

# Potential of anti-inflammatory treatment for cystic fibrosis lung disease

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**Abstract:** Cystic fibrosis (CF) is the most common life-shortening genetic disorder in Caucasians. With improved diagnosis and treatment, survival has steadily increased. Unfortunately, the overwhelming majority of patients still die from respiratory failure caused by structural damage resulting from airway obstruction, recurrent infection, and inflammation. Here, we discuss the role of inflammation and the development of anti-inflammatory therapies to treat CF lung disease. The inflammatory host response is the least addressed component of CF airway disease at this time. Current challenges in both preclinical and clinical investigation make the identification of suitable anti-inflammatory drugs more difficult. Despite this, many researchers are making significant progress toward this goal and the CF research community has reason to believe that new therapies will emerge from these efforts.

**Keywords:** therapy, cystic fibrosis, survival, inflammation

## Introduction

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations on chromosome 7 resulting in impaired chloride transport through the cystic fibrosis transmembrane conductance regulator (CFTR) protein.<sup>1</sup> In exocrine glands, this channel is concentrated in the apical membrane of epithelial cells and helps to regulate ion and water content at the luminal surface. For reasons that are not completely understood, CFTR deficiency causes greater activity of the epithelial sodium channel (ENaC) leading to increased absorption of sodium and water from the luminal surface. In the lung, this dysfunctional conductance of chloride and sodium ions reduces the water content and height of the airway surface liquid layer (ASL). An abnormal ASL impairs the mucociliary escalator causing accumulation of tenacious secretions within the airways. These secretions trap bacteria and other pathogens and initiate a self-perpetuating cycle of airway obstruction, endobronchial infection, and exuberant inflammation. It is not clear whether or not exuberant inflammation in the human CF airway exists independent of airway obstruction and infection. Regardless, evidence indicates that the inflammatory host response in the airway begins early in life, is of greater magnitude than is observed in patients without CF and persists beyond apparent eradication of infectious stimuli.<sup>2-8</sup>

For many years, regular use of pancreatic enzyme supplements and improved nutrition have greatly improved health outcomes for patients with CF. Therefore, pulmonary manifestations are currently the center of health impairment for most of these patients. For over 40 years, prescribed therapies have focused on improving

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airway clearance of secretions and treating endobronchial infection. However, in recent decades, there has been a growing appreciation of the role of inflammation in CF lung disease. Excessive airway inflammation is a key component of the pathophysiology of CF, and researchers are working to identify effective and well-tolerated anti-inflammatory therapies.

Both prospective and retrospective studies of anti-inflammatory medications in CF demonstrate preserved lung function measured by spirometry – a value closely correlated with survival.<sup>9–14</sup> The majority of these studies do not show an increased risk of infectious complications in subjects receiving anti-inflammatory drugs. This paper reviews these studies, as well as ongoing research addressing the negative impacts of airway inflammation in CF.

## Inflammation in CF lung disease

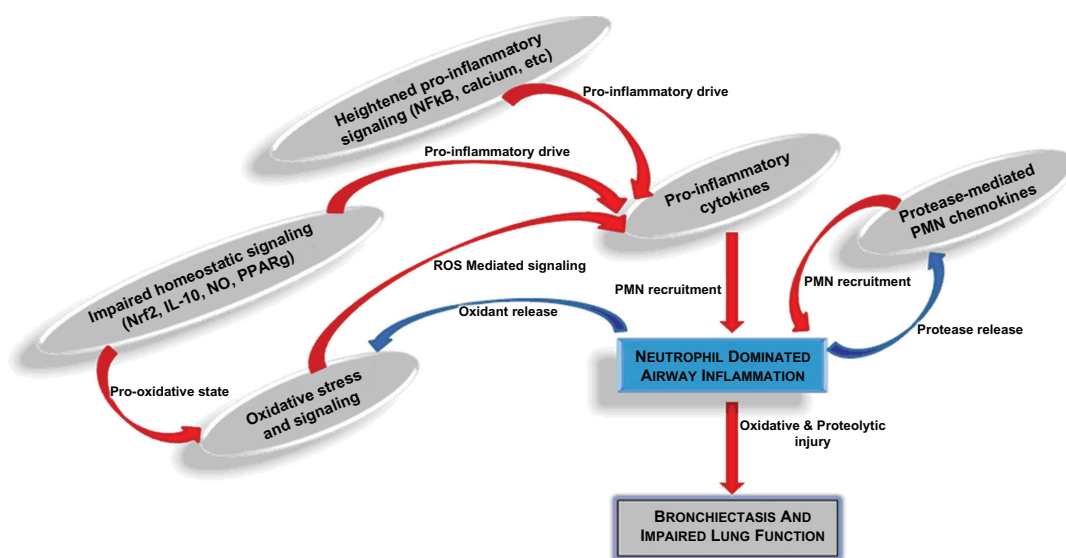
At birth, the lungs of neonates with CF are structurally normal and not inflamed. However, during early infancy, secretions begin to plug the bronchioles and ducts of submucosal glands.<sup>2</sup> Bronchoalveolar lavage (BAL) studies from infants with CF show high concentrations of neutrophils and proinflammatory mediators in the airways, often in the absence of identifiable pathogens.<sup>3,6,7</sup> When bacteria are found, the inflammatory response relative to the bacterial burden is greater in CF infants compared with infants without CF.<sup>8,15</sup> This neutrophil dominated host response is typically sequestered in and around the airways but spreads to involve

the local airway wall and supporting structures in advanced stages. The alveoli are spared until late in the disease course, making CF largely an airways disease. The upper limit of normal for BAL from healthy lung is <3% neutrophils. BAL from CF lungs typically contains 10, or even 20 times this degree of neutrophilia (Figure 1).

