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# Emerging Treatments in Cystic Fibrosis

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## Abstract

There are a number of potential drugs for the treatment of cystic fibrosis (CF) currently undergoing clinical studies. A number of antibacterials formulated for delivery by inhalation are at various stages of study; these include dry-powder inhaler versions of colistin, tobramycin and ciprofloxacin, and formulations of azteonam, amikacin, levofloxacin, ciprofloxacin and fosfomycin/tobramycin for nebulization. Clinical trials of anti-inflammatory agents, including glutathione, phosphodiesterase-5 inhibitors such as sildenafil, oral acetylcysteine, simvastatin, methotrexate, docosahexaenoic acid, hydroxychloroquine, pioglitazone and α1-antitrypsin, are ongoing. Ion channel modulating agents, such as lancovutide (Moli1901, duramycin) and denufosol, which activate alternate (non-CF transmembrane regulator [CFTR]) chloride channels, and GS 9411, a sodium channel antagonist, are now at the stages of clinical study and if successful, will offer a new category of therapeutic agent for the treatment of CF. Correction of the underlying gene effect, either by agents that help to correct the dysfunctional CFTR, such as ataluren, VX-770 and VX-809, or by gene transfer (gene therapy), is a particularly exciting prospect as a new therapy for CF and clinical studies are ongoing. This article reviews the exciting potential drug treatments for CF currently being evaluated in clinical studies, and also highlights some of the challenges faced by research and clinical teams in assessing the efficacy of potential new therapies for CF.

Cystic fibrosis (CF) is an autosomal recessive multi-system condition characterized by recurrent respiratory tract infections, pancreatic malabsorption and male infertility. It is caused by defects of the gene that encodes the CF transmembrane regulator (CFTR), a glycoprotein found on epithelial cells throughout the body. CFTR is a chloride channel that regulates fluid and electrolyte transport both directly and through interactions with other apical membrane proteins involved in conductance pathways. In the lungs, defective CFTR results in depletion of the airway surface liquid (ASL) height and impairment of the mucociliary transport mechanism.[1]

Over the past few decades, there have been remarkable improvements in the care and clinical outcome for people with CF. Conventional therapy has reduced the rate of lung function decline,[2] improved nutritional status[3] and increased survival, with a median age for survival of patients born this decade predicted to be 40 years.[4] Progress cannot be related to any individual intervention but is a result of the combination of multiple therapies delivered as a package of care by skilled multidisciplinary teams.

This article reviews recent achievements and highlights exciting potential new therapeutic

agents (table I) for the treatment of CF currently being evaluated in clinical studies.

## 1. Anti-Infectives

The majority of CF-related morbidity and mortality is a result of chronic pulmonary sepsis. The major pathogen in CF lung disease is *Pseudomonas aeruginosa*.

### 1.1 Anti-Pseudomonal Vaccines

A phase III study of a flagella-based *P. aeruginosa* vaccine failed to demonstrate a reduction in rates of chronic *P. aeruginosa* infection, although rates were low in both vaccinated and control groups.[5] A recent Cochrane database review concluded that vaccines against *P. aeruginosa* cannot be recommended.[6] Importantly, the review discussed a recently completed trial of an octovalent *O*-polysaccharide toxin A conjugate vaccine (Aerugen®), after which, clinical development was suspended and no data made public.[7]

### 1.2 Inhaled Antibacterials

Inhaled antibacterials are recommended as a component of care for maintenance therapy against chronic *P. aeruginosa* infection.[8] Nebulized colistin has been the main inhaled antibacterial used for the past few decades, and there is considerable experience with this treatment, particularly in European CF centres. In the past decade, preservative-free preparations of tobramycin (TOBI®, Bramitob®) have been introduced as an alternative nebulized antibacterial specifically formulated and licensed for inhalation. Large multicentre studies have demonstrated improvements in lung function, and reduced rates of exacerbations and hospitalization.[9,10]

In recent years, there have been considerable technological advances in nebulizer-compressor systems, with new devices such as the I-neb® Adaptive Aerosol Delivery (AAD®) System and the eFlow®rapid allowing an increase in the efficiency of drug delivery and offering reduced treatment times for patients.

