Modifying disease in cystic fibrosis: current and future therapies on the horizon

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Purpose of review
Recent therapies directed at proximal targets within cystic fibrosis (CF) pathophysiology hold potential to modulate disease. This review highlights recent clinical trials and future therapies focused on these early steps of disease.

Recent findings
Recent approval of a CF transmembrane conductance regulator (CFTR) protein modulator, ivacaftor (Kalydeco), has ignited a wave of investigations for other modulators directed at CFTR mutation classes. Gene replacement therapy continues to be pursued at a slower pace in early phase clinical trials. Airway surface liquid strategies such as dry-powder mannitol and alternate ion channel regulation are discussed as genotype-independent methods of early modulation.

Summary
The breadth of therapies for early targets of CF holds considerable hope to modify the natural history of this disease. Ongoing focus to develop novel markers of early disease state is paramount. The progress of drug development requires concurrent attention on a spectrum of targets to achieve maximal impact.

Keywords
airway surface liquid, cystic fibrosis, cystic fibrosis transmembrane conductance regulator protein modulator, gene therapy

INTRODUCTION
An estimated 70,000 people worldwide have cystic fibrosis (CF), with over 800 people diagnosed per year in the United States [1,2]. CF survival has made dramatic improvement in the last 40 years, but mortality continues to be premature with the expected median survival of 37 years. Most of the mortality is attributed to progression of lung disease and respiratory failure.

The basic genetic defect in CF is altered function and/or lack of expression of the CF transmembrane conductance regulator (CFTR) protein [3]. Dysfunction of this cAMP-activated ion channel protein leads to abnormal transport of salt and water in epithelial cells of various tissues. In the lung, these changes likely incite alterations in composition and quality of the periciliary airway surface liquid (ASL) layer. Depletion of ASL impairs airway defenses, ultimately leading to a perpetuating cycle of mucus obstruction, infection, and inflammation (Fig. 1) [5].

The mainstays of therapies have focused on minimizing this cycle of injury by fighting airway infection and mucus accumulation. These therapies are treating pathophysiologic consequences of CFTR dysfunction and may be too late to modulate the disease process. Therapies aimed at early steps are promising to halt disease progression. This review will describe the status of recent and future therapies directed at these early targets in CF.

GENE AND STEM CELL THERAPY
Immediate excitement was generated with the discovery of the CF gene in 1989 [6], particularly with hope toward gene therapy to replace a normal copy of the CFTR gene into airway cells. Multiple frustrations have impeded the success of gene replacement therapy, including failure to identify the optimal delivery method or target cell(s) within
the airway and an efficient vector that is sustainable within the immunogenic lung environment [7]. Recently, the UK CF Gene Therapy Consortium has launched a phase 2B clinical trial for repeated application of a lipid-based vector complexed with a CFTR-expressing plasmid [8]. This trial will assess clinical benefit of nebulized CFTR gene therapy in multiple doses over 1 year, with anticipated results in June 2014 (www.cfgenetherapy.org.uk). Prior clinical studies of adenoviral and adeno-associated viral vectors have been previously reviewed with overall discouraging results [9,10]. Recently, preclinical studies have explored lentivirus as a more efficient vector in the murine model [11] and adenovirus in the porcine model [12]. Cell-based therapies such as human amnionic mesenchymal stem cells show potential in vitro, with production of CFTR mRNA in co-culture with airway epithelium [13]. Stem cell replacement therapy may represent a future therapeutic alternative, but remains in its infant stages. The challenges facing gene and cell-based therapy are daunting, but such therapy would potentially be beneficial to all CF patients regardless of genotype status.

