

## Emerging Therapies for Cystic Fibrosis Lung Disease

a report by

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### Cystic Fibrosis and Current Care

Cystic fibrosis (CF) is a common, life-shortening disease affecting tens of thousands of people in the US and worldwide (Cystic Fibrosis Foundation (CFF) registry statistics, 2005). It is an autosomal recessive disorder caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a membrane localized traffic adenosine tri-phosphate (ATP)-ase protein that functions as a chloride channel. CFTR is also a regulator of many ion transport pathways (including non-CFTR chloride channels, sodium, bicarbonate, ATP, glutathione, and, potentially, other small molecules), and this protein helps to establish the content of the airway surface liquid (ASL) and of submucosal glandular secretions.<sup>1-7</sup> Loss of CFTR activity in the airways initiates a chain of events including reduction in the ASL volume, dehydration and thickening of glandular mucus secretions, airway obstruction, chronic bacterial infection, and relentless inflammation, which together contribute to the development of bronchiectasis and eventual respiratory failure. Advances in supportive care have led to a steady increase in the average life expectancy of people with CF, with recent statistics demonstrating a median survival of over 37 years (CFF registry statistics, 2005). CF remains a devastating illness for many patients, with substantial numbers succumbing to the disease in childhood and early adulthood.

Pulmonary therapies are aimed at treating disease symptoms, including:

- regular chest clearance (through daily chest percussion—predominately by hand or vest);
- mucolytics (e.g. nebulized recombinant human deoxyribonuclease (rhDNase));
- antibiotics (including cycled use of tobramycin for nebulization (TOBI®) in patients with respiratory tract cultures positive for *Pseudomonas*

*aeruginosa*, extending to oral and/or intravenous antibiotics for acute pulmonary exacerbations); and

- macrolide therapy, bronchodilators, and anti-inflammatories (oral and inhaled).<sup>8-14</sup>

Cumulatively, these therapies have led to a dramatic increase in the survival of CF patients. They also can create substantial and often confusing treatment regimens for patients who require regular daily attention for clear clinical benefits. Compliance can be extremely challenging across all age groups of CF patients and their families for a variety of reasons, and the limited number of comparative trials (that might simplify treatment) leaves CF care-givers with little evidence to guide choices between therapies. The result is often an escalating care plan as new therapies arise or symptoms increase, with the potential to further complicate daily care. Accumulating evidence from patient outcomes in several European CF care centers, and more recently from studies performed in the US, are leading to a more preventive, pre-symptomatic approach to CF care.<sup>15-22</sup> This shift in care philosophy is well timed with the adoption of CF newborn screening in the US and abroad, and will be of significant benefit to CF patients and their families.<sup>23,24</sup> It also raises many questions regarding how to adapt therapies to the infant and toddler CF population, and how to demonstrate efficacy in these difficult to study patients.

### Understanding of Cystic Fibrosis Airway Biology— Application to New Pulmonary Strategies

The CFTR gene was identified in 1989, and since that historic achievement an immense amount of work has been performed to understand how this single genetic defect results in the complex clinical picture that characterizes CF.<sup>1-3</sup> While there are many questions that remain to be answered regarding the steps between genetic defect and airway disease, the breadth of knowledge gained regarding CF pathogenesis has helped researchers to identify numerous strategies directed toward central causes of CF. These approaches include: normalization of sodium, chloride, and/or mucociliary transport in the airways; direct gene transfer to the airway epithelia; and restoration of mutant CFTR function through genotype-specific, small-molecule therapies. Each of these therapeutic strategies has advanced to clinical trials in CF patients, and positive results have the potential to extend CF therapy from 'symptom-based' to treatment of the underlying causes of CF lung disease.

### Normalization of Sodium, Chloride, and/or Mucociliary Transport

Normally, the composition and volume of the ASL layer that bathes and maximizes ciliary function is delicately balanced between sodium



