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

Thida Ong and Bonnie W. Ramsey

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

Department of Pediatrics, Seattle Children's Hospital and the University of Washington School of Medicine, Seattle, Washington, USA

Correspondence to Thida Ong, MD, Seattle Children's Hospital, Mail-stop OC.7720, Seattle, WA 98105, USA. Tel: +1 206 987 2174; e-mail: thida.ong@seattlechildrens.org

Curr Opin Pulm Med 2013, 19:645–651
DOI:10.1097/MCP.0b013e328365ab5f

KEY POINTS

- Multiple trials are testing therapies focused on proximal targets in CF pathophysiology in areas of gene therapy, CFTR protein modulation, and airway surface liquid modification.
- CFTR protein modulation holds promise as a major future therapy, however concomitant work addressing multiple therapeutic targets must continue.
- Sensitive early markers of disease are critical to clinical trial design of these innovative therapies.

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.

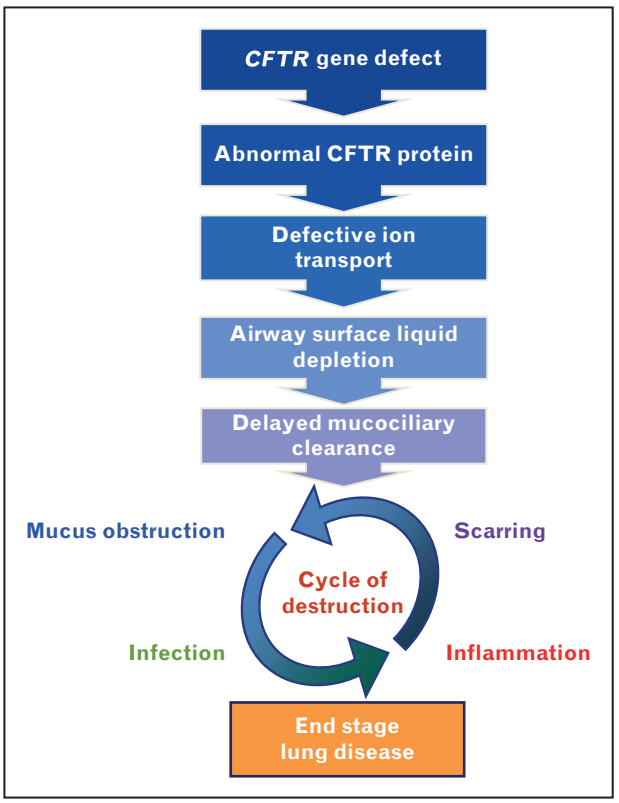

FIGURE 1. Pathophysiology of cystic fibrosis. Adapted with permission from [4].

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 (FEV_1) 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 FEV_1 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

