Modulator Therapy for Cystic Fibrosis: An Exploration of Current Research

Jessalyn Rombocos

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Gary Isaacs, Ph.D.
Thesis Chair

_____

Daniel Howell, Ph.D.
Committee Member

_____

Cindy Goodrich, EdD
Assistant Honors Director

_____

Date

Abstract

Developing a drug therapy that addresses the root cause of cystic fibrosis (CF) by increasing CFTR protein levels has long been a research challenge. After genetic therapy failed because a suitable delivery system could not be found, researchers began searching for small organic molecules that could act as chaperones for CFTR. These molecules, known as modulators, allowed CFTR to be assembled correctly and function similarly to wild type CFTR. Since 2012, four modulator drugs have been developed, tested, and approved by the FDA. In October 2019, Trikafta was approved as the first triple-combination modulator drug and has completely revolutionized CF therapy. This paper details the research challenges, successes, and failures that led to the development of modulator therapies.

Modulator Therapy for Cystic Fibrosis: An Exploration of Current Research

Cystic Fibrosis Introduction

Cystic fibrosis (CF) is a genetic autosomal disease caused by a mutation in a key protein. CF is the most common deadly genetic mutation in Caucasians and impacts 70,000 people worldwide, who have a predicted median survival of 44.4 years old (up from 32.8 years old in 2003) (Bompadre, Li, & Hwang, 2008; CFF, 2019). CF is caused by a monogenic autosomal recessive mutation that prohibits the cystic fibrosis transmembrane conductance regulator (CFTR) protein from functioning normally. CFTR is essential for maintaining homeostasis in the body by facilitating water movement through secretion of chloride, bicarbonate, and other anions. The CFTR gene was cloned in 1989 which led to rapid increases in knowledge of pathophysiology caused by mutant CFTR. Since then, CF research has focused on understanding the role of CFTR in the body and developing treatments such as genetic therapy and modulators which can reestablish CFTR function to the body.

CF affects many different organs in the body including the lungs, kidneys, gastrointestinal and reproductive tracts, liver, and pancreas (Griesenbach, Pytel, & Alton, 2015). Common symptoms of CF include salty-tasting skin, persistent sputum-producing coughing, frequent lung infections, and greasy, bulky stools (CFF, 2019). Most recent data from the Cystic Fibrosis Foundation 2018 Patient Registry list the median age at death to be 30.8 years for individuals with CF with a median predicted survival age of 44.4 years for those born between 2014-2018 (CFF, 2019). While CF affects the whole body, more than 90% of fatalities are due to lung disease; therefore, this paper will focus primarily on the lungs and treatments to help improve lung function (Burney & Davies, 2012).

This paper briefly explains how CF effects the body as well as several important CF mutations to give a background into the research behind modulators. This background gives insight into the research advancements and challenges of modulator research. In addition, this paper lists all phase 2 and 3 placebo-controlled clinical trials that tested modulators. By compiling details from all clinical trials on numerous different drugs, one can see trends in clinical results and clearly see the successes and failures of various modulators.

CFTR's impact on the body: a story of inflammation and mucus

A mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) protein makes it impossible for the cell to maintain homeostasis. The CFTR gene codes for an ATP-binding cassette (ABC) transporter which creates a channel for chloride, bicarbonate, and other anions to flow along their respective concentration gradients (Callebaut, Chong, & Forman-Kay, 2018). The CFTR protein is made of five domains: two membrane spanning domains (MSD1 and MSD2) each of which are composed of six transmembrane segments, two nucleotide binding domains (NBD1 and NBD2,) and a regulatory domain (R) (Figure 1) (Farinha & Canato, 2017). The two nucleotide binding domains dimerize in a head-to-tail configuration which requires two ATP to open the channel (Y. Wang, Wrennall, Cai, Li, & Sheppard, 2014). In addition, the R region contains a number of protein kinase A phosphorylation sites that react to an increase in cAMP levels. The phosphorylation causes the R-region to stop sterically interfering with NBD dimerization (Meng, Clews, Kargas, Wang, & Ford, 2017). In order to open the channel, the R region must be properly phosphorylated by cAMP and one ATP must have bound to each of the two nucleotide binding domains. In other words, the opening of the CFTR channel is not a simple switch but instead responds to a variety of signals and requires at least two activating

factors to open. This level of complexity means that a small mutation in the DNA can render the entire protein nonfunctional (Meng et al., 2017).

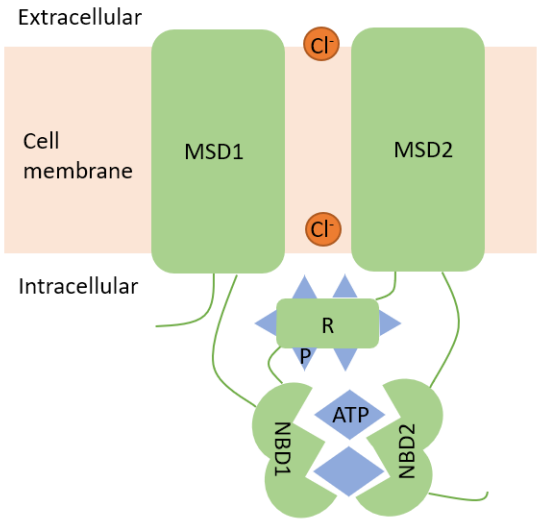

Figure 1. Cartoon map of CFTR showing five domains and signaling molecules. CFTR is made up of two membrane spanning domains (MSD), two nucleotide binding domains (NBD), and a regulatory region (R) (Farinha & Canato, 2017). In order to open, the R doming must be phosphorylated (P) and two ATP must have bound to the NBDs allowing them to dimerize (Meng et al., 2017; Y. Wang et al., 2014). Once open, CFTR acts as channel for chloride and other small ions. Figure adapted from (Fajac & Wainwright, 2017).

A normal CFTR protein functions to maintain water balance across the cell membrane. When properly triggered by cAMP and ATP, the channel opens and allows chloride and bicarbonate to flow along their concentration gradient, which for most situations, means that these ions flow out of the cell. CFTR is the main channel for Cl^- exit, which in turn triggers Na^+ secretion to balance ion charges (Saint-Criq & Gray, 2017). The additional salt ions outside the cell membrane draws water outside the cell through aquaporins. This water rehydrates airway surface liquid (ASL) which is essential to the function of the mucociliarly escalator and to preventing buildup of fluid and pathogens in the lungs. In addition, CFTR controls other Cl^- and

Na^+ channels including the epithelial sodium channel (ENaC) (Collawn & Matalon, 2014). CFTR functions as the master control to secrete salt and water from the cell, and without CFTR the cell becomes very ineffective at secreting water (Collawn & Matalon, 2014).

