Cystic fibrosis: Insight into CFTR pathophysiology and pharmacotherapy

Bob Lubamba, Barbara Dhooghe, Sabrina Noel, Teresinha Leal

Louvain Centre for Toxicology and Applied Pharmacology, Institut de Recherche Expérimentale et Clinique, Centre des Sciences de la Santé, Université catholique de Louvain, Ave Hippocrate 10, B-1200 Brussels, Belgium

A B S T R A C T

Cystic fibrosis is the most common life-threatening recessively inherited disease in Caucasians. Due to early provision of care in specialized reference centers and more comprehensive care, survival has improved over time. Despite great advances in supportive care and in our understanding of its pathophysiology, there is still no cure for the disease. Therapeutic strategies aimed at rescuing the abnormal protein are either being sought after or under investigation. This review highlights salient insights into pathophysiology and candidate molecules suitable for CFTR pharmacotherapy. Clinical trials using Ataluren, VX-809 and ivacaftor have provided encouraging data. Preclinical data with inhibitors of phosphodiesterase type 5, such as sildenafil and analogs, have highlighted their potential for CFTR pharmacotherapy. Because sildenafil and analogs are in clinical use for other clinical applications, research on this class of drugs might speed up the development of new therapies for CF.

© 2012 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.
pancreatic insufficiency and intestinal malabsorption. While many organs are affected in CF, pulmonary disease is the major cause of morbidity and mortality.

Approximately 80,000 people in the world are diagnosed with CF. However, CF shows wide geographic and ethnic variations, with prevalence ranging from 1 in 1700 to 7700 live births in Europe (the highest incidence is found in the Republic of Ireland while in Finland CF is extremely rare). CF is found to be rare in persons of non-Caucasian origin. The most prevalent CF-causing mutation in the Caucasian population, accounting for 86% of CF alleles (www.cftr2.org), is the F508del mutation, a deletion of the amino acid phenylalanine at position 508 of the encoded protein. Yet, presently over 1900 different CFTR mutations have been identified (www.genet.sickkids.on.ca).

Due to early referral to specialized, multidisciplinary reference centers for CF [3] and more comprehensive care, survival has improved over time. While in 1938, 70% of CF babies died within the first year of life [4], the median life expectancy of patients in the US reached 37 years in 2008 [5]. However, despite great advances in supportive care and in our understanding of pathophysiology of the disease, there is still no cure for CF.

Historical overview

Eighteenth century German and Swiss literatures warned: “Wehe dem Kind, das beim Kuß auf die Stirn salzig schmeckt, es ist verhezt und muss bald sterben”, which can be translated as: “Woe to that child which when kissed on the forehead tastes salty; he is bewitched and soon must die”. This adage is an early reference to the genetic disease recognized today as CF. The first clear description of CF was made in 1938 by Dorothy Andersen, a pathologist at the New York Babies Hospital; her paper entitled “Cystic fibrosis of the pancreas and its relation to celiac disease” [4] firmly established CF as a diagnosis separate and apart from celiac disease. Chronic respiratory infection was one of the major symptoms and antibiotics were therefore introduced in the treatment of CF in the 1940s. When investigations evidenced that salty tasting skin occurs as a result of increased secretion of chloride and sodium by CF sweat glands, the use of the diagnostic pilocarpine sweat test was instigated in 1959 [6]. In the 1980s more knowledge on the underlying pathophysiology of the disease was brought about with the description of chloride impermeability of the CF sweat gland [7] and of altered chloride and sodium transport across CF respiratory epithelia [8]. The gene was cloned in 1989 [1,2]. The subsequent decades have witnessed enormous progress toward understanding the molecular biology of the CFTR gene. The basic defect in CF was firmly established as a loss of function and/or expression of the CFTR protein. However the exact role of transepithelial ion transport in the pathogenesis of the disease is still not completely understood. Finding new medicines to fight CF and ultimately a cure has been the driving force of the Drug Development Pipeline of the CF Foundation (http://www.cff.org/research/DrugDevelopmentPipeline/). Despite the multimodal approach that has been adopted, CFTR modulation appears to be the most promising strategy. Present research is focusing on different pathophysiological aspects of the disease and on therapeutic strategies aimed at rescuing the abnormal protein. Currently, candidate molecules suitable for CFTR pharmacotherapy are either being sought after or under investigation. Based on the high prevalence of F508del-CFTR mutation, strategies to rescue the functional status of this particular mutant will benefit most of the CF population.

Structure and function of CFTR gene and protein

The CFTR gene is located in the long arm of chromosome 7 (7q31.2) [9,10]. The encoded protein functions mainly as an adenine 3′,5′-cyclic monophosphate (cAMP)-regulated chloride channel in a variety of polarized epithelia. The CF gene is large, spans approximately 250 kb, and contains 27 exons. The encoded mRNA is about 6.5 kb long and is translated into a protein product of 1480 amino acids. On the basis of the DNA sequence of the gene, a CFTR protein structure was postulated (Fig. 1). The amino acid sequence of CFTR protein shows significant homology to the family of ATP-binding cassette (ABC) transporters. The predicted protein structure is composed of two repeated units, each consisting of a membrane-spanning domain (MSD) comprising of six hydrophobic transmembrane helices, followed by a nucleotide-binding domain (NBD) that interacts with ATP. Ten of the 12 transmembrane helices contain one or more charged amino acids, and two potential glycosylation sites are found between helices 7 and 8. The two repeated units are linked by a single regulatory (R) domain that contains 9 of the 10 consensus sites for phosphorylation by protein kinase A (PKA) and 7 of the phosphorylation sites for protein kinase C (PKC). The R domain separates the two MSDs and interacts physically with the NBD1 [11–13]. The R domain is unique for CFTR as it is not present in the other members of the ABC superfamily. The protein domains assemble to line the pore of the anion-selective channel [14] through which chloride flows across the plasma membrane [15]. Anion flow through the channel is believed to be gated by cAMP-dependent PKA phosphorylation of the R domain [11,12,16,17] and by the interaction of ATP to NBD sites that induces conformational changes in the protein, finally resulting in its opening and closing statuses [15,18,19]. CFTR is mainly located at the apical membrane of polarized epithelial tissues [20,21], although it is also found in a number of non-epithelial tissues such as cardiac myocytes [22,23], smooth muscle [24–26], erythrocytes [27,28] and immune cells such as macrophages [29].