In addition to being excessive, the inflammatory response is also prolonged, leading many researchers to postulate that the exaggerated inflammatory state is largely a function of impaired resolution. This may explain why inflammation can be detected in apparently sterile BAL samples of infants with CF, reflecting an ongoing response to prior infection.<sup>3</sup> As it is central to the host inflammatory response in the CF airway, anti-inflammatory therapies must address, either directly or indirectly, the neutrophil itself or its products.

## Nuclear factor kappa-B transcriptional regulation

Many believe that the heightened inflammatory response in CF is closely related to abnormal CFTR function. This theory is supported by data showing increased basal and inducible inflammation in human airway cells with either genetic or chemical CFTR impairment and decreased inflammatory response in CF epithelial cells treated with CFTR correctors.<sup>16,17</sup> Likewise, transgenic mice with CFTR impairment show altered inflammatory response to pertinent airway challenge when compared with wild-type mice.<sup>18,19</sup> Much of the mechanistic research has focused on



**Figure 1** Complex interactions contributing to CF airway inflammation. Several proinflammatory mechanisms have been identified in research of cystic fibrosis (CF) airway disease. Heightened proinflammatory signaling pathways, impaired redox-regulation and anti-inflammatory signaling pathways, and perpetual proteolytic and oxidative stress are some of the most well-described mechanisms driving the neutrophil-dominated host response. This neutrophilic inflammation, along with ineffective airway clearance and chronic airway infection, lead to progressive bronchiectasis and impaired lung function.

**Abbreviations:** PMN, polymorphonuclear cell, ie, neutrophil; ROS, reactive oxygen species; NO, nitric oxide.

the intracellular transcription factor nuclear factor kappa-B (NFκB). The majority of proinflammatory cytokines believed to be important in CF-related airway inflammation are regulated by NFκB activity. These include interleukin (IL)-8, tumor necrosis factor-α (TNF-α), IL-1β, IL-6, and granulocyte-macrophage colony-stimulating factor, and so on.<sup>4</sup> Cellular interaction with bacteria, bacterial products, and proinflammatory cytokines causes NFκB to translocate to the nucleus and promote transcription of multiple proinflammatory gene products. It is unclear whether or not this transcription factor is inherently altered in CF. The observed increase in NFκB activation may be a response to the abundant stimuli present in the CF lung. Alternatively, there is evidence of increased Iκ-kinase (IKK) activity in CFTR-deficient epithelial cells. IKK is an important step in the canonical activation pathway for NFκB and over activity of this enzyme would increase NFκB-driven proinflammatory signaling.<sup>20–22</sup>

Studies also suggest that the local environment of airway secretions surrounding CF respiratory epithelial cells enhances NFκB activation by increasing local calcium-dependant signaling of this transcription factor.<sup>23</sup> These data would indicate that improved airway clearance can indirectly reduce inflammation in the CF airway. Similarly, inflammatory products (eg, DNA from necrotic neutrophils, mucus production) add to the viscosity of airway secretions and further impair airway clearance. Therefore, impaired airway clearance and the exaggerated inflammatory state in the CF airway are closely linked. Effective therapies for either abnormality will likely improve both aspects of CF lung disease. Furthermore, whether or not increased activation of NFκB is a primary or secondary process in CF cells, its central role as a regulator of many downstream inflammatory responses makes it an obvious target for anti-inflammatory treatment.

## Anti-inflammatory signaling

Studies indicate that other immunoregulatory signaling pathways may be abnormal in CF. IL-10, an important anti-inflammatory cytokine produced by many cell types in the lung is reduced.<sup>24,25</sup> Lipopolysaccharide (LPS) and TNF-α, which are abundant in the CF airway, stimulate IL-10 production in subjects without CF.<sup>26</sup> However, studies of BAL from patients with CF unexpectedly contain relatively little IL-10 as compared with that from patients without CF.<sup>4,27</sup> Both IL-10 and nitric oxide (NO), which is also low in CF, limit NFκB activation by preserving IκB. By this and other mechanisms, mediators such as IL-10 induce

neutrophil apoptosis, decrease antigen presentation and T-cell stimulation, and help to terminate the inflammatory response.<sup>28,29</sup> Therefore, impairment of such immunoregulatory signaling may contribute to both the heightened and prolonged airway inflammatory response.

PPARγ is a transcription factor that, among other functions, also limits NFκB activity and is reduced in CF human airway epithelial cells.<sup>30,31</sup> Abnormalities in the fatty acid content of CF cells, with deficiencies of both docosahexaenoic acid (DHA) and linoleic acid, may contribute to impaired PPARγ signaling.<sup>30</sup> Similarly, lipoxin A4 (LXA4) is an important endogenous anti-inflammatory lipid mediator that helps mitigate the acute inflammatory response.<sup>32</sup> It has been reported that LXA4 concentrations are reduced in BAL fluid from stable CF patients compared with non-CF patients with pulmonary inflammation.<sup>32</sup> Although the reasons for the decreased LXA4 concentrations have not been elucidated, administration of a metabolically stable lipoxin analog in cell culture and mouse models of chronic airway infection and inflammation decreased IL-8 production and neutrophilic inflammation without increasing infectious burden.<sup>32</sup> The role of LXA4 deficiency in CF lung disease remains controversial and a more recent study found no difference in LXA4 concentration in BAL from CF patients compared with non-CF disease-matched controls with neutrophilic airway inflammation.<sup>33</sup>

