Ion channel regulators for the treatment of cystic fibrosis

Cystic fibrosis is a life-shortening hereditary disease caused by mutations in the cystic fibrosis transmembrane conductance regulator gene. The main clinical manifestations are chronic infections of the airways, malabsorption due to pancreatic insufficiency, male infertility and salt loss syndromes. The cystic fibrosis transmembrane conductance regulator protein functions as a chloride channel in the apical cell membrane and is thought to inhibit the epithelial sodium channel. In the lung, absence or dysfunction of the cystic fibrosis transmembrane conductance regulator protein leads to dehydration of the airway surface liquid and disturbed mucociliary clearance. After decades of symptomatic therapy to combat the relentless disease progression, there is now hope that interference with the apical ion channel functions will bring a major improvement in the disease course. We discuss the ion channel regulators currently under clinical evaluation in cystic fibrosis, as well as some of the challenges associated with finding disease-modifying therapies. In this article we give a brief description of the clinical and epidemiological aspects of the disease cystic fibrosis. We discuss the pathophysiology of cystic fibrosis, the ion channel regulators under clinical development as potential disease-modifying therapies and some of the challenges associated with developing groundbreaking therapies for this orphan disease.

KEYWORDS: ataluren ▪ CFTR ▪ CFTR correctors ▪ CFTR potentiatiors ▪ cystic fibrosis ▪ denufosol ▪ ENaC ▪ ENaC inhibitors ▪ ion channel regulators

Christiane De Boeck[†1] & Harry Cuppens[2]

[1]University Hospital Leuven Belgium, Department of Pediatrics, Cystic Fibrosis Reference Center, Herestraat 49, 3000 Leuven, Belgium
[2]University of Leuven Belgium, Center for Human Genetics, O&N I, Herestraat 49 – bus 602, 3000 Leuven, Belgium
[†]Author for correspondence:
Tel.: +32 1634 3820
Fax: +32 1634 3842
christiane.deboeck@uz.kuleuven.ac.be

The disease cystic fibrosis

Cystic fibrosis (CF) is a life-shortening, autosomal, recessive disease. The main clinical manifestations are chronic infections of the upper and lower airways, malabsorption due to pancreatic insufficiency, male infertility and salt loss syndromes [1]. The most important symptom is lung infection since more than 90% of subjects die from respiratory insufficiency or, in recent decades, from the complications of lung transplant. The current median age of death in patients with CF is only 26–28 years [2]. Great strides have been made in the prognosis for patients and instrumental in this have been:

- Follow-up in specialized CF centers;
- Detailed CF patient registries recognizing the main clinical problems;
- Treatment guidelines based on results of comparative effectiveness research, as well as randomized controlled trials.

Cystic fibrosis occurs everywhere in the world but the incidence is higher in the Caucasian population [3,101]. CF is classified as a rare disease since it occurs in less than 1 in 2000 subjects. Across Europe the incidence varies, even within one country, for example in France, the incidence of CF is as high as of 1 in 2700 in Brittany and less than 1 in 7700 in the Pyrenees [4].

In several European countries a national program of screening for CF at birth has been instituted. Since the lung is normal at birth, this offers the possibility of optimal follow-up and treatment. In many countries, patients are still diagnosed via symptoms. Most patients are detected during infancy, because of failure to thrive and repeated chest infections. The sweat test remains the best diagnostic test [5]. From diagnosis on, a preventive follow-up and treatment are instituted [6]. This includes:

- Optimization of nutritional status
- Chest physiotherapy
- Use of mucolytic drugs
- Bacterial surveillance
- Intensive treatment of airway infections

Still, repeated cycles of lung infection occur and lead to structural lung damage and eventually respiratory insufficiency [7]. Some patients have a milder disease phenotype. They present later in life, they have fewer organ systems involved, they typically have preservation of pancreatic function and they have an intermediate sweat test result [8].

part of future medicine fsg

More than 20 years ago, the CF transmembrane conductance regulator (*CFTR*) gene was discovered on the long arm of chromosome 7. Worldwide, F508del is the most frequent mutation and is seen in 70% of the *CFTR* genes derived from CF patients [9]. In the mean time, over 1800 different *CFTR* mutations have been identified [102]. Some mutations are seen in 1–2% of CF subjects, but most mutations are uncommon and each account for less than 1% of the mutant *CFTR* genes. Of course, for many mutations, although rare, there are specific hotspots of occurrence in different parts of the world [3]. In some individuals with 'CF-like disease symptoms', such as chronic pancreatitis, idiopathic bronchiectasis or male infertility due to absence or obstruction of the vas deferens, one or two *CFTR* mutations have been detected. Patients with these partial disease manifestations are grouped as atypical CF or CFTR-related disorders [5,10].