CFTR function as a chloride channel

As a transepithelial anion channel, CFTR provides a pathway for chloride, gluconate and bicarbonate transport [30–33]. After identification of the gene [1,2], functional studies confirmed CFTR as the affected gene in CF disease and its protein product as an epithelial chloride channel. Transfection of functional wild-type CFTR into cultured CF respiratory and digestive epithelial cells corrected the chloride transport defect [34–37]. Conclusive evidence was brought by the demonstration that insertion of wild-type CFTR into artificial bilayer membranes generates chloride channels with characteristic properties of CFTR-associated conductances [38]. These properties are: 1) CFTR activity is regulated by cAMP-dependent phosphorylation and intracellular nucleotides [39,40]; 2) the anion conductance

Fig. 1. Predicted topology and protein structure of the cystic fibrosis transmembrane conductance regulator (CFTR), an integral transmembrane glycoprotein composed of five distinct structural domains: two MSDs (Membrane Spanning Domains); two NBDs (Nucleotide Binding Domains) and one central Regulatory R domain. Each MSD has six hydrophobic α-helices. The protein is N-glycosylated at the 4th extracellular loop (MSD2); C: C terminus; N: N terminus.
and permeability selectivity is of $\text{Br}^– > \text{Cl}^– > I^– > F^–$ [41,42]; 3) the current–voltage relationship of CFTR is linear [41]; and 4) CFTR has a small single-channel conductance in the 6–11 pS range [41,42]. Over the past few years, it has become apparent that a bicarbonate pathway, also defective in patients with CF, is crucial to normal expansion of mucin that, in CF, remains aggregated, poorly solubilized and less transportable [43].

The best known modulator of the CFTR chloride channel is intracellular cAMP. In addition, it has been shown that cyclic-GMP-dependent protein kinases might be involved in phosphorylation and activation of CFTR in the intestine [44].

**CFTR function as a regulator**

Although CFTR is known to function as an apical epithelial chloride channel, deregulated sodium transport is an additional, well-described phenomenon that is proposed to play a major role in the pathophysiology of CF lung disease. Accordingly, it has been shown that stimulation of CFTR by cAMP agonists inhibits the amiloride-sensitive epithelial Na$^+$ channel, ENaC [45] and that ENaC activity is increased in CF respiratory epithelia [8]. A critical role for increased sodium conductance in the development of CF lung disease is supported by studies on a mouse model genetically modified to over-express ENaC [46,47]. An additional role for CFTR has been assigned in the regulation of the outwardly rectifying chloride channel (ORCC) that can only be activated by PKA and ATP when CFTR is functionally intact [48]. CFTR can also control many other ion channels, such as the Ca$^{2+}$-activated chloride conductance (CaCC), the renal outer medullar K$^+$ (ROMK) channels, the sodium/proton exchanger NHE3, and an aquaporin channel [49–57]. Moreover, CFTR was shown to be expressed in intracellular vesicles where it may play a role in intracellular and intravesicular pH regulations [58,59]. CFTR also seems to control exocytosis/endocytosis processes [60,61] and to regulate ATP export, proinflammatory cytokine expression and possibly other cellular functions [62,63].

**Pathophysiology of CFTR gene and protein**

Under normal circumstances, the CFTR gene undergoes transcription and is translated into a CFTR protein that traffics to the cell membrane where it fully functions as a chloride channel (Fig. 2). In CF, the majority of CFTR mutations involve changes in three or fewer nucleotides and result in amino acid substitutions, frame shifts, splice site, or nonsense mutations. The most common and first identified mutation, the F508del, corresponds to a three base pair deletion that codes for phenylalanine at position 508 of the CFTR protein. However, the relative frequency of the F508del mutation in families carrying the CF gene varies between groups. An increasing South East–North West gradient has been noticed for the relative frequency of F508del across European countries: the highest frequency of 82% is reached in Denmark but the mutation is much less frequent in Mediterranean regions, where less than 50% of chromosomes with the CFTR gene have this mutation [64]. The overall frequency of non-F508del mutations is low, except for some rare alleles that segregate with a specific ethnic group. For instance, the W1282X, a stop codon mutation, accounts for 48% of CF chromosomes in Ashkenazi Jews [65] and 23% of French Canadian CF chromosomes carry the 621+1G>T variant [66,67]. It is the presence of the F508del mutation that increases the frequency of CF in Caucasian population relative to other races.