## T helper 17

Recent work proposes a greater role for adaptive immunity in CF as a subset of T cells known as T helper (TH)-17 cells appear to be involved in CF pulmonary inflammation.<sup>34</sup> IL-23 and IL-17 are proinflammatory cytokines involved in TH-17 cell signaling and are elevated in CF airways (human and mouse) in response to common stimuli.<sup>35,36</sup> IL-17 appears to prime CF airway epithelial cells to greater IL-8 release in response to *Pseudomonas*, and may promote MUC5AC gene transcription through NFκB activation.<sup>37,38</sup> Viscous secretions at the apical surface of airway epithelia trap bacteria and may magnify the likelihood of antigen presentation to dendritic cells and macrophages which, in turn, trigger the IL-23/IL-17 signaling pathway. IL-17 is important in recruiting neutrophils to the airway in response to bacterial products such as LPS and the relative importance of IL-17 in the CF airway is a focus of continued research.<sup>39</sup> These findings further support the interconnected nature of impaired airway clearance, bacterial infection, and inflammatory response.

## Nrf2 transcriptional regulation

Finally, recent studies in CF cell and mouse models have uncovered a deficiency of nuclear factor E2-related factor 2 (Nrf2), a transcription factor functional in respiratory epithelia and pivotal to mitigating the acute inflammatory response.<sup>40</sup> Nrf2 regulates a large number of antioxidant and cytoprotective genes. Therefore, impairment of this transcription factor may underlie both the prooxidative and hyperinflammatory state. Several studies find an increased oxidative state in the CF airway, and reactive oxygen species, as intracellular messengers, are complicit in NFκB activation in respiratory epithelia.<sup>41</sup> Data from CF models show low levels of NO resulting from a deficiency of inducible NO synthase in epithelial cells,<sup>42,43</sup> and low concentrations of glutathione in extracellular lining fluid, possibly due to deficient transport of this antioxidant through CFTR channels.<sup>44,45</sup> Many prooxidative and inflammatory signaling pathways are interrelated and, therefore, compounds with direct or indirect antioxidant properties may be effective anti-inflammatories. In support of this theory are data showing that Nrf2 agonists reduce the airway inflammatory response in CF cell and mouse models, making Nrf2 signaling and antioxidant gene regulation another promising area for therapeutic development.<sup>46</sup>

Experiments utilizing Nrf2-knockout mice and bone marrow adoptive transfer show that Nrf2 function in the airway plays a pivotal role in both inflammatory response and structural damage after elastase-induced injury.<sup>47</sup> A significant protease/antiprotease imbalance exists in the CF airway, largely driven by excess free neutrophil elastase. This not only is a major contributor to progressive airway damage, but also likely helps to perpetuate the neutrophilic inflammatory response. Certain proteases (eg, MMP-8, MMP-9) abundant in the CF airway are capable of cleaving small peptides from collagen.<sup>48</sup> These peptides can then serve as chemoattractants for neutrophil recruitment to the airway, demonstrating an important role of proteases in the inflammatory host response. Though further increased during pulmonary exacerbation, protease levels in the CF airway typically remain elevated even during times of relative health. Recent work highlights the ability of neutrophil elastase to cleave and activate MMP-9 while cleaving and inactivating its inhibitor TIMP-1.<sup>49</sup> This reaffirms the neutrophil and its products as a central regulator and therapeutic target in the excessive and perpetual inflammatory state. Similarly, 2 neutrophil serine protease inhibitors, secretory leukoprotease inhibitor and elafin, are both cleaved and inactivated by abundant neutrophil elastase in the infected CF airway.<sup>50,51</sup>

In addition to augmenting the proteolytic imbalance, elastase also impairs the antimicrobial host defense properties of these proteins, again underscoring the need to address excessive inflammation in a comprehensive treatment strategy.

Ultimately, there is evidence that many altered immunoregulatory pathways contribute to the inflammatory response in CF. Although no unified pathway directly connecting CFTR dysfunction to exuberant inflammation has been found, the combined effects of numerous dysregulated proinflammatory and anti-inflammatory signaling events are a significant part of the pathophysiology in CF lung disease. It may ultimately be less important to determine whether this is directly related to CFTR impairment or more indirectly due to the altered airway environment developing from both abnormal CFTR and ENaC channel activity. Despite inconsistencies in preclinical models, the majority of the CF research community view inflammation as a valid therapeutic target. This view is supported by greater lung health afforded from either systemic corticosteroids or high-dose ibuprofen in clinical trials.<sup>10,11,13</sup> However, neither of these well-studied anti-inflammatories is prescribed for the majority of patients with CF secondary to concern over unacceptable side effects.<sup>52</sup> Therefore, research continues to search for more suitable agents that will likely benefit the majority of patients with CF by addressing a fundamental component of ongoing airway injury.