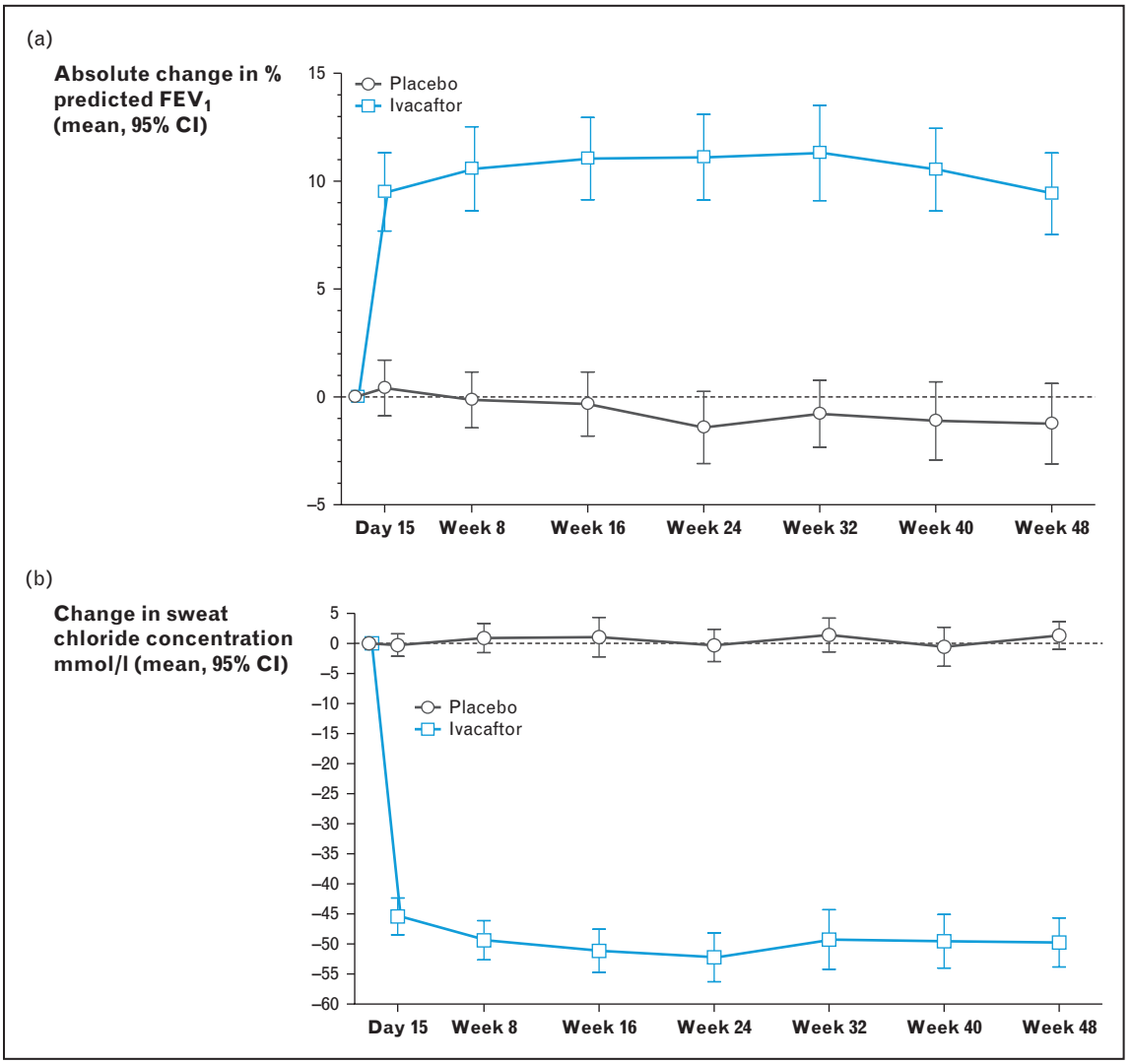

FIGURE 2. Results from phase 3 trial of ivacaftor versus placebo through 48 weeks. (a) Absolute mean change from baseline in percentage of predicted forced expiratory volume in 1 s (FEV_1). (b) Mean change from baseline in the concentration of sweat chloride (mmol/l). Adapted with permission from [21].

arm [22▪]. In the preliminary results of the open-label extension trial of ivacaftor, FEV_1 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_1 [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_1 declined −2% relative to placebo groups, but following 28 subsequent days of VX-809 and ivacaftor combination therapy, the relative change in FEV_1 improved 8.6% compared with placebo. In a subgroup of heterozygous F508del patients, a smaller, but significant FEV_1 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_1 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_1, 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_1 [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 [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 [48▪]. Recently, a phase 3 trial evaluated 7% hypertonic saline in 321 patients younger than 6 years over a 48-week period [49▪▪]. Although the main outcome of pulmonary exacerbation rate was not significantly different in both groups, subgroup analysis suggested a treatment group improvement of $FEV_{0.5,}$ 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_1 of 4% in the European trial [50] and 3.8% in the American trial [51▪]. Pooled analysis of both studies suggests a sustained benefit in FEV_1 for patients older than 12 years [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_1 from baseline [53], and over a 48-week time period denufosol had no improvement in FEV_1 or reduction in the incidence of pulmonary exacerbations [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 [55▪]. Some compounds such as 552-02 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 [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 [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 [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 [57▪,62]. Lung clearance index from multiple breath washout techniques also holds promise of early disease detection [63▪,64▪,65]. Biomarkers from bronchoalveolar lavage, exhaled breath condensate, and induced sputum are being explored to assess microbiologic milieu and inflammatory status [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 [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,

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.

Acknowledgements

T.O. is funded in part by the National Institutes of Health. B.W.R. receives grant funding from the Cystic Fibrosis Foundation and the National Institutes of Health.

