Targeting cystic fibrosis inflammation in the age of CFTR modulators: focus on macrophages

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CFTR modulators have revolutionised the way that cystic fibrosis is treated and can drastically improve patient quality of life, but questions remain over their long-term effects on the inflammatory processes that underpin cystic fibrosis chronic lung disease https://bit.ly/3lbtUig

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ABSTRACT Cystic fibrosis (CF) is a life-shortening, multi-organ, autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The most prominent clinical manifestation in CF is the development of progressive lung disease characterised by an intense, chronic inflammatory airway response that culminates in respiratory failure and, ultimately, death. In recent years, a new class of therapeutics that have the potential to correct the underlying defect in CF, known as CFTR modulators, have revolutionised the field. Despite the exciting success of these drugs, their impact on airway inflammation, and its long-term consequences, remains undetermined. In addition, studies querying the absolute requirement for infection as a driver of CF inflammation have challenged the traditional consensus on CF pathogenesis, and also emphasise the need to prioritise complementary anti-inflammatory treatments in CF. Macrophages, often overlooked in CF research despite their integral role in other chronic inflammatory pathologies, have increasingly become recognised as key players in the initiation, perpetuation and resolution of CF lung inflammation, perhaps as a direct result of CFTR dysfunction. These findings suggest that macrophages may be an important target for novel anti-inflammatory interventional strategies to effectively treat CF lung function decline. This review will consider evidence for the efficacy of anti-inflammatory drugs in the treatment of CF, the potential role of macrophages, and the significance of targeting these pathways at a time when rectifying the basic defect in CF, through use of novel CFTR modulator therapies, is becoming increasingly viable.
Background

*Lung inflammation in cystic fibrosis: an intrinsic element to a lethal process*

Loss of functional cystic fibrosis transmembrane conductance regulator (CFTR) in cystic fibrosis (CF) causes clinical disease in multiple organ systems [1]. The primary cause of morbidity and mortality in the vast majority of CF cases, however, is the progressive loss of lung function that occurs as a result of recurring airway infections and an intense, non-resolving inflammatory response that, over time, causes structural damage to the airways, bronchiectasis and respiratory failure [2]. Despite decades of research, the mechanisms that drive this inflammatory response are still poorly understood. The classic model of disease progression in the CF lung points towards loss of CFTR expression in epithelial cells leading to thickening of the mucus layer and chronic retention of the pathogen as the primary, if not solitary, cause of infection and lung disease in CF (figure 1).

More recently, consensus has shifted and a more complex picture of what instigates and drives lung disease in CF has been proposed, challenging the simplicity of this classic model. As well as missing the role of CFTR in maintaining proper salt/water balance in the airway, the loss of crucial, CFTR-dependent bicarbonate secretion from bronchial epithelial cells leads to a drop in pH in the mucus layer, which compromises the antimicrobial activity of the airway surface liquid and contributes to the early bacterial clearance defect seen in new-born CF pigs [3, 4]. In addition, in the 1990s several clinical studies on infants identified markers of airway inflammation in bronchoalveolar lavage fluid (BALF) from CF children as young as 4 weeks old. These markers included high neutrophil and macrophage counts and high levels of interleukin (IL)-8; crucially, they were present prior to colonisation of the lungs and in the absence of infection [5, 6]. Further studies have demonstrated that raised markers of airway inflammation are a hallmark of disease in children and many of these markers persist into adulthood [7, 8]. A similar increase in inflammatory markers in BALF from children with CF has been shown to correlate with increased mucin production, despite the same samples exhibiting similar, or even lower, levels of bacterial burden when compared to healthy controls [9]. A recent study in the CF pig also indicates that CFTR has an important role in normal fetal airway development [10]. In a ferret model of the disease, correction of CFTR function following administration of CFTR modulators *in utero* helped to rectify developmental defects associated with CF that affect the pancreas, gut and reproductive organs [11]. Mucus plugging of small airways also contributes to the development of steep oxygen gradients and, in recent years, the switch to glycolytic metabolism in response to hypoxia has been shown to have significant effects on immune cell function in macrophages in particular [12–15]. Such observations call into question the absolute requirement for infection as a stimulus for lung disease in CF. Questions, instead, are raised as to what links defective CFTR to the onset of sterile inflammation.

One important concept, which is gaining credence in the field of CF research, is that immune cells may be intrinsically dysregulated in CF and pre-programmed to contribute to an excessive inflammatory response.