## Prior clinical trials of anti-inflammatory therapy in CF Corticosteroids

Though several potential anti-inflammatory drugs have been studied in clinical trials of patients with CF, results from prior trials with 3 medications largely drive this field of research: systemic corticosteroids, high-dose nonsteroidal anti-inflammatory drugs (NSAIDs), and azithromycin. Nearly 3 decades ago, investigators noted that patients with CF who also had hypogammaglobulinemia treated with systemic corticosteroids had better lung function than those without hypogammaglobulinemia (and therefore not treated with steroids).<sup>53</sup> This led to 2 large clinical trials of oral steroids in patients with CF. An initial study enrolled 21 patients aged 1–12 years with mild to moderate lung disease, given with either placebo or prednisone 1–2 mg/kg by mouth every other day.<sup>9</sup> Subjects receiving prednisone showed better lung function, improved weight gain, and few hospitalizations over a 4-year observational period. No apparent adverse events were reported during this study, which led to a larger, multicenter trial, enrolling

285 patients aged 6–14 years with mild to moderate lung disease.<sup>54</sup> Prednisone 1 mg/kg or 2 mg/kg by mouth every other day was compared with a placebo group, and again showed beneficial effects of corticosteroids on lung function – particularly in subjects infected with *Pseudomonas aeruginosa*. However, significant adverse events occurred in those receiving prednisone, including growth impairment, cataracts, and glucose intolerance. The high-dose (2 mg/kg) group was halted half way through the 4-year study due to a high incidence of adverse events.<sup>55</sup> Follow-up analysis 6 years after completion of the trial found persistent growth deficits,<sup>56</sup> and a separate analysis 5 years after the initial smaller study found growth deficits in 14/17 subjects in the prednisone arm who completed the study.<sup>12</sup> For these reasons, regular use of systemic corticosteroids is not recommended for patients with CF, despite the beneficial effects on pulmonary health. It is, however, worth noting that those receiving prednisone in these trials did not show greater risk of infection. Though unacceptable as a long-term therapy for most patients, shorter courses of systemic steroids may be useful,<sup>57</sup> particularly for those with a prominent asthmatic phenotype or during treatment for acute pulmonary exacerbation when the inflammatory response is further increased.

The use of inhaled corticosteroids (ICS) to treat CF lung disease has been a topic of much interest in recent years. This therapy continues to be prescribed to a large number of patients, likely due to a relatively high incidence of asthma-like symptoms in patients with CF and the perceived safety and familiarity among health care providers. However, in clinical trials subjects with CF, ICS have not consistently proven effective. A recent Cochrane Database Systematic Review of clinical trials found no conclusive benefits of ICS in CF and no apparent adverse events when discontinuing ICS.<sup>58</sup> Authors also noted that data are not available to determine if long-term use of ICS will reduce lung inflammation in CF, and that this question is unlikely to be answered by a clinical trial at this point.

## Ibuprofen

Seeking to identify an anti-inflammatory drug that might be better tolerated, Konstan et al<sup>59</sup> in Cleveland began studying the use of high-dose ibuprofen in animal models, and then in clinical trials of patients with CF. A 4-year double-blind placebo-controlled trial in 85 subjects aged 5–39 years with mild lung disease showed that those receiving the NSAID had less decline in pulmonary function, preserved body weight, and fewer hospitalizations.<sup>11</sup> Individual pharmacokinetics

targeting a peak plasma concentration of 50–100 µg/mL (typical dose 20–30 mg/kg) were used in this study. This is 2–3 times higher than commonly prescribed doses and *in vitro* studies indicate that, at this concentration, ibuprofen may inhibit pertinent proinflammatory signaling events in the cell (ie, NFκB, AP-1 activation).<sup>60,61</sup> A second trial was conducted in Canada with 142 subjects over 2 years and showed preserved forced vital capacity and a trend toward preserved forced expiratory volume in 1 second (FEV<sub>1</sub>) in the ibuprofen arm compared with placebo.<sup>13</sup> The incidence of adverse events between placebo and ibuprofen did not significantly differ in these trials, and in the Canadian study a greater number of subjects receiving placebo dropped out of the study due to adverse events that could be attributable to NSAID therapy. This observation likely underscores the reason that fewer than 10% of patients with CF in the United States currently receive high-dose ibuprofen. The underlying disease often manifests with symptoms that overlap with NSAID toxicity (eg, abdominal pain, epistaxis, hemoptysis, gastrointestinal hemorrhage) and confusion or concern of these apparently rare, though at times dramatic, events is the reason most cited by practitioners for not prescribing this therapy.<sup>52</sup> The need for individual pharmacokinetics has also been cited as a limitation to ibuprofen use.

## Azithromycin

Azithromycin is a commonly prescribed therapy for CF and likely has immunomodulatory effects. Data indicating that regular use of macrolides afforded better pulmonary health in another chronic lung disease, diffuse panbronchiolitis, led to studies in CF, including a trial of azithromycin in 185 patients with CF who had mild to moderate lung disease and were infected with *P. aeruginosa*.<sup>62</sup> This multicenter, placebo-controlled trial found that subjects given azithromycin thrice weekly had better lung function, weight gain, quality of life, and fewer pulmonary exacerbations than those given placebo. Studies conducted in the United States and abroad similarly showed that chronic azithromycin provides clinically relevant health benefits, though more consistently so in those infected with *P. aeruginosa*.<sup>63,64</sup> Many mechanistic studies have since been done to better understand how azithromycin is beneficial. From data available to date, it appears that there is a combined effect of both anti-inflammatory and anti-infective properties. Azithromycin can inhibit neutrophil recruitment and oxidative burst, as well as proinflammatory cytokine production.<sup>65–67</sup> This macrolide antibiotic also inhibits several virulence factors of *P. aeruginosa*, which may be more susceptible to azithromycin in the stationary growth

phase.<sup>68</sup> The question of mechanism is further complicated by recent studies in both CF mouse and human models showing that treatment with azithromycin causes increased rather than decreased release of pivotal proinflammatory cytokines from primary airway epithelial cells.<sup>69,70</sup> Similarly, sputum concentrations of IL-8 were not significantly altered in the largest US clinical trial of azithromycin in CF patients and patients receiving this treatment continue to have significant airway neutrophilia.<sup>62</sup>