Conflicts of interest

B.W.R., in her capacity as director of the Cystic Fibrosis Foundation Therapeutics Development Network Coordinating Center, has received grants from the following companies in the past 3 years: AlgiPharma AS; Amgen, Inc; Aradigm Corporation; Axcan Pharma, Inc; Bayer Healthcare AG; Chiesi Pharmaceuticals Inc; CSL Behring LLC; Gilead Sciences, Inc; GlaxoSmithKine, Inc; Inspire Pharmaceuticals, Inc; KaloBios, Inc; MerLion Pharmaceuticals GmbH; Mpex Pharmaceuticals, Inc; MPM Asset Management LLC; N30 Pharmaceuticals LLC; Novartis Pharmaceuticals Corp; Pharmaxis Ltd; PTC Therapeutics, Inc; Solvay Pharmaceuticals, Inc; Insmed, Inc; Vectura Ltd; and Vertex Pharmaceuticals Incorporated.

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

1. Rowe SM, Borowitz DS, Burns JL, *et al.* Progress in cystic fibrosis and the CF ■ Therapeutics Development Network. Thorax 2012; 67:882–890.

Excellent review of the breadth of current therapies in CF.

2. Cystic Fibrosis Foundation. National Patient Registry Annual Data report. Bethesda, Maryland, USA; 2011.
3. Welsh MJ, Ramsey BW, Accurso F, Cutting GR. Cystiv fibrosis. In: Scriver CR, Beaudet AL, Valle D, Sly WS, editors. The metabolic and molecular bases of inherited diseases, 8th ed New York: McGraw-Hill; 2001. pp. 5121–5188.
4. North American Cystic Fibrosis Conference. Reversing the basic defect: a vision for the future. 2012. http://www.nacfconference.org/plen.archive.html. [Accessed on 5 April 2013]
5. Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. N Engl J Med 2005; 352:1992–2001.
6. Kerem B, Rommens JM, Buchanan JA, *et al.* Identification of the cystic fibrosis gene: genetic analysis. Science 1989; 245:1073–1080.
7. Prickett M, Jain M. Gene therapy in cystic fibrosis. Transl Res 2013; ■ 161:255–264.

Summarizes current status of gene therapy trials and limitations in progress.

8. Alton EW, Boyd AC, Cheng SH, *et al.* A randomised, double-blind, placebo-controlled phase IIB clinical trial of repeated application of gene therapy in patients with cystic fibrosis. Thorax. doi:10.1136/thoraxjnl-2013-203309. [Epub ahead of print]
9. Griesenbach U, Alton EW. Progress in gene and cell therapy for cystic fibrosis lung disease. Curr Pharm Des 2012; 18:642–662.
10. Griesenbach U, Alton EW. Expert opinion in biological therapy: update on developments in lung gene transfer. Expert Opin Biol Ther 2013; 13:345–360.
11. Griesenbach U, Inoue M, Meng C, *et al.* Assessment of F/HN-pseudotyped lentivirus as a clinically relevant vector for lung gene therapy. Am J Respir Crit Care Med 2012; 186:846–856.
12. Potash AE, Wallen TJ, Karp PH, *et al.* Adenoviral gene transfer corrects the ion transport defect in the sinus epithelia of a porcine CF model. Mol Ther 2013; 21:947–953.
13. Paracchini V, Carbone A, Colombo F, *et al.* Amniotic mesenchymal stem cells: a new source for hepatocyte-like cells and induction of CFTR expression by coculture with cystic fibrosis airway epithelial cells. J Biomed Biotechnol 2012; 2012:575471.
14. Ferec C, Cutting GR. Assessing the disease-liability of mutations in CFTR. ■ Cold Spring Harb Perspect Med 2012; 2:a009480.

Review of CFTR mutations in association with phenotypes of CF and CFTR-related disorders.

15. Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell 1993; 73:1251–1254.
16. Sloane PA, Rowe SM. Cystic fibrosis transmembrane conductance regulator protein repair as a therapeutic strategy in cystic fibrosis. Curr Opin Pulm Med 2010; 16:591–597.
17. Rowntree RK, Harris A. The phenotypic consequences of CFTR mutations. Ann Hum Genet 2003; 67:471–485.
18. Johns Hopkins University (CFTR2 Team): Clinical and Functional Translation of CFTR (CFTR2). www.cftr2.org. [Accessed on 4 August 2013]
19. Zielenski J, Tsui LC. Cystic fibrosis: genotypic and phenotypic variations. Annu Rev Genet 1995; 29:777–807.
20. Haardt M, Benharouga M, Lechardeur D, *et al.* C-terminal truncations destabilize the cystic fibrosis transmembrane conductance regulator without impairing its biogenesis. A novel class of mutation. J Biol Chem 1999; 274: 21873–21877.
21. Ramsey BW, Davies J, McElvaney NG, *et al.* A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N Engl J Med 2011; 365:1663–1672.
22. Davies JC, Wainwright CE, Canny GJ, *et al.* Efficacy and safety of ivacaftor in ■ patients aged 6 to 11 years with cystic fibrosis with a G551D mutation. Am J Respir Crit Care Med 2013; 187:1219–1225.

Phase 3 trial of ivacaftor in children with G551D CF with sustained benefit in lung function.

23. Mckone E, Li H, Gilmartin G, Davies JC. Long-term safety and efficacy of ivacaftor in persons with cystic fibrosis who have the G551D-CFTR mutation. Pediatr Pulmonol Suppl 2012; S35:296.
24. Flume PA, Liou TG, Borowitz DS, *et al.* Ivacaftor in subjects with cystic fibrosis who are homozygous for the F508del-CFTR mutation. Chest 2012; 142: 718–724.
25. Yu H, Burton B, Huang CJ, *et al.* Ivacaftor potentiation of multiple CFTR channels with gating mutations. J Cyst Fibros 2012; 11:237–245.
26. Van Goor F, Yu H, Burton B, *et al.* Ivacaftor potentiates mutant CFTR forms associated with residual CFTR function. J Cyst Fibros 2012; 11:S31.
27. Pilot Study Testing the Effect of Ivacaftor on Lung Function in Subjects With Cystic Fibrosis and Residual CFTR Function. 2012. http://www.clinicaltrials.gov/ct2/show/nct01685801. [Accessed on 24 April 2013]
28. Study of Ivacaftor in Subjects With Cystic Fibrosis Who Have a Non-G551D CFTR Gating Mutation (KONNECTION). 2013. www.clinicaltrials.gov/ct2/show/nct01614470. [Accessed on 2 May 2013]
29. Study of Ivacaftor in Subjects With Cystic Fibrosis Who Have the R117H-CFTR Mutation (KONDUCT). 2013. http://www.clinicaltrials.gov/ct2/show/nct01614457. [Accessed on 2 May 2013]
30. Van Goor F, Hadida S, Grootenhuis PD, *et al.* Correction of the F508del- ■■ CFTR protein processing defect in vitro by the investigational drug VX-809. Proc Natl Acad Sci U S A 2011; 108:18843–18848.

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.

31. Clancy JP, Rowe SM, Accurso FJ, *et al.* Results of a phase IIa study of VX-809, ■ an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation. Thorax 2012; 67:12–18.

The findings of this clinical trial demonstrate VX-809 monotherapy improves F508del-CFTR bioactivity by sweat test.

32. Vertex Pharmaceuticals Inc. Final data from phase 2 combination study of VX-809 and KALYDECO (ivacaftor) showed statistically significant improvement inlung function in people with cystic fibrosis who have two copies of the F508del mutation. http://investors.vrtx.com/releasedetail.cfm?ReleaseID=687394. [Accessed on 5 April 2013]
33. Boyle MP, Bell S, Konstan M, *et al.* The investigational CFTR corrector, VX-809 (lumacaftor) co-administered with the oral potentiator ivacaftor improved CFTR and lung function in F508DEL homozygous patients: phase II study results. Pediatr Pulmonol Suppl 2012; S35:315.
34. A Study of Lumacaftor in Combination With Ivacaftor in Cystic Fibrosis Subjects Aged 12 Years and Older Who Are Homozygous for the F508del-CFTR Mutation (TRANSPORT). 2013. http://clinicaltrials.gov/ct2/show/NCT01807949. [Accessed on 24 April 2013]
35. A Study of Lumacaftor in Combination With Ivacaftor in Cystic Fibrosis Subjects Aged 12 Years and Older Who Are Homozygous for the F508del-CFTR Mutation (TRAFFIC). 2013. http://clinicaltrials.gov/ct2/show/NCT01807923. [Accessed on 24 April 2013]

36. Van Goor F, Straley KS, Cao D, *et al.* Rescue of DeltaF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. Am J Physiol Lung Cell Mol Physiol 2006; 290:L1117–1130.