![FIGURE 1 The classic model of lung disease in cystic fibrosis. CFTR: cystic fibrosis transmembrane conductance regulator.](https://doi.org/10.1183/13993003.03502-2020)
in the lung irrespective of infection, hypoxia or changes in pH. In line with this concept is the observation that CFTR is also expressed in non-epithelial cells, including lymphocytes, neutrophils, monocytes and macrophages, whilst whole-cell patch clamp recording has shown functional CFTR ion conductance activity in human monocytes and both human and porcine macrophages [16–19]. Knowledge of the exact function of CFTR in immune cells, and whether CFTR ion channel activity at the cell surface specifically affects immune function or not, is still lacking. However, the development of mouse models that allow the conditional knockout of CFTR in a tissue-specific manner has helped shed some light on the contribution of CFTR to immune function. These models have shown that loss of myeloid-specific CFTR expression causes an increase in neutrophil and macrophage counts in the lung during steady state, as well as decreased survival and elevated lung pathology following Pseudomonas aeruginosa infection [20]. In addition, bone marrow (BM) chimera experiments, where BM was transplanted between wild-type (WT) and Cfr-null mice following total-body irradiation, demonstrated that the milieu of pro-inflammatory cytokines found in the CF lung exists as a consequence of CFTR deficiency in immune cells rather than epithelial cells [21]. It was noted that several of the cytokines (including IL-1α and IL-6) found at elevated levels in the CF-BM-receiving WT mice, 6 h after the mice were nebulised with lipopolysaccharide (LPS), are produced primarily by macrophages in the lung, suggesting an intrinsic abnormality in these cells linked to loss of CFTR. Given the importance of macrophages, particularly in the earliest stages of inflammation and in its proper resolution, a significant primary functional defect in these cells could be a key driver of early and progressive lung disease in CF and consequently a potential target for treatment (figure 2).

**Lung inflammation in CF: involvement of macrophages**

Macrophages are the most plastic of all immune cells and their immense functional diversity allows them to play a part in orchestrating all major stages of the inflammatory response, from initiation to resolution and repair. In steady-state airways, resident alveolar macrophages constitute over 90% of immune cells present and function as so-called sentinels of the respiratory host defence. The airways are constantly exposed to inhaled particles, ranging from harmless antigens to deadly pathogens, and it falls on alveolar macrophages to remain in an inherently immunosuppressive state so as not to respond aggressively to innocuous antigens, whilst being ready to mount a quick and effective response to a genuine threat. Central to such a response is the release of cytokines and chemokines and the recruitment of neutrophils en masse and monocyte-derived macrophages (MDMs), which, in a chronically inflamed tissue such as the CF airway, can replenish and replace the resident macrophage population and more effectively drive inflammation [22]. Depending on the nature of the tissue microenvironment and the stage of inflammation, macrophages can adopt an array of immune phenotypes that are broadly classified as being pro-inflammatory "M1"-like or more anti-inflammatory, pro-resolution "M2"-like, although in truth this

classification is inherently reductive and understates much of the true diversity of macrophage functionality.

There is now a wealth of evidence pinpointing various functional defects in CF macrophages (as reviewed extensively in [23, 24]). Infections with *Staphylococcus aureus* and *Haemophilus influenzae* in the airways of infants and with *P. aeruginosa*, *Burkholderia cepacia* and several other pathogens later in life are commonplace in CF. Macrophages are the earliest immune responders to infection and have been shown to display defective bacterial clearance in CF, arising from both impaired pathogen internalisation and intracellular killing. Improper control of early infections by alveolar macrophages, as evidenced by the ability of *S. aureus* to survive intracellularly within Cfr-deficient alveolar macrophages, resulting in pneumonia after transplantation of said cells into uninfected CF mice, may contribute to initiating early disease in the airways and providing fertile ground for the growth of opportunistic pathogens such as *P. aeruginosa* [25]. Studies from as early as the 1970s demonstrated that culturing alveolar macrophages in serum derived from CF patients had a negative effect on their ability to phagocytose *Pseudomonas* [26, 27]. Such observations introduced the idea that the CF microenvironment fundamentally alters macrophage function. The unique composition of the diseased CF airway provides a cauldron of factors capable of shaping the function of both resident and highly plastic infiltrating macrophage populations. Numerous, non-resolving bacterial infections and a landscape of heightened inflammatory cytokine, protease and reactive oxygen species (ROS) levels concentrated within the mucus layer help shape macrophage dysfunction in CF, whilst mucus layer hypoxia caused by mucus plugging and elevated epithelial O₂ consumption likely favours an activated M1-like macrophage phenotype [12, 28, 29]. There is also the potential that direct macrophage–mucin interaction can alter macrophage function, as is seen with the pro-apoptotic effects of Muc5b binding to airway eosinophils [30]. Exposure of macrophages to such an environment would indicate that defective bacterial killing and their other functional impairments in CF are acquired and, although this is likely to be largely the case, there is also now mounting evidence for intrinsic dysfunction in CFTR-defective macrophages. Indeed, the aforementioned phagocytic phenotype has recently been attributed to dysregulated calcium signalling, defective fusion of the phagosome and lysosome, compromised autophagic activity and alkalinisation of the phagosome leading to impaired bacterial killing, although the latter remains controversial [25, 31–34].

