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The impact of CFTR modulator therapies on CF airway microbiology

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Abstract

Major historical advances in cystic fibrosis (CF) respiratory clinical care, including mechanical airway clearance and inhaled medications, have aimed to address the consequences of cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction. In contrast, CFTR modulator therapies instead target the underlying protein defect that leads to CF lung disease. The extent to which these therapies might reduce susceptibility to chronic lung infections remains to be seen. However, by improving airway clearance, reducing the requirement for antibiotics, and in some cases, through direct antimicrobial effects, CFTR modulators are likely to result in substantial changes in CF airway microbiology. These changes could contribute substantially to the clinical benefit associated with modulator therapies, as well as providing an important indicator of treatment efficacy and residual pathophysiology. Indeed, the widespread introduction of modulator therapies might require us to re-consider our models of CF airway microbiology.

CFTR dysfunction and CF disease

CF arises due to mutational dysfunction in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. CFTR transports anions, including chloride and bicarbonate, across the epithelial cell membrane through a cAMP regulated channel, and reduction in this function leads to ion imbalance and dehydration of the epithelial surface [1]. The main clinical manifestations of CF include dehydrated, tenacious, and adherent airway and gut mucus, obstructed pancreatic and biliary ducts, and excessive fluid loss from the skin. While CFTR dysfunction results in multisystem disease, the greatest morbidity typically occurs in

Conflict of interest statement

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the respiratory tract, where mucus obstruction of the airways leads to inflammation and chronic infection, and ultimately respiratory failure.

The evolving nature of CF clinical care

When first described in 1938 [2], CF commonly resulted in death during infancy due to intestinal blockage (meconium ileus), malnutrition arising from malabsorption of fat and protein, or respiratory infection. Early diagnosis through sweat testing, the development of a structured approach to clinical management, and the establishment of specialist treatment centres, greatly improved the duration and quality of life [3]. The subsequent introduction of inhaled hypertonic saline and recombinant DNase therapies to improve respiratory function and airway clearance [4, 5], oral and inhaled antibiotics for the treatment of chronic infections [6, 7], and the long-term use of macrolides to reduce exacerbation rates [8], all helped to slow the rate of respiratory decline. Indeed, in large part due to these improvements in CF therapy, it is now common for patients to live into their fifth decade and beyond [9]. Until recently, therapeutic advances such as these have always targeted the consequences, rather than the cause, of CFTR dysfunction. Restoring CFTR function itself has long represented a holy grail of CF clinical research. In the past decade, this goal has been increasingly attained as a number of CFTR modulators have been developed and, in some cases, introduced to patients. These small molecule drugs act through a range of mechanisms (Box 1) and have varying levels of demonstrated efficacy, related in large part to the genetic diversity of CFTR itself.

Over 2000 variants in the CFTR gene have been identified, of which more than 300 are known to cause disease [10]. These variants can be grouped into six classes based on their effects [11], differing in both the severity of the associated disease and potential for pharmacological correction (Box 2). Most of the modulator therapies that have been developed and tested to date target class II mutations (CFTR protein is not trafficked through the endoplasmic reticulum) and class III mutations (CFTR protein reaches the cell surface but fails to regulate chloride ion transport appropriately due to a gating abnormality).

The first CFTR modulator therapy to be approved for prescription in 2012 was ivacaftor, a drug potentiating the abnormal “gating” (opening) of the CFTR channel conferred by class III mutations such as the G551D mutation, which is carried on at least one allele by 4 to 5% of CF patients [12, 13]. In phase 3 clinical studies, ivacaftor was shown to markedly improve CFTR function (based on reduced sweat chloride), and consequently, clinical parameters such as lung function [14]. Subsequently, ivacaftor has also proven useful in the treatment of CFTR with less severe channel dysfunction (class IV mutations); currently, ivacaftor has been approved for treating patients with 38 different class III and IV CFTR mutations [15]. The enthusiasm generated by these remarkable developments was tempered by the fact that most people with CF carry class II CFTR mutations—most commonly, Phe508del. This and other class II mutations result in insufficient protein trafficking to the cell surface due to protein misfolding, cellular stress, and proteosomic activity. Moreover, any CFTR that does arrive at the cell surface is typically unstable and unable to gate. This combination of problems has been approached with a combination of modulators: A CFTR corrector, which leads to improved protein delivery to the epithelial cell surface, and a potentiator to improve

channel function. In 2015, the combination of the corrector lumacaftor and the potentiator ivacaftor successfully completed phase 3 trials in patients homozygous for Phe508del [16]. However, compared to ivacaftor alone in patients with class III mutations, such as G551D, the effects of lumacaftor/ivacaftor dual therapy on lung function and rates of exacerbation in this population were relatively modest. Similarly modest clinical effects have been observed with another corrector/potentiator combination, tezacaftor/ivacaftor, both in patients homozygous for the Phe508del CFTR mutation [17] and in those with one Phe508del mutation and a second "residual function" mutation (any class other than the most severe, class I, which results in no functional protein) [18].