**Table 1**

<table>
<thead>
<tr>
<th>Class</th>
<th>Mutation prototypes</th>
<th>Consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Severe CF phenotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>G542X, W1282X, R553X, 3950delT</td>
<td>CFTR is not synthesized because of stop codons or splicing defects</td>
</tr>
<tr>
<td>II</td>
<td>F508del, N1303K</td>
<td>CFTR is synthesized but in an immature form (only partly glycosylated, misfolded, not released from the endoplasmic reticulum) and is mostly degraded by the ubiquitin–proteasomal pathway</td>
</tr>
<tr>
<td>III</td>
<td>G551D</td>
<td>CFTR is synthesized and transported to the plasma membrane, but its activation and regulation by ATP or cAMP are disrupted</td>
</tr>
<tr>
<td><strong>Milder CF phenotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>R334W, G341E, R347P, D1152H</td>
<td>CFTR is synthesized and expressed at the plasma membrane, but chloride conductance is reduced</td>
</tr>
<tr>
<td>V</td>
<td>3849 + 10 kb C&gt;T, 3272–26 A&gt;G</td>
<td>CFTR synthesis or processing is partly defective</td>
</tr>
<tr>
<td><strong>Severe CF phenotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>1811 + 1.6 kb A&gt;G</td>
<td>CFTR is synthesized, but membrane stability or conductance of ions other than chloride is reduced</td>
</tr>
</tbody>
</table>
CF mutations can disrupt CFTR function through a variety of mechanisms, ranging from complete loss of protein synthesis to normal apical membrane expression of a protein with poor chloride conductance [11,68,69]. The understanding of the mechanisms of dysfunction of CFTR mutations may have direct relevance to tailor new therapeutic strategies. The six major mechanisms by which CFTR function is altered are summarized in Table 1 and illustrated in Fig. 3. The exact assignment of most mutations in this scheme is not yet completely established. It is important to recognize that specific mutations may have characteristics of more than one class. The F508del, the prototype of class II mutation, causes the protein to misfold leading to premature degradation and failure to reach the apical membrane except for special experimental conditions, such as low temperature. Mutations belonging to classes I–III promote reduced or no functional CFTR at the apical membrane and are associated with more severe phenotypes. Mutations belonging to classes IV and V are associated with some residual CFTR-mediated channel function and milder phenotypes. Although they have been investigated only in a limited number of patients, most class VI mutations should be considered as severe [70].

In F508del-CF cells (Fig. 3B), most mutant protein chains do not pass quality control in the endoplasmic reticulum, and those that are misfolded remain thermally unstable, only partially functional, and are rapidly endocytosed and degraded [71,72]. As a consequence, F508del protein can be occasionally detected by immunohisto- or cytochemistry studies inside the cytoplasm, usually close to the nucleus.

Clinical manifestations in CF

A simplified cascade of pathophysiology in CF lung disease is summarized in Fig. 4. CF respiratory phenotype is characterized by a vicious cycle of obstruction, inflammation and infection that progressively damages the airway tissue leading to respiratory failure and death. CF disease has a complex phenotype with variable disease severity and a broad clinical spectrum that reflects the underlying pathology of target organs and systems [73]. Clinically, typical or classical CF [74] is characterized by accumulation of dehydrated and hyperviscous mucus that compromises mucociliary clearance and makes CF airways more vulnerable to infection and inflammation, ultimately leading to...
airway destruction, respiratory failure and death. The intestinal tract shows malabsorption and pancreatic insufficiency that is observed in at least 85% of patients and leads to steatorrhea and failure to thrive. Early gastrointestinal manifestations include meconium ileus that occurs in 10–17% of patients within the first days of life. Sweat glands are most consistently affected in classical CF and produce sweat with elevated chloride (>60 mmol/L) and sodium concentrations [74]. About 97 to 98% of CF males are infertile because of atrophic or absent vasa deferentia.

The most prominent symptoms in the CF respiratory tract are cough, tachypnea and wheezing due to recurrent to chronic bronchopulmonary infections. Most CF patients have chronic rhinitis, and nasal polyps occur in about 15 to 20% of patients, however lower respiratory tract disease usually dominates the clinical picture. Structural bronchopulmonary changes occur much earlier in life than previously thought and can be detected shortly after diagnosis in CF newborns [75–77]. Recurrent exacerbations, typically triggered by acute bacterial infections, promote progressive loss of lung tissue architecture with bronchiectasis. Colonization with *Pseudomonas aeruginosa*, the most common pathogen isolated from CF airways, is particularly difficult to eradicate and is associated with acceleration of decline in lung function and with poorer prognosis [78]. Hyperinflation of the lungs starts early in the course of the disease. Atelectasis, pneumothorax, and hemoptysis are serious complications in advanced stages. Other clinical features of pulmonary involvement include a barrel-chest deformity, use of accessory muscles of respiration, hypertrophic pulmonary osteoarthropathy, digital clubbing, decreased exercise tolerance, and, in end-stage, lung disease, pulmonary arterial hypertension, cor pulmonale, and respiratory failure with cyanosis. The natural history of CF lung disease includes changes in airway microbiology with age: initially *Haemophilus influenzae, Staphylococcus aureus* then *P. aeruginosa* are usually detected. Initial colonization by *P. aeruginosa* by nonmucoid isolates may convert to the antibiotic-resistant mucoid phenotype. Among other clinically relevant pathogens, the multiresistant *Burkholderia cepacia* complex bacteria may have a potential impact on lung transplantation. The high transmissibility of particular strains has led to segregated clinics to minimize patient cross contamination. In addition to infections with *P. aeruginosa* and with other bacteria, up to 50% of CF patients have positive growth of *Aspergillus fumigatus* in their sputum, and some exhibit the syndrome of allergic bronchopulmonary aspergillosis [79].