As the inflammatory response in the airways progresses, activated monocytes and MDMs are recruited whilst neutrophils accumulate to form the predominant immune cell population in the CF lung [35]. In CF, macrophages appear uniquely programmed to exacerbate this response. The LPS-mediated response in CF monocytes and macrophages is dysregulated, leading to the overproduction of several pro-inflammatory cytokines, including, crucially, potent neutrophil chemoattractant IL-8 [28, 36–38]. Additionally, neutrophil extracellular trap production has been shown to stimulate exaggerated IL-8 and tumor necrosis factor-α (TNF-α) production in CF MDMs, creating a self-perpetuating pro-inflammatory loop in the airway [39]. Importantly, in CF there also appears to be a breakdown in the resolution phase of inflammation [20, 40]. An integral part of successful resolution is the effective clearance of apoptotic neutrophils by local macrophages, known as efferocytosis, to prevent secondary necrosis and subsequent release of neutrophils inflammatory content and any viable bacteria. The high protease content of the CF airway, neutrophil elastase in particular, appears to intrude on this process through cleavage of surface receptors; more fundamentally, loss of CFTR appears to impede the ability of MDMs to differentiate into a reparative, anti-inflammatory M2-like state [41–43]. Such a polarisation imbalance could be key in progressing airway inflammation in CF to a chronic state. The specialised pro-resolving mediator (SPM) superfamily are highly effective lipid regulators of this transition and mediate effective resolution through a multitude of functions, which include halting neutrophil recruitment and activation, augmenting macrophage efferocytosis in the case of lipoxin A₄ (LXA₄), and promoting tissue repair and regeneration [44]. LXA₄ has been found at significantly reduced levels in the airways of CF patients compared to healthy controls, whilst a similar reduction is seen in expression of the LXA₄ receptor ALX/FPR2 in patient-derived MDMs and CFBE41o- cells (human CF bronchial epithelial cell line homozygous for the ΔF508 mutation), indicating a direct role for CFTR dysfunction [45–47].

Progressive infection and inflammation in the lower airways that is sustained over a prolonged period leads to irreversible structural damage to the airways, the development of bronchiectasis and ultimately a rapid decline in lung function. The whole process of tissue repair in CF appears to be ineffective in restoring the basic architecture of the damaged airways. The resident macrophage population in the lung, supplemented by recruited monocytes from the blood, produces an abundance of growth factors and cytokines that promote epithelial proliferation and angiogenesis, stimulate fibroblasts and facilitate tissue repair and regeneration [48]. As well as secondary contributors to airway damage in CF through recruitment of neutrophils and other drivers of inflammation, macrophages have also been implicated in the direct destruction of the airway epithelium in CF. Specifically, whole-genome expression analysis of lung tissue...
revolutionised the field. What role, then, will anti-inflammatories in CF play in the future?

Although there is a clear current requirement for effective anti-inflammatories in CF, much of the momentum surrounding CF therapeutics currently lies behind the development of CFTR modulators. These aim to correct the basic defect in CF and could, therefore, remove the need for drugs aimed at treating the consequences of CF lung disease entirely. Despite successes so far, such a scenario seems improbable, owing to the level of redundancy in the processes underpinning established chronic inflammation and the fact that the majority of CFTR mutations have not yet been shown to be completely amenable to modulator therapy. Nevertheless, such therapies are showing remarkable promise and have revolutionised the field. What role, then, will anti-inflammatories in CF play in the future?