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absorption, which is believed to drive chloride and water transport from the luminal to the blood compartment, and chloride secretion, which helps to hydrate the luminal compartment.<sup>25-33</sup> Evidence also suggests that the source of this fluid is in large part in the submucosal glands, where CFTR expression is significantly higher than in surface epithelial cells.<sup>34-37</sup> In the CF airway, loss of CFTR function leads to loss of cyclic adenosine monophosphate (cAMP)-stimulated chloride conductance via CFTR and the outwardly rectified chloride channel (ORCC), which is positively linked to CFTR,<sup>38</sup> alteration of submucosal gland secretions, and enhanced sodium absorption from the epithelial surface. The increase in sodium transport is believed to drive dehydration of the ASL compartment, which perturbs normal mucociliary clearance and sets the stage for the development of mucus plaques that adhere to the underlying epithelium.<sup>32,39</sup> This mucus stasis has been postulated to make the CF airway susceptible to bacterial infection, and potentially begin the cascade of CF lung disease. This system is not without potential back-up ion transport pathways that are protective. Work by Boucher and colleagues supports a model in which ATP released from the airway epithelium in response to shear stress (such as the breathing cycle or coughing) can bind to P<sub>2Y2</sub> receptors that are normally found on the apical surface of airway epithelial cells.<sup>32,40-44</sup> Stimulation of these G-protein-coupled receptors raises cell calcium, which in turn activates calcium-activated chloride channels (CaCCs) and can partially restore mucociliary clearance. However, over time this system is believed to fail (possibly due to accumulated damage and/or negative effects of recurrent viral infections), leading to the typical sequelae of CF lung disease.<sup>32,45</sup>

In an attempt to discover new strategies to normalize mucociliary clearance, sodium and chloride transport has recently been studied in CF patients, or is in later-phase clinical trials. Normalization of sodium transport through use of blockers of the epithelial Na channel (ENaC) has been shown to reduce the excessive sodium transport seen in CF patients to within the range of non-CF subjects, and to enhance mucociliary clearance in pre-clinical studies.<sup>46-49</sup> A double-blind cross-over study of nebulized amiloride four times a day for 24 weeks compared with placebo demonstrated improvements in sputum rheology and loss of forced vital capacity (FVC) while on amiloride, but failed to demonstrate improvements in forced expiratory volume in the first second (FEV<sub>1</sub>).<sup>48,50</sup> Channel blockers designed with higher channel affinity and longer half-life on the airway surface are under development,<sup>51,52</sup> and have begun to enter clinical trials in small, dose-ranging studies (Clinical trials.gov NCT00274313).

Agents that maximize the activity of CaCCs through stimulation of P<sub>2Y2</sub> receptors and/or other mechanisms (with and without ENaC blockade) have been shown to activate chloride conductance in CF pre-clinical model systems and in CF patients,<sup>53-56</sup> and recent phase II results provided evidence of biologic effect with improved FEV<sub>1</sub> in denufosol-treated subjects compared with placebo.<sup>57</sup> A large randomized, double-blind, placebo-controlled trial of denufosol in CF patients with mild lung disease is under way. Finally, two recently completed and complementary trials have led to the use of nebulized hypertonic saline (HTS) in many CF patients.<sup>58,59</sup> Elkins and colleagues recently reported that subjects treated with twice-daily HTS for one year had improved lung function and reduced pulmonary exacerbations compared with saline-treated controls in a blinded, randomized trial. In the complementary study reported by Donaldson, eight CF patients treated with HTS four times a day for two

weeks had improved FEV<sub>1</sub> and mucociliary clearance compared with saline-treated controls. Interestingly, the study demonstrated a durable effect of HTS, as subjects treated for two weeks in the study retained increases in mucociliary clearance up to eight hours post-dosing compared with controls. However, amiloride eliminated the positive effect of HTS on both outcome measures, presumably by inhibiting the capacity of the hypertonic stimulus to draw fluid into the ASL compartment. Based

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on these and other promising results, additional hypertonic therapies (such as use of mannitol as an inhaled dry powder) have begun to be explored.<sup>60,61</sup> Hopefully, trials such as these will lead to improvements in treatment compliance, as only about 60% of subjects randomized to the HTS arm in the Australian investigation remained on the study drug for the entire test period.

#### Gene Transfer

Following discovery of the CFTR gene, work rapidly advanced to develop gene transfer strategies that could deliver normal CFTR complementary DNA (cDNA) to the airway epithelia of CF patients. Pre-clinical studies suggested that modest gene transfer efficiency (<10%) could be capable of restoring important features of CFTR-dependent ion transport, and led to high degrees of enthusiasm regarding the potential to rapidly realize a therapeutic effect.<sup>62</sup> Unfortunately, significant gene transfer to the human airway epithelium has proved difficult, with limitations in efficiency and host inflammation seen with both viral (such as replication-deficient adenovirus) and non-viral (lipid-based agents complexed with plasmid cDNA) vectors.<sup>63-72</sup> More recent studies have identified many of these barriers, and have led to new approaches that hold promise for this strategy. One is the development of adeno-associated viral vectors (AAVs) that can be adapted to CFTR-gene delivery.<sup>73,74</sup> This virus is non-pathogenic and stimulates a modest host response following airway exposure.