Though not always reflected in spirometry, the clinical benefits of azithromycin therapy in CF appear to be significant. However, the complexities noted above combined with an increased incidence of nausea and vomiting and increased antibiotic resistance<sup>71</sup> (eg, *Staphylococcus aureus*, nontuberculous mycobacteria) with chronic use, lead many to conclude that this therapy is useful but not the ideal anti-inflammatory treatment for CF. Recent data from a large clinical trial suggest that azithromycin use for 24 weeks may not significantly affect pulmonary function in children without chronic *P. aeruginosa* infection.<sup>72</sup> Despite the fact that FEV<sub>1</sub>, the primary outcome measure, was not affected, other potentially important exploratory outcomes were improved with azithromycin use (eg, rate of pulmonary exacerbation).

There are many challenges to identifying a more suitable anti-inflammatory therapy. Despite what has been learned from prior research, the proper molecular targets are not clear and there is significant redundancy in proinflammatory signaling within the lung. Highly specific drugs targeting individual pathways have often proven ineffective or potentially toxic. Those with the greatest proven clinical efficacy appear to have broad, rather nonspecific effects, which may predispose to more side effects. Despite these challenges, ongoing research is identifying multiple new and promising therapeutic targets, and there is significant hope that more effective and well-tolerated therapies will be identified in the near future.

## Challenges in preclinical models when testing new therapies

An essential step in developing new anti-inflammatory therapies is use of preclinical models to identify molecular targets and test drug activity. Although these models have provided fundamental information for our understanding of the role of inflammation in CF lung disease, each model under current use has limitations for the evaluation of candidate therapies. Available cell culture and animal models are unable to reproduce the chronic airway impaction and infection underlying the perpetual inflammatory response. As highlighted earlier, the interrelated nature of multiple

processes in the CF airway (chronic infection, airway obstruction, and host inflammatory response) is clear. Therefore, improved methods, likely employing better animal models, will be necessary to more accurately predict clinical utility when testing promising candidate therapies.

## Cell lines and cultured epithelial cells

A comprehensive list of established cell lines used in CF research is beyond the scope of this article, but has been reviewed.<sup>73</sup> Use of immortalized cell lines from patients with CF has been especially useful to study the effects of CFTR dysfunction and to test drugs that may improve CFTR function.<sup>73,74</sup> Some features of the CF inflammatory response have been demonstrated in primary and immortalized CF cells, such as an increased inflammatory response following *P. aeruginosa* exposure,<sup>75,76</sup> and higher levels of proinflammatory cytokines and nuclear transcription factors involved in the inflammatory pathway.<sup>25,77–80</sup> However, it is unlikely that the immunologic response to chronic bacterial infection seen in the CF lung can be fully reproduced in either cultured primary cells or in immortalized cell lines. For example, phenotypic characteristics that are expressed by a particular clone from an immortalized cell line may not completely reflect that of the original mixed population of epithelial cells from which it was derived. Furthermore, the transformation process and cell passaging (or in the case of primary cells, transferring the cells into culture) can lead to differential expression of phenotype. Finally, cells *in vitro* have a limited ability to tolerate live bacterial challenge, and the strain of *P. aeruginosa* that is used in an experiment can influence the inflammatory response.<sup>73</sup> *In vitro* cell culture studies have been extremely useful in CF inflammation research and models are being improved to better recapitulate the human airway. However, it appears unlikely that these methods, in isolation, will be adequate to accurately predict the most promising drugs for clinical trials.

## Animal models

The mouse, which has 78% sequence homology with humans, has been used extensively in CF research.<sup>81</sup> Mice are relatively inexpensive, easy to produce and to manipulate genetically, and widely available compared with other CF animal models. However, there are differences in airway morphology between the mouse and human.<sup>82,83</sup> More importantly, although many CF mouse models show exaggerated inflammatory response in the airway and certain mice demonstrate aspects of altered pulmonary physiology consistent with CF lung disease, none develops the full spectrum of chronically inflamed

and infected airways.<sup>84</sup> Furthermore, the phenotype of each mutant mouse is dictated not only by the *Cfir* mutation, but also by the genetic background and environment of the animal, making proper controls essential in these studies.<sup>84</sup> For many reasons, the CF mouse has been a useful *in vivo* mammalian model for inflammation research; however, present limitations must be considered when using these animals to determine clinical promise of new therapies.

Because of the limitations posed by the mouse model, researchers have investigated use of other animal models with the hope that lung function, size, and architecture would be more like that of humans. The sheep, ferret, and the pig, each having >90% sequence homology with humans, are models under development.<sup>85–88</sup> Early data from the CFTR  $-/-$  pig shows that the newborn piglets have nasal voltage measurements consistent with CFTR dysfunction and pancreatic exocrine insufficiency.<sup>89</sup> Recent reports from 5 CF pigs surviving to several months of age indicates that they may develop a lung phenotype similar to that seen in humans.<sup>90</sup> If survival for these animals can be improved and this model does consistently develop lung disease similar to that of humans, the model will be humans, it will be an invaluable resource for testing new drug therapies. Practical considerations of cost and broad availability to the research community will also need to be addressed in this process. Finally, it should be noted that thus far, survival of CF piglets has required intestinal surgery at birth performed by skilled investigators.