The specific CF genotype mainly determines the pancreatic phenotype. The lung disease severity varies greatly even between patients carrying the same mutations. Indeed, the CF phenotype is influenced by other genetic characteristics, called gene modifiers [11] and by environmental factors, such as exposure to tobacco smoke [12], age at diagnosis, intensity of treatment.

CF pathophysiology

The *CFTR* gene codes for the CFTR protein that mainly functions as a chloride channel in the apical cell membrane [13]. CFTR plays an important role in directing sodium chloride and water transport across epithelial membranes. In patients with CF, the airways have near absent CFTR-mediated chloride secretion and an elevated sodium absorption, attributed to absence of downregulation of epithelial sodium channel (ENaC) by CFTR [14]. The nasal potential difference test indirectly measures these ion fluxes [15]. In CF, the imbalance in ion secretion leads to airway surface liquid volume depletion and impairment of mucociliary clearance. Airway obstruction and propensity to chronic lung infection then follow. This theory of airway liquid dehydration as the starting point of CF pathophysiology has recently been challenged by new data in the CF pig model [16]. No decrease in airway surface liquid height was found in newborn animals. In addition, the authors found no evidence of ENaC hyperfunction in addition to the impaired chloride secretion.

Apart from the chloride secretory defect at the apical membrane, other factors are involved in the CF pathology. Defects in innate immunity in the airway have been described and they are in part related to the altered ion fluxes [17]. In addition, a complex increased inflammatory response has long been described [18]; this adds to the CF pathology and may be a distinct therapeutic target.

At present, most CF therapies target the end of the pathophysiologic cascade by treating the lung infection and decreasing the airway obstruction. The improved knowledge of the CF pathophysiology has started an intensive translational research program to detect novel treatment options. Therapies that improve the ion transport dysregulation indeed have the potential to more dramatically improve the disease course, since they interfere very upstream in the pathophysiologic cascade. There are several possible strategies. Some of these are *CFTR* mutation specific, such as restoration of the CFTR chloride secretory function by CFTR correctors, CFTR potentiators or suppressors of nonsense mutations. Other strategies can be applied to patients irrespective of their specific *CFTR* mutations, such as restoration of the CFTR protein by gene therapy, stimulating chloride secretion via alternative chloride channels, decreasing sodium absorption via inhibition of ENaC, or attracting water to the dehydrated mucosal surface by inhaling drugs such as mannitol or hypertonic saline. The latter two drugs and gene therapy cannot be considered as ion channel regulators and will not be discussed further.

CFTR mutation classes

Normal *CFTR* gene transcription is initiated at the promoter region. The mRNA transcript is translated from the start codon up to the termination codon by ribosomes on the endoplasmic reticulum (ER) to 'band A' CFTR protein. Here it is also folded in its correct tertiary structure and undergoes early glycosylation, to 'band B' CFTR. It then attains its full glycosylation in the Golgi apparatus to 'band C' CFTR. These band-type proteins are detected by protein electrophoresis and near absence of band C is typical for CF cell lines. The CFTR protein is finally incorporated in the apical membrane of the epithelial cell. When activated by phosphorylation and ATP binding and hydrolysis, the protein undergoes a conformational change, thereby allowing chloride transport. Rapid cycles of opening and closing (gating) characterize a normal 'wild-type' CFTR protein [19].

The total CFTR chloride flux at the mucosal cell surface is best described by the product of