Anti-inflammatory therapies for CF: a focus on macrophages

Corticosteroids and nonsteroidal anti-inflammatory drugs

Developing an effective anti-inflammatory therapy to treat CF has proven frustrating. Corticosteroids represent potent treatments for several diseases characterised by excessive, non-resolving inflammation, including rheumatoid arthritis, inflammatory bowel disease and multiple sclerosis. However, long-term administration can result in serious adverse effects. Justification for the use of corticosteroids requires that the therapeutic benefits provided outweigh the risks of adverse effects and CF has proven to be a disease for which this balance negates their use as treatment. Two large clinical trials of the systemic corticosteroid prednisone in children with CF underlined the therapeutic potential on offer from this kind of treatment for lung inflammation, but also marked the endpoint for corticosteroids in CF. Both trials, published in 1985 and 1995, reported improved lung function, weight gain and fewer hospitalisations in those given prednisone on alternate days when compared with placebo [51, 52]. However, one of the groups from the latter study was halted after 2 years because of a high incidence of adverse events, including cataracts and abnormalities in glucose metabolism. A subsequent follow-up study analysing the long-term effects of prednisone treatment in CF children found a strong association between the treatment and suppression of growth, even years after the treatment was stopped [53]. Owing to the risks highlighted by these trials and other toxicities known to be associated with long-term use, systemic corticosteroids are not recommended for clinical use to treat CF lung inflammation. Nevertheless, these trials underline the potential efficacy of targeting inflammation in CF [54].

The pitfalls of steroid use in CF have made nonsteroidal anti-inflammatory drugs (NSAIDs) an attractive alternative. Two major clinical trials in patients with CF found that high-dose ibuprofen taken twice daily for either 4 years [55] or 2 years [56] resulted in a significantly slower decline in lung function and fewer admissions to hospital and safeguarded against severe weight loss, all without any serious adverse effects. A 2018 matched cohort study into the long-term effects of high-dose ibuprofen use in CF children corroborated the improved lung function over a 2-year treatment period and showed greater long-term survival over a 16-year follow-up than the control cohort [57]. Accordingly, ibuprofen is currently the only anti-inflammatory drug recommended for clinical use in CF [54]. However, only a small minority of patients actually receive this treatment, owing in part to difficulties in tailoring treatment to individuals in order to reach the peak plasma concentration, established in the two aforementioned clinical trials, and the potential toxic side effects of long-term NSAID use on renal function in a patient group already burdened with regular use of nephrotoxic agents such as aminoglycoside antibiotics [58].

Targeting inflammation with higher precision

The advent of biological therapeutics now makes it possible to target even the most subtle components of the inflammatory cascade with high precision. Such treatments have proven effective in tackling chronic inflammation in diseases such as rheumatoid arthritis and inflammatory bowel disease and are slowly reducing the use of corticosteroids [59, 60]. However, a key distinction between CF and these diseases is that the inflammatory response in the CF lungs, although exaggerated, remains a vital process that is fundamentally protective to the host. Treating inflammation in CF thus represents a dangerous balancing act. Making the right choice in terms of which node of this response to target is crucial so as not to make the patient any more vulnerable to the resident bacteria and other infectious agents colonising the CF airways. One target of immunomodulatory therapy that has shown promise in CF is the arachidonate metabolism pathway. Leukotriene B4 (LTB4), a potent neutrophil chemoattractant, is produced in abundance by airway macrophages following stimulation with P. aeruginosa. BIIL 284 BS (amelubant), a small molecule inhibitor of the LTB4 receptor, inhibits the action of this chemoattractant [61, 62]. However, despite much promise garnered from results of preclinical trials and animal studies, a
placebo-controlled phase II clinical trial of BIIL 284 BS in patients with CF resulted in early termination. This was owing to a disproportionate incidence of pulmonary-related serious adverse events and exacerbations associated with increased airway inflammation [63, 64]. The outcome of this trial underlines the risks involved in aggressively targeting the inflammatory response in the CF lung and potentially damaging host control of the bacterial population. A more recent phase II trial of eicosanoid modulators aims to address the “unmet need” remaining after the BIIL 284 BS trial, for a safe and effective anti-inflammatory therapy, with a once-daily leukotriene A4 (LTA4) hydroxylase inhibitor known as CTX-4430 (acebilustat), which blocks the conversion of LTA4 to LTB4. Unlike BIIL 284, which directly blocks receptor activity, acebilustat modulates LTB4 formation and thus allows for more precise regulation of LTB4 levels through carefully designed dosing that does not completely eradicate LTB4 activity and leave the host susceptible to infection [65]. The extent to which this results in improved clinical outcomes remains to be seen.