It is likely that over the next few years there will be an increasing availability of preparations of current anti-pseudomonal antibacterials formulated and licensed for inhalation. Dry-powder inhaler versions of the two established nebulized antibacterials, colistin as colistimethate sodium (Colobreathe) and tobramycin (Tobramycin Inhalation Powder [TIP]), have been developed and the results of recent phase III studies are awaited. An European phase IIa study of another aminoglycoside antibacterial, liposomal amikacin for inhalation (Arikace™), reported good safety and tolerability, and a reduction in rates of hospita-

**Table I.** Examples of potential therapeutic agents undergoing clinical studies for the treatment of cystic fibrosis (CF)

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|---|
| **Inhaled antibacterials** |
| Dry-powder tobramycin: tobramycin inhalation powder [TIP] (Novartis) |
| Dry-powder colistin: Colobreathe (Forest) |
| Aztreonam lyseine for inhalation (Gilead) |
| Ciprofloxacin: Bay Q3939 (Bayer Schering), inhaled liposomal ciprofloxacin hydrochloride (Aradigm Corporation) |
| Levofloxacin: MP-376 (Mpex Pharmaceuticals) |
| Fosfomycin/tobramycin [GS 9310/11] (Gilead) |
| Liposomal amikacin for inhalation: Arikase™ (Transave Inc.) |
| **Anti-inflammatory agents** |
| Glutathione |
| Phosphodiesterase-5 inhibitors |
| Acetylcysteine: HE-3286 (Hollis-Eden Pharmaceuticals) |
| Simvastatin |
| Methotrexate |
| Docosahexaenoic acid |
| Hydroxychloroquine |
| Pioglitazone |
| α1-Antitrypsin |
| **Osmotic agents** |
| Mannitol: Bronchitol (Pharmaxis) |
| **Ion channel modifiers** |
| Lancovutide [Moli1901] (Lantibio) |
| Denufosol (Inspire Pharmaceuticals) |
| GS9411 (Gilead) |
| Cobiprostone [SPI-8811] (Sucampo Pharmaceuticals) |
| **CF transmembrane regulator modulators** |
| Ataluren (PTC Therapeutics) |
| VX-770 (Vertex Pharmaceuticals) |
| VX-809 (Vertex Pharmaceuticals) |
| **Gene therapy vectors** |
| Cationic liposomes |
| Lentiviruses |
| **Pancreatic enzymes** |
| Meripase® (Meristem Therapeutics) |
| Trizytek® (Altus Pharmaceuticals) |

lizations, improvements in lung function and reductions in sputum density of *P. aeruginosa*.[11]

Formulations of a number of other antibacterials, with activity against *P. aeruginosa* and other bacterial pathogens, for delivery by the inhalation route, are at various phases of clinical studies. In a phase III study involving 211 patients with CF, aztreonam lysine administered by nebulization at a dose of 75 mg two or three times daily for 28 days reduced both the frequency of infective exacerbations and sputum *P. aeruginosa* density, and improved lung function and quality of life.[12] The subsequent open-label study suggested a greater benefit with the three-times-daily than the twice-daily treatment arm.[13] Further phase III studies are ongoing. Phase II studies of the inhaled fluoroquinolones levofloxacin and ciprofloxacin are being conducted. In addition, an inhaled combination antibacterial of fosfomycin/tobramycin for inhalation has completed phase I studies.[14-17]

A novel anti-infective in a phase I study is an antibody, KB001, that interferes with the mechanism of *P. aeruginosa* infection by blocking type III secretion apparatus.[18]

### 1.3 Oral Macrolides

There have been a number of randomized controlled trials that have shown benefit from the use of macrolides, particularly azithromycin, in patients with CF, including reductions in hospitalization, and improvements in lung function and quality of life.[19-21] Consequently, azithromycin has now become firmly established as a component of long-term treatment for many patients with CF. Although the true mode of action is unknown, anti-inflammatory effects are thought to play a part. Unresolved issues include the optimal dose and frequency of administration, and whether the initial benefits are sustained long term.