## Challenges for design of clinical trials when testing new therapies

Until an adequate animal model of CF lung disease is developed, much of the efficacy and safety data must be generated in human subjects with CF for new therapies that show promise in current preclinical models. As an orphan disease with only approximately 30,000 patients in the United States and 70,000 worldwide, the number of patients available to participate in clinical trials is limited.<sup>91–93</sup> Furthermore, because it is a progressive, multisystem disease, there is wide variability in disease burden between patients. Potential confounders that must be considered in trial design and analysis include but are not limited to pulmonary function, gender, bacterial infection, presence of CF-related diabetes, mutation class, pancreatic function, and nutritional status.<sup>74</sup>

Large, placebo-controlled, double-blinded, multicenter clinical trials are necessary to provide adequate data for Federal Drug Administration (FDA) approval. However, to demonstrate that these large trials are warranted, smaller

trials that evaluate efficacy and safety of a candidate drug must be conducted first. One such trial design is the proof of concept.<sup>81</sup> A successful proof of concept study provides positive outcome data that is specific to the biological process the drug is intended to effect. It should also provide preliminary data that reflects that the drug is safe. In addition, because pharmacokinetics often differ in patients with CF,<sup>94–96</sup> it is important to establish appropriate dosing prior to progressing to large trials of a new drug. In consideration of all these factors, design of proof of concept trials must include stringent inclusion and exclusion criteria, collection of outcome measures that support the biologic effects of the drug, and pharmacokinetic data that suggests the chosen dose is adequate for patients with CF. These requirements must be balanced so that the cost and burden to subjects will not prevent completion of the study. Study design of these initial trials is critical as CF studies, similar to those of many orphan diseases, can easily become underpowered to detect significant differences in the primary outcomes. This makes it difficult to justify larger, more costly studies and promising new therapies may be lost to future development.

One of the most significant challenges to testing new therapies in CF is determining appropriate outcome measures. Outcomes used in clinical trials for treatment with anti-inflammatory therapies have traditionally included clinical efficacy measures such as time to next exacerbation, or surrogate end points such as pulmonary function (usually FEV<sub>1</sub> and/or rate of decline of FEV<sub>1</sub>).<sup>10,11,13,14,97,98</sup> As a result of improvements in clinical care, the rate of decline of lung function in patients with CF has slowed substantially.<sup>99</sup> In addition, rates of exacerbation in patients with relatively less lung damage are lower than in those with more severe disease.<sup>100</sup> However, it is these patients who may benefit most from anti-inflammatory treatment to prevent pulmonary decline over time. Thus, using lung function or pulmonary exacerbations as primary end points in clinical trials requires a large number of patients over a significant length of time to detect differences in these clinical parameters.<sup>101</sup> The FDA is increasingly accepting and encouraging the use of measures designed to assess Patient Reported Outcomes (PROs) such as health-related quality of life (eg, the Cystic Fibrosis Questionnaire-Revised).<sup>102</sup> However, this may be problematic when studying a chronic therapy aimed at reducing the rate of decline in health over a lifetime. As in the case of FEV<sub>1</sub> and pulmonary exacerbations, either prohibitively long follow up or large numbers of subjects may be needed to detect a significant difference in PROs.<sup>74</sup>

Given the limited numbers of potential research subjects and the need to maximize limited resources, early phase studies must delicately balance the risk of underpowered results with the burden of subject participation and cost. For new anti-inflammatory therapies, other outcome measures may be more sensitive for detecting short-term response to treatment. In recognition of the need to develop and implement novel outcome measures for use in CF clinical research, the Cystic Fibrosis Foundation (CFF) Therapeutics Development Network (TDN) established outcome-measures working groups to make recommendations.<sup>103</sup> Mayer-Hamblett et al<sup>103</sup> also defined standards by which new outcome measures should be evaluated: a new outcome measure should be biologically relevant, sensitive and specific to treatment effects, reproducible, and feasible. The remainder of this portion of the review focuses on potential outcome measures for use in trials of new anti-inflammatory therapies.

### Systemic biomarkers

Because a systemic biomarker of inflammation would be readily obtainable (in blood or urine) from a patient of any age and with any severity of pulmonary disease, researchers have evaluated such measures in patients with stable disease and in those experiencing exacerbations.<sup>104–119</sup> Although, some systemic markers have shown response to systemic antibiotic therapy,<sup>104,110,112,115,120</sup> there is concern that approach may not be sufficiently sensitive to detect changes that are occurring in the lung in response to a new anti-inflammatory therapy (Table 1).<sup>121</sup>

### Bronchoalveolar lavage

The lung or, more precisely, the airway is the most active site of CF-related inflammation; thus, there has been substantial

focus on developing biomarkers that are measurable in airway samples. Bronchoscopy with BAL is considered the gold standard for evaluation of lung inflammation, and has been used to evaluate response to therapy in many CF clinical trials.<sup>122–131</sup> However, this procedure is invasive, resource intensive and samples only a small portion of the lung.<sup>121</sup> Two less invasive means of capturing airway specimens are exhaled breath condensate (EBC) and sputum collection.