**Effect of current therapies on inflammation and macrophage function**

Despite the fact that ibuprofen is presently the only anti-inflammatory recommended for clinical use in CF, several prominent therapies currently in use have been shown to exert anti-inflammatory effects themselves. Dornase alfa is a mucolytic agent that cleaves extracellular DNA produced by degrading neutrophils in the CF airways. This results in thinning of the viscous mucus layer, reduced exacerbations and improved lung function [66]. More recently, dornase alfa has also been shown to reduce the neutrophil count in the BALF of CF patients [67]. It also results in reduced levels of neutrophil products that contribute to exacerbation of lung inflammation and tissue damage, including IL-8, neutrophil elastase and MMP-9, leading to some demands to reduce the age at which this therapy can be offered as standard [67].

Antibiotics play a vital role in managing bacterial infections in CF and have a very clear secondary effect on lung inflammation. A 2003 clinical trial demonstrated that azithromycin taken three times weekly resulted in improved lung function, reduced exacerbations, fewer hospitalisations and improved weight gain in *P. aeruginosa*-infected CF patients, compared to placebo [68]. In a further placebo-controlled trial in children aged 6–18 years who were not infected with *P. aeruginosa*, azithromycin use was also shown to reduce pulmonary exacerbations and improve weight gain, although there was no change in lung function [69]. The precise mechanism of action through which this macrolide mediates these effects is unclear, but there is strong evidence to suggest that it has potent immunomodulatory properties. Azithromycin drastically reduces airway neutrophilia in patients suffering from chronic allograft rejection following lung transplant, and a similar effect was shown in the serum of CF patients, with a corresponding decrease in calprotectin and acute-phase inflammatory mediators, C-reactive protein and serum amyloid A [70, 71]. In terms of macrophage-mediated inflammation, treatment with azithromycin or other macrolides significantly reduced the production of TNF-α, IL-1β and IL-6 by sputum cells derived from patients with chronic obstructive pulmonary disease and produced a similar effect in macrophages cultured from azithromycin-treated CF mice [72, 73]. Azithromycin administration *in vitro* also reduced the expression of IL-12p40, a subunit of the primary cytokine involved in priming T-helper 1 cells, in RAW264.7 macrophages stimulated with LPS and interferon-γ [74]. Azithromycin treatment *in vitro* has also been shown to upregulate IL-10 expression in BM-derived macrophages, which is seen as the hallmark of so-called immunosuppressive, alternatively activated M2-like macrophages [75]. Further evidence that macrolides may promote differentiation of these cells came from a group investigating the immunomodulatory properties of azithromycin in lupus, who found that treatment caused a significant increase in the expression of M2 markers Fizz-1 and IL-10 as well as improved phagocytic activity in patient MDMs [76]. This suggests that one effect of azithromycin in CF may be to blunt primary macrophage responses to the inflammatory CF airway, and the subsequent adaptive immune response, by promoting alternative activation of differentiating macrophages and possibly also contributing to partial correction of the defective phagocytic phenotype. By contrast, an increased susceptibility to nontuberculous mycobacteria (NTM) infection has been reported in CF patients undergoing long-term azithromycin use. An investigation into the effects of the macrolide on cell autophagy (an important immune defence mechanism against mycobacterial infection) found that azithromycin treatment impaired intracellular killing of multiple strains of mycobacteria by human MDMs, which displayed defective autophagy, and promoted infection with azithromycin-resistant *M. abscessus in vivo* [77, 78]. It should be noted, however, that an association between azithromycin use and NTM infection is disputed, with several studies finding no such correlation, and one study even reporting that azithromycin use is protective against NTM infection in CF adults [79–81].