37. Study of VX-661 Alone and in Combination With VX-770 in Subjects Homozygous to the F508del-CFTR Mutation. 2013. http://clinicaltrials.gov/ct2/show/NCT01531673. [Accessed on 2 May 2013]

38. Treatment with VX-661 and Ivacaftor in a Phase 2 Study Resulted in Statistically Significant Improvements in Lung Function in People with Cystic Fibrosis Who Have Two Copies of the F508del Mutation. 2013. http://investors.vrtx.com/releasedetail.cfm?ReleaseID=757597. [Accessed on 29 May 2013]

39. Zaman K, Fraser-Butler M, Bennett D. Novel s-nitrosothiols have potential therapeutic uses for cystic fibrosis. Curr Pharm Des 2013; 19:3509–3520.

40. Green LS, Chun LE, Patton AK, *et al.* Mechanism of inhibition for N6022, a first-in-class drug targeting S-nitrosoglutathione reductase. Biochemistry 2012; 51:2157–2168.

41. Safety and Pharmacokinetic Study of N6022 in Subjects With Cystic Fibrosis Homozygous for the F508del-CFTR Mutation. April 22, 2013. http://clinicaltrials.gov/ct2/show/NCT01746784. [Accessed on 2 May 2013]

42. Wilschanski M, Yahav Y, Yaacov Y, *et al.* Gentamicin-induced correction of CFTR function in patients with cystic fibrosis and CFTR stop mutations. N Engl J Med 2003; 349:1433–1441.

43. Clancy JP, Rowe SM, Bebok Z, *et al.* No detectable improvements in cystic fibrosis transmembrane conductance regulator by nasal aminoglycosides in patients with cystic fibrosis with stop mutations. Am J Respir Cell Mol Biol 2007; 37:57–66.

44. Du M, Liu X, Welch EM, *et al.* PTC124 is an orally bioavailable compound that promotes suppression of the human CFTR-G542X nonsense allele in a CF mouse model. Proc Natl Acad Sci U S A 2008; 105:2064–2069.

45. Kerem E, Hirawat S, Armoni S, *et al.* Effectiveness of PTC124 treatment of cystic fibrosis caused by nonsense mutations: a prospective phase II trial. Lancet 2008; 372:719–727.

46. Sermet-Gaudelus I, Boeck KD, Casimir GJ, *et al.* Ataluren (PTC124) induces cystic fibrosis transmembrane conductance regulator protein expression and activity in children with nonsense mutation cystic fibrosis. Am J Respir Crit Care Med 2010; 182:1262–1272.

47. PTC Therapeutics I. http://ptct.client.shareholder.com/releasedetail.cfm?ReleaseID=681445. [Accessed on 5 April 2013]

48. ▪ Mogayzel PJ Jr, Naureckas ET, Robinson KA, *et al.* Cystic fibrosis pulmonary guidelines. Chronic medications for maintenance of lung health. Am J Respir Crit Care Med 2013; 187:680–689.

Summarizes current pulmonary therapy guidelines based on systematic literature review.

49. ▪▪ Rosenfeld M, Ratjen F, Brumback L, *et al.* Inhaled hypertonic saline in infants and children younger than 6 years with cystic fibrosis: the ISIS randomized controlled trial. JAMA 2012; 307:2269–2277.

Phase 3 trial assessing safety and efficacy of inhaled hypertonic saline in infants and children.

50. Bilton D, Robinson P, Cooper P, *et al.* Inhaled dry powder mannitol in cystic fibrosis: an efficacy and safety study. Eur Respir J 2011; 38:1071–1080.

51. ▪ Aitken ML, Bellon G, De Boeck K, *et al.* Long-term inhaled dry powder mannitol in cystic fibrosis: an international randomized study. Am J Respir Crit Care Med 2012; 185:645–652.

International phase 3 trial demonstrating improved lung function in the treatment arm for inhaled mannitol.

52. Bilton D, Bellon G, Charlton B, *et al.* Pooled analysis of two large randomised phase III inhaled mannitol studies in cystic fibrosis. J Cyst Fibros 2013; 12:367–376.

53. Accurso FJ, Moss RB, Wilmott RW, *et al.* Denufosol tetrasodium in patients with cystic fibrosis and normal to mildly impaired lung function. Am J Respir Crit Care Med 2011; 183:627–634.