CFTR is primarily expressed on exocrine tissues, where one of the main functions of the tissue is to secrete water. The height of ASL is directly determined by the number of Na^+ and Cl^- ions in the extracellular fluid (Boucher, 2003). In normal lungs, ASL is a low viscosity fluid composed primarily of mucin fiber and a small amount of actin, DNA, and other macromolecules (Ong, Mei, Cao, Lee, & Chung, 2019). Without CFTR, Cl^- cannot be secreted into the extracellular fluid, which results in low levels of osmotic pressure drawing water out of the cell, eventually causing the ASL to lose height and volume while at the same time becoming more viscous. Equally important is the fact that without CFTR, ENaC is not properly regulated which results in constant Na^+ absorption (Saint-Criq & Gray, 2017). By facilitating absorption of salt, ENaC and CFTR work together to control the viscosity and height ASL. Because CF patients lack CFTR, they cannot counteract ENaC, which results in hyperabsorption of sodium, further dehydrating ASL.

Tall, low viscosity ASL is required for proper mucociliary clearance in the lungs (Somayaji, Ramos, Kapnadak, Aitken, & Goss, 2017). In CF patients, the ASL has high viscosity and cannot be easily cleared from the lungs. When the lungs lack effective muco-ciliary clearance, thickened mucus can accumulate in distal airways and cause blockage of airways in the form of mucus adhesions, mucus plaques, and plugs (Randell, Boucher, & University of North Carolina Virtual Lung, 2006). If mucus is not cleared from the lungs, it creates barriers to immune system function and provides a perfect place for bacteria to grow (Randell et al., 2006).

Bacteria thrive in thick mucus. From eating mucinoses, to forming biofilms, which prevent immune cells from killing bacteria, the thick mucus in CF lungs is hospitable to bacteria (Randell et al., 2006). Furthermore, the thickened ASL restricts bacterial motility leading to high local bacterial colony densities while simultaneously inhibiting neutrophil and macrophage migration (Matsui et al., 2005). The protein defect in CF results in lungs primed for large scale bacterial infection.

In addition, inflammation is increased by lack of HCO_3^- transport through CFTR. Beyond forming a Cl^- channel, CFTR can also transport other small anions including HCO_3^-. In the lungs, HCO_3^- is responsible for reducing pH of ASL. Acidic ASL not only inhibits antimicrobial peptides, proteins, and lipids which are inactivated at acidic pH, it also increases inflammation of the lung tissue (Stoltz, Meyerholz, & Welsh, 2015). Furthermore, CF macrophages and neutrophils tend to be hyperinflammatory with exaggerated responses to bacterial infection leading to recruitment of more neutrophils (Conese, 2011; Ralhan et al., 2016). The increased level of neutrophils and increased secretion of proinflammatory cytokines from macrophages create a death spiral of chronic infection (Bruscia et al., 2009). Moreover, studies have found that airway phlegm in CF patients contains little intact mucin but rather consists of bacteria, inflammatory cells, and F-actin; the ASL is more characteristic of pus rather than mucus (Flume & Van Devanter, 2012).

Chronic lung infections and high levels of inflammation lead to irreversible lung damage by scarring lung tissue. This scarring can lead to advanced lung disease that slowly becomes respiratory failure. Eventually these repeat infections and high inflammation levels destroy the lungs completely, and a double lung transplant is required.

Without functional CFTR, a person is not able to survive, and even with modern medical advances, CF patients have a much shorter life expectancy because CFTR is so vital to maintain homeostasis. Death is usually caused by lung failure, which is a result of repeated lung infections and high levels of inflammation. The lack of one protein completely destroys homeostasis and prevents the body from adequately regulating osmotic pressure, clearing the lungs, or effectively fighting bacteria.

CFTR Mutations

Because CF is a recessive, monogenic disease, one common way to classify CF patients is by their mutations. Of the 281 identified disease causing mutations, the F508del mutation is homologous in 70% of CF patients, and the G551D mutation is present in another 4% (Fajac & Wainwright, 2017; Hart & Harrison, 2017). The mutations fall into six broad categories. Class I mutations are caused by a premature stop codon which results in no full-length CFTR. Caused by a processing defect, class II mutations result in nonfunctional CFTR that are usually destroyed in the endoplasmic reticulum (ER). Class III are gating mutations caused by a regulation defect where the channel will not open when signaled by cAMP and ATP. Class IV is a result of decreased conductance; class V represents reduced synthesis, and class VI results in increased turnover in membrane (Bosch & De Boeck, 2016). Also known as residual function (RF) or minimal function (MF), classes IV-VI cause less severe CF as some proteins are usually able to survive and maintain some level of homeostasis. The F508del mutation, a class II mutation, and G551D, a class III mutation, are particularly significant because modulator treatments are currently available for both of them.

The F508del mutation is caused by a deletion of a phenylalanine (F) at codon 508. This mutation is the most common mutation seen in CF patients, and it has been estimated that 95%

of patients possess at least one allele with this mutation (Meng et al., 2017). Codon 508 alters the secondary structure of the protein, and without this phenylalanine, the connection between MSD2 and NBD1 is very unstable (Bartoszewski et al., 2010; Meng et al., 2017). Endoplasmic reticulum (ER) quality control mechanisms such as the 26S proteasome register this instability and destroy the faulty protein (Carlile et al., 2018; Clancy, 2018). In addition, even if F508del reaches the plasma membrane, it also exhibits gating defects and protein instability in the plasma membrane (Clancy, 2018). Because this mutation results in very low levels of CFTR proteins to reach the cell membrane, it is considered a class II mutation.

The second mutation of note is the G551D mutation, which is caused by an aspartic acid (D) substitution for glycine (G) at codon 551 located within NBD1 (Derand, Bulteau-Pignoux, & Becq, 2003). As a result, the NBD1 section is unable to bind and hydrolyze ATP. The protein folds correctly, goes to the plasma membrane, and the R region is correctly phosphorylated, but the NBD1 section is unable to dimerize with NBD2 because it cannot hydrolyze ATP (Bompadre et al., 2008). Therefore, the protein rarely opens because it is unable to bind to the energy source it needs to open the gate, making this mutation a class III mutation. While the G551D mutation is not as common as F508del because the protein makes it all the way to the surface and there is only one section that is malfunctioning, many researchers believed that correcting this mutation should be an easier fix.