Historically, limitations of this vector have included low efficiency of gene transfer, packaging constraints, proteosomal degradation, and difficulties in attaining high titers to allow human airway dosing. Advances in AAV technology led to several proof-of-principle studies in the upper and lower airways of CF patients, and two randomized, placebo-controlled lower airway phase II studies of AAV (serotype 2) carrying the full CFTR cDNA have been completed.<sup>67</sup> While one study found statistically significant increases in FEV<sub>1</sub> and decreases in sputum interleukin-8 (IL-8) levels in treated patients compared with controls, the larger follow-up study failed to meet its primary efficacy end-point. Importantly, however, both studies demonstrated that repeated AAV-CFTR dosing was well tolerated by patients, which had been a significant limitation of previous (non-AAV) lower airway gene transfer strategies. Recent work has indicated that other AAV serotypes (e.g. AAV5) are potentially better vector choices, as they

have the capacity to enter airway cells by apical receptors (as opposed to AAV2 serotype receptors that are located on the basolateral membrane).<sup>75</sup> Thus, the favorable safety profile and a better understanding of basic AAV biology may lead to subsequent studies with the potential to demonstrate improved efficacy.

In the UK, researchers are optimizing non-viral vector strategies for lower airway gene transfer in preparation for large, multidose protocols.<sup>70,72</sup> Cationic lipids complexed with CFTR cDNA have been shown to successfully produce gene transfer to the lower airway epithelium of CF patients with modest transgene delivery.<sup>64,68</sup> Nebulized lower airway delivery has been associated with flu-like symptoms in research subjects, and the source of these symptoms is believed to be CpG sequences associated with bacterial production of CFTR cDNA.<sup>64,68,70</sup> Furthermore, limitations in the durability of transgene expression are being addressed by examining eukaryotic, epithelium-specific promoters. Together, these refinements have led to enhanced transgene efficiency and durability of expression in pre-clinical animal models, and human studies are anticipated. Additional non-viral gene transfer strategies, such as DNA nanoparticles, have shown promise in upper airway studies in small numbers of CF patients, and suggest that improvements in non-viral technologies may overcome many of the historic limitations of related gene transfer approaches.<sup>76</sup>

Ongoing questions related to gene transfer include whether extrachromosomal CFTR expression can substitute for expression of the endogenous gene, and the realization that current gene therapy strategies do not address absent CFTR expression in other organ systems that produce disease. Also important is the knowledge that disease in some organs is potentially reversible, such as the deeper airway glands, the hepatobiliary and gastrointestinal (GI) tract, the sweat glands, and potentially the pancreas in young patients.

## Restorative Cystic Fibrosis Transmembrane Conductance Regulator Therapies

An attractive strategy to treat CF is to restore mutant CFTR activity at the genetic or protein level through the use of small-molecule agents. The genetics of CF are well characterized, with over 1,400 separate CF mutations identified.<sup>4,77</sup> These can be broadly classified into six groups:

- class I (biosynthetic defects);
- class II (folding and maturation defects);
- class III (regulatory defects);
- class IV (conduction defects);
- class V (low expression of functional CFTR); and
- class VI (defects that alter recycling at the cell membrane).

The majority of CF patients carry at least one copy of the DF508 CFTR (class II mutation), while certain ethnic groups are enriched for other mutations (such as non-sense mutations—class I—in patients of Ashkenazi descent). ‘Genotype-specific’ strategies that use small molecules to overcome defects unique to specific classes of mutations have the potential to treat underlying

mechanisms of CF, and possibly to be extended to other genetic disorders that share common themes with CF. The first successful proof-of-principle study using this strategy was reported by Rubenstein and Zeitlin, in which a small group of CF patients homozygous for DF508 CFTR were treated with 4-phenyl butyrate (4-PBu), a molecule shown to overcome DF508 CFTR trafficking defects using *in vitro* models.<sup>78,79</sup> Patients treated with high-dose

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4-PBu demonstrated improvements in nasal ion transport (using the nasal potential difference (NPD) assay) compared with controls, and provided momentum to develop more selective and tolerable agents.