Currently, a number of other modulator drugs or combinations are in development or undergoing clinical trials [Box 3]. These include "next generation" correctors in combination with both first generation correctors, such as tezacaftor, and ivacaftor to further improve efficacy [19]. Phase 2 studies of the triple combinations 445/tezacaftor/ivacaftor [20] and 659/teacaftor/ivacaftor [21] have both shown substantial improvements in their primary endpoint of $ppFEV_1$ improvement, with both demonstrating a greater than 10% improvement in $ppFEV_1$ over that achieved with the dual combination alone. Early data from phase 3 studies support this improvement suggesting that triple therapy is likely to be available to patients within the next few years. Because of this the CF Foundation (CFF) is planning a robust prospective study (PROMISE) which aims to examine the short- and long-term clinical impact of triple therapy on people with CF Phe508del

CFTR dysfunction, airway microbiology, and lung disease

The extent to which CFTR dysfunction leads directly to inflammation and airway damage is unclear [22–25]. However, a close link does exist between the establishment of chronic lung infection, as defined by the detection of high abundances of known pathogens in respiratory samples, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus,* and respiratory decline [26, 27]. Current evidence suggests that reduced airway clearance and the accumulation of respiratory secretions increases susceptibility to such opportunistic pathogens. In addition, members of the upper respiratory commensal microbiota are also detected in the lower respiratory tract, particularly in the early stages of disease [28–31].

Ecological principles dictate that the characteristics of the CF lung microbiota are shaped both by exposure from the environment and by the selective pressures of the lower airways, including the physicochemical characteristics of airway secretions, host immune response, and exposure to antimicrobials and other medications [32]. Accordingly, the composition of the CF lung microbiota changes with increasing inflammation and disease progression [33–35]. While lung disease in infants is commonly associated with high relative abundances of *S. aureus* and *Haemophilus influenzae*, the contribution of these pathogens to microbiota constituency tends to wane as they are supplanted by *P. aeruginosa, Burkholderia* spp., and *Achromobacter* spp. in older patients with more severe disease [36, 37]. These changes in culturable bacteria reflect the evolving composition of the wider airway microbiota with disease progression, with a broad trend towards reduced diversity, with initially high prevalence and relative abundance of common oropharyngeal commensal taxa giving way to more adaptable opportunists [32].

Our clinical experience with CFTR modulator therapies is still in its early stages. While increasingly effective modulator therapies are being tested and made available to a larger proportion of the CF population, we are just beginning to learn how they will impact the trajectory of disease in these patients. Understanding the relationship between these innovations and CF airway microbiology in particular is important for a number of reasons. Firstly, in addressing the underlying pathophysiology, successful CFTR-targeting therapies might see a reversion in lower airway microbiology to levels and composition seen in healthy individuals. Changes in microbiota characteristics might therefore provide a valuable indicator of the degree and nature of improvement in pathophysiology, and the extent of residual, irreversible, airway damage. Secondly, microbiological changes might be necessary, at least in part, to achieve clinical efficacy, with the extent of change correlating to clinical benefit; in that case, if infection is not improved despite restoration of CFTR therapy, antibiotics or other treatments may continue to be a cornerstone of respiratory maintenance therapy. Thirdly, the introduction of other pivotal CF therapies has seen a shift in the microbiological characteristics of CF lung disease at the level of the patient population [38].

Potential CFTR-mediated impacts of modulator therapies on airway microbiology

Our understanding of how CFTR regulates airway physiology and defences remains incomplete. Predicting how CFTR modulators will impact airway microbiology is therefore difficult. Effects on mucus hydration and mucociliary clearance [13, 39, 40] that might be predicted to affect pathogen abundance could conceivably be rapid and persistent. Other effects, such as the pH or chemical content of airway secretions, that might be predicted to directly modulate pathogen behaviour, could be comparatively transient, diminishing with the emergence of different microbial subpopulations or with other adaptations. More severe structural airway damage, such as advanced bronchiectasis, might persist despite modulator therapy. As bronchiectasis alone is a risk factor for chronic airways infection [41], the microbiota would be unlikely to “normalise” in the setting of such irreversible airway damage. Furthermore, it is anticipated that effective modulator therapy will lead to a diminished requirement for antibiotics, reducing one of the more potent selective pressures on the lung microbiota [34]. Changes in airway microbiology are therefore likely to be variable, reflecting factors such as patient age, severity of disease, associated changes in treatment, and the extent and reversibility of airway damage.

Potential CFTR-independent impacts of modulator therapies on airway microbiology: Antimicrobial effects

In addition to indirectly changing airway microbiology by addressing CFTR dysfunction, there is growing evidence that some CFTR modulators have direct antimicrobial properties. For example, the structure of ivacaftor includes a quinoline ring; quinoline derivatives and analogues frequently have antibacterial properties through interference with DNA replication [42]. Indeed, ivacaftor has been shown to be a weak inhibitor of bacterial DNA

gyrase and topoisomerase IV, while lumacaftor increases the cellular production of damaging reactive oxygen species [43].

In vitro, ivacaftor has been shown to reduce *S. aureus* viability in a dose-dependent fashion, with a similar but less robust effect on *P. aeruginosa,* as well as inhibiting the growth of *S. aureus* and *Streptococcus pneumoniae* respiratory isolates [44]. Ivacaftor also exhibits positive interactions with vancomycin, trimethoprim sulfamethoxazole, moxifloxacin, and linezolid in their effects on *S. aureus* and *S. pneumoniae* [44]. Schneider *et al* showed ivacaftor, lumacaftor, and polymyxin B to each be ineffective in killing CF isolates of *P. aeruginosa* in isolation [43]. However, the combination of these medications exhibited a synergistic antimicrobial effect, with a 100-fold decrease in bacterial count after 24 hours. Investigation of the mechanistic basis of this effect indicated damage to both the outer and inner cell membranes of *P. aeruginosa,* which did not occur with any of the individual drugs in isolation [43]. In separate studies, ivacaftor has also been shown to act in synergy with tobramycin in killing *S. aureus* and *Streptococcus* species [45], and with ciprofloxacin in inhibiting the growth of *P. aeruginosa* [46].