CF Treatment Overview

There are two main drives with CF lung treatments. The first is downstream treatments. These strive to treat the symptoms of CF. The goal is to clear out sputum, also known as mucus, from the lungs via airway clearance techniques, to control and prevent bacterial infections via antibiotics, and to reduce inflammation in the lungs. Together these treatments can improve lung

function, but they are not without their drawbacks. Airway clearance techniques can take hours every day, certain bacteria are antibiotic resistant, and only so much inflammation can be reduced. In addition, as CF is a chronic, progressive disease, these treatments are not able to keep up with the progression of the disease. For many years, these downstream therapies were the only option to help those with CF, and each new advancement and discovery added years to CF patients' lives. But with modern research, new therapies are now available that can address the root cause of CF: the lack of CFTR.

The second drive for CF treatment is to establish CFTR protein function to the lungs and other organs and thereby return the body to normal homeostasis. The two methods that have been proposed to accomplish this goal is genetic therapy and modulators. By delivering wildtype DNA or RNA to cells, genetic therapy allows cells to make wtCFTR using normal cell processes. However, genetic therapy has faced some serious research development challenges in identifying a suitable vector. Since 1989 there have been over 25 human clinical trials testing genetic therapy using various viral and non-viral vectors, yet none of these trials have been successful (Alton et al., 2016; Griesenbach & Alton, 2011; Griesenbach et al., 2015). Researchers have been unable to design a vector that can bypass the body's defenses and successfully deliver genetic material. While this is a testament to the amazing design of the human body's immune system, it means that genetic therapy has not proved an effective treatment for CF. After the failures of genetic therapy, researchers moved onto studying modulators, which fix the mutated CFTR by acting as readthrough agents or binding to the mutated CFTR to correct structural or gating insufficiencies. These small organic molecules do not require a vector for delivery, and thus did not face the same research challenges. In fact, four

different modulators have been developed which turn mutated CFTR into functioning CFTR allowing CF cells to maintain homeostasis.

Modulator Therapy Introduction

Modulators help to increase or restore function of mutated CFTR by directly interacting with mutated CFTR and are thereby inherently mutation specific. Depending on the mechanism of action, modulators are put into three different categories: potentiators, correctors, and amplifiers. Potentiators keep CFTR channels open once they are in the plasma membrane (Guimbellot, Sharma, & Rowe, 2017). Correctors act as chaperones to prevent misfolding of CFTR in the ER and to transport CFTR to the plasma membrane (Guimbellot et al., 2017). Amplifiers increase the amount of CFTR protein the cell produces (Giuliano et al., 2018). There are three ways to improve activity of CFTR: by increasing the number of CFTR channels on the plasma membrane, increasing the time that the channels are open, or increasing the size or conductance of the channel (Clancy, 2018). Modulators can improve CFTR function in the cell by increasing any one of these variables.

Currently there are four CF modulator therapies available to patients: Ivacaftor, Lumacaftor/Ivacaftor, Tezacaftor/Ivacaftor, and Elexacaftor/Tezacaftor/Ivacaftor. Ivacaftor was the first drug approved by FDA on January 31, 2012 for treatment of CF patients with the G551D mutation (FDA, 2012). Ivacaftor is a potentiator which increases the time a CFTR channel is open and works for a large variety of mutations. Lumacaftor, Tezacaftor, and Elexacaftor are all correctors that were primarily designed to correct mutated F508del CFTR. When combined with Ivacaftor, these drugs form a combination therapy, where the corrector is responsible for the folding of CFTR, which allows it to be trafficked to the plasma membrane while Ivacaftor increases the possibility that the channel is open. The most recent addition to

these drug therapies is Trikafta (Elexacaftor/Tezacaftor/Ivacaftor) which was approved by the FDA on October 21, 2019 and is the first second-generation, triple-combination modulator therapy (FDA, 2019).

The tables in the next few sections list all phase 2 or 3 placebo-controlled clinical trials using modulators published up to December 31, 2019. Journal articles were found primarily via pubmed, proquest, and google scholar searches of individual drug names as well as searches on clinicaltrials.gov, FDA.gov, and searches of pharmaceutical websites. In order to be included, the trial must be placebo controlled and report results either in $ppFEV_1$ change or sweat chloride change. FEV_1 is one of several tests that measures pulmonary function. FEV_1 measures the forced expiratory volume at the end of the first second of measurement during a large exhale from the patient. $ppFEV_1$ is the percent predicted FEV_1, which compares a patient's results to the predicted normal values for their age, sex, height, weight, and ethnicity; the result is expressed as a percentage of the normal value. For CF patients, $ppFEV_1$ is one of the most common measures of lung function and how well their lungs are working. Improvement in $ppFEV_1$ is usually the main endpoint for clinical trials of modulator therapies because it shows quantitatively that the drug is helping improve lung function. Most clinical trials required an initial $ppFEV_1$ between 40-90% to participate as a $ppFEV_1$ lower than that signifies late-stage pulmonary disease and the potential need for a lung transplant (Pettit & Fellner, 2014; Ramos et al., 2019).

The second commonly used endpoint for clinical trials is change in sweat chloride. The sweat test is used to diagnose CF and is a relatively simple test where sweat is stimulated using pilocarpine iontophoresis, collected, and then the chloride concentration is measured within the sweat (LeGrys et al., 2007). A result of <30 mmol/L is the normal result and indicates that CF is unlikely, 30-59 mmol/L requires more testing, and >60 mmol/L indicates CF (Farrell et al.,

2017). High chloride sweat content is present in CF patients because the body is unable to reabsorb chloride from the sweat (Quinton, 2007). A decrease in chloride content from the sweat test would indicate that CFTR receptors are being expressed on the cell surface and functioning properly.

Note, the tables below list the relative change in $ppFEV_1$ and sweat chloride. That is to say the treatment result is compared to the placebo result and the relative change is reported to most clearly show how the treatment compared to the placebo. These tables allow easy comparison of different modulator drugs and show the process of developing drugs that provide high levels of clinical benefit.