the number of CFTR channels present at the cell surface, the channel open probability (gating) and the individual channel current (dependent on the channel's conductance). Individual *CFTR* mutations are therefore classified in five groups according to which of these characteristics is primarily disturbed (TABLE 1) [20]. In class 1 we group mutations that result in no functional CFTR protein being synthesized. These include nonsense mutations, frame-shift mutations, large deletions or insertions, as well as several splice-site mutations. Of special interest are nonsense mutations, such as premature termination codons that account for approximately 9% of the CF alleles worldwide, but in Israel this is as high as 50% [21]. Class 2 groups the mutations that lead to misfolded CFTR proteins that are targeted for degradation in the proteasome, so that hardly any, or no, mature CFTR protein is found in the apical cell membrane. The mutation F508del is the typical example of a class 2 mutations. In many countries more than 90% of subjects with CF carry F508del on at least one CF allele [22,101]. Class 3 groups the mutations with abnormal gating properties so that no CFTR function occurs. The G551D mutation seen in approximately 3% of patients worldwide belongs to class 3 [23,101]. *CFTR* mutations leading to defective chloride conductance are grouped into class 4. Class 5 mutations result in a decreased amount of otherwise normal CFTR. It contains mutant splice site mutations that are only partially affected (e.g., 3849 + 10 Kb C→T) or mutant *CFTR* that still matures to some extent (e.g., A455E). Class 4 and 5 mutations are rare apart from R117H, which is detected in up to 10% of asymptomatic subjects after newborn screening [24]. Patients carrying two *CFTR* mutations of class 1–3 (either in homozygosity or compound heterozygosity) nearly always have pancreatic insufficiency and as a group have more severe lung disease compared with a group of subjects carrying at least one class 4 or 5 mutation.

Nonsense mutation read-through aims at selectively inducing ribosomal read-through of mRNA at the site of a premature nonsense mutation. CFTR potentiators aim at improving CFTR channel gating and are thus the solution for subjects with CF and at least one class 3 mutation. CFTR correctors aim at rescuing the mutant CFTR protein from degradation and allowing it to be expressed at the cell membrane. However, these mutant proteins may still have abnormal gating properties. Since correct CFTR trafficking involves many steps, it is anticipated that correcting abnormal CFTR trafficking is more difficult than correcting abnormal CFTR gating by CFTR potentiators [25]. The assignment of CFTR mutations to one individual class is artificial, since many mutations have characteristics of more than one mutation class. Combining therapeutic strategies may thus yield more effect than improving individual components. Similarly, many investigational drugs have more than one drug target and therefore, reducing them to one drug category is an oversimplification.

CFTR correctors & potentiators under investigation

Strategies aimed at correcting the defect in class 2 mutations are extremely important, since they are responsible for the majority of CF disease around the world. Increased CFTR degradation results from mutations leading to protein instability or protein misfolding [26]. The search for small molecules that correct the folding defect was already suggested by Thomas in 1993 and can be helped by structure-based

Table 1. Different classes of *CFTR* mutations.

CFTR mutation class	Main defect	*CFTR* mutations with mainly this dysfunction	Possible therapy
1	Severely defective protein synthesis	Nonsense mutations (e.g., G542X, W1282X, E60X) Large deletions/insertions Frameshift mutations	Nonsense read-through
2	Defective trafficking	F508del, N1303K, G85E, R560T	CFTR correctors
3	Defective gating	G551D S1251N	CFTR potentiator
4	Defective conductance	R117H	
5	Decreased protein synthesis	A455E 3849 + 10 Kb C→T	

CFTR: CF transmembrane conductance regulator.

in vitro virtual screening [26,27]. VRT-325 has been shown to enhance folding of mutant F508del-CFTR in human embryonic kidney cells (HEK 293) [28]. Decreasing histone deacetylase activity to interfere with transcriptional and post-translational regulation of synthesis, folding and trafficking of F508del-CFTR protein is another strategy under evaluation in cell-based assays [29]. Decreasing misfolded protein degradation is, however, the most researched option and has reached the clinical phase of development. Since correct CFTR trafficking is a multistep process, correctors are classified into three groups:

- Nonspecific chemical chaperones;
- Molecules that influence the concentration of or the interaction with, natural chaperones;
- Protein-specific pharmacological chaperones [30].

Since chemical chaperones are nonspecific, they require a high concentration and have limited potential for clinical use. Most pharmacological chaperones are small molecules discovered through high-throughput screening.

VX-809 is a pharmacochaperone that partially restores the defective cellular processing and trafficking of F508del-CFTR so that increased amounts appear at the cell membrane. In *in vitro* cultures, VX-809 could rescue F508del-CFTR [31]. In F508del homozygous adult CF patients (n = 89), the safety and tolerability of VX-809 was documented in a 28-day trial. There was a modest effect on sweat chloride in patients treated with the high drug dose: the mean drop in sweat chloride was 6 mmol/l in patients taking 100 mg a day, and 8 mmol/l in patients taking 200 mg a day [32]. From the modest effect of VX-809 seen in patients so far, either better correctors should be developed, or the combined efficacy of a corrector with a potentiator compound should be evaluated. The tolerability and safety of combining the corrector VX-809 plus the potentiator VX-770 in increasing doses is currently being explored in F508del homozygous subjects.