As discussed above, several established and prospective therapies for CF have notable impacts on macrophage function. Recent observations suggest that this is also true for novel CFTR modulators. The CFTR-corrector lumacaftor is able to augment the phagocytic killing of *P. aeruginosa* by human
CFTR-deficient MDMs when administered in vitro, suggesting a key role in macrophage CFTR expression [82]. A similar recovery of phagocytic ability was shown using the CFTR-potentiator ivacaftor, which also improved killing of P. aeruginosa by human CF MDMs and reduced inflammatory cytokine secretion by Burkholderia cenocepacia-infected CF MDMs [83]. A recent study on the impact of different combinations of CFTR modulator on CF monocyte function in vitro showed that modulators could significantly reduce secretion of the inflammasome products IL-1β and IL-18 after activation of NLRP3 with LPS and ATP treatment, with the combination of ivacaftor and tezacaftor (another CFTR-corrector) being particularly effective [84]. Another prospective therapeutic is lenabasum (also known as anabasum, resunah, JBT-101 and ajulemic acid), a synthetic oral cannabinoid receptor type 2 agonist. In a phase IIa clinical trial, lenabasum significantly increased the time to the first pulmonary exacerbation and caused a sharp drop in the rate of exacerbations (although the overall number of events was low). The study also showed a clear reduction in sputum levels of inflammatory biomarkers, including neutrophil count, neutrophil elastase, IL-8 and IgG [85]. The protective and pro-resolution properties of lenabasum are well established [86], including a direct effect on macrophage function. In vitro treatment of human MDMs significantly reduced IL-6 gene expression and protein release after LPS treatment in a dose-dependent manner and had a comparable effect on IL-1β release by human monocytes [87, 88]. It was recently shown that lenabasum causes a substantial decrease in the production of multiple key LPS-induced pro-inflammatory mediators (IL-6, IL-8 and TNF-α) by primary airway macrophages from CF lungs, whilst increasing synthesis of pro-resolution eicosanoids [89]. Treating differentiating human CF monocytes with lenabasum in vitro leads to a sharp reduction in M1-like polarisation in a dose-dependent manner and dampens the release of pro-inflammatory mediators by LPS-treated CF MDMs whilst also rescuing defective phagocytic activity in the differentiated CF cells [90]. Despite these experimental effects in vitro, the difficulty of translating such results into clinical benefits is further underlined by the fact that the recent phase IIb study of lenabasum for treatment of CF failed to meet its primary endpoint of significantly reducing the rate of pulmonary exacerbation [91].

A major risk with targeting macrophages directly, as with any global anti-inflammatory strategy, is that such treatments may weaken one of the immune system’s foremost defensive lines against infection. This is particularly worrisome in CF patients, who are so susceptible to deadly, drug-resistant bacterial infections and exacerbations. A more nuanced approach may be able to exploit the protective qualities of macrophages, harnessing their pro-resolution and pro-repair functions to help the host’s internal inflammatory dimmer switch. It is not yet clear how this could be achieved therapeutically but there is evidence in mouse models for the possibility of macrophage adoptive transfers, BM transplants in CF and stem cell therapy to put in place an appropriate antimicrobial response whilst halting the progression of chronic disease [92–94]. The aforementioned SPM superfamily represents another potential avenue for engaging pro-resolution pathways therapeutically, with a recent study showing that treatment of P. aeruginosa-infected CF mice with SPM resolvin D1 (RvD1) drastically reduced bacterial burden and neutrophilic inflammation whilst improving the rate of airway macrophage efferocytosis of neutrophils and T-cells [95]. RvD1 treatment also improved phagocytic killing of P. aeruginosa by CF airway macrophages, an effect replicated upon treatment of sputum phagocytes derived from CF patients, and caused a significant, simultaneous reduction in the secretion of the pro-inflammatory cytokines IL-8 and IL-6.