Understanding origins and mechanisms of inflammation in CF is critical for designing effective interventions. Studies focused on the role of immune pathways and the corresponding cell populations and chemical factors involved in CF lung disease have demonstrated that inflammatory responses are exaggerated in CF and that pulmonary inflammation starts early in infancy and can be evidenced even in the absence of any detectable infection [80]. CF pulmonary inflammation is characterized by increased [80,81] and potentially defective [82,83] neutrophils, elevated interleukin (IL)-8 concentration and neutrophil elastase [84]. Among other critical mediators for sustaining chronic neutrophil influx in the CF lung, tumor necrosis factor (TNF)-α, IL-6 and IL-1β, complement-derived chemotactants and leukotriene B4 can be listed [85–87]. Neutrophil breakdown releases massive amount of proteases that destroy the local host defenses, induce oxidative stress, increase viscosity of endobronchial secretions, worsen mucociliary clearance and promote lung tissue destruction [88,89]. More recently, it has been demonstrated that macrophages play a critical role in triggering and orchestration of inflammatory responses in CF: macrophage cells [29] and blood monocytes [90] express a functional CFTR protein, and macrophage cell density is increased in CF airways [91]. Moreover, it has been evidenced that cell differentiation processes leading to generation of cell population subtypes playing distinct roles in inflammatory response (M1/M2 polarization) are altered in CF macrophages [92].

Patients displaying classic characteristics of CF from infancy usually have a relatively poor prognosis [74]. Additionally, there has been a growing recognition of atypical, milder, paucisymptomatic disease cases of CF presenting in adolescence or adulthood and displaying normal (<30 mmol/L) or borderline (30–60 mmol/L) sweat chloride concentration [74,93] and a better prognosis for survival [94]. Atypical CF and CF-related disorders, including congenital absence of vasa deferens, isolated idiopathic pancreatitis, allergic bronchoalveolar aspergillosis, chronic rhinosinusitis, nasal polyposis, and idiopathic bronchiectasis [95], have made the diagnosis of CF less straightforward for clinicians and biologists. In such cases, diagnostic confirmation requires detection of one disease-causing mutation on each CFTR gene [95] and direct quantification of CFTR dysfunction by measuring in vivo nasal potential difference [96] or ex vivo intestinal current in

![Pathophysiological cascade of CF lung disease.](image-url)
rectal biopsy specimens [97]. Although CF has been recognized as a monogenic disorder, it has become increasingly evident that modifier genes [98] and environmental factors play a substantial role in determining disease severity.

Despite the clear link between abnormal chloride transport and CF, the pathogenesis of CF is complex and still debated. Studies have suggested that the initiating event in the pathogenesis of the CF airways disease is reduced airway surface liquid (ASL) volume, which develops, in a large part, as a consequence of abnormal ion transport [8]. Insights that have been provided from the development of animal models of CF disease illustrate the complexity of the pathophysiology of CF. Much has been learned through CF mouse models however the species does not spontaneously recapitulate a major lung phenotype as seen in CF patients. Interestingly, the CF pig model does not demonstrate sodium transport or ASL abnormalities [99].

**Strategies for CF treatment**

In recent years, growing efforts have been made to tailor intervention strategies that target the basic defects of CF rather than its symptoms. Following the discovery of the CF gene, some expectations arose that gene therapy, especially targeting topically the airways, would soon provide a treatment [100,101]. In some trials, transgene expression has been detected, but no effective clinical benefit has thus far been recorded [102]. Access of the transfecting agents to the surface epithelial cells is hampered by the overlying mucus while transfection of the submucosal glands poses special problems. The work continues in an attempt to overcome these technical difficulties, as gene therapy may yet provide the best solution. However, in the recent years, the focus has turned toward pharmacotherapy as it is more likely to yield more immediate results and help reduce the burden of the disease. The emphasis on pharmacotherapy for CF is to develop agents that rescue CF cell physiology or to press into service alternative chloride channels, also expressed in CF cells, to act as surrogates for CFTR.

**Therapy directed at CFTR defects**

As CFTR gene mutations have different functional consequences, pharmacotherapy should be tailored to target specific mutation classes.

**Therapies directed at class I defects**

Aminoglycosides and Ataluren (PTC 124) are examples of therapies directed at this class of mutations. Almost 15 years ago, it was described that gentamicin, a potent bactericidal aminoglycoside antibiotic, is able to induce read-through of premature stop codons resulting in the synthesis of a full-length and functional CFTR protein [103]. More recently, beneficial effects of topical nasal application [104,105] and intravenous administration [106,107] of gentamicin have been demonstrated in CF patients displaying at least one class I mutation. The effects, even though controversial [108], consisted in improvement of CFTR-dependent chloride transport, as assessed by nasal potential difference, a diagnostic test that has been used to evaluate therapeutic efficacy of potential drugs during clinical trials [96]. Moreover, the dose regimens required to achieve a significant effect on chloride transport would not be suitable for prolonged clinical use due to the adverse effects of aminoglycosides, mainly nephro- and oto/vestibular toxicities.