The antimicrobial mechanisms exhibited by these CFTR modulating drugs exploit highly conserved aspects of bacterial physiology, and their effects are therefore unlikely to be limited to pathogens such as *P. aeruginosa* and *S. aureus.*

Treatment-associated changes in airway microbiology: Examining the evidence

To date, CFTR modulator studies have focused largely on clinical outcomes, with assessments of changes in microbiology limited to a number of relatively small cohorts, measuring different outcomes. Bernarde *et al* assessed airway microbiology in three children with CF and G551D mutations (average age 12 years) without chronic *P. aeruginosa* infections, following them for an average of 13 months after the initiation of ivacaftor treatment [47]. Analysis was performed on a least six sequential sputum samples, including determination of bacterial load by quantitative PCR (qPCR) and bacterial community composition by 16S rRNA gene amplicon sequencing (16S sequencing). No significant changes were identified with treatment, either in relation to bacterial load or overall bacterial community composition.

The fact that none of the patients assessed by Bernarde *et al* had chronic *P. aeruginosa* infections is significant. Of all microbial species associated with the CF airways, *P. aeruginosa* has been most strongly correlated with respiratory deterioration [48–50] (other pathogens have also been associated with decline, but their dynamics and clinical associations are less well-defined). Despite its clinical importance, the basis of this association of *P. aeruginosa* with the CF airways is still not fully understood. Determining the relationship between CFTR dysfunction and *P. aeruginosa* infection in patients with CF more precisely, and understanding the effect of restoring CFTR function on the presence or abundance of *P. aeruginosa,* is therefore critical.

Rowe *et al* assessed the effects of ivacaftor therapy on *P. aeruginosa* sputum levels and other outcomes in 133 patients aged 6 years and above [13]. Significant improvements were seen in sweat chloride, lung function (FEV_1 % predicted), body mass, and mucociliary clearance. In addition, the investigators observed a significant reduction in the rate of culture detection of *P. aeruginosa* 6 months after initiating therapy.

A culture-independent assessment of the effects of ivacaftor on *P. aeruginosa* burden in the study population provided a more nuanced view. *P. aeruginosa*-specific qPCR did not indicate a significant decrease in sputum abundance; similarly, sputum total bacterial abundance was not observed to change with a broad-range (bacterial 16S rRNA gene) qPCR assay. To more comprehensively analyse these dynamics, the investigators performed sputum inflammatory marker and microbiota analysis on a subset of 14 subjects. After microbiota characterisation by 16S sequencing, the reads that aligned to bacterial genera known to contain traditional CF pathogens (*Pseudomonas*, *Staphylococcus*, *Haemophilus*, *Stenotrophomonas, Achromobacter,* and *Burkholderia)* were assessed. No significant changes in any inflammatory markers or in bacterial diversity were observed with ivacaftor treatment. However, a non-significant downward trend was observed in the combined relative abundance of traditional CF bacterial pathogens, with a significant increase in the relative abundance of *Prevotella.* Consistent with these findings, a subsequent analysis of the study data showed that the observed decrease in culture detection of *P. aeruginosa* occurred primarily among subjects with baseline intermittent, rather than chronic, *P. aeruginosa* infection, perhaps reflecting lower initial sputum relative abundances in those with sputum conversion and little change in those with higher initial abundances [51].

However, the results of a subsequent study indicated that ivacaftor may decrease sputum *P. aeruginosa* abundances even in chronic infection. Hisert *et al* assessed the impact of ivacaftor in 12 adult patients with G551D mutations chronically infected with *P. aeruginosa* [40]. Lung function, markers of inflammation, chest computed tomography scans, and sputum bacterial content (by 16S sequencing, species-specific and broad-range qPCR, and culture) were assessed before initiation of ivacaftor therapy, and longitudinally after days, weeks, and years of treatment. Significant improvements in sweat chloride concentrations were evident after two days, indicative of improved CFTR function. Lung function also improved after two days, with continued improvements on Day 7, and Day 400, while levels of neutrophil elastase, IL-8, IL-1b, arginase-1, myeloperoxidase, and calprotectin in sputum supernatants fell during the first week.

Eight of the subjects had chronic *P. aeruginosa* lung infections. In these, sputum pseudomonal load began to decrease at Day 2, and average sputum *P. aeruginosa* colony forming units (CFUs) by culture declined by 10-fold by Day 7. Similar decreases were identified by qPCR in both *P. aeruginosa* and total bacterial load. Microbiota composition analysis showed that decreases in the relative abundance of *P. aeruginosa* were generally accompanied by reciprocal increases in the relative abundance of nonconventional organisms (including *Streptococcus, Prevotella, Veillonella,* and other taxa) and in the overall diversity of microbiota present. However, no subject became consistently culture-negative for *P. aeruginosa,* and after the first year *P. aeruginosa* density rebounded as diversity declined.