**CFTR modulators**

It is increasingly likely that future therapies for CF will need to be developed to complement CFTR modulators. CFTR modulators currently available for clinical use can be divided into two types: correctors and potentiators. CFTR potentiators augment the ion channel function of CFTR proteins that are expressed on the cell surface. The modulator ivacaftor has been shown to effect a substantial improvement in lung function (10.6% increase in predicted forced expiratory volume in 1 s (FEV₁) after 24 weeks) and weight gain, and a reduction in pulmonary exacerbations in patients with CFTR gating mutations (notably Gly551Asp/G551D) [96]. Such mutations only affect ∼3% of CF patients. However, individuals with the more common CFTR trafficking mutations (including the most prevalent Phe508del/ΔF508) appear to be amenable to therapies combining modulators such as ivacaftor with CFTR correctors, which chaperone misfolded CFTR proteins to the cell surface where potentiators can then augment channel function. The corrector lumacaftor, when given in combination with ivacaftor, generated encouraging, albeit more modest, improvements in lung function (~3% increase) and a decreased rate of exacerbations when given to patients homozygous for Phe508del [97, 98]. Combining ivacaftor with the corrector tezacaftor produces similar improvements [99]. These results provided a framework for the search for more effective combinations of modulators, leading to the development of triple combination therapies that aim to target the up to 30% of patients heterozygous for ΔF508 and a class I minimal-function mutation (e.g. Gly542X/ G542X) who do not benefit from the aforementioned double-combination therapies, and who often
present with the most severe disease [100]. One such therapy, combining tezacaftor/ivacaftor with the next-generation CFTR-correction elexacaftor, has produced some astonishing clinical results. Elexacaftor serves much the same function as the first-generation correctors (e.g. lumacaftor and tezacaftor) of chaperoning the CFTR protein to the cell surface. However, it uses a different mechanism of action, thereby potentially offering a synergistic effect when combining the two. A phase III trial of elexacaftor/tezacaftor/ivacaftor in patients aged ≥12 years who were homozygous for ΔF508 and a minimal-function mutation saw a 14.3% increase in predicted lung function (FEV1) and a 63% reduction in the rate of pulmonary exacerbation at 24 weeks, relative to the placebo control group [101]. A separate phase III trial of patients homozygous for ΔF508 (roughly 50% of the CF population) showed similarly drastic improvements, with a 10% increase in predicted FEV1 after 4 weeks in patients receiving the triple therapy compared to the group receiving only tezacaftor/ivacaftor [102]. Taken together, the results of these trials suggest that improvements akin to those produced in the original ivacaftor trial can now be brought to the 90% of CF patients with one or two ΔF508 mutations. Elexacaftor/tezacaftor/ivacaftor received US Food and Drug Administration (FDA) approval in October 2019, with the process of extending provision of the drug to Europe and the rest of the world underway [103].

It is clear that CFTR modulators have immense potential for improving the quality of life for people with CF. However, even those patients who react well to the most successful treatment to date, ivacaftor, still require the standard CF disease treatments (antibiotics, mucolytic agents, pancreatic enzyme supplements). Furthermore, a longitudinal cohort study following CF patients with at least one Gly551Asp (G551D) allele for 6 months after commencement of ivacaftor treatment found no evidence of any dampening of airway inflammation. Levels of sputum inflammatory markers did not decrease despite a clear downward trend of the relative abundance of Pseudomonas and several other common CF pathogens [104, 105]. A 2019 study found high baseline sputum levels of inflammatory markers, including IL-1β, IL-8 and neutrophil elastase, which remained unchanged after 6 months of ivacaftor treatment [106]. In direct contrast, a recent study of 12 patients with G551D mutations undergoing ivacaftor treatment found a decrease in sputum inflammatory markers, despite the P. aeruginosa burden in the sputum persisting over 2 years of treatment (following an initial decline) [107]. It should be noted that inflammation in this study, although significantly dampened, did remain at a high level. The same group carried out a proteomics study of monocytes isolated from patients 1 week after undergoing ivacaftor treatment and found that the drug elicited a reduction in several markers of inflammation and leukocyte migration [108]. In a separate study, serum levels of the pro-inflammatory cytokines IL-1β, IL-8 and TNF-α were also reduced following 3 months of treatment with ivacaftor/tezacaftor, likely as a result of less secretion by peripheral blood mononuclear cells, which showed blunted production from patients in the treatment group in response to inflammasome activation with LPS and ATP in vitro [84]. However, again in contrast, whole-blood RNA sequencing has shown significant overexpression of an array of inflammatory genes in the circulation of ΔF508 homozygous patients compared to non-CF controls, and these levels remained unchanged after 6 months of lumacaftor/ivacaftor treatment [109]. The emerging data regarding the effects of modulator treatment on inflammation point towards a possible dampening of the inflammatory state of the circulation in CF and a subsequent curtailment of the hyperinflammatory phenotype of circulating monocytes, but there is still a lack of clear evidence showing that these changes translate, in the long-term, to improvements in the inflammatory milieu of the airways, where it is needed most (table 1). The effect of these modulators when administered in vitro appears to be far more clear-cut, with CFTR correction inducing a direct reversal of the dysfunctional phagocytic and inflammatory responses of human CF macrophages [82, 83].