A drug discovery program using high-throughput screening assays has identified Ataluren (PTC 124), a small molecule able to allow complete translation of proteins containing nonsense mutations leading to insertion of a stop codon (UGA, UAG, or UAA) into the protein-coding region of mRNA. Oral treatment with Ataluren was shown to bring beneficial effects to CF adults [109] and children [110] displaying at least one CFTR nonsense mutation. Ataluren induced a tendency toward normalization or a significant correcting effect of chloride transport by respiratory cells [109,110]. In addition, treatment with Ataluren allowed an increased CFTR expression at the apical membrane of nasal cells [110]. Interestingly, variable responses were found among patients with different genotypes, indicating that even CFTR class-specific treatment may not necessarily work for all patients within a given mutation class [111]. A limiting factor appears to be the amount of CFTR mRNA to be translated. This amount depends on a cell mechanism called nonsense-mediated mRNA decay (NMD) which functions as an mRNA quality control program able to detect nonsense mutations and to prevent the expression of truncated or erroneous proteins. In some CF patients, NMD is particularly active and leads to an accumulation of truncated CFTR proteins in the endoplasmic reticulum which finally activate the unfolded protein response (UPR). The interplay between the two cell processes, NMD and UPR, should have important consequences on the response to read-through drugs such as Ataluren. A phase III extension study with Ataluren (http://clinicaltrials.gov/ct2/shows/NCT01140451) that should enroll 208 patients is currently ongoing. If hopes with Ataluren treatment are confirmed, this should be a therapeutic option for a small fraction of the CF population, as class I mutations are highly prevalent in Israel [65] but are found in only about 5% of the CF population in other countries.

**Table 2**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Mechanisms of action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VX-809</td>
<td>Phosphodiesterase type 5 inhibitors</td>
<td>[114] [154,155]</td>
</tr>
<tr>
<td>VRT-325</td>
<td>Phosphodiesterase type 5 inhibitors</td>
<td>[115,120,121,136]</td>
</tr>
<tr>
<td>Sildenafil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vardenafil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tadalafil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KM11060</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miglustat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-PBA</td>
<td>Activator of transcription of several genes; chaperone protein inhibitor</td>
<td>[146–149]</td>
</tr>
<tr>
<td>Correctors 2a, 3a, 4a, 4b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzoquinolizinium (MFP-07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curcumin</td>
<td>SERCA inhibitor</td>
<td>[157,158]</td>
</tr>
</tbody>
</table>

4-PBA: 4-phenylbutyrate; SERCA: sarcoplasmic/endoplasmic reticulum calcium pump.

**Therapies directed at class II defects**

Treatment directed at class II mutations, including the clinically relevant F508del mutation, should be able to increase the amount of protein expressed in the plasma membrane of target cells by correcting defective trafficking of the mutant protein. However, it has been recognized that rescuing F508del-CFTR to the plasma membrane does not completely correct chloride transport abnormalities, as F508del protein additionally displays reduced channel activity [112] and reduced residence time in the plasma membrane because of increased protein internalization and recycling [58–61,71,72]. Therefore, an effective treatment for patients with F508del mutation should additionally enhance CFTR channel gating and membrane stability. A compound able to enhance F508del-CFTR trafficking to the membrane has been termed “corrector” while a compound able to increase its PKA-regulated open probability has been named “potentiator”. Candidate drugs that specifically increase the residence time of CFTR protein in the apical membrane have not yet been clearly identified but studies on modulation of endocytic activity of misfolded integral proteins with formation of intracellular aggregates and autophagy, as well as the consequences of these deregulated processes in CF lung disease, are under investigation [113]. Clearly, a single therapeutic agent with properties of both correctors and
potentiators would be preferable for CF therapy or a combination therapy with a corrector and a potentiator agent has been considered.

A particularly interesting corrector is the compound identified by Vertex Pharmaceuticals, VX-809. This corrector can promote maturation of CFTR processing mutants in human bronchial epithelial cells in culture. Oral administration of VX-809 for 4 weeks to 89 patients homozygous for the F508del mutation was well tolerated and a positive impact of the treatment was shown on CFTR function in the sweat glands. Indeed, sweat chloride contents decreased in a modest, though statistically significant fashion, with treatment in a dose-dependent manner [114]. However treatment with VX-809 neither improved the CFTR chloride transport in the nasal epithelium, as measured by the nasal potential difference, nor the respiratory function. An exploratory phase II clinical trial to evaluate combination regimens of VX-809 with a potentiator, ivacaftor (VX-770), in patients with F508del mutation is currently ongoing (http://clinicaltrials.gov/ct2/show/NCT01225211).

Phosphodiesterase type 5 (PDE5) inhibitors, such as sildenafil (Viagra®), have been shown to be able to promote maturation and to correct trafficking of CFTR mutants [115]. Because sildenafil has already been approved for clinical use in the treatment of erectile dysfunction [116] and pulmonary arterial hypertension [117], it can be speculated that research on this class of drugs might speed up the development of new therapies for CF.