While concerning, the significant increase in sputum *P. aeruginosa* levels during the second treatment year in the Hisert study suggests a number of potential explanations, each with different implications. For example, it is possible that decreased symptoms led to reduced adherence to maintenance therapies, such as suppressive antibiotics, airway clearance, or even ivacaftor itself [52]. Alternatively, these dynamics could represent microbiological adaptation to the ivacaftor-treated CF airway, such as the emergence of *P. aeruginosa* genetic variants that are capable of surviving or even thriving after CFTR is restored. The therapeutic approaches to these two possibilities are different, and both warrant further investigation.

While the effects reported by Rowe *et al* and Hisert *et al* focused on *P. aeruginosa,* the two studies' aggregate results point to an improved ability to clear lower airway bacteria. In Hisert *et al.,* the decrease in *P. aeruginosa* relative abundance led to a relative increase in the apparent contribution of upper respiratory microbiota to sputum composition profiles. Indeed, in Hisert *et al.,* qPCR-based determination of absolute levels of members of the genera *Streptococcus* and *Prevotella* identified no significant increases with therapy. However, among the four patients who did not have *P. aeruginosa* infections, a decline in total sputum bacterial load was observed in the first week of therapy, suggesting a more general effect on sputum microbial clearance that is most marked in specific taxa. This point is further supported by the observation that the relative sputum abundance of *Burkholderia* species fell in the two subjects with chronic *Burkholderia* infections, while a comparable change was not observed in patients with *Staphylococcus*-dominated infections. However, due to the small study size, the non-pseudomonas infected subgroup was insufficient to rigorously define the effects on dominant, non-*P. aeruginosa* bacteria, or to determine the independent relationship between clinical improvement and increased clearance of *P. aeruginosa* alone.

A subsequent study by Peleg *et al* investigated the impact of ivacaftor on CF respiratory microbiology during the early treatment period [53]. Subjects were assessed through a 4-month double-blind, placebo-controlled, crossover study, with 28 days of active treatment. A combination of qPCR and 16S sequencing defined sputum microbiota composition, total bacterial load, and *P. aeruginosa* load at five key time points in 20 adult patients with at least one G551D mutation (including two homozygotes). Nineteen of the subjects were culture-positive for *P. aeruginosa*, ten for *S. aureus* (methicillin-sensitive or resistant), and one for *Mycobacterium abscessus* complex.

No significant difference in fold change was observed either for total sputum bacterial load or *P. aeruginosa* load during ivacaftor therapy or placebo, as compared to respective pre-treatment levels. Nor were any significant changes in sputum microbiota composition identified following ivacaftor treatment or placebo. However, it was noted that sputum microbiota composition variation was significantly greater between subjects than within subjects over time. A significant change in microbiota composition was however associated with any change in antibiotic exposure, regardless of whether ivacaftor or placebo was administered. In a small, subgroup analysis of subjects without changes in magnitude of antibiotic exposure during the study, a significant reduction in total bacterial load was observed during treatment with ivacaftor.

These findings highlight the potential for the short-term impact of ivacaftor therapy on sputum microbiota composition in patients with G551D mutations to be modest compared to the effects of antibiotics, which may mask microbiological effects of restored CFTR function.

Impact of modulator therapies on pathogen virulence

The studies described above focused on the microbiological impact of modulator therapies on the levels and types of microbes in lower airway secretions. However, these therapies also have the potential to influence clinically-relevant behaviours of microbes within the airways beyond mere viability or growth.

P. aeruginosa undergoes substantial genetic and gene expression changes during the establishment of chronic infection of the CF lower airways. These changes are accelerated in part by hypermutation [54, 55], and diversification driven by niche heterogeneity [56]. Among these adaptations is a coordinated down-regulation in expression of genes related to central metabolism, motility and virulence, and concurrently upregulated expression of genes affecting membrane permeability and efflux [57–62]. Despite the substantial inflammation associated with chronic CF airway infection, characterized by an abundant neutrophilic response [63], chronic *P. aeruginosa* isolates exhibit paradoxically reduced expression of pathogenicity traits and pro-inflammatory characteristics [64–66].

As these genetic changes are associated with worsening respiratory disease, one might expect that, by improving respiratory physiology, modulator therapies might result in the reestablishment of airway conditions that select more virulent pathogen characteristics. In short, beneficial changes in pathogen behaviour observed during chronic infection could conceivably be reversed. However, no investigations of the impact of CFTR modulator therapy on *in vivo* gene expression of CF pathogens have been undertaken to date.

Consequences of modulator therapies for CF population microbiology

The introduction of new therapeutic strategies has occurred in the context of ongoing changes in CF respiratory microbiology on the level of the patient population. For example, shifts in the culture prevalence of CF pathogens, including *P. aeruginosa, S. aureus, H. influenzae,* and *Burkholderia cepacia* complex (BCC), *Stenotrophomonas maltophilia, Achromobacter xylosoxidans,* and nontuberculous mycobacteria, are evident in registry [67, 68] and cohort [69, 70] studies spanning these successive advances. Such changes are likely to reflect, in part, improved identification, increasing prevalence of individuals with milder disease, and survivor effects [38, 68]. However, they are also likely to relate to the direct impact of disease-modifying therapies, such as inhaled DNase, hypertonic saline, and antibiotics such as tobramycin and long-term macrolide therapy, on the airway environment.