## 2. Anti-Inflammatory Agents

The exaggerated and persistent inflammatory response in CF airways leads to lung damage and, therefore, anti-inflammatory therapies are components of the therapeutic regimen for CF lung disease. Adverse effects associated with anti-inflammatory agents need to be minimized, and there has to be a careful balance between reducing the damage associated with the chronic inflammatory response in CF without over-suppressing the beneficial protective effect of the host inflammatory defence. Some anti-inflammatories previously studied include corticosteroids, NSAIDs and leukotriene receptor antagonists.

A placebo-controlled, multicentre study of 224 children with CF showed that oral prednisolone led to a reduction in rate of decline in lung function for patients with *P. aeruginosa* infection, but was associated with significant adverse effects, including growth retardation, cataract formation and the development of glucose intolerance.[22] Although inhaled corticosteroids are widely prescribed in CF, a recent placebo-controlled multicentre study found no difference in its primary outcome measure of time to respiratory exacerbation following withdrawal of inhaled corticosteroids in patients with CF,[23] and the role of inhaled corticosteroids in CF remains unresolved.

NSAIDs have been investigated for the treatment of CF lung disease. A recent retrospective study has suggested an association between reduced rate of forced expiratory volume in 1 second ($FEV_1$) decline and use of ibuprofen; however, there was a higher rate of gastrointestinal bleeding in the ibuprofen-treated group.[24] A phase III study of high-dose ibuprofen failed to demonstrate a significant change in the rate of decline in $FEV_1$, the primary endpoint.[25]

Agents currently being explored as anti-inflammatory treatments for CF include inhaled glutathione, phosphodiesterase-5 inhibitors such as sildenafil, an oral acetylcysteine HE-3286, simvastatin, methotrexate, docosahexaenoic acid, hydroxychloroquine, pioglitazone and α1-antitrypsin, although the results from early studies with the latter three agents have not shown clinical improvements.[26-28]

A recent study of a leukotriene $B_4$ receptor antagonist was terminated early by the safety monitoring committee because of a significant increase in exacerbation rates in the active treatment group.[29] This perhaps highlights the challenge of anti-inflammatory therapy in CF, in

achieving a delicate balance between reducing the damage from a heightened inflammatory response while conserving the protective effects of the host against infections.

A number of studies have convincingly demonstrated short-term clinical benefits of macrolides for the treatment of CF.[19-21] Administration of azithromycin has been shown to reduce systemic markers of inflammation.[30] At present, macrolides probably offer the best option as an anti-inflammatory therapy for CF.

## 3. Mucolytics

Sputum in CF contains considerable amounts of neutrophils in addition to bacteria, and is thick and viscous. Dornase alfa (recombinant human DNase) breaks down the large amounts of the DNA in CF sputum and reduces sputum viscosity, improves pulmonary function and decreases the number of exacerbations.[31,32] It is firmly established in the treatment regimen of many patients; however, careful assessment of an initial therapeutic trial is mandatory, as individual patient response is highly variable.[33]

Acetylcysteine has been investigated as a treatment because it may affect the rheological properties of sputum. However, studies of both oral and inhaled acetylcysteine have not shown evidence of clinical benefit in CF.[34]

## 4. Improving Airway Hydration

In CF, the ASL is depleted through the imbalance of defective chloride secretion and increased sodium absorption. Replenishing the ASL is another therapeutic target, either through drugs that influence ion channels or osmotic agents that may replenish the ASL.

### 4.1 Osmotic Agents

Nebulized 7% hypertonic saline is now established as part of the treatment regimen for many patients with CF, following the recent publication of two clinical studies demonstrating beneficial effects in increased rates of mucus clearance[35] and improvements in lung function and a reduction in pulmonary exacerbation rates with use of hypertonic saline.[36]

A dry-powder preparation for inhalation of the osmotic agent mannitol (Bronchitol) is currently being evaluated for the treatment of CF lung disease. In a 2-week crossover trial in 39 patients with mild to moderate CF lung disease, inhaled mannitol 420 mg twice daily demonstrated improvement in lung function parameters with a mean $FEV_1$ increase of 7% above baseline.[37] As with all inhaled treatments, there should be close supervision of the initial test dose by a specialist physiotherapist experienced in CF care. In a study of 38 children with CF, 9 (24%) had a decrease in $FEV_1$ of ≥15% during a bronchial provocation challenge to mannitol.[38] The phase III study of this dry-powder preparation of mannitol for the treatment of CF lung disease has recently been completed, the full results of which are awaited.[39]