Under the scope of translational research and development with the specific goals of exploring CF lung pathology [91,92,118] and trancheal dysmophogenesis [119], of testing fundamental therapeutic strategies [120–122] and of characterizing imbalanced fatty acid metabolism in CF [123–125], genetically modified CF mice have been used. The Cftrtm1lux mouse model generated by the Erasmus Medical Center of Rotterdam [126] has the advantage of harboring a specific clinically relevant mutation and it displays several advantages over the two other F508del that have also been described [127,128]. Indeed, the survival rate of the Cftrtm2Cam model [127] is markedly reduced with less than 5% progression to maturity. Even though the survival rate of the Cftrtm1lux model [128] is better (40% progression to maturity), a decrease in mutant mRNA levels at the intestinal tract has been characterized while in our Cftrtm1lux model [126] mRNA levels are preserved in all relevant target organs and tissues. Thanks to the Cftrtm1lux mouse model, it has been confirmed that treatments with PDE5 inhibitors represents a potential fundamental therapy for CF. When applied to F508del-CF mice at clinical doses, by intraperitoneal injection [121] or by local deposition at the nasal mucosa [120], sildenafil, vardenafil and/or taladafil correct CFTR-dependent chloride transport at the nasal epithelium of F508del homozygous mice but not of Cftr knockout mice. The effect was identified by means of the nasal potential difference, a technique that has been applied in mice [120–122,129] as well as in patients [93,110,130–133]. The mechanism of action of PDE5 inhibitors is not completely understood. In vitro studies have suggested that the drugs can reverse hyperacidiﬁcation of endosomes and reduce ENaC-dependent sodium hyperabsorption [134]. A more potent structural analog of sildenafil, KM1060, was recently identiﬁed [135]. Interestingly, it was also shown that vardenafil is able to attenuate inﬂammatory responses in CF mice [136]. However, the mechanism of action of vardenafil as an anti-inﬂammatory agent in CF as well as the target-effector cells involved in these responses are not clear and deserve further studies. As lung inﬂammation plays a major role in morbi-mortality in CF, identifying a molecule that combines ability to correct the basic ion transport defect and to reduce deregulated inﬂammatory responses in CF is very exciting and promising. A clinical trial aiming at investigating safety and eﬃcacy of sildenafil in CF lung disease is listed on clinicaltrials.gov/ (NCT00659929). The study is examining whether sildenafil can lower markers of the airway inﬂammation, such as sputum IL-8 and elastase, in patients with CF.

Miglustat, an N-alkylated iminosugar (n-butyldeoxyxojirimycin, NB-DNJ, Zavesca®), is an orally available drug approved for non-neuropathic, type I Gaucher disease [137–139] and for the other glycosphingolipidoses [140] as well as viral infections [141,142]. The efficacy of miglustat to rescue endogenous F508del-CFTR and to down regulate sodium transport has been demonstrated in several CF experimental models such as F508del mutant airway epithelial cells in culture or isolated tissues from mice [122,143–145]. The suspected mechanism of action is the disruption of the interaction between F508del-CFTR and endoplasmic reticulum calnexin [144,145]. Oral treatment with miglustat has been evaluated recently in 11 CF patients homozygous for the F508del mutation during a pilot phase IIa clinical trial (http://clinicaltrials.gov/ct2/show/NCT00742092). Patients were assessed in two 8-day cycles of oral placebo or miglustat 200 mg t.i.d. separated by a 14-day washout period [133]. No statistically signiﬁcant effects on chloride transport were observed on the basis of the nasal potential difference [133]. The study allowed pointing out that inclusion of CF patients with some residual chloride transport at baseline conditions and poor reproducibility of the nasal potential test are limiting aspects to draw deﬁnitive conclusions on the impact of miglustat on chloride secretion by the respiratory tract. Safety and tolerance proﬁles were acceptable and diarrhoea, due to inhibition of intestinal disaccharidases, such as sucrase, maltase and lactase, was the most frequent adverse event recorded [133].

Preclinical studies with sodium–4-phenylbutyrate (4-PBA), a histone deacetylase inhibitor known as an activator of transcription of a variety of genes, have shown that the drug is able to traffic F508del protein to the cell membrane and to restore CFTR chloride function at the plasma membrane of lung cells in vivo and in vitro [146]. The correcting eﬀect of 4-PBA on CFTR mutants may be due to increased transcription of the protein resulting in more mutated CFTR escaping the ubiquitin–proteasomal pathway and becoming available to be addressed to the cell membrane [147]. A pilot clinical trial with oral 4-PBA showed improved nasal potential diﬀerence in F508del patients consistent with increased apical CFTR activity and no changes in sweat chloride concentrations [148]. More recently, loss of enthusiasm for therapy with 4-PBA in CF has arisen with the demonstration that treatment of human bronchial epithelial cell lines with clinical doses of 4-PBA promotes a marked increase in proinflammatory responses with production of IL-8 cytokine. The induced IL-8 overproduction was associated with a strong increase of several regulatory factors involved in activator protein-1 [149], a major transcriptional pathway playing a central role in early inflammatory processes in CF lung disease [150].

Some other correctors were shown to be relatively speciﬁc for rescuing F508del-CFTR in preclinical experiments [151–153]. For example, corr-4a, a bisaminomethyl bithiazole compound, [153], the thiazole derivative corr-2b and the pyrazole derivative VRT-532 promote maturation of F508del-CFTR. An interesting molecule that can correct mutants of CFTR and of its sister protein, P-gp seems to be the quinazoline derivative VRT-325 [154,155]. Drugs able to correct a number of misprocessed proteins including CFTR have been called pharmacological chaperones. Dormer et al. demonstrated that the benzo(c)quinolinizinium MPB-07, an agent that augments the activity of F508del-CFTR at the cell surface, rescues CFTR biosynthesis in nasal epithelial cells from CF patients homozygous for the F508del

<table>
<thead>
<tr>
<th>Drug</th>
<th>Families</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>VX-770</td>
<td>Pyrazole</td>
<td>[159,160,162]</td>
</tr>
<tr>
<td>NS004</td>
<td>Benzimidazoline</td>
<td>[163,164]</td>
</tr>
<tr>
<td>Genistein</td>
<td>Flavonoid</td>
<td>[163,165]</td>
</tr>
<tr>
<td>Phloxine B</td>
<td>Phloxine</td>
<td>[166]</td>
</tr>
<tr>
<td>GPact-11a</td>
<td></td>
<td>[167]</td>
</tr>
</tbody>
</table>
Table 4
Candidates for alternative anion conductance in cystic fibrosis.