Ivacaftor: The First Modulator

Currently four modulator regimens have been approved by the FDA. The first of these was Ivacaftor, also known as Kalydeco or VX-770, which was originally approved for patients with at least one G551D allele. Since its original FDA approval, Ivacaftor has been approved for 33 more mutations including most of the gating mutations (Eisenman, 2017). Developed by Vertex Pharmaceuticals, Ivacaftor is a small organic molecule that was initially found using high throughput screening assays (Van Goor et al., 2009). Using human bronchial epithelial (HBE) cells from CF patients with the G551D mutation, F508del mutation, and control non-CF cells, Ivacaftor's effect was tested. The study showed Ivacaftor increased CFTR channel open probability and increased chloride secretion to approximately 50% of normal chloride secretion in wtCFTR cells (Van Goor et al., 2009). In addition, Ivacaftor prevented dehydration of ASL and increased cilia beating (Van Goor et al., 2009). After this very promising test, Ivacaftor moved to human clinical trials (Table 1).

Table 1: *Ivacaftor Clinical Trials*

Reference	NAME Details	Participants	N	Treatment	Percent Change in $ppFEV_1$	Sweat chloride change (mmol/L)
(Accurso et al., 2010)		- G551D ≥1 allele - Age ≥ 18	20	IVA 25, 75, or 150 mg BID 2 x 14 day periods	25 mg: 4.2% 75 mg: 9.3% 150 mg: 9.8%	25 mg: -37.3 75 mg: -44.8 150 mg: -46.3
(Accurso et al., 2010)		- G551D ≥1 allele - Age ≥ 18	19	IVA 150 or 250 mg BID 28 days	150 mg: 1.4% 250 mg: -3.1%	150 mg: -64.5 250 mg: -43.0
(Ramsey et al., 2011)	STRIVE	- G551D ≥1 allele - Age ≥ 12	161	150 mg IVA BID 48 wks	10.5%	-48.1
(Flume et al., 2012)	DISCOVER	- F508del homo - Age ≥ 12	140	150 mg IVA BID 16 wks	1.9%	- 2.9
(J. C. Davies et al., 2013)	ENVISION	- G551D ≥1 allele - Age 6-11	52	150 mg IVA BID 48 wks	10%	-54.3
(J. Davies et al., 2013)	Crossover 2x2[a]	- G551D≥1 allele - Age ≥ 6 - $ppFEV_1 \geq 90\%$	21	IVA 150 mg BID - 28 days IVA or PBO - 28 days WO	8.67%	
(De Boeck et al., 2014)	KONNECTION, Crossover 2x2[a]	- Non G551D gating mutation ≥1 allele - Age ≥ 6	39	IVA 150 mg BID - 8 wks IVA or PBO - 4-8 wks WO	10.68%	-49.17
(Moss et al., 2015)	KONDUCT	- R117H ≥1 allele - Age ≥ 6	69	150 mg IVA BID 24 wks	2.1%	-24.0
(Nick et al., 2019)	N-of-1 study, Crossover 2x2[a]	- 2 RF mutations - Age ≥ 12	21	IVA 150 mg BID - 2 wks IVA and 2 wks of PBO (order random) - 4-8 wks WO	2.3%	

Note. All placebo controlled, phase 2 or phase 3 clinical trials testing ivacaftor by itself were found through a literature search and compiled. Relative results comparing treatment and placebo are listed in the last two columns. homo = homozygous; IVA = ivacaftor; PBO = placebo; WO = washout period; RF = residual function.

[a]Crossover 2x2 means that participants received treatment (IVA or PBO) for first period, then took nothing during washout period, followed by opposite treatment (PBO if they received IVA first time, or IVA if PBO was received first time) and a second washout period. This study design allows testing of ivacaftor against placebo in a single individual.

Table 1 demonstrates that Ivacaftor produces significant results both in ppFEV$_1$ change and sweat chloride change for individuals with at least one G551D allele. Ivacaftor works by binding directly to the CFTR protein and causes spontaneous ATP-independent opening of the channel (Eckford, Li, Ramjeesingh, & Bear, 2012; Jih & Hwang, 2013). When Ivacaftor is bound, phosphorylation of the regulatory region of CFTR is required, but ATP is not required to dimerize NBD1 and NBD2 domains. This corrects the G551D mutation, which does not respond to ATP and thus rarely opens. Ivacaftor has little effect on homologous F508del because Ivacaftor only increases the open probability of CFTR channels already in the plasma membrane while F508del CFTR never reaches the plasma membrane (Flume et al., 2012). In addition, a 2019 study used a n-of-1 study method to test Ivacaftor's effect on rare residual mutations (Nick et al., 2019). N-of-1 studies use a crossover method of receiving placebo or treatment successively to test results of treatment when only one participant can be found with the given mutation. This is very helpful in research because it opens up possibilities of FDA approval of modulators for those with very rare CF mutations. Because Ivacaftor also increases the likelihood that a wtCFTR channel will be open, it has been approved for 33 different mutations including, gating, residual function, splice, and conduction mutations (Eisenman, 2017). While Ivacaftor may only cause clinical change in certain mutations that make up a small percentage of total CF patients, Ivacaftor is the gold standard in modulator therapy for reliably providing large amounts of clinical change (FDA, 2017).

Lumacaftor: Modulator Treatment for F508del Homozygous Mutation

The next modulator to be developed was Lumacaftor, also known as VX-809, which was also developed by Vertex Pharmaceuticals. Falling into the category of correctors, Lumacaftor was designed to prevent misfolding of F508del CFTR. The F508del mutation is the most

common mutation and is homologous in about 70% of CF patients (Fajac & Wainwright, 2017). Lumacaftor works cotranslationally by altering the protein conformation of MSD1, which in turn allows a more stable connection between MSD1 and NBD1, thus partially correcting the F508del mutation (Ren et al., 2013). An initial clinical trial was completed using just Lumacaftor for homologous F508del CF patients, but the results showed that Lumacaftor by itself had no effect on ppFEV$_1$ or sweat chloride (Table 2) (Clancy et al., 2012). This is due to the fact that F508del CFTR exhibits not only folding defects but also gating defects (Clancy, 2018). However, when Lumacaftor was combined with Ivacaftor to create a dual modulator treatment, the combined therapy established some CFTR function. Lumacaftor would facilitate correct folding of the CFTR and Ivacaftor would increase the time it was open.

Lumacaftor/Ivacaftor, also known as Orkambi, has some clinical benefit for F508del homozygous patients but no effect for heterozygous F508del patients (Boyle et al., 2014). In addition, researchers found that a large daily dose of Lumacaftor was more effective than splitting the dose into two daily doses (Ratjen et al., 2017; S. M. Rowe, McColley, et al., 2017). Compared to Ivacaftor's effect on G551D mutation, Lumacaftor/Ivacaftor results in a much more modest improvement in lung function. The change is more consistent with therapies that treat downstream symptoms of CF (Deeks, 2016). Yet, Lumacaftor/Ivacaftor does cause a significant reduction in the rate of FEV$_1$ decline thereby allowing a stabilization in ppFEV$_1$ (Talamo Guevara & McColley, 2017). Because of this and the modest improvement in lung function, Lumacaftor/Ivacaftor was approved by the FDA in 2015 (FDA, 2015).