### 4.2 Ion Channel Modulating Agents

A novel therapeutic strategy for CF is the modulation of non-CFTR ion channels in epithelial cells. Lancovutide (Moli1901, duramycin) activates an alternative chloride channel in epithelial cells by elevating intracellular calcium levels, and thus, may potentially compensate for CFTR deficiency in the airway epithelium and increase the volume of the ASL. In a phase I study, lancovutide increased chloride permeability in the nasal epithelium of both healthy controls and CF patients.[40] In a small phase II study of 18 adult patients with CF and a $FEV_1$ of >60% predicted, a dose of nebulized lancovutide administered was generally well tolerated and, at higher doses, produced improvements in $FEV_1$ that were sustained at follow-up 26 days later.[41]

Denufosol is a drug that stimulates the purigenic $P2Y_2$ receptor on epithelial cells, increases chloride secretion through activation of an alternative non-CFTR chloride channel, inhibits sodium absorption, enhances mucin secretion from goblet cells and stimulates cilia beat frequency. In a 28-day phase II study, nebulized denufosol three times daily was generally well tolerated and led to an improvement in lung

function of 0.14 L from placebo.[42] Inspire Pharmaceuticals recently issued a press release[43] stating that in the first phase III TIGER (Transport of Ions to Generate Epithelial Rehydration)-1 study, nebulized denufosol 60 mg versus placebo given three times daily for 24 weeks in 352 patients with mild lung disease ($FEV_1$ ≥75% predicated) successfully achieved its primary efficacy endpoint, an improvement in $FEV_1$ compared with placebo. Denufosol is currently being investigated in a second phase III study (TIGER-2).

Other compounds of potential therapeutic interest include GS9411, an inhaled epithelial sodium channel antagonists[44] and cobiprostone (SPI-8811), an agent that bypasses transport of defective chloride channels. Cobiprostone can be given orally and is currently being investigated in a phase IIa study.[45]

## 5. Agents to Correct Dysfunctional Non-Cystic Fibrosis Transmembrane Regulator

There are many (>1500) different gene mutations for CF, and these fall into different classes (I–V) reflecting their effect on CFTR: (I) defective protein synthesis; (II) impaired processing; (III) defective regulation; (IV) impaired function; and (V) reduced synthesis of normal functioning CFTR. Recently, much attention has been turned to the development of disease-modifying agents that may correct the dysfunctional CFTR, such as by addressing the trafficking of CFTR to the apical cell membrane or the function of the defective CFTR. These agents may offer benefit, although for some this may be limited to specific classes of mutations.

In approximately 10% of patients, CF results from single point alterations in DNA that, when transcribed into messenger RNA, introduces a premature stop codon, halting the ribosomal translation process and producing a truncated, non-functional protein (class I mutations). Phase I and II studies have been conducted on ataluren (PTC124), an orally bioavailable drug that can cause ribosomal read-through of premature stop mutations in patients with class I (stop) mutations and correct the processing of the CFTR gene, allowing increased production of functional CFTR protein. A phase II study conducted in Israel demonstrated an overall improvement in nasal transepithelial potential differences in adult patients with class I mutations treated with ataluren, although not all patients responded to treatment.[46] A phase IIb study is being initiated.

VX-770 is an oral drug that acts on malfunctioning CFTR at the cell surface, creating a more effective opening of the CFTR chloride channel. An interim analysis of a phase IIa trial involving 20 patients with the class III G551D mutation (that accounts for approximately 2% of CF mutations) found that patients receiving VX-770 demonstrated improvements in both nasal potential difference and sweat chloride measurements, consistent with improvements in CFTR function.[47] For those patients taking the higher dose of VX-770 twice daily for 14 days, there was a 10.5% increase in $FEV_1$. A phase IIb study is due to begin in 2009.