**MUTATION CLASSIFICATION**

Genotype status has moved to the forefront in consideration of therapies modifying the CFTR protein. Over 1900 mutations within the CFTR gene are identified, but only a small percentage account for the majority of disease phenotypes [14]. The majority of these mutations are classified within a framework of functional categories [15] developed to describe CFTR ion channel dysfunction and recently reviewed in this journal [16]. Class I mutations result in an absence of functional CFTR protein from a truncated and unstable mRNA. Mostly, these mutations are premature termination codons or nonsense mutations and are more predominant in the Ashkenazi Jew population [14]. Class I mutations also include frame-shift mutations, or mRNA splicing defects [17]. Class II mutations such as F508del also have reduced quantity of CFTR channel expression, but from aberrant processing and maturation. F508del is the most common mutation homozygous in nearly 50% of patients [1]. Faulty channel functioning occurs in class III and IV mutations. Class III mutations lead to reduction in the gating mechanism or channel opening of the CFTR protein. The most common allele in this group is G551D, accounting for 4% of the CF population [18]. Class IV mutations allow limited chloride and bicarbonate ion transport and are referred to as conductance. The production and function of CFTR can be normal in class V and VI mutations, but class V mutations result in reduced quantity of channel produced from abnormal transcriptional regulation and class VI mutations result in a high turnover at the apical surface [19,20].

**CF TRANSMEMBRANE CONDUCTANCE REGULATOR PROTEIN MODULATION THERAPY**

The most promising and provoking therapy to date has been with the introduction of small-molecule candidate drugs aimed to improve function of
the abnormal CFTR protein. These therapies are directed at gene class specific mutations. These small-molecule therapies, known as CFTR modulators, are functionally categorized as potentiators, correctors, and read-through agents.

**CF transmembrane conductance regulator potentiators**

Potentiators work to improve gating and conductance defects, as in class III and IV mutations, by augmenting the open configuration of the CFTR ion channel. In 2012, ivacaftor (marketed as Kalydeco) became the first approved therapy in both the US and the European Union as a CFTR channel potentiator. Ivacaftor therapy is specific to CF patients with the gating mutation, G551D. Rapid approval was obtained following the landmark global phase 3 trial of oral ivacaftor for 48 weeks in patients 12 years and older with at least one copy of the G551D mutation [21]. Of 161 patients, 83 patients in the treatment arm demonstrated a 10% increase in percentage of predicted forced expiratory volume in one second (FEV₁) with a treatment effect noted by day 15 of therapy. In addition to this primary outcome, there was a decrease in pulmonary exacerbations and hospitalizations, improved reported respiratory symptom scores, weight gain, and a reduction in sweat chloride. Figure 2 summarizes sustained increase in FEV₁ and baseline change in sweat chloride. A second study of similar design but involving younger patients, age 6–11 years, also demonstrated improved lung function, weight gain, and a reduction in sweat chloride in the treatment arm.

![Figure 2](image-url)
Cystic fibrosis arm [22*]. In the preliminary results of the open-label extension trial of ivacaftor, FEV<sub>1</sub> treatment effect was sustained over 60 weeks with a treatment difference of 9.4% [23].

The impact of these trials is significant for 4% of the CF population, those with the G551D mutation. In a phase 2 study with homozygous F508del patients, ivacaftor resulted in no difference in sweat chloride or FEV<sub>1</sub> [24]. Other class III gating mutations may benefit from ivacaftor monotherapy based on in-vitro studies [25]. Ivacaftor has also shown some benefit in potentiating class IV conductance in mutations such as R117H [26]. Ongoing phase 2 and 3 trials are testing ivacaftor monotherapy in other class III mutations and class IV and V mutations, however this only accounts for a small percentage of the CF population [27–29].

**CF transmembrane conductance regulator correctors**

A therapeutic target of great interest is a class of small molecule compounds, termed correctors, that can impact the most common mutant, F508del-CFTR. As with class II mutations, F508del-CFTR results in an unstable protein leading to rapid degradation and minimal functioning channel present at the cell surface. Corrector compounds aim to increase the quantity of functional CFTR protein at the apical surface.

VX-809 (lumacaftor) is a CFTR corrector shown to be effective *in vitro* for improved chloride channel function and quantity of CFTR protein [30**]. VX-809 has been tested in a phase 2a trial for homozygous F508del-CFTR patients. For 28 days, 89 randomized patients demonstrated similar adverse events in both treatment and placebo groups and some dose-dependent effect was found in reduction of sweat chloride levels as a marker of CFTR activity [31*]. However, the sweat chloride treatment effect of VX-809 at the maximum dose of 200 mg (−8.21 mmol/l) in F508del homozygous patients was much less than that seen with ivacaftor in the patients with at least one copy of the G551D mutation (−47.9 mmol/l) [21].