### Exhaled breath condensate

EBC collection allows measurement of markers even in those CF patients who do not produce sputum. Markers of inflammation, NO, and pH have been studied in the EBC of adult and pediatric patients with CF.<sup>132–139</sup> In some studies, pH and markers of inflammation in EBC from patients with CF who are experiencing an exacerbation have been shown to respond to antibiotic therapy.<sup>132,136,138</sup> In response to the increasing interest in use of EBC to study pulmonary disease, the American Thoracic and European Respiratory Societies collaborated to make recommendations for optimal collection and analysis techniques.<sup>6</sup> These efforts are promising, though concerns over sensitivity and specificity of this technique have been expressed. Many traditional inflammatory indices may not be volatile enough to accurately measure by this technique.

### Sputum biomarkers

More extensive work has been done by CF researchers to evaluate the use of sputum biomarkers to sample lower airway secretions and response to therapy. Because patients with mild disease rarely produce sputum when stable, some studies have utilized sputum induction. For example, Ordonez et al<sup>140</sup> studied pretherapy and posttherapy inflammatory markers in induced sputum following treatment with IV antibiotics.

**Table 1** Current and potential outcome measures for use in clinical trials of new anti-inflammatory therapies in CF

Outcome measure	Examples	Reference(s)
Clinical efficacy	FEV <sub>1</sub> , rate of decline of FEV <sub>1</sub> , rate of exacerbations	10, 11, 13, 14, 97, 98
Patient reported outcome	CF-HQR	102
BAL markers	Neutrophils, bacterial count, elastase IL-8, TNF- $\alpha$ , soluble ICAM-1, LB4	122–131
EBC markers	NO, pH, MMP-9	132–139
Sputum markers	White cell count, bacterial count, elastase IL-6, IL-8, IL-10, TNF- $\alpha$	140–143
Systemic markers	CRP, NE-APC, TNF- $\alpha$ , IL-1, IL-2, IL-8, EGF	104–119
CT scan	CT score, airway trapping, airway wall thickening	145–152
MRI	Structural changes, lung perfusion/pulmonary blood flow, ventilation defects	154–158

**Note:** Table does not include exhaustive list of examples for each outcome measure. For comprehensive review of outcome measures in clinical trials for CF patients/candidate biomarkers for CF airway inflammation. See references 74 and 121.

**Abbreviations:** FEV<sub>1</sub>, forced expiratory volume in 1 second; IL, interleukin; TNF, tumor necrosis factor; L, leukotriene; NO, nitric oxide; MMP, matrix metalloprotein; CRP, C-reactive protein; NE-APC, neutrophil antiproteinase complex; EGF, endothelial growth factor; CT, computed tomography; MRI, magnetic resonance imaging; CF, cystic fibrosis.



Total sputum white cell count, active elastase, IL-8, and *P. aeruginosa* counts were significantly decreased after antibiotic therapy.<sup>140</sup> Although sputum induction was used in this study, expectorated sputum has also been shown to reflect airway inflammation in CF.<sup>141,142</sup> Sagel et al<sup>143</sup> demonstrated that in patients with CF, measurement of inflammatory markers is comparable in induced and expectorated sputum. Thus, either method can be successfully employed in clinical trials in which sputum biomarkers will be an end point.

To assess the association between sputum biomarkers and clinical parameters, Mayer-Hamblett et al<sup>101</sup> constructed a database of sputum biomarkers and pulmonary function data from 4 multicenter CF clinical trials to demonstrate the strength of association between expectorated sputum biomarkers and lung disease severity. Both elastase and IL-8 were negatively correlated with lung function in this diverse group of CF patients. Of the markers tested, sputum elastase showed the strongest correlation with FEV<sub>1</sub> (correlation, 0.35; 95% confidence interval [CI], -0.46 to -0.22). On average, CF patients whose sputum elastase measurements differed by 0.5 logs differed in their FEV<sub>1</sub> values by -7.3% (95% CI, -9.7 to -4.6). Finally, sputum elastase was shown to have a significant longitudinal association with FEV<sub>1</sub>: a -2.9% decline in FEV<sub>1</sub> for every log increase in elastase. Thus, differences in sputum elastase correlated well with clinically meaningful differences in lung function, providing a useful biomarker, particularly for studies in which the intervention would likely preserve function through mechanisms that would also reduce the airway elastase concentration.<sup>101</sup>

Aware that differences in collection of sputum and analysis of biomarkers may lead to variability in results, and thus decrease the reproducibility of test results, the CFFTDN created standard operating procedures (SOPs) for both collection of sputum by induction and processing of sputum.<sup>121</sup> In addition, core laboratories were established for analysis of sputum inflammatory biomarkers and sputum cytology (The Children's Hospital/University of Colorado Health Sciences Center and Case Western Reserve University, respectively.) Use of SOPs and core laboratories for centralized analysis will insure consistency of results from single and multicenter studies.

## Computed tomography (CT)

Although induced sputum and EBC can be collected from children (as young as 10 years for induced sputum<sup>121</sup> and 3–4 years for EBC),<sup>144</sup> neither procedure is feasible in very young children and infants. Imaging such as CT and magnetic resonance imaging (MRI) would be applicable to subjects of all ages. Furthermore, therapies aimed at correcting the

basic defects in CF are hoped to prevent or delay the onset of functional lung disease. Airway imaging would allow researchers to serially assess the presence and progression of structural lung damage.

