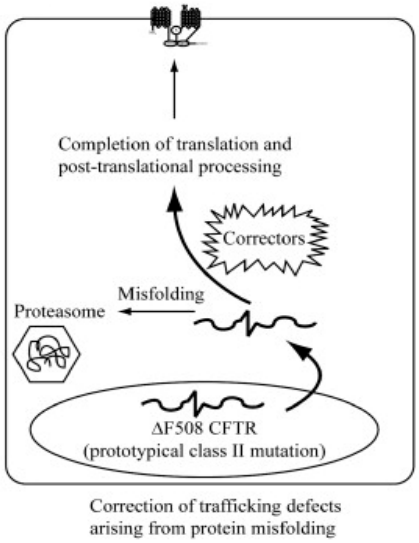

Figure 17. Schematic depiction of CFTR correctors improving trafficking of CFTR to cell surface from Kreindler JL. Pharmacology &Therapeutics 2010; 125: 219-29.

Combination strategy for the F508del mutation — In vitro experiments have shown that, in addition to being misfolded, F508del CFTR does not activate normally even when the protein is induced to traffic correctly to the cell surface. Thus, the F508del mutation manifests both Class II and Class III defect properties. Therefore, it is possible that both functional defects could be

addressed by combined treatment with VX-809 and VX-770. A phase 2 clinical trial to assess the efficacy of combined treatment with VX-809 and VX-770 in adult patients with homozygous or heterozygous F508del mutations demonstrated a significant but modest improvement in lung function (improvement in FEV1% of 8.5, p = 0.002) among homozygous patients treated with the drug combination for 56 days, as compared to those given placebo.[46] Among patients in the treatment arm, 35 percent (13/37) achieved an absolute improvement in FEV1 of 5 percent or more, and 19 percent (7/37) achieved an absolute improvement in FEV1 of 10 percent or more, as compared to no patients in the placebo arm (0/11). This preliminary analysis focused only on patients with homozygous mutations in the treatment arm, although both heterozygotes and homozygotes were included in the placebo arm. The phase III trial of VX-770+VX-809 to treat CF patient with F508del homozygous genotypes is set to begin in the next few months.

As these high throughput screening programs continue on to find additional compounds to treat these and other CFTR class mutations, there remain several unanswered questions including how much CFTR function is enough to overcome the phenotypic problems, how many combinations of drugs will it take to get the best efficacy, and what outcome measures to follow (sweat test, FEV1%, etc.). Clinically, the question remains as to whether patient should and will continue to need to do hours of breathing treatments to remain healthy.

UTSW CF CLINICAL AND RESEARCH PROGRAM

We have the 17th largest CF program in the United States and service the larger Dallas/Ft. Worth metroplex, Midland, Odessa, Waco, Amarillo, El Paso, and other areas with approximately 430 patients. UTSW was named a Therapeutics Development Network site in January of 2011 to actively participate in multi-center CF clinical trials. We currently have over 12 clinical trials active or in start up. In addition, we have active investigator initiated research occurring on campus: Phil Thomas, Drew Feranchak, Carolyn Cannon, David Greenberg, myself.

SUMMARY

CF is the most common autosomal recessive genetic disease in the U.S. It is a multisystem life shortening disorder of which respiratory complications contribute to the greatest cause of morbidity and mortality. There have been huge advancements in understand the disease that have led to improved therapies to target the underlying pathogenesis and most recently to target the underlying genetic mutations. As a result the life expectancy continues to increase. We have an active clinical and research program at UTSW to help care for and treat these patients.

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