Although corrector therapy may propagate production of CFTR, the functionality of the channel improves with potentiation and studies of combination testing with ivacaftor (Kalydeco) are in progress. A second VX-809 phase 2 study recently evaluated the safety and efficacy of 28 days of VX-809 600 mg once daily monotherapy followed by an additional 28 days of combination therapy with 600 mg daily VX-809 and 250 mg twice daily ivacaftor in homozygous F508del adults [32,33]. After 28 days of VX-809 monotherapy, FEV<sub>1</sub> declined −2% relative to placebo groups, but following 28 subsequent days of VX-809 and ivacaftor combination therapy, the relative change in FEV<sub>1</sub> improved 8.6% compared with placebo. In a subgroup of heterozygous F508del patients, a smaller, but significant FEV<sub>1</sub> improvement was noted for combination therapy compared with homozygous patients. Based on these Phase II findings, two international Phase III trials of combination therapy started enrollment in May 2013 in homozygous F508del patients [34,35].

Future correctors being studied include VX-661 and N6022. VX-661 *in vitro* may also be a corrector of protein misfolding and or trafficking [36]. A phase 2 dose escalation study of VX-661 monotherapy (doses 10, 30, 100, 150 mg) and in combination with ivacaftor (150 mg twice daily) has been completed in 128 homozygous F508del adult patients [37]. Initial results from the phase 2 VX-661 trial demonstrated a 9% improvement in FEV<sub>1</sub> with the 100 mg dose and a 7.5% improvement with the 150 mg dose compared with placebo [38]. N6022 targets an alternate corrector pathway thought to stimulate maturation of the CFTR protein by regulation of cell metabolism. Although exact mechanisms are unknown, N6022 preserves levels of the signaling molecules, S-nitrosothiols. In airway cell culture models, S-nitrosothiols increase CFTR function and potentially affect airway inflammation [39,40]. This intravenous available agent is currently undergoing phase 1 trials for homozygous F508del patients [41].

**Read-through agents**

In patients with a nonsense mutation of CFTR, small-molecule therapeutics are being developed that prevent aberrant truncation of protein by ‘read-through’ of a premature termination codon. The first therapeutic agent evaluated in CF patients was the aminoglycoside gentamicin, which showed promising early results [42]. Intranasal gentamicin did not improve CFTR function by nasal potential difference or increase localization of CFTR by nasal cell immunocytochemistry, suggesting the need to focus on alternative therapeutic agents [43]. A novel small molecule, PTC124 (ataluren), has also been in development. Initial studies in the CF mouse model [44] as well as both adults [45] and children [46] with class I mutations demonstrated suppression of the nonsense alleles and activation of CFTR expression and chloride channel function. Recently, a 48-week randomized placebo-controlled phase 3 trial of PTC124 in 238 patients older than 6 years with at least one copy of a nonsense mutation in CFTR was completed. Unfortunately, neither the primary endpoint, change in percentage of predicted FEV<sub>1</sub>, nor
the secondary endpoint, rate of pulmonary exacerbation, demonstrated a statistically significant difference between the PTC124 and placebo treatment groups. A small subset of patients not treated with inhaled antibiotics, however, did demonstrate some improvement in FEV\textsubscript{1} \cite{47}. The strong foundation of laboratory and early clinical research using the read-through approach has paved the way for other new compounds to emerge in the near future \cite{1*}.

**AIRWAY SURFACE LIQUID MODIFICATION**

Genotype-specific therapies will likely not encompass 100% of the CF population, and therefore consideration must be given to other therapies, which impact the primary physiologic consequences of CFTR dysfunction. One of the most important approaches is normalizing airway hydration through osmotic improvement of mucus or by restoration of the airway surface liquid (ASL) layer through alternate ion channel regulation.