Two separate longitudinal studies in patients with CF using every 2 or 3 year CT scans showed that scored CT images were more sensitive than spirometry for detecting disease progression.<sup>145,146</sup> In addition, Brody et al<sup>147</sup> showed that there was correlation between the frequency of pulmonary exacerbations and CT score. Small intervention studies in patients with CF have demonstrated improvement in overall CT score following treatment.<sup>148,149</sup>

In addition to evaluation of general structural changes, CT scan can be used to reproducibly measure airway wall thickness. Airway wall thickness can be reproducibly measured by CT scan.<sup>150–152</sup> Because airway wall thickness is thought to correspond to airway inflammation, this measurement may be useful to monitor the efficacy of anti-inflammatory therapies. Airway wall thickness has been successfully used to demonstrate response to inhaled steroids in asthmatic subjects.<sup>153</sup> More data are needed to determine if this outcome measure will be useful in anti-inflammatory studies in CF lung disease. Investigators are faced with concerns over cumulative radiation exposure and potential need for sedation in the very young when designing these experiments.

## Magnetic resonance imaging

Using proton-MRI sequences, structural changes such as bronchial wall thickening, mucus plugging, bronchiectasis, consolidation, and segmental destruction can be visualized.<sup>154</sup> Although the spatial and temporal resolution of MRI of the lung is inferior to that of CT, the lack of ionizing radiation makes MRI appealing as a repeated measure in clinical trials. Use of intravenous or inhaled contrast expands the potential of MRI in assessing CF lung disease. For example, with gadolinium-based intravenous contrast, MRI can examine lung perfusion and pulmonary blood flow.<sup>155,156</sup> Inhaled hyperpolarized gas can provide a high MRI signal from airspaces of the lung. No ionizing radiation is used for these contrast studies and reported adverse events are mild.<sup>157</sup> Using ventilation MRI imaging, Mentore et al<sup>158</sup> demonstrated correlation between ventilation defects and spirometry. Furthermore, MRI imaging correlated with treatment and could detect abnormalities in subjects with normal spirometry. As in the case of sputum biomarkers, in order to use imaging techniques as outcome measures in clinical trials, SOPs will need to be created, and centralized reading centers where a common scoring system is used must be developed.<sup>159</sup>

## Future directions

### CFF pipeline-drug discovery program/CF therapeutics development network

Through research funded by the CFF, both ibuprofen and azithromycin were shown to be beneficial in CF as described earlier. The CFF TDN is sponsoring a number of trials of potential new anti-inflammatory drugs, including both novel compounds and drugs that are FDA approved for other indications.<sup>160</sup> Based on promising preclinical data, 3 FDA-approved drugs were recently tested in proof of concept trials including the immunosuppressant hydroxychloroquine, the HMG CoA reductase inhibitor, simvastatin and the PPAR gamma agonist, Pioglitazone.<sup>161–163</sup> Although results of safety and efficacy of these 3 therapies were inconclusive, consideration is being given for longer trials.<sup>164</sup>

Another FDA-approved therapy, the phosphodiesterase inhibitor, sildenafil, is being studied through the CF TDN for its potential as an anti-inflammatory. Toward et al<sup>165</sup> demonstrated that pretreatment with sildenafil inhibited LPS-induced airway hyperreactivity, white cell influx, and NO dysfunction in 2 guinea pig models of airway disease.<sup>165</sup> In CF respiratory epithelial cells, Poschet et al<sup>166</sup> showed that sildenafil exposure could reverse the excessive proinflammatory response to *P. aeruginosa*. In separate work, DBA/2 mice sensitive to *P. aeruginosa* were fed a diet with or without sildenafil. Following aerosol-delivered *P. aeruginosa* respiratory infection, myeloperoxidase in lung homogenates (a reflection of neutrophil infiltration) was reduced by 42% ± 11% ( $P = 0.047$ ) in animals who received sildenafil-containing diets.<sup>167</sup> Based on these data, sildenafil is currently being tested in a phase 2 trial.

A novel drug that is under investigation through the CF TDN is SB656933, a selective chemokine receptor (CXCR2) antagonist. This once-daily oral medication is also in a phase 2 multicenter study. The CFF is also funding investigation of drugs that may combat the pathogenic result of excessive inflammation by targeting antioxidant deficiencies. The ability of *N*-acetylcysteine (an oral agent that replenishes glutathione levels) to modulate inflammation in CF was studied by Tirouvanziam et al.<sup>168</sup> In this proof of concept study, CF patients were treated for 4 weeks, and inflammatory markers in expectorated sputum were assessed. In addition to increased glutathione levels in blood and in neutrophils, researchers found that sputum elastase was significantly decreased following treatment with *N*-acetylcysteine. Based on these results, a multicenter phase 2b is underway. Targeting similar mechanisms, a phase 2 trial of inhaled glutathione is being conducted in Germany. Finally,

a multicenter study of DHA supplementation is also ongoing. As discussed earlier, DHA supports anti-inflammatory signaling that may be impaired in CF (eg, PPAR $\gamma$ ).

In summary, preclinical models of CF have provided invaluable information about the pathophysiology of CF lung disease, highlighting many molecular mechanisms which promote an exaggerated host inflammatory response. However, significant limitations of these models present challenges when trying to identify promising anti-inflammatory therapies for clinical trials. Similarly, inherent qualities of CF (eg, variable disease burden, relatively slow progression, limited number of potential subjects, etc) require thoughtful planning for early human studies to balance patient burden and cost with risk of underpowered data. Improving preclinical models of CF lung disease and developing useful and more dynamic surrogates of pulmonary function (ie, biomarkers) will help to overcome these challenges and significantly accelerate progress toward identifying new anti-inflammatory therapies. The CF research community continues to identify and test promising drugs, while many patients benefit from those already in use.

## Disclosure

The authors report no conflicts of interest in this work.

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