Tezacaftor: a Lumacaftor Replacement

Tezacaftor or VX-661 has a similar structure to Lumacaftor and was developed as a replacement for Lumacaftor. Tezacaftor is a broader-acting CFTR corrector that enables cellular

Table 2: *Lumacaftor Clinical Trials*

Reference	NAME Details	Participants	N	Treatment	Percent Change in $ppFEV_1$	Sweat chloride change (mmol/L)
(Clancy et al., 2012)		- F508del homo - Age≥18	89	LUM 25 mg, 50 mg, 100 mg, or 200 mg QD 28 days	25 mg: -2.46% 50 mg: -2.15% 100 mg: .32% 200 mg: .47%	25 mg: 0.1 50 mg: -4.61 100 mg: -6.13 200 mg: -8.21
(Boyle et al., 2014)	Cohort 1	- F508del homo - Age ≥18	62	- LUM 200 mg QD for 21 days - IVA 150 mg or 250 mg BID was added for last 7 days	150 mg: 2.7% 250 mg: .2%	150 mg: -5 250 mg: -10.9
(Boyle et al., 2014)	Cohort 2	- F508del homo and F508del het - Age ≥18	109	- LUM 200 mg, 400 mg, or 600 mg QD for 56 days - IVA 250 mg BID added in for last 28 days	200 mg: 3.8% 400 mg: 2.7% 600 mg (homo): 5.6% 600 mg (het): .3%	200 mg: -5.12 400 mg: -9.8 600 mg (homo): -9.6 600 mg (het): -6.0
(Boyle et al., 2014)	Cohort 3	- F508del homo - Age ≥18	15	- LUM 400 mg BID for 56 days - IVA 250 mg BID added in for last 28 days	4.2%	-11.1
(Wainwright et al., 2015)	TRAFFIC TRANS-PORT	- F508del homo - Age≥12	1108	- LUM 400 mg QD + IVA 250 mg BID - LUM 600 mg BID + IVA 250 mg BID 24 wks	400 mg: 4.8% 600 mg: 5.6%	
(Ratjen et al., 2017)		- F508del homo - Age 6-11	204	LUM 200 mg BID + IVA 250 mg BID 24 wks	2.4%	-20.8
(Rowe et al., 2017b)		- F508del het - Age ≥18	126	LUM 400 mg BID + IVA 250 mg BID 56 days	.6%	-11

Note. All placebo controlled, phase 2 or phase 3 clinical trials testing lumacaftor by itself or in combination with ivacaftor were found through a literature search and compiled. Relative results over the entire study comparing treatment and placebo are listed in the last two columns. homo = homozygous; het = heterozygous; LUM = lumacaftor; IVA = ivacaftor.

production and facilitates trafficking of multiple mutant forms of CFTR including F508del (S. M. Rowe, Daines, et al., 2017). Furthermore, because Tezacaftor does not induce CYP3A4 enzymes, there are less drug-drug interactions than Lumacaftor (Donaldson et al., 2018). This means Tezacaftor is safer and improves lung function in more mutations than Lumacaftor. Clinical trials showed that Tezacaftor/Ivacaftor treatment resulted in similar levels of change in $ppFEV_1$ and sweat chloride as Lumacaftor/Ivacaftor treatment (Table 3).

Based on these clinical trials, symdeko therapy was created which uses Tezacaftor/ Ivacaftor, which was approved by the FDA in February 2018 for patients with one allele of F508del and an Ivacaftor-responsive residual function allele (FDA, 2018; S. M. Rowe, Daines, et al., 2017). This meant that modulator therapy was now available for more people with CF. For homologous F508del patients, there is similar clinical benefits from both Lumacaftor/Ivacaftor and Tezacaftor/Ivacaftor, but Tezacaftor/Ivacaftor is responsible for less adverse side effects (Kirby, 2018).

Trikafta: The Challenge of Finding a Triple-Combination Modulator

The next step in modulator development was to create triple-combination modulators also known as second generation modulators. It was theorized that adding a second corrector to the Tezacaftor/Ivacaftor combination with a complementary mechanism of action would better restore CFTR function (Heijerman et al., 2019). In addition, the goal was to develop a modulator that would provide modulator therapy to the 30% of CF patients who are heterozygous for F508del and a minimal function mutation (Davies et al., 2018). Minimal-function mutations include nonsense, insertion/deletion, splicing, and several severe protein misfolding mutations (Davies et al., 2018). The goal was to develop a modulator regimen that could correct CFTR function from the one F508del allele regardless of what the second allele is. Vertex

Table 3: *Tezacaftor Clinical Trials*

NAME Reference	Participants	N	Treatment	Percent Change in ppFEV$_1$	Sweat chloride change (mmol/L)
(Donaldson et al., 2018)	- F508del homo - Age ≥ 18	131	TEZ monotherapy: TEZ 10, 30, 100, or 150 mg QD Combination therapy: TEZ 10, 30, 100, or 150 mg QD + IVA 150 mg BID 28 days	TEZ monotherapy: - 10 mg = 3.63% - 30 mg = 1.77% - 100 mg = 1.74% - 150 mg = 2.68% Combination therapy: - 10 mg = 1.44% - 30 mg = 3.04% - 100 mg = 3.89% - 150 mg = 3.75%	TEZ monotherapy: - 10 mg = 4.78 - 30 mg = -3.9 - 100 mg = -19.57 - 150 mg = -9.6 Combination therapy: - 10 mg = -4.2 - 30 mg = -5.14 - 100 mg = -5.18 150 mg = -1.77
(Donaldson et al., 2018)	- F508del homo - Age ≥ 18	67	- TEZ 100 mg QD + IVA 150 mg BID - TEZ 100 mg QD + IVA 50 mg BID - LUM 50 mg BID + IVA 150 mg BID 28 days	TEZ 100 + IVA 150 = 2.19% TEZ 50 + IVA 150 = .84% LUM 100 + IVA 50 = -.53%	TEZ 100 + IVA 150 = -4.13 TEZ 50 + IVA 150 = -6.7 LUM 100 + IVA 50 = -4.88
(Donaldson et al., 2018)	- F508del/ G551D - Age ≥ 12	18	TEZ 100 mg QD + IVA 150 mg BID 28 days	3.2%	-17.2
EVOLVE (Taylor-Cousar et al., 2017)	- F508del homo - Age≥12	510	TEZ 100 mg QD + 150 mg IVA BID 24 wks	6.8%	
EXPAND (Rowe et al., 2017a)	- F508del + RF - Age≥12	248	Crossover 3x2[a] - TEZ 100 mg QD + IVA 150 mg BID - IVA 150 mg BID 8 wks treatment 8 wks WO	TEZ + IVA = 6.8% IVA = 4.7%	TEZ + IVA = -9.5 IVA = -4.5