**Osmotic mucus improvement**

Inhaled hypertonic saline is frequently used in therapeutic practice as an airway hydrator for patients older than 6 years \cite{48*}. Recently, a phase 3 trial evaluated 7% hypertonic saline in 321 patients younger than 6 years over a 48-week period \cite{49**}. Although the main outcome of pulmonary exacerbation rate was not significantly different in both groups, subgroup analysis suggested a treatment group improvement of FEV\textsubscript{0.5}, emphasizing the critical importance of trial design for sensitive biomarkers of early disease.

Inhaled dry-powder mannitol (Bronchitol) has demonstrated a relative improvement in FEV\textsubscript{1} of 4% in the European trial \cite{50} and 3.8% in the American trial \cite{51*}. Pooled analysis of both studies suggests a sustained benefit in FEV\textsubscript{1} for patients older than 12 years \cite{52}. Inhaled mannitol is approved and available in Australia and the European Union.

**Alternate ion channel therapy to restore airway surface liquid**

Denufosol tetrasodium is nebulized therapy that increases chloride secretion through a calcium-activated chloride channel, suggesting a therapeutic role to increase airway surface liquid independent of CFTR. Unfortunately, the results of phase 3 trials were disappointing. Compared with placebo, 24 weeks of nebulized denufosol demonstrated a modest improvement in FEV\textsubscript{1} from baseline \cite{53}, and over a 48-week time period denufosol had no improvement in FEV\textsubscript{1} or reduction in the incidence of pulmonary exacerbations \cite{54}.

Another strategy to increase airway surface liquid is to downregulate the hyperactive epithelial sodium channel (ENaC). Wild-type CFTR inhibits ENaC activity by uncertain mechanisms, implying mutant CFTR promotes hyperactive sodium absorption and further reduction of airway surface liquid \cite{55*}. Some compounds such as SSZ-02 and GS-9411 have reached early phase trials. Short-interfering RNA (siRNA) targeting ENaC subunits in vitro have been shown to improve airway surface liquid depth and may be a possible future target to modulate airway surface liquid through alternate ion channels \cite{56}.

**BIOMARKERS AND CLINICAL ENDPOINTS**

Trials of early innovative therapies will require more sensitive markers of disease. Important information from the Australian Respiratory Early Surveillance Team for CF (AREST CF) describes early evidence of CF lung disease in young children diagnosed by newborn screening \cite{57,58,59,60**}. The US National Heart, Lung, and Blood Institute prioritized research to develop biomarkers of early lung disease to reflect CF pathophysiology, clinical outcome, and response to therapy \cite{61*}.

Ideal markers are minimally invasive, cost-effective, and obtainable within young patients. Early markers of airway obstruction such as hyperinflation suggest CT or MRI to monitor disease state \cite{57,62}. Lung clearance index from multiple breath washout techniques also holds promise of early disease detection \cite{63*,64*,65}. Biomarkers from bronchoalveolar lavage, exhaled breath condensate, and induced sputum are being explored to assess microbiologic milieu and inflammatory status \cite{63*,66–69}.

Specific to CFTR modulators, chloride secretion measurements are used as a benchmark of CFTR function. Nasal potential difference, sweat chloride testing, and intestinal conductance measurements by rectal biopsy have been reviewed previously \cite{70,71}. However, only future trials will determine if chloride and conductance values translate to meaningful clinical outcomes.

**CONCLUSION**

There is a flurry of activity in clinical trials with therapies aimed at proximal events in CF pathophysiology. Ultimately, it remains to be seen if any of these early modulators will show sustained benefit and derail disease progression. The hope,
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however, remains palpable with the possibility to trivialize CF mortality and revolutionize this disease as we know it. Drug development is a slow and unpredictable science and requires continued commitment in a multifaceted approach that historically has shown tremendous success. As we forge ahead, we must continue every level of attack to alter the course of this disease.

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

2. Excellent review of the breadth of current therapies in CF.
14. Forc B, Cutting GR. Assessing the disease-liability of mutations in CFTR.
32. Investigation of VX-809 on F508del-CFTR processing by human bronchial epithelial cell culture. This study extends evidence of VX-809 as a CFTR corrector for F508del patients.
34. The findings of this clinical trial demonstrate VX-809 monotherapy improves F508del-CFTR bioactivity by sweat test.
38. Ferec C, Cutting GR. Assessing the disease-liability of mutations in CFTR.