Premature stop mutations are a common minority of CF-causing mutations, occurring in 7–10% of the general CF population, and in much higher numbers of CF patients of Ashkenazi Jewish descent.<sup>3,80,81</sup> Studies piloted by Bedwell and Howard examined the capacity of aminoglycosides to restore activity to CFTR mutations caused by premature stop mutations using elegant *in vitro* systems.<sup>82,83</sup> Aminoglycoside antibiotics bind to the small eukaryotic ribosomal subunit (18S), and certain members of this class have the capacity to interrupt the normal proofreading function of the ribosomal complex.<sup>84,85</sup> The result is that this interaction can lead to insertion of a near cognate transfer ribonucleic acid (tRNA) at a premature stop codon, allowing the ribosomal machinery to continue translation and produce full-length CFTR protein. This work has been extended to animal models<sup>84,86</sup> and to four proof-of-principle studies in CF patients, with three demonstrating biologic activity and improvements in CFTR-specific ion transport in the airways of CF patients possessing premature stop mutations compared with controls.<sup>80,81,87,88</sup> The strongest effects have been demonstrated in Israeli patients compared with subjects studied in the US. The reasons for this discrepancy are unclear, but could be related to higher CFTR messenger ribonucleic acid (mRNA) substrate levels in responders, and theoretically enriched in Israeli populations,<sup>89</sup> or unique features of late-CFTR premature stop codons—e.g. W1282X CFTR, which has been the predominate mutation studied in the Israeli CF population—which may make them more susceptible to restorative strategies compared with early stop mutations in the CFTR that predominate in the US.<sup>90</sup> This strategy has also led to the development of PTC124, an orally available molecule identified by high throughput screening by PTC Therapeutics.<sup>91,92</sup> This agent has demonstrated activity in pre-clinical *in vitro* systems and in two animal models of premature stop codon-mediated disease (CF and muscular dystrophy (MD)). PTC124 is undergoing phase II studies in CF patients and MD patients with premature stop codon-mediated disease. The results of the CF studies have been particularly encouraging in the Israeli population, where many patients demonstrated improvements in CFTR-specific airway ion transport (assayed by the NPD) while on study drug.<sup>93</sup> Larger, placebo-

controlled studies are needed to confirm this activity and to demonstrate treatment effects using clinical efficacy outcome measures.

Measures that have been identified to correct DF508 CFTR trafficking (such as growth at low temperature, treatment with osmotic agents) are generally not feasible for *in vivo* examination, and although agents that enhance the activity of surface localized, mutant CFTR have been studied intensely *in vitro*, limitations of these agents have been barriers to direct extension to clinical trials. Intense HTS efforts to identify agents that overcome trafficking defects in DF508 CFTR ('correctors') and agents that increase the activity of mutant CFTR that is found at the cell membrane ('potentiators') have been fruitful, identifying several lead compounds that are currently being characterized in pre-clinical model systems.<sup>94-100</sup> Vertex Pharmaceuticals is optimizing and characterizing lead compounds in both of these classes, and one potentiator compound (VX-770) is projected to enter phase II studies in CF patients (summer 2007) who carry select mutations found at the cell membrane (such as G551D CFTR, a class III mutation). It appears that these two strategies may require combined use to demonstrate maximal benefits, as several studies indicate that DF508 CFTR that has been treated to localize to the cell membrane has significantly abnormal channel gating and reduced chloride conductance.<sup>101-103</sup> It is also unclear whether restoration of mutant CFTR activity can lead to improvements in clinical outcome measures, but indirect

evidence from recent genotype/phenotype correlation studies in subjects with varying levels of CFTR function indicate that increasing levels of CFTR activity correlate with biomarkers of CFTR activity (NPD findings and sweat chloride concentrations) and clinical manifestations such as pulmonary, pancreatic, and vas deferens function.<sup>104</sup>

## Conclusions

CF therapy currently stands on a threshold, moving from a symptom-based to a pre-symptomatic approach that targets underlying causes of CF lung disease. Strategies directed at normalizing ion transport and mucociliary clearance, optimizing gene transfer, and restoring function to mutant CFTR have advanced well into clinical studies in CF patients, and the potential of these approaches to ameliorate disease manifestations appears significant. Clinical researchers will be challenged to prioritize agents for investigation and to develop meaningful and clinically relevant biomarkers that accelerate the development of the most promising agents in these different classes. Optimism is high that some or all of these strategies will yield meaningful improvements in CF care and outcomes in the near future. ■

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