VX-809, an agent currently at phase I study, assists the movement of defective CFTR to the epithelial cell membrane and improves its function as a chloride channel. It is aimed at the most prevalent CF mutation, the delta-F508 mutation (class II). A phase IIa study started in 2009.

## 6. Gene Therapy

The gene defect for CF was discovered in 1989 and since this time there has been a drive to explore the possibility of treatment by replacing the defective CFTR with wild-type CFTR. While clinical trials have achieved delivery of the gene, significant and long-lasting effects on CFTR function have as yet not been achieved.[48,49] The UK Gene Therapy Consortium (http://www.cfgenetherapy.org.uk) was established in 2001 and has developed an impressive research programme. The two options currently being explored as a suitable vector to deliver a functional and long-lasting gene are cationic liposomes, which form complexes with DNA and enter the cell, and lentiviruses, which are retroviruses with the ability to integrate into chromosomal DNA and potentially provide stable and long-lasting expression. The UK Gene Therapy Consortium

is aiming to begin large clinical trials in the near future.[50]

## 7. Exocrine Pancreatic Insufficiency

One of the characteristics of CF is exocrine pancreatic insufficiency, affecting approximately 85–90% of patients. The current treatment for exocrine pancreatic insufficiency is porcine-derived exocrine pancreatic enzyme preparations. For many patients, this involves taking a considerable number of capsules with each meal and even then normal absorption is not always achieved. Phase II studies have been completed for one porcine-free recombinant pancreatic replacement enzyme, Merispase®,[51] and another, Trizytek™, is presently being evaluated in a phase III study.[52]

## 8. Treatments for Other Complications of Cystic Fibrosis

With increased life expectancy, other complications are being more frequently encountered, such as low bone mineral density and CF-related diabetes mellitus. Clinical trials are required to identify the optimal treatment strategies for these conditions.

## 9. Challenges

The number of promising drugs for the treatment of CF is increasing, and at the preclinical stage of testing, the use of high-throughput screening is likely to ensure that further agents with therapeutic promise will continue to emerge. This in itself presents challenges in assessing the efficacy of new therapies. The number of suitable participants for enrollment in trials of therapies in this orphan disease is limited. In assessing lung disease, the traditional outcome primary outcome measure has been decline in $FEV_1$ and this has been shown to be predictive of survival.[53] However, the decline in $FEV_1$ has been considerably slowed by improvements with current clinical care[2] and increasing numbers of trial participants are required to demonstrate statistically significant differences in $FEV_1$ change for phase III clinical studies. New surrogate markers of lung disease, such as the lung clearance index[54,55] and high resolution CT scan changes,[56] are being explored as potential suitable endpoints for clinical trials. Measurements of biological and physiological markers such as changes in nasal potential differences, sweat sodium levels, and cellular CFTR expression with agents that modify CFTR function or ion channels, may be included as outcome measures in studies. There remains a need to ensure that these measurements are sufficiently standardized and to establish how they correlate with clinical outcome. International clinical trial networks are being formed to assist and coordinate the evaluation of new treatments for CF.[57]

This review has focused on *P. aeruginosa* as the predominant CF pathogen. However, the pathogen that strikes most fear in families and individuals is the *Burkholderia cepacia* complex. This bacterial opportunist, which has emerged in the last few decades, is inherently multiresistant and, in some patients, causes cepacia syndrome, a fulminating lethal pneumonia. Another recent report, highlights yet another challenge posed by the *B. cepacia* complex, namely the production of large amounts of bacterial exopolysaccharide and a change to a mucoid colonial form, when the bacteria are exposed to sugar alcohols; these include the osmolyte mannitol[58] mentioned in section 4.1. This report has caused concern[59] and suggests that the present policy of excluding individuals infected with *B. cepacia* complex from clinical trials of mannitol therapy should continue until further information is available.

The affordability of those therapies that gain a treatment license will be a continual challenge for CF centres. There is often little information about the relative efficacies of established and emerging treatments, and for individual patients with a high treatment burden, which therapies should be prioritized. However, these will be welcome challenges in an exciting new era of care.

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