Other compounds aimed at correcting post-translational modification are under investigation. Among those having reached clinical development is miglustat, the α-1-2 glucosidase inhibitor, that is already used in Gaucher's disease [33]. *In vitro* studies have shown that even low concentrations of this compound decrease calnexin complexes, restore chloride secretion in nasal cell lines and lead to *CFTR* expression in intestinal crypts in F508del mice [34]. Miglustat is thus an example of the second type of correctors, a molecule that interacts with the ER chaperone calnexin. A pilot trial in patients with CF is reported in [103], but the results are not available.

Class 3 'gating' mutations are rare. The most common class 3 mutation G551D occurs in only approximately 3% of CF patients worldwide. Several other mutations with abnormal gating have been identified [35]. The relative frequency of all these mutations combined has not been assessed yet. Excellent results have been obtained in patients with CF carrying the G551D mutation during treatment with the CFTR channel potentiator VX-770. A robust improvement in sweat chloride and nasal CFTR chloride secretion was seen in a Phase II trial, during which patients were exposed to increasing drug doses for 14 days. An additional group of patients was exposed to 150, 250 mg or placebo during 28 days. The improvement in sweat chloride seen was remarkable: several patients normalized their sweat chloride throughout the treatment duration. In the 28-day treatment period, an improvement in forced expiratory volume (FEV_1) in 1 s was seen [36]. The recently performed Phase III trial in patients 12 years and older has equally impressive results: a mean improvement of 10.6% predicted in FEV_1 in a study population with a mean baseline FEV_1 of 63.5% predicted, a mean weight gain of 3.1 kg, a decrease of sweat chloride concentration from a mean of 100 mmol/l to below 60 mmol/l, and a 55% decrease in likelihood of experiencing a pulmonary exacerbation. The intermediate results of the VX-770 trial in 52 children 6–11 years of age demonstrate the same impressive efficacy: a mean improvement in FEV_1 of 12.7% predicted or 17.4% improvement from baseline, double the weight gain compared with the placebo group and a drop in sweat chloride from a mean of 104 to 60 mmol/l [104].

Other small molecules with potential CFTR corrector and/or potentiator activity are under evaluation, such as VX-661, which is expected to start the first phase of clinical evaluation in late 2011. Sildenafil showed some efficacy in a mouse model [37]. The high doses required for correction of F508del-CFTR make it an unlikely candidate for clinical use. However, a clinical evaluation of 10-week duration in adult patients with CF was announced on the clinicaltrials.gov website [103]. Other chaperones that showed promise but failed in subsequent evaluations are curcumin [38] and phenylbutyrate [39]. The compound genistein

has many actions, including CFTR potentiator activity, but not to levels of wild-type CFTR [40]. Pilot clinical trials with curcumin, phenylbutyrate and genistein in combination are reported as completed on the clinicaltrials.gov website [103], but the results have not been published in the scientific literature. High throughput screening of large and diverse chemical libraries for direct activators or stimulators of CFTR has identified different potent compounds – mainly other flavonoids, benzoquinolizinium compounds and phenylglycines – which may in the future reach clinical development. Finally, effort has increased in the search for naturally derived compounds, with the idea to bypass time and cost-intensive validation procedures. For example, vitamin C is under investigation [41].

Suppression of premature nonsense mutations

At the end of a transcript, a termination codon signals the end of protein translation. However, as a result of a mutation, termination codons can also be positioned within the normal gene sequence. These nonsense mutations then cause premature cessation of translation. Small molecules such as aminoglycosides are known to read through premature nonsense mutations by incorporating a random amino acid at the site of the nonsense codon. In this way translation continues to the end of the gene sequence, so that a full length protein is made. Aminoglycosides are potentially nephrotoxic but were important as proof of concept. Ataluren, formerly named PTC124, resulted from the search for small molecules with similar action.