Note. All placebo controlled, phase 2 or phase 3 clinical trials testing tezacaftor by itself or in combination with ivacaftor were found through a literature search and compiled. Relative results comparing treatment and placebo are listed in the last two columns. Homo = homozygous; TEZ = tezacaftor; LUM = lumacaftor; IVA = ivacaftor; WO = washout; RF = residual function.

[a]3 sets of a different treatment or placebo followed by washout.

Pharmaceuticals initially identified two very similar modulators that could be added to the Tezacaftor/Ivacaftor combination. To test which drug was better, Vertex completed separate phase 2 and phase 3 studies on VX-659 and VX-445 and compared the results (Table 4).

VX-659 and VX-445 are correctors that decrease misfolding of CFTR and promotes trafficking of CFTR from ER to plasma membrane (Davies et al., 2018). More importantly, they have an additive effect to Tezacaftor (Davies et al., 2018; Keating et al., 2018). Clinical trials showed that both VX-659 and VX-445 triple combination therapy showed drastic lung improvement in both homozygous and heterozygous patients. This level of benefit is similar to Ivacaftor's benefit for the G511D mutation and has the ability to drastically improve a patient's quality of life.

After running parallel phase 3 studies of VX-659 and VX-445, Vertex decided that VX-445, also known as Elexacaftor, was the more effective drug. Both studies included a 24 week test for patients with one F508del mutation and one MF mutation along with a 4 week test for homozygous patients. Both programs met their primary and secondary endpoints, and both of the triple combinations were generally well tolerated with a low amount of adverse effects (Vertex, 2019b). Based on all the data, Vertex submitted Elexacaftor in combination with Tezacaftor/ Ivacaftor for FDA approval. On October 21, 2019, Trikafta (Elexacaftor/Ivacaftor/ Tezacaftor) was approved by the FDA for patients with at least one F508del mutation (Arnold, 2019). This drug opens up life-saving modulator treatment to over 90% of the CF population. It is available to patients who previously were ineligible for modulator therapy and greatly improves lung efficiency over previous modulator drugs. For the 27,000 people in the United States that this drug is approved for, Trikafta means additional years of life, greatly improved quality of life, less hours spent in airway clearance, less fear to pulmonary exacerbations, and a healthier body.

Table 4: *Triple Combination Drug Trials*

Reference	Participants	N	Treatment	Percent Change in $ppFEV_1$	Sweat chloride change (mmol/L)
(Davies et al., 2018)	- F508del + MF - Age ≥ 18	63	VX-659 80, 240, or 400 mg QD + TEZ 100 mg QD + IVA 150 mg BID 4 wks	80 mg = 9.8% 240 mg = 11.6% 400 mg = 12.9%	80 mg = -48.6 240 mg = -46.7 400 mg = -54.3
(Davies et al., 2018)	- F508del homo - Age ≥ 18	29	VX-659 400 mg QD+ TEZ 100 mg QD + IVA 150 mg BID (placebo group received TEZ +IVA) 4 wks	9.7%	-45.2
(Davies et al., 2018)	- F508del + MF - Age ≥ 18	25	VX-659 400 mg QD + TEZ 100 mg QD + VX-561 200 mg QD 4 wks	17.2%	-36.8
(Vertex, 2019a)[a]	- F508del + MF - Age ≥ 12	385	VX-659 400 mg + TEZ 100 mg QD + IVA 150 mg BID 24 wks	14% at week 4	
(Vertex, 2019a)[a]	- F508del homo - Age ≥ 12	111	VX-659 400 mg + TEZ 100 mg QD + IVA 150 mg BID (placebo group received TEZ +IVA) 4 wks	10%	
(Keating et al., 2018)	- F508del + MF - Age ≥ 18	95	VX-445 50, 100, or 200 mg QD + TEZ 100 mg QD + IVA 150 mg BID 4 wks	50 mg =11.1% 100 mg = 7.9% 200 mg = 13.8%	50 mg = -36 100 mg = -31 200 mg = -36.9
(Keating et al., 2018)	- F508del homo - Age ≥ 18	28	VX-445 200 mg QD+ TEZ 100 mg QD + IVA 150 mg BID (placebo group received TEZ +IVA) 4 wks	10.6%	-40.4
(Keating et al., 2018)	- F508del + MF - Age ≥ 18	29	VX-445 200 mg QD + TEZ 100 mg QD + VX-561 200 mg QD 4 wks	10.5%	-34.6
(Middleton et al., 2019)	- F508del + MF - Age ≥ 12	403	VX-445 200 mg + TEZ 100 mg QD + IVA 150 mg BID 24 wks	14.3%	-41.8
(Heijerman et al., 2019)	- F508del homo - Age ≥ 12	107	VX-445 200 mg QD+ TEZ 100 mg QD + IVA 150 mg BID (placebo group received TEZ +IVA) 4 wks	10%	-45.1

Note. All placebo controlled, phase 2 or phase 3 clinical trials testing VX-445 or VX-659 published before Dec 2019 were found through a literature search and compiled. Relative results comparing treatment and placebo are listed in the last two columns. MF = minimal function; homo = homozygous TEZ = tezacaftor; IVA = ivacaftor.

[a]Vertex pharmaceuticals did not actually publish a journal article phase 3 testing of VX-659 because it was never submitted for FDA approval or researched further. These results are from a press release from Vertex Pharmaceuticals on the phase 3 trial results. They are included to allow easy comparison between VX-659 and VX-445.

While it is important to note that Trikafta is not a cure for CF, and if at any time a patient is unable to continue the therapy, they will shortly return to the same baseline before modulator treatment, Trikafta is a major research accomplishment in developing a therapy that establishes CFTR function in the vast majority of CF patients (Trimble & Donaldson, 2018).