Comparable changes are likely to occur with the increasing introduction of CFTR modulator therapies. It is therefore possible that CF airway microbiology will undergo substantial change in the course of the next decade.

Conclusions

This a hugely exciting time for those involved in cystic fibrosis care. The development of CFTR modulator therapies that can provide benefit to an increasing proportion of those with CF has the potential to change the fundamental clinical characteristics of CF disease. Understanding the impact of these therapies on CF airway microbiology is critical. It will not only inform our basic understanding of CFTR function and mechanisms of airway infection, but will help to inform the strategies used to manage chronic CF airway infections in the post-modulator era, particularly through the use of antibiotics.

References

1. Gentzsch M, Mall MA. Ion Channel Modulators in Cystic Fibrosis. Chest 2018; 154(2):383–393. [PubMed: 29750923]
2. Andersen DH. CYSTIC FIBROSIS OF THE PANCREAS AND ITS RELATION TO CELIAC DISEASEA CLINICAL AND PATHOLOGIC STUDY. Am J Dis Child 1938;56(2):344–399.
3. Castellani C, Duff AJA, Bell SC, Heijerman HGM, Munck A, Ratjen F, et al. ECFS best practice guidelines: the 2018 revision. J Cyst Fibros 2018; 17(2):153–178. [PubMed: 29506920]
4. Fuchs HJ, Borowitz DS, Christiansen DH, Morris EM, Nash ML, Ramsey BW, et al. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. N Engl J Med 1994;331(10):637–642. [PubMed: 7503821]
5. Wark PA, McDonald V. Nebulised hypertonic saline for cystic fibrosis. Cochrane Database Syst Rev 2003;(1):CD001506.
6. Ramsey BW, Pepe MS, Quan JM, Otto KL, Montgomery AB, Williams-Warren J, et al.: Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic Fibrosis Inhaled Tobramycin Study Group. N Engl J Med 1999;340(1):23–30. [PubMed: 9878641]
7. McCoy KS, Quittner AL, Oermann CM, Gibson RL, Retsch-Bogart GZ, Montgomery AB. Inhaled aztreonam lysine for chronic airway Pseudomonas aeruginosa in cystic fibrosis. Am J Respir Crit Care Med 2008;178(9):921–928. [PubMed: 18658109]
8. Southern KW, Barker PM, Solis-Moya A, Patel L. Macrolide antibiotics for cystic fibrosis. Cochrane Database Syst Rev 2012;11:CD002203.
9. Cystic Fibrosis Foundation Patient Registry. 2017 Annual Data Report. Cystic Fibrosis Foundation. 2018; Bethesda, Maryland.
10. Ivanov M, Matsvay A, Glazova O, Krasovskiy S, Usacheva M, Amelina E, et al. Targeted sequencing reveals complex, phenotype-correlated genotypes in cystic fibrosis. BMC Med Genomics 2018;11(Suppl 1):13. [PubMed: 29504914]
11. Marson FAL, Bertuzzo CS, Ribeiro JD. Classification of CFTR mutation classes. Lancet Respir Med 2016, 4(8):e37–e38. [PubMed: 27377414]
12. McKone EF, Emerson SS, Edwards KL, Aitken ML. Effect of genotype on phenotype and mortality in cystic fibrosis: a retrospective cohort study. Lancet 2003;361(9370):1671–1676. [PubMed: 12767731]
13. Rowe SM, Heltshe SL, Gonska T, Donaldson SH, Borowitz D, Gelfond D et al. Clinical mechanism of the cystic fibrosis transmembrane conductance regulator potentiator ivacaftor in G551D-mediated cystic fibrosis. Am J Respir Crit Care Med 2014; 190(2):175–184. [PubMed: 24927234]
14. Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Drevinek P et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N Engl J Med 2011;365(18):1663–1672. [PubMed: 22047557]
15. Skilton M, Krishan A, Patel S, Sinha IP, Southern KW. Potentiators (specific therapies for class III and IV mutations) for cystic fibrosis. Cochrane Database Syst Rev 2019;1:CD009841.

16. Wainwright CE, Elborn JS, Ramsey BW, Marigowda G, Huang X, Cipolli M et al. Lumacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del CFTR. N Engl J Med 2015;373(3):220–231. [PubMed: 25981758]
17. Taylor-Cousar JL, Munck A, McKone EF, van der Ent CK, Moeller A, Simard C et al. Tezacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del. N Engl J Med 2017;377(21):2013–2023. [PubMed: 29099344]
18. Rowe SM, Daines C, Ringshausen FC, Kerem E, Wilson J, Tullis E et al. Tezacaftor-Ivacaftor in Residual-Function Heterozygotes with Cystic Fibrosis. N Engl J Med 2017;377(21):2024–2035. [PubMed: 29099333]
19. Clancy JP, Cotton CU, Donaldson SH, Solomon GM, VanDevanter DR, Boyle MP et al. CFTR modulator theratyping: Current status, gaps and future directions. J Cyst Fibros 2019; 18(1):22–34. [PubMed: 29934203]
20. Keating D, Marigowda G, Burr L, Daines C, Mall MA, McKone EF, et al. VX-445-Tezacaftor-Ivacaftor in Patients with Cystic Fibrosis and One or Two Phe508del Alleles. N Engl J Med. 2018;379(17):1612–1620. [PubMed: 30334692]
21. Davies JC, Moskowitz SM, Brown C, Horsley A, Mall MA, McKone EF et al. VX-659-Tezacaftor-Ivacaftor in Patients with Cystic Fibrosis and One or Two Phe508del Alleles. N Engl J Med 2018;379(17):1599–1611. [PubMed: 30334693]
22. Pillarisetti N, Williamson E, Linnane B, Skoric B, Robertson CF, Robinson P et al. Infection, inflammation, and lung function decline in infants with cystic fibrosis. Am J Respir Crit Care Med 2011;184(1):75–81. [PubMed: 21493738]
23. Ranganathan SC, Parsons F, Gangell C, Brennan S, Stick SM, Sly PD et al. Evolution of pulmonary inflammation and nutritional status in infants and young children with cystic fibrosis. Thorax 2011;66(5):408–413. [PubMed: 21398685]
24. Sagel SD, Wagner BD, Anthony MM, Emmett P, Zemanick ET. Sputum biomarkers of inflammation and lung function decline in children with cystic fibrosis. Am J Respir Crit Care Med 2012;186(9):857–865. [PubMed: 22904182]
25. Elizur A, Cannon CL, Ferkol TW. Airway inflammation in cystic fibrosis. Chest 2008;133(2):489–495. [PubMed: 18252915]
26. Ratjen F, Doring G. Cystic fibrosis. Lancet 2003;361(9358):681–689. [PubMed: 12606185]
27. Treggiari MM, Rosenfeld M, Retsch-Bogart G, Gibson R, Ramsey B. Approach to eradication of initial Pseudomonas aeruginosa infection in children with cystic fibrosis. Pediatr Pulmonol 2007;42(9):751–756. [PubMed: 17647287]
28. Frayman KB, Armstrong DS, Carzino R, Ferkol TW, Grimwood K, Storch GA et al. The lower airway microbiota in early cystic fibrosis lung disease: a longitudinal analysis. Thorax 2017;72(12):1104–1112. [PubMed: 28280235]
29. Muhlebach MS, Zorn BT, Esther CR, Hatch JE, Murray CP, Turkovic L, et al. Initial acquisition and succession of the cystic fibrosis lung microbiome is associated with disease progression in infants and preschool children. PLoS Pathog 2018;14(1):e1006798.
30. Frayman KB, Wylie KM, Armstrong DS, Carzino R, Davis SD, Ferkol TW, et al. Differences in the lower airway microbiota of infants with and without cystic fibrosis. J Cyst Fibros 2018 pii: S1569–1993(18)30941-X
31. Pittman JE, Wylie KM, Akers K, Storch GA, Hatch J, Quante J et al. Association of Antibiotics, Airway Microbiome, and Inflammation in Infants with Cystic Fibrosis. Ann Am Thorac Soc 2017; 14(10):1548–1555. [PubMed: 28708417]
32. Rogers GB, Hoffman LR, Carroll MP, Bruce KD. Interpreting infective microbiota: the importance of an ecological perspective. Trends Microbiol 2013;21(6):271–276. [PubMed: 23598051]
33. Zemanick ET, Wagner BD, Robertson CE, Ahrens RC, Chmiel JF, Clancy JP et al. Airway microbiota across age and disease spectrum in cystic fibrosis. Eur Respir J 2017;50(5) pii: 1700832.
34. Zhao J, Schloss PD, Kalikin LM, Carmody LA, Foster BK, Petrosino JF et al. Decade-long bacterial community dynamics in cystic fibrosis airways. Proc Natl Acad Sci U S A 2012; 109(15):5809–5814. [PubMed: 22451929]

35. Klepac-Ceraj V, Lemon KP, Martin TR, Allgaier M, Kembel SW, Knapp AA et al. Relationship between cystic fibrosis respiratory tract bacterial communities and age, genotype, antibiotics and Pseudomonas aeruginosa. Environ Microbiol 2010;12(5):1293–1303. [PubMed: 20192960]
36. Goss CH, Burns JL. Exacerbations in cystic fibrosis. 1: Epidemiology and pathogenesis. Thorax 2007;62(4):360–367. [PubMed: 17387214]
37. Cystic Fibrosis Foundation Patient Registry, 2011 Annual Data Report. Cystic Fibrosis Foundation 2011 Bethesda, Maryland.
38. Lipuma JJ. The changing microbial epidemiology in cystic fibrosis. Clinical microbiology reviews 2010;23(2):299–323. [PubMed: 20375354]
39. Donaldson SH, Pilewski JM, Griese M, Cooke J, Viswanathan L, Tullis E, et al. Tezacaftor/Ivacaftor in Subjects with Cystic Fibrosis and Phe508del /Phe508del - CFTR or Phe508del / G551D-CFTR. Am J Respir Crit Care Med 2018;197(2):214–224. [PubMed: 28930490]
40. Hisert KB, Heltshe SL, Pope C, Jorth P, Wu X, Edwards RM et al. Restoring Cystic Fibrosis Transmembrane Conductance Regulator Function Reduces Airway Bacteria and Inflammation in People with Cystic Fibrosis and Chronic Lung Infections. Am J Respir Crit Care Med 2017;195(12):1617–1628. [PubMed: 28222269]
41. Angrill J, Agusti C, de Celis R, Rano A, Gonzalez J, Sole T et al. Bacterial colonisation in patients with bronchiectasis: microbiological pattern and risk factors. Thorax 2002;57(1):15–19. [PubMed: 11809984]
42. Heeb S, Fletcher MP, Chhabra SR, Diggle SP, Williams P, Camara M. Quinolones: from antibiotics to autoinducers. FEMS Microbiol Rev 2011;35(2):247–274. [PubMed: 20738404]
43. Schneider EK, Azad MA, Han ML, Tony Zhou Q, Wang J, Huang JX et al. An “Unlikely” Pair: The Antimicrobial Synergy of Polymyxin B in Combination with the Cystic Fibrosis Transmembrane Conductance Regulator Drugs KALYDECO and ORKAMBI. ACS Infect Dis 2016;2(7):478–488. [PubMed: 27626100]
44. Reznikov LR, Abou Alaiwa MH, Dohrn CL, Gansemer ND, Diekema DJ et al. Antibacterial properties of the CFTR potentiator ivacaftor. J Cyst Fibros 2014; 13(5):515–519. [PubMed: 24618508]
45. Payne JE, Dubois AV, Ingram RJ, Weldon S, Taggart CC, Elborn JS, et al. Activity of innate antimicrobial peptides and ivacaftor against clinical cystic fibrosis respiratory pathogens. Int J Antimicrob Agents 2017;50(3):427–435. [PubMed: 28666755]
46. Cho DY, Lim DJ, Mackey C, Skinner D, Zhang S, McCormick J et al. Ivacaftor, a Cystic Fibrosis Transmembrane Conductance Regulator Potentiator, Enhances Ciprofloxacin Activity Against Pseudomonas aeruginosa. Am J Rhinol Allergy 2019;33(2):129–136. [PubMed: 30585080]
47. Bernarde C, Keravec M, Mounier J, Gouriou S, Rault G, Ferec C et al. Impact of the CFTR-potentiator ivacaftor on airway microbiota in cystic fibrosis patients carrying a G551D mutation. PLoS One 2015;10(4):e0124124.
48. Konstan MW, Morgan WJ, Butler SM, Pasta DJ, Craib ML, Silva SJ et al. Risk factors for rate of decline in forced expiratory volume in one second in children and adolescents with cystic fibrosis. J Pediatr 2007; 151(2):134–139, 139 e131. [PubMed: 17643762]
49. Emerson J, Rosenfeld M, McNamara S, Ramsey B, Gibson RL. Pseudomonas aeruginosa and other predictors of mortality and morbidity in young children with cystic fibrosis. Pediatr Pulmonol 2002;34(2):91–100. [PubMed: 12112774]
50. Nixon GM, Armstrong DS, Carzino R, Carlin JB, Olinsky A, Robertson CF et al. Clinical outcome after early Pseudomonas aeruginosa infection in cystic fibrosis. J Pediatr 2001;138(5):699–704. [PubMed: 11343046]
51. Heltshe SL, Mayer-Hamblett N, Burns JL, Khan U, Baines A, Ramsey BW et al. Pseudomonas aeruginosa in cystic fibrosis patients with G551D-CFTR treated with ivacaftor. Clin Infect Dis 2015;60(5):703–712. [PubMed: 25425629]
52. Siracusa CM, Ryan J, Burns L, Wang Y, Zhang N, Clancy JP et al. Electronic monitoring reveals highly variable adherence patterns in patients prescribed ivacaftor. J Cyst Fibros 2015;14(5):621–626. [PubMed: 26074007]