After successful Phase I and II programs, ataluren is now being evaluated in a large, worldwide Phase III study. In adult CF patients carrying at least one nonsense mutation and having an FEV_1 above 40% predicted, open-label administration of ataluren over 14 days was associated with a significant improvement in CFTR chloride transport measured in the nasal epithelium [21]. The same improvement in chloride transport was documented in children with CF carrying at least one nonsense codon mutation and the effect was seen with a low dose, as well as with a high dose of ataluren [42]. A subset of Israeli patients (n = 19) took ataluren for a period of 12 weeks. This chronic intake was well tolerated. It was associated with a sustained improvement in nasal chloride secretion and a trend towards improvement in lung function [43]. Soon it will be clear whether ataluren provides a significant clinical benefit. The results of the currently running Phase III study are awaited in early 2012: more than 200 patients carrying at least one nonsense mutation are included in this double-blind evaluation of ataluren, an oral drug taken three-times a day. The study duration is 48 weeks, followed by an open-label extension of 48 weeks. The primary outcome is FEV_1 measured at week 48. Multiple secondary outcomes are being evaluated, such as the occurrence of pulmonary exacerbations, cough frequency measured by Respimat®, chest computed tomography score and quality of life. In addition to close monitoring of safety, exploratory biomarkers are also measured, such as chloride secretion in the nose, in the sweat gland and parameters of inflammation in the blood [103].

Activators of alternative chloride channels

Other chloride channels than CFTR are known to contribute to mucosal chloride fluxes. The Ca^{2+}-activated chloride channel was discovered by serendipity, around the time the *CFTR* gene was identified. In subjects with CF, superfusion of the nasal mucosa with extracellular nucleotides resulted in nasal chloride secretion [44]. When it later became apparent that CFTR protein is absent on the nasal mucosa of subjects with CF, the search for these alternative chloride channels and their therapeutic potential was launched. Classic purinergic stimulators, such as ATP provoke bronchosconstriction and have a short half-life; they are thus not ideal drug candidates [44]. Denufosol was shown to stimulate alternative chloride secretion. In the first Phase III trial in subjects with mild lung disease, the drug was shown to have a small but sustained positive effect on lung function [45]. Unfortunately, these results were not reproduced in the second Phase III study with denufosol [105]. It is unclear whether this is as result of incomplete understanding of the contribution of alternative chloride channel function to overall mucosal chloride transport in CF, to insufficient potency of the drug, or to trial design failure. The latter is indeed not impossible, since showing clinical improvement in a cohort of CF subjects with mild disease is a major challenge.

Duramycin or Moli1901, a polycyclic peptide with antibiotic properties, was thought to stimulate chloride secretion via the Ca^{2+} activated chloride channel. However, in human bronchial epithelial cell cultures Moli1901 did not function as a specific stimulator of alternative chloride channels [46]. Aspecific membrane

changes, induced by different concentrations of Moli1901, seemed to explain the drug's variable effect on epithelial chloride secretion.

In 2008, the TMEM16A protein was found to be the alternative Ca^{2+}-activated chloride channel [47,48]. Further knowledge of its expression and function will elucidate whether the strategy of stimulating alternative chloride channels in patients with CF has therapeutic potential. And if so, at what level of disease severity: as a preventative strategy in subjects with mild lung disease, or as aid in rescue in subjects with advanced disease.

In the human airway, still other chloride channels, such as the CIC-2, a member of the pH- and voltage-activated chloride channels, are expressed [49]. Lubiprostone, one of several novel prostones, prostaglandin E-derived molecules that target the CIC-2 channels, is approved by the US FDA for the treatment of idiopathic constipation [50]. The drug might have promise in CF, since CIC-2 is overexpressed in immortalized CF cell lines [51] and application of lubiprostone in the CF murine nasal airway, improves chloride secretion [52].

Modulation of ENaC

Even if ENaC is not primarily involved in the CF pathophysiology, manipulation of ENaC function may have therapeutic potential [53]. Together with CFTR, ENaC is a major player in epithelial ion fluxes. Therefore, decreasing sodium absorption has the theoretical potential of increasing airway hydration. The disease pseudohypoaldosteronism may even be seen as an important 'proof-of-concept'. In this disease, loss of ENaC function leads to increased airway fluid secretion and is associated with recurrent airway infection and airway obstruction [54]. In addition, several data link ENaC overexpression to CF-like lung disease such as in the β-ENaC-subunit overexpressing mouse model [55]. Also, further data in humans point towards a role for ENaC, since ENaC mutations have been identified in subjects with CF-like disease [56,57].