An important point to consider, which may prevent this effect from translating to a substantial clinical difference, is that one of the key factors that allows inflammation to transition to a chronic state is the mediators which drive spiralling inflammation exhibit a significant level of redundancy. If an inflammatory milieu, such as that seen in the CF airways, persists and progresses for long enough, it may achieve a state of self-perpetuation, at which point even the direct removal of the initial stimulus, in this case the loss of CFTR function, is not enough to reverse the process [110]. This can be seen in non-CF bronchiectasis, which shares several key hallmarks with CF lung disease despite normal CFTR function. In this condition, once disease chronicity is established, the structural damage to the airway architecture itself may be sufficient to maintain infection and inflammation [111, 112]. CFTR modulators are achieving life-altering clinical improvements for patients after an initial drastic improvement in mucus clearance; however, these drugs are still in their relative infancy and, without directly combating chronic inflammation, it may be that modulators will shift the burden of disease to later in life, leading to a CF population that is living longer, with a higher quality of life, but nonetheless still succumbing to the same consequences of severe lung disease further down the line. The prospect of combining effective CFTR modulators and anti-inflammatories, alongside the standard treatment regimen for CF, creates an exciting outlook for the future of CF therapeutics.
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<th>Drug name (compound name)</th>
<th>Modulator class</th>
<th>Genotypes amenable</th>
<th>Patients approved for treatment %</th>
<th>Clinical response</th>
<th>Effects on lung inflammation</th>
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<td>Ivacaftor (VX-770), also known as Kalydeco®</td>
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<td>(\geq 1) copy gating mutation (e.g. G551D, G178R)</td>
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<td>10.6% increase in predicted lung function (FEV1) at 24 weeks</td>
<td>55% reduction in risk of pulmonary exacerbation (compared to placebo control group)</td>
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<td>Lumacaftor (VX-809)/ Ivacaftor, also known as Orkambi®</td>
<td>Corrector + potentiator</td>
<td>(\Delta F508/\Delta F508)</td>
<td>~50</td>
<td>3% increase in FEV1, 30% decrease in risk of pulmonary exacerbation, 8% decrease in sweat chloride</td>
<td>No change to circulating inflammatory gene expression at 6 months post-lumacaftor/ ivacaftor</td>
<td>[98, 109]</td>
</tr>
<tr>
<td>Tezacaftor (VX-661)/ Ivacaftor also known as Symdeko®/ Symkevi®</td>
<td>Corrector + potentiator</td>
<td>(\Delta F508/\Delta F508) and patients heterozygous for (\Delta F508) and residual function mutation (e.g. P67L)</td>
<td>~55</td>
<td>6.8% increase in FEV1, 35% decrease in risk of pulmonary exacerbation, 10% decrease in sweat chloride</td>
<td>Unknown</td>
<td>[110]</td>
</tr>
<tr>
<td>Elexacaftor (VX-445)/ Ivacaftor/tezacaftor also known as Trikafta®/Kaftrio®</td>
<td>Next-generation corrector + first-generation corrector + potentiator</td>
<td>(\geq 1) copy (\Delta F508)</td>
<td>~90</td>
<td>10% ((\Delta F508/\Delta F508)) and 14.3% ((\Delta F508/minimal function)) increase in FEV1, 63% decrease in risk of pulmonary exacerbation, 43–47% decrease in sweat chloride</td>
<td>Unknown</td>
<td>[99, 100]</td>
</tr>
</tbody>
</table>

CFTR: cystic fibrosis transmembrane conductance regulator; FEV1: forced expiratory volume in 1 s.
Concluding remarks

Decades on from the first observations that the inflammatory response in the CF airways is dangerously disproportionate to the degree of bacterial invasion, it is now accepted that inflammation is a vital target of any effort to curtail progressive lung function decline in CF. Despite this, little progress in terms of specific treatments has been made. There have been exciting breakthroughs in CFTR modulator treatments in recent years and the prospect of correcting the basic defect in CF now seems more realistic. Whilst, ostensibly, therapies such as these must be the major focus of ongoing efforts in CF research, analyses of CFTR modulator clinical trials have, if anything, further underlined the importance of concurrently targeting inflammation. It is now apparent that macrophages are important instigators and drivers of excessive CF airway inflammation and have an intrinsic impairment, likely related to CFTR function, that implicates them in the major stages of lung disease progression. Macrophages represent crucial mediators of the early response to infection and the ensuing inflammatory response as well as the resolution of the response and repair of any subsequently damaged tissue. Given that all of these processes appear to be dysregulated in CF, macrophages are now seen as important potential targets, with data showing that several promising treatments currently undergoing clinical trials affect macrophage function. For the full potential of CFTR modulators to be realised, their use will need to be complemented by an effective, nuanced, safe and, as of yet, elusive immunomodulatory intervention, for which macrophages may well prove to be a central component.

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