Failed Modulator Developments

However not every clinical trial is a success nor does every drug tested make it to market. During the research process there are numerous drugs that are developed and tested, but at some point, they fail, and research on that drug is stopped. Oftentimes *in vitro* studies or even phase 1 studies show very promising results, but during phase 2 or phase 3, clinical benefit is not achieved, and the drug is abandoned. When this happens, sometimes journal articles are written and explain the failure and why it happened, but other times the failure is not recorded, and the pharmaceutical company simply switches to another project. Currently there are two failed drugs that made it to phase 2 or 3 in placebo-controlled trials that published at least an abstract.

In 2014 PTC Therapeutics tested Ataluren in a phase 3 clinical trial (Table 5) (Kerem et al., 2014). Ataluren is an amplifier which was designed to prevent nonsense mutations (Class I) from happening (Guimbellot et al., 2017). While Ataluren performed well in early clinical trials, results from phase 3 clinical trials were not statistically significant. Because Ataluren has a similar structure to tobramycin (a commonly used antibiotic), it was hypothesized that chronic tobramycin use inhibited Ataluren. However, even excluding patients who used tobramycin, a retrospective analysis found that Ataluren still did not cause clinically significant change (Kerem et al., 2014).

Another drug that was developed then disregarded was QBW251, a potentiator, which was developed by Novartis Institutes. While the phase 2 trial showed somewhat positive results

for patients with gating or residual function mutations, it seems that QBW251 has since been abandoned as a CF treatment (Table 5) (Kazani, 2016). Instead QBW251 was tested in 2018 in people with COPD and may have applications in other respiratory diseases (S. Rowe et al., 2018).

Analyzing failures gives insight into the time, money, and risks that medical research requires. Each of these failures as well as the hundreds of potential drugs that were abandoned after phase 1 trials represent years of development, hundreds of recruited patients willing to try an experimental drug, and millions of dollars. Yet without these failures, new lifesaving drugs would not be developed. Failures are a reality of any development process, and in pharmaceutical development, failures show the challenges of developing drugs that cause the human body to drastically change the way it functions.

Current Modulatory Summary

From initial FDA approval of Ivacaftor in 2012, a large amount of research and development has gone into modulator therapies. Because CFTR modulators are inherently mutation specific, one of the major research drives is to expand currently approved drugs to new mutations. This process was started in 2017 when the FDA approved Ivacaftor for 23 additional mutations based solely on *in vitro* trials (FDA, 2017). This decision is very beneficial because some mutations are so rare that there are simply not enough people to do a clinical trial, but the access to modulator therapy can greatly improve and extend their lives. While n-of-1 studies help to expand access to modulator treatments, the approval of additional mutation based solely on in vitro trials means that lifesaving treatment is available to more people. Even with Ivacaftor being approved for these additional mutations, modulator therapy was still only available for a small amount of the CF population before the development of Lumacaftor.

Table 5: *Failed Drug Trials*

Reference	Participants	Treatment	Percent Change in ppFEV$_1$	Sweat chloride change (mmol/L)
(Kerem et al., 2014)	- Class 1 mutation - Age ≥ 6 - N=238	Ataluren (10 mg/kg at breakfast, 10 mg/kg at lunch, 20 mg/kg at dinner) 48 wks	3% 5.7% (for patients not using chronic inhaled tobramycin)	
(Kazani, 2016)	- GM or RF ≥ 1 allele - N=26	QBW251 150 or 450 mg BID 2 wks	450 mg = 7.3%	150 mg = -16.9
(Kazani, 2016)	- F508del homo - N=14	QBW251 450 mg BID 2 wks	No change	No change

Note. Placebo controlled, phase 2 or phase 3 clinical trials testing drugs that did not proceed further published before Dec 2019 were found through a literature search and compiled. Relative results comparing treatment and placebo are listed in the last two columns. GM = gating mutation; RF = residual function; homo = homozygous.

The second research drive was to combine modulators to develop new drugs to better remedy the F508del mutation. Both Lumacaftor and Tezacaftor were unable to produce clinically significant results by themselves, but when combined with Ivacaftor, these drugs were able to restore some clinical function. The development of Lumacaftor/Ivacaftor meant that modulator treatment was available to the 70% of CF patients homozygous for F508del (Fajac & Wainwright, 2017). However, Lumacaftor/Ivacaftor only produced a modest improvement in lung function (Table 2).

This led to the third research drive, which is the development of new drugs that are safer and produce more clinical benefit. Tezacaftor was developed to try to solve this problem, and because of its increased safety and fewer drug-related interactions, it has replaced Lumacaftor. In addition, Tezacaftor/Ivacaftor treatment was not limited to people F508del homozygous mutations but also produced benefit in some F508del heterozygous patients (Table 3).

The fourth research drive was to create a triple combination therapy. This was done by adding a second corrector (Elexacaftor) to the Tezacaftor/Ivacaftor combination that amplified Tezacaftor's effect. By adding a third drug, the effect of the modulator treatment is multiplied as more CFTR is folded correctly and is more stable which allows for transport from ER to the cell surface. Furthermore, this second-generation modulator opened up treatment to all CF patients with at least one F508del mutation, meaning that 90% of CF patients now have access to modulator treatment. In the future this research drive will continue as new drugs are developed to allow more CFTR to be produced until CFTR levels in CF patients resemble normal levels. Until that goal has been accomplished, there is still much more research that can be done to develop new modulators.

Overall, modulator therapy is a growing field of CF treatment that has the possibility of changing a CF diagnosis from a fatal life sentence to a tolerable disease. Modulators will never be able to completely cure CF, but the hope is that they will restore enough CFTR function to allow patients to live normal lives. Modulators are beginning to drastically change what life with CF looks like. While hours of daily treatment, immune compromised states, pulmonary exhibitions, and long hospital stays are still part of daily life for many CF patients, this does not have to be the case. Modulators have the potential to restore CFTR function to normal non-CF levels, but they still need more development and research to fully complete this goal. Modulators are the route to fulfilling CF patients' hope that one day there will be no need for lung transplants, weeks of IV antibiotic treatment, or the fear of a mother that they will bury their child because of this chronic disease. There is a hope that in years to come, Trikafta will be looked back upon as the drug that started the progression of changing CF from a life sentence to a livable disease.