A first clinical trial in CF using the ENaC blocker amiloride did not show a therapeutic benefit [58]. The explanation may be that amiloride has a short-lived action. The data in ENaC CF mice [55] stimulated renewed interest in exploring this therapeutic venue, especially since a long acting ENaC blocker has been identified [59]. Another route being explored, is blocking ENaC function by epithelial transfection with antisense oligonucleotides [60].

Challenges in developing disease-modifying therapies

Although patients with CF die from respiratory disease, CF is a multisystem disorder. Therefore, to correct CF, systemic therapy has more promise than inhaled therapies only targeting the airways. The reverse side of the coin will be increased risk of toxicity. For corrector compounds, the question is whether there will be long-term safety and specificity issues through interference with the ER quality control mechanism. Also for premature nonsense mutation read-through, this same issue of specificity and lack of long-term toxicity is crucial.

What should be expected from disease-modifying therapies?

The dysfunction associated with F508del-CFTR is complex; there are redundant ubiquitination pathways in the ER, the mutant protein if rescued has decreased membrane stability and there is increased peripheral lysosomal degradation [61]. Therefore, interfering with one degradation system might thus be insufficient. Interfering with the proteasome function rather than the ER trafficking has therefore been suggested [62]. There is also ongoing discussion whether, to have a true impact on the CF disease course, it is sufficient to improve chloride secretion or whether other basic cell defects, such as the inherent proinflammatory state linked to the abnormal protein trafficking, should be corrected as well [62].

The strategy of activating alternative chloride channels or suppressing ENaC function has the theoretical advantage of applicability in all subjects with CF. CFTR correctors, CFTR potentiators and suppressors of premature nonsense mutations are indeed prime examples of individualized therapy. Although all of these compounds are under study in subjects with CF, each compound is only indicated in a subset of patients carrying specific *CFTR* mutations. The fact that many subjects carry two different *CFTR* mutations often belonging to different mutation classes adds an extra layer of complexity. A patient who is double heterozygote for a nonsense mutation and mutation F508del is, at least in theory, a candidate for combination therapy with all three drug classes. Unless treatment of one mutation leads to sufficient correction of *CFTR* activity, the safety and tolerability, as well as the added or synergistic efficacy of combination therapy should be evaluated. Compounds with dual corrector and potentiator activity (called CoPo) belonging to the cyanoquinolone class have been detected

[63]. If sufficiently effective, CoPo compounds might simplify this issue. With more than 1800 *CFTR* mutations described, gaining insight in the relative potency of correctors, potentiators and CoPo compounds on different *CFTR* mutations in the same mutation class is needed.

The current slow rate of lung disease progression of only 1–2% FEV_1 per year documents the relative success of intensive patient management, but is a drawback for proving clinical benefit in Phase III clinical trials [64]. Large patient numbers are needed when these outcomes are used [64]. For mutation-specific therapies these patient numbers are often not available. Hence, the substitution of FEV_1 and markers of clinical benefit (pulmonary exacerbations, quality of life) as outcome parameter by biomarkers of CFTR function (i.e., sweat chloride, nasal potential difference measurement) should be considered. Safety and efficacy can be followed up during Phase IV pharmacovigilance studies. This possible strategy is also discussed in the EMA guideline for clinical trials in small populations [106].

In addition, targeting the right patient cohort for disease-modifying therapies is critical: it is not certain that patients with advanced disease and widespread bronchiectasis will benefit sufficiently. In a β-ENaC overexpressing mouse model, the sodium channel blocker amiloride slowed development of lung disease when administered in newborn mice, but not when administered to mice with established lung disease [65]. In that respect the first results from VX-770 surprisingly showed approximately equal benefit in patients with mild versus advanced CF lung disease [66].

Lastly, factors other than the specific mutation determine the CF disease severity [11,12]. It is likely that genetic differences between subjects in drug handling or differences in intermediate cell processes, such as nonsense mediated mRNA decay will influence drug efficacy [21].

Executive summary

The disease cystic fibrosis

- Cystic fibrosis (CF) is a life-shortening multisystem disease occurring all over the world, but the incidence is higher in the Caucasian population.
- Each patient with CF has two CF-causing mutations, one on each CF transmembrane conductance regulator (*CFTR*) gene.
- A minority of subjects with two *CFTR* mutations have milder, partial or even no disease.
- In patients with CF, respiratory insufficiency is the main cause of death.