54. Ratjen F, Durham T, Navratil T, *et al.* Long term effects of denufosol tetrasodium in patients with cystic fibrosis. J Cyst Fibros 2012; 11:539–549.

55. ▪ Qadri YJ, Rooj AK, Fuller CM. ENaCs and ASICs as therapeutic targets. Am J Physiol Cell Physiol 2012; 302:C943–C965.

Summarizes ENaC hyperactivity associated with CF as a basis for a therapeutic target.

56. Gianotti A, Melani R, Caci E, *et al.* ENaC silencing as a strategy to correct the airway surface fluid deficit in cystic fibrosis. Am J Respir Cell Mol Biol 2013; 49:445–452.

57. ▪ Mott LS, Park J, Gangell CL, *et al.* Distribution of early structural lung changes due to cystic fibrosis detected with chest computed tomography. J Pediatr 2013.

Evidence of heterogeneous bronchiectasis in young children with CF.

58. Sly PD, Brennan S, Gangell C, *et al.* Lung disease at diagnosis in infants with cystic fibrosis detected by newborn screening. Am J Respir Crit Care Med 2009; 180:146–152.

59. Pillarisetti N, Williamson E, Linnane B, *et al.* Infection, inflammation, and lung function decline in infants with cystic fibrosis. Am J Respir Crit Care Med 2011; 184:75–81.

60. ▪▪ Sly PD, Gangell CL, Chen L, *et al.* Risk factors for bronchiectasis in children with cystic fibrosis. N Engl J Med 2013; 368:1963–1970.

Summarizes longitudinal data for early risk factors of bronchiectasis in newborn screen diagnosed patients up to 3 years old.

61. ▪ Ramsey BW, Banks-Schlegel S, Accurso FJ, *et al.* Future directions in early cystic fibrosis lung disease research: an NHLBI workshop report. Am J Respir Crit Care Med 2012; 185:887–892.

Summary of NHLBI priorities in early CF lung disease.

62. Wielputz MO, Eichinger M, Puderbach M. Magnetic resonance imaging of cystic fibrosis lung disease. J Thorac Imaging 2013; 28:151–159.

63. ▪ Belessis Y, Dixon B, Hawkins G, *et al.* Early cystic fibrosis lung disease detected by bronchoalveolar lavage and lung clearance index. Am J Respir Crit Care Med 2012; 185:862–873.

Suggests lung clearance index as a useful marker of lung disease in young children.

64. ▪ Stocks J, Thia LP, Sonnappa S. Evaluation and use of childhood lung function tests in cystic fibrosis. Curr Opin Pulm Med 2012; 18:602–608.

Review of multiple spirometric measures to evaluate CF in children.

65. Jensen R, Stanojevic S, Gibney K, *et al.* Multiple breath nitrogen washout: a feasible alternative to mass spectrometry. PLoS One 2013; 8:e56868.

66. Hunter RC, Klepac-Ceraj V, Lorenzi MM, *et al.* Phenazine content in the cystic fibrosis respiratory tract negatively correlates with lung function and microbial complexity. Am J Respir Cell Mol Biol 2012; 47:738–745.

67. Patel K, Davis SD, Johnson R, Esther CR Jr. Exhaled breath condensate purines correlate with lung function in infants and preschoolers. Pediatr Pulmonol 2013; 48:182–187.

68. Griffin PE, Roddam LF, Belessis YC, *et al.* Expression of PPARgamma and paraoxonase 2 correlated with Pseudomonas aeruginosa infection in cystic fibrosis. PLoS One 2012; 7:e42241.

69. Sagel SD, Wagner BD, Anthony MM, *et al.* Sputum biomarkers of inflammation and lung function decline in children with cystic fibrosis. Am J Respir Crit Care Med 2012; 186:857–865.

70. Rowe SM, Accurso F, Clancy JP. Detection of cystic fibrosis transmembrane conductance regulator activity in early-phase clinical trials. Proc Am Thorac Soc 2007; 4:387–398.

71. Sousa M, Servidoni MF, Vinagre AM, *et al.* Measurements of CFTR-mediated Cl- secretion in human rectal biopsies constitute a robust biomarker for cystic fibrosis diagnosis and prognosis. PLoS One 2012; 7:e47708.