Future Modulator Research

Even with the amazing accomplishment that Trikafta is, research is not done. Vertex Pharmaceuticals and several other companies are working hard to create new, more effective, and safer modulators. To begin with, Vertex Pharmaceuticals is testing a deuterated form of Ivacaftor known as VX-561 (formerly known as CTP-565). Deuteration is the process of replacing one or more hydrogen atoms with deuterium. This form of Ivacaftor is more stable in vitro and would be taken once daily rather than the current form which is taken twice a day (Harbeson et al., 2017). VX-561 was actually included in the two phase 2 clinical trials testing VX-659 and VX-445 and showed promising results (Davies et al., 2018; Keating et al., 2018). VX-561 is beginning its own phase 2 clinical trials and may replace Ivacaftor in the future.

Vertex Pharmaceuticals is the only company to develop and receive approval for a modulator therapy for CF, but they are not the only company developing modulator therapies (Table 6) (Chaudary, 2018). A cooperation between AbbVie and Galapagos Cystic Fibrosis Collaboration has led to the development of several novel modulators. To begin with, GLPG222 is a corrector that was found to be 25-fold more potent than Lumacaftor in cell line testing (X. Wang et al., 2018). However, in human trials GLPG2222 unfortunately only produced minor improvements (Bell et al., 2019). The second possibility is GLPG 2737, another corrector which resembles Elexacaftor and VX-659. GLPG2737 demonstrated 3 fold improvement of rescue of F508del homozygous CFTR activity when compared with Lumacaftor/Ivacaftor treatment (van Koningsbruggen-Rietschel et al., 2019). While the lung efficiency improvement in human trials was only modest, it is important to note the patients were already receiving Lumacaftor/Ivacaftor treatment (van Koningsbruggen-Rietschel et al., 2019). The addition of GLPG2737 further improved their lung function. This fact warrants further research into GLPG2737 as an Elexacaftor alternative. In addition, AbbVie/Galapagos recently completed a phase 1 study combining GLPG 2222, GLPG 2737, and GLPG 2451 to test their own triple-combination drugs (Clinical Trials, 2019). In the next few years, AbbVie/Galapagos may release either their own triple combination therapy or novel drugs to increase the benefit of existing modulator therapies.

Flatley Discovery Lab and Proteostasis Therapeutics have both developed correctors and potentiators than can be used in place of or in addition to various Vertex drugs. Both of these companies recently completed phase 2 clinical trials with these drugs, but as of December 2019 have not published journal articles, so results of these trials are not reported here. In the next couple of years, it is likely that another pharmaceutical company may be able to create a competing modulator.

Table 6: *New Drug Research*

NAME Reference	Participants	Treatment	Percent Change in $ppFEV_1$	Sweat chloride change (mmol/L)
(Bell et al., 2019)	- F508del homo - Age ≥ 18 - N=59	GLPG2222 50 mg, 100 mg, 200 mg, 400 mg, QD 29 days	50 mg: 1.1% 100 mg: .7% 200 mg: 1% 400 mg: 2.3%	50 mg: -3.3 100 mg: - 4.1 200 mg: -15.8 400 mg: -6.3
(Bell et al., 2019)	- F508del + GM - Age ≥ 18 - N=36	GLPG2222 150 mg, 300 mg QD, + IVA 150 mg BID 29 days	150 mg: .2% 300 mg: 3%	150 mg: -10.4 300 mg: -11.6
PELICAN (van Koningsbrugg en-Rietschel et al., 2019)	- F508del homo - Age ≥ 18 - N=22	GLPG2737 75 mg BID + LUM 400 mg + IVA 250 mg BID 28 days	3.4%	-19.6

Note. All placebo controlled, phase 2 or phase 3 clinical trials testing new modulator drugs published before Dec 2019 were found through a literature search and compiled. Relative results comparing treatment and placebo are listed in the last two columns. Homo = homozygous; IVA = Ivacaftor; LUM = Lumacaftor; GM = gating mutation.

Furthermore, research is not isolated to correcting CFTR; in fact, Spyryx Bioscience, Inc. has developed a ENaC modulator known as SPX-101 (Couroux et al., 2019). By promoting channel internalization, SPX-101 regulates ENaC activity, which reduces Na^+ absorption from ASL (Couroux et al., 2019). This novel approach illustrates that much more research can still be done in the development of modulators. While only in phase 1 testing, SPX-101 creates an entirely new class of modulators that lessen the effects of CF in a completely novel method.

The process of developing a new modulator therapy is not easy. From intensive scanning through thousands of molecules to find a potential modulator, to cell culture testing, to *in vitro* models, to *in vivo* testing in animal models, there are a lot of steps before a therapy even reaches human clinical trials. From there, phase 1 safety trials are performed with a small number of non-CF and CF volunteers. Phase 2 trials test the safety and efficiency of the drug at different doses and are followed by a phase 3 trial where a larger group of CF patients take the drug for an extended period of time. Ideally each of these trials is placebo controlled, so the results of the drug can be easily evaluated. In short, the development of modulator therapy for CF is a time and money intensive process but well worth the effort.

Conclusion

The research into modulators has produced several life changing drugs. As of the end of 2019, modulator therapy is available for 90% of the CF population, and clinical results show outstanding improvements in lung function. This is a major accomplishment! But there is still more work to be done. Modulators can be developed for all CF patients and be improved upon so that they are more effective and have less side effects.

Modulators are a revolutionary therapy and will drastically change the clinical approach to treating CF. Rather than facing continued failures as genetic therapy research has, modulators

have been proven to cause clinical change without major adverse effects. Modulators are able to significantly fix the root cause of CF by reestablishing CFTR function rather than only treating symptoms as downstream therapies do. When combined with downstream therapies, modulators can create a therapy regimen that prolongs and improves the life of CF patients.

CF modulator therapy has come a long way from when Ivacaftor was released in 2012, yet there is still further modulator therapy can go. The second generation, triple combination modulators were a significant improvement and opened the doors for more people to receive effective modulator treatment. But researchers are nowhere close of finding the limits of modulators. Trikafta uses two correctors and a potentiator, but there is another whole class of modulators that needs to be explored more fully: amplifiers; as well as modulators that interact with ENaC. CF research is just finding the tip of the iceberg of what can be done through modulator therapy.

Modulators are the CF treatment of the future. The ultimate goal of CF research is to improve and lengthen the lives of CF patients. Research into modulators have shown very promising results including significant clinical benefit, increase lung efficiency, decreased pulmonary exacerbations, and longer and healthier lives for people with CF. Modulator research provides hope that one day a treatment or combination of treatments will allow CF patients to live normal lives without worrying about the effects of what was once a chronic, progressive, deadly disease. Modulators are the path to a brighter future for those with cystic fibrosis.

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