CF pathophysiology

- Cystic fibrosis is an ion channel disease, caused by absence or dysfunction of the apical CFTR chloride channel.
- At present it is less clear if loss of CFTR function also causes increased function of epithelial sodium channel (ENaC).
- In the CF lung, decreased airway liquid and impaired mucociliary clearance lead to chronic infection.
- The current CF treatments decrease disease progression but do not address the basic pathophysiology.
- CF treatments under development include ion channel regulators: to correct CFTR function, to stimulate alternative chloride channels or to decrease ENaC function.

CFTR *mutation classes*

- *CFTR* mutations are grouped according to how mutations disrupt the translation from gene to protein.
- Many *CFTR* mutations have characteristics of more than one mutation class.

CFTR correctors & potentiators under investigation

- CFTR correctors promote normal trafficking of mutant CFTR.
- CFTR potentiators increase channel opening.
- The potent CFTR potentiator VX-770 is of proven clinical benefit for subjects with CF carrying at least one G551D mutation.
- Correcting abnormal CFTR trafficking has proven more difficult than correcting abnormal CFTR gating by CFTR potentiators.

Suppression of premature nonsense mutations

- Suppressors of nonsense mutations over-read premature nonsense mutations.
- Ataluren improved nasal chloride secretion in Phase II clinical trials and is at present being evaluated for clinical efficacy in a Phase III trial.
- The long known Ca^{2+}-activated alternative chloride channel has been identified as the TMEM16A protein.

Activators of alternative chloride channels

- Denufosol is an activator of the alternative chloride channel but failed to consistently demonstrate a clinical benefit during Phase III clinical trials.
- CIC-2 chloride channels are also being explored as a therapeutic target.

Modulation of ENaC

- Together with CFTR, ENaC is the major determinant of epithelial ion fluxes.
- Blocking sodium absorption via ENaC should theoretically improve CF airway hydration.
- An effective and safe long-acting ENaC blocker has not yet been developed.

Conclusion

Cystic fibrosis is a disease marked by impaired epithelial chloride secretion through the CFTR channel. Traditionally it is also thought to be associated with increased sodium absortion via ENaC. The latter is, at present, somewhat debated. The primary therapeutic goal is restoration of CFTR chloride secretion. Much is known about how specific *CFTR* mutations disrupt the normal transition from gene to CFTR protein. Therefore, *CFTR* mutation class specific therapies are being developed as a new form of individualized treatment aimed at correcting the chloride transport defect. As expected, correcting abnormal CFTR trafficking proves harder than activating CFTR present at the cell membrane. The CFTR potentiator VX-770 has proven efficacy for the class 3 mutation G551D. Combined treatment with CFTR correctors and potentiators, and treatment with suppressors of nonsense mutations are under evaluation for patients with other CFTR mutations. Given the successes obtained so far, many high-throughput screening programs aim at finding new, additional or better potentiators and correctors for defective CFTR [67,68].

If the CFTR chloride transport cannot be restored, alternative strategies consist of stimulation of chloride secretion via alternative chloride channels or blocking ENaC function. Apart from denufosol, a drug that failed to show consistent clinical efficacy during the last phase of its clinical development, these alternative approaches are only slowly progressing to the clinical phase of development.

Future perspective

In the next 5–10 years, it will become clear whether activating CFTR at the cell membrane is sufficient to greatly improve the course of CF lung disease and whether other disease manifestations are affected, such as progression to CF-related diabetes. It will become evident whether gene therapy for CF is successful. If not, now that a potent CFTR potentiator has been identified, the search for small molecules that function as CFTR correctors will intensify even further. Improved knowledge of the basic physiology of the alternative chloride channel TMEM16A is needed to estimate whether or not development of stimulators of alternative chloride channels have potential in CF therapy. The importance of ENaC in early and late stage CF lung disease will be clarified. Even if ENaC dysfunction is not primary in CF, development of potent, long-acting ENaC blockers should be a valid treatment option to improve airway hydration in CF.

Financial & competing interests disclosure

C De Boeck has been a consultant for Galapagos, ABlynx and Janssens pharma, a menber of advisory boards for Vertex, Gilead, Pharmaxis, Boehringer Ingelh, Baxter, Bayer, Biovitrum, Inspire and PTC, and has worked as an investigator/principal investigator for several companies. H Cuppens has a research collaboration with Galapagos. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Proesmans M, Vermeulen F, de Boeck K. What's new in