<table>
<thead>
<tr>
<th>Class</th>
<th>Protein</th>
<th>Expression</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(^{2+}) activated chloride</td>
<td>Bestrophins (Best1–4)</td>
<td>Lung, colon, liver, kidney</td>
<td>Cl(^{-}) secretion in mouse airways</td>
<td>[169]</td>
</tr>
<tr>
<td>channels (CaCC)</td>
<td>Tweety (TTYH)</td>
<td>Excitable tissues</td>
<td>Cl(^{-}) channel</td>
<td>[170,171]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Secretory epithelia (lung, colon, pancreas, liver, kidney, stomach)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Broad</td>
<td></td>
<td>[175]</td>
</tr>
<tr>
<td>Voltage-gated chloride</td>
<td>SLC26A9</td>
<td>Epithelial cells (lung, Gl tract, kidney)</td>
<td>Participates in CFTR-dependent Cl(^{-})</td>
<td>[176–178]</td>
</tr>
<tr>
<td>channels (CLC)</td>
<td></td>
<td></td>
<td>secretion in airway cells</td>
<td></td>
</tr>
<tr>
<td>Cl(^{-})/HCO(_3) exchange</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5
Drugs that activate alternative chloride channels.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Alternative chloride channels</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP analog</td>
<td>CaCC channel</td>
<td>[179,180]</td>
</tr>
<tr>
<td>Denufusol tetrasodium (INS37217)</td>
<td>CaCC channel</td>
<td>[181–184]</td>
</tr>
<tr>
<td>Lubiprostone</td>
<td>ClC-2 channel</td>
<td>[192–195]</td>
</tr>
<tr>
<td>NO-4995</td>
<td>CaCC channel</td>
<td>[196–202]</td>
</tr>
</tbody>
</table>

mutation [156]. Curcumin has been proposed to act as an F508del corrector in F508del-CF mice [157], however further research with curcumin showed inconsistent data [158].

Some insights into correctors of CFTR are summarized in Table 2.

Therapies directed at class III and IV defects

Though class III mutations, which reduce the PKA-dependent open probability of the CFTR channel, are relatively rare, studies of compounds that activate CFTR channel gating (potentiators) may have a role beyond the class III patient group. The G551D, a prototype of class III mutation, is the third most common mutation found in patients with CF; however, it accounts for only about 2–3% of cases worldwide.

Ivacaftor was shown to stimulate chloride transport in epithelial cells expressing G551D-CFTR [159] which is a severe gating defective mutant with normal expression at the cell membrane. Encouraging data have been collected recently for ivacaftor during a multicentric clinical trial enrolling 39 CF patients who carry at least one copy of the G551D mutation. Oral treatment for 4 weeks with the potentiator was shown to display a good efficacy and similar safety profile as compared with a placebo group [160]. A significant effect on chloride transport, as measured by nasal potential difference, and on sweat chloride concentration was observed in the ivacaftor treated group [160]. The effect on the sweat test was dose-dependent and showed a quasi-normalization of sweat chloride contents in the majority of the patients. The mechanism of action of ivacaftor is not completely understood. Studies showed that the drug can restore cAMP-dependent chloride transport across airway epithelial cells of normal or CF airway epithelia to these agents increases transcellular chloride secretion in mouse airways (http://clinicaltrials.gov/ct2/show/NCT01225211).

Other potentiator compounds acting through different mechanisms have been investigated at preclinical experiments. Some (true) potentiators are able to augment CFTR gating in response to CAMP signaling while others induce CFTR channel gating when administered alone; the latter have been considered as ‘gating activators’. Benzimidazolone (NS004) activates F508del-CFTR at nanomolar concentrations [163]. NS004 was shown to be able to activate wild-type and G551D-CFTR mutant chloride channels [164]. The flavonoid genistein [163,165] has been extensively used in experimental protocols to treat class III and class IV defects. The mechanism of action of genistein was initially thought to be via inhibition of enzymes in the regulatory pathway, specifically tyrosine kinase. More recently, it has been proposed that genistein interacts directly with CFTR at an NBD site, which in turn leads to a larger open probability of the chloride channel. Flavonoids also have other properties that would make them good candidates as therapeutic candidates in CF. Al-Nakkash et al. [163] proposed that genistein and benzimidazolone stimulate CFTR by a common mechanism. The drugs inhibit ATP hydrolysis at NBD-2 to stabilize the open channel configuration [164]. Phloxine B is a potent modulator of CFTR, with complex effects on channel activity. Low micromolar concentrations of phloxine B stimulate CFTR chloride currents, by interacting with NBDs and by prolonging channel openings [166]. It has been suggested that Phloxine B interacts directly with CFTR at multiple sites to modulate channel activity [166]. GPact-11a is a novel water-soluble activator of CFTR chloride channels in human airway epithelial cell lines and activates rescued F508del-CFTR in nasal, tracheal and pancreatic human CF epithelial cells [167].

Some insights into potentiators of CFTR are summarized in Table 3.

Therapies directed at class V and VI defects

Class V and VI mutations affect mRNA and CFTR stability, respectively. Although relatively less common, subjects are more likely to be compound heterozygotes with F508del mutation. Treatment options include increasing mRNA and maximal activation of the normal CFTR. Therapeutics such as 4-PBA [146–149], milrinone or genistein [163,165] may prove useful.