53. Peleg AY, Choo JM, Langan KM, Edgeworth D, Keating D, Wilson J et al. Antibiotic exposure and interpersonal variance mask the effect of ivacaftor on respiratory microbiota composition. J Cyst Fibros 2018; 17(1):50–56. [PubMed: 29042177]
54. Mena A, Smith EE, Burns JL, Speert DP, Moskowitz SM, Perez JL et al. Genetic adaptation of Pseudomonas aeruginosa to the airways of cystic fibrosis patients is catalyzed by hypermutation. J Bacteriol 2008;190(24):7910–7917. [PubMed: 18849421]
55. Cramer N, Klockgether J, Wrasman K, Schmidt M, Davenport CF, Tummler B. Microevolution of the major common Pseudomonas aeruginosa clones C and PA14 in cystic fibrosis lungs. Environ Microbiol 2011;13(7):1690–1704. [PubMed: 21492363]
56. Markussen T, Marvig RL, Gomez-Lozano M, Aanaes K, Burleigh AE, Hoiby N et al. Environmental heterogeneity drives within-host diversification and evolution of Pseudomonas aeruginosa. MBio 2014;5(5):e01592–01514.
57. Anderson GG, Moreau-Marquis S, Stanton BA, O'Toole GA. In vitro analysis of tobramycin-treated Pseudomonas aeruginosa biofilms on cystic fibrosis-derived airway epithelial cells. Infect Immun 2008;76(4):1423–1433. [PubMed: 18212077]
58. Aspedon A, Palmer K, Whiteley M. Microarray analysis of the osmotic stress response in Pseudomonas aeruginosa. J Bacteriol 2006;188(7):2721–2725. [PubMed: 16547062]
59. Bagge N, Schuster M, Hentzer M, Ciofu O, Givskov M, Greenberg EP et al. Pseudomonas aeruginosa biofilms exposed to imipenem exhibit changes in global gene expression and beta-lactamase and alginate production. Antimicrob Agents Chemother 2004;48(4):1175–1187. [PubMed: 15047518]
60. Cirz RT, O'Neill BM, Hammond JA, Head SR, Romesberg FE. Defining the Pseudomonas aeruginosa SOS response and its role in the global response to the antibiotic ciprofloxacin. J Bacteriol 2006;188(20):7101–7110. [PubMed: 17015649]
61. Jones AK, Fulcher NB, Balzer GJ, Urbanowski ML, Pritchett CL, Schurr MJ et al. Activation of the Pseudomonas aeruginosa AlgU regulon through mucA mutation inhibits cyclic AMP/Vfr signaling. J Bacteriol 2010;192(21):5709–5717. [PubMed: 20817772]
62. Wu W, Badrane H, Arora S, Baker HV, Jin S. MucA-mediated coordination of type III secretion and alginate synthesis in Pseudomonas aeruginosa. J Bacteriol 2004;186(22):7575–7585. [PubMed: 15516570]
63. Folkesson A, Jelsbak L, Yang L, Johansen HK, Ciofu O, Hoiby N et al. Adaptation of Pseudomonas aeruginosa to the cystic fibrosis airway: an evolutionary perspective. Nat Rev Microbiol 2012;10(12):841–851. [PubMed: 23147702]
64. Smith EE, Buckley DG, Wu Z, Saenphimmachak C, Hoffman LR, D'Argenio DA et al. Genetic adaptation by Pseudomonas aeruginosa to the airways of cystic fibrosis patients. Proc Natl Acad Sci U S A 2006;103(22):8487–8492. [PubMed: 16687478]
65. Lore NI, Cigana C, De Fino I, Riva C, Juhas M, Schwager S et al. Cystic fibrosis-niche adaptation of Pseudomonas aeruginosa reduces virulence in multiple infection hosts. PLoS One 2012;7(4):e35648.
66. Huse HK, Kwon T, Zlosnik JE, Speert DP, Marcotte EM, Whiteley M. Parallel evolution in Pseudomonas aeruginosa over 39,000 generations in vivo. MBio 2010;1(4).
67. Emerson J, McNamara S, Buccat AM, Worrell K, Burns JL. Changes in cystic fibrosis sputum microbiology in the United States between 1995 and 2008. Pediatr Pulmonol 2010;45(4):363–370. [PubMed: 20232473]
68. Millar FA, Simmonds NJ, Hodson ME. Trends in pathogens colonising the respiratory tract of adult patients with cystic fibrosis, 1985–2005. J Cyst Fibros 2009;8(6):386–391. [PubMed: 19740710]
69. Acosta N, Whelan FJ, Somayaji R, Poonja A, Surette MG, Rabin HR et al. The Evolving Cystic Fibrosis Microbiome: A Comparative Cohort Study Spanning 16 Years. Ann Am Thorac Soc 2017;14(8):1288–1297. [PubMed: 28541746]
70. Crull MR, Ramos KJ, Caldwell E, Mayer-Hamblett N, Aitken ML, Goss CH. Change in Pseudomonas aeruginosa prevalence in cystic fibrosis adults over time. BMC Pulm Med 2016; 16(1):176. [PubMed: 27927212]

Box 1:

Types of CFTR modulator

CFTR modulators are small molecules that aim to improve the function of mutant CFTR proteins. These can act through a number of different mechanisms, with efficacy for different classes of mutation.

- Potentiators: Improve the channel gating of CFTR variants (increase open channel probability)
- Correctors: Augment trafficking of CFTR processing variants to the plasma membrane
- Stabilisers: Increase the residence time of variant CFTR at the plasma membrane
- Amplifiers: Increase the amount of variant CFTR available for subsequent modulation by protein-active small molecules
- Read-through agents of in-frame premature termination codons (PTCs) - suppress PTCs, produce translational readthrough by the ribosome and subsequent full-length protein.

See descriptions by Clancy et al, 2019 [19].

Box 2:

Classes of CFTR mutation

Class I mutations: Result in a failure to CFTR protein and are not considered to be readily rescuable.

Class II mutations: Include the F508del mutation (present in approximately 90% of the CF population), result in a protein that is not trafficked through the endoplasmic reticulum to the cell surface.

Class III mutations: Include the G551Asp mutation (present in approximately 5% of patients with CF) produce a protein that is trafficked to the cell surface, but which fails to regulate chloride ion transport appropriately due to a gating abnormality.

Class IV mutations: Allow for trafficking to the surface, but chloride ions cannot pass due to a conductance defect.

Class V and VI mutations: Lead to normal CFTR protein to reach the surface and open, but there is either less protein (Class V) or less stability at the surface (Class VI).

Box 3.

Existing and emerging modulator therapies

Approximately 90% of patients have a Class II mutation, which includes the F508 mutated gene. Therapies currently available for patients who are homozygous for Phe508del include:

- lumacaftor/ivacaftor [16]
- tezacaftor/ivacaftor [17]

Promising results have also been seen in phase 2 studies of the triple combinations 445/tezacaftor/ivacaftor [20] or 659/tezacaftor/ivacaftor [21].

Triple combinations also seem effective in heterozygote patients expressing at least one Phe508del gene.

Class III and IV CFTR mutated protein respond to the corrector ivacaftor alone [14].

- Combination dual therapy in this group did not demonstrate an additional clinical benefit.