Therapy of alternative chloride channels

Another method of preventing defect of CFTR is to use alternative chloride channels to act as surrogates for CFTR [168–178]. Several candidates for alternative anion conductance in epithelial cells have been identified (Table 4). Although the existence of epithelial CaCC channels has been known for more than 20 years, the molecular identity of these proteins remains obscure. One of the candidates is TMEM16A (ANO1), a CaCC channel expressed in secretory epithelia, smooth muscle, and other tissues. Recently, Verkman et al. have discovered small-molecule activators and potentiators of TMEM16 that do not elevate intracellular calcium concentration ([Ca\(^{2+}\)]\(_i\)) [168]. In addition to producing a more sustained activation of chloride conductance (whereas P2Y2 agonists generally produce only transient elevation of [Ca\(^{2+}\)]\(_i\)), direct-acting CaCC activators also have the theoretical advantage over Ca\(^{2+}\) agonists based on a higher target specificity.

ATP or UTP analogs have been pointed out as potential drugs for CF therapy. Accordingly, it has been established that both purine (ATP) and pyrimidine (UTP) triphosphate nucleotides are able to activate chloride transport across airway epithelial cells. Exposure of normal or CF airway epithelia to these agents increases transepithelial chloride transport via increases in [Ca\(^{2+}\)]\(_i\) that secondarily
activate CaCC channels present in the apical membrane [179]. UTP analogs inhibit sodium entry in both wild-type and F508del-CFTR cells from human bronchial epithelia [180].

Denufosol tetrasodium (INS37217) is a novel ion channel regulator designed to correct ion transport defect in CF after binding to a P2Y2 receptor located at the apical membranes [181]. Preclinical experiments showed that denufosol increases CaCC-dependent chloride secretion, inhibits sodium absorption via ENaC, increases mucosal hydration and stimulates ciliary beat frequency of respiratory epithelial cells [181,182]. Initial phase II and III clinical trials with inhaled denufosol claimed encouraging results independently from the dose used and the genotype [182,183]. However, initial results have been only partly confirmed during a larger (352 CF patients) phase III clinical trial [184]. Indeed, a slight improvement was shown on FEV1 but not on other measures of lung function or on other monitored outcome parameters [184].

Duramycin (Moli-1901) is a poly cyclic peptide antibiotic derived from Streptomyces cinnamonum. Duramycin may increase chloride transport via CaCC channels that compensates for the CFTR deficiency in the CF airway epithelia [185,186]. However the exact mechanism by which duramycin stimulates transepithelial chloride transport is not completely elucidated. It has been suggested that the effect of duramycin is independent of CaCC currents and is rather related to unspecific changes of the cell membrane or its components [187]. Phase I [188] and phase II [189] clinical trials showed that inhaled Moli-1901 appeared to be safe and multiple escalating doses have sustained beneficial effects on pulmonary function. The demonstration of absence of detectable inflammatory response during pharmakokinetic studies in healthy volunteers qualified the substance for intrapulmonary administration in CF patients [190].

Lubiprostone is a laxative medication that activates ClC-2 channels [191] and has the potential to be an effective treatment for constipation in adults with CF. The drug has the potential to activate non-PKA dependent chloride secretion in cells from the gastrointestinal tract [192]. However recent studies have shown that the drug is also efficient in airway epithelial cells [193,194]. A pilot clinical trial conducted in seven CF adults with constipation showed improved overall symptoms of constipation [195]. Data on the impact on nutritional status and pulmonary function from larger population size studies are needed.

INO-4995, a synthetic analog of the intracellular signaling molecule, D-myo-inositol 3,4,5,6-tetraakisphosphate, inhibits sodium and fluid absorption across CF airway epithelia, thus alleviating the critical pathophysiology [196]. INO-4995 modulates ion channel activities, including chloride and potassium channels [197–199]. A particular inositol phosphate, Ins(3,4,5,6)P4, targets chloride channels in epithelia [200,201]. It has been demonstrated that the potency of INO-4995 on ion transport across the nasal mucosa in CF knockout mice corresponded to effects on fluid secretion and amiloride responses in humans. The exact effect of treatment with INO-4995 on fluid and sodium absorption was maintained after multiple doses and repeated daily exposure lowered the required effective doses [202]; this enhanced potency with repetitive treatment should be taken into consideration when developing dosing strategies for subsequent studies and clinical trials.

Some insights into therapy with surrogates for CFTR are summarized in Table 5.

Conclusion

Recent years have witnessed major advances in CF supportive care and in our understanding of CFTR pathophysiology. The basic defect in CF is reduced airway surface liquid volume related to faulty chloride and sodium transport across a variety of excocrine epithelia. Present research is focusing on the development of therapeutic strategies aimed at rescuing the abnormal protein that is either synthesized in reduced amounts or has poor anion conductance. Mutation-specific pharmacological approaches are currently the most favored strategies. Promising clinical data have been provided with novel candidate molecules, Ataluren, VX-809 or ivacaftor, tailored to treat CF patients with class I, II or III mutations. Based on its high prevalence, strategies to rescue the functional status of the F508del mutant will benefit most of the CF population. As PDE5 inhibitors, sildenafil, vardenafil and tadafalil, are able to correct transepithelial ion transport abnormalities and to limit exaggerated inflammatory responses related to F508del-CF protein, the drugs are promising compounds for fundamental pharmacotherapy in CF. Since sildenafil and analogs are in clinical use, research on the drugs might accelerate the development of new therapies for CF.

Acknowledgments

BL is a PhD fellow with the Fonds Spéciaux de Recherche (FSR; Académie Universitaire Louvain). SN is a postdoctoral researcher with the FSR and Marie Curie actions of the European Commission. TL is an associate researcher with the Fonds de la Recherche Scientifique Médicale (FRSM). This study was supported by the French CF Association, Vaincre la Mucoviscidose, the FSR and the Foundation St Luc (‘St Luc University Hospital and Université catholique de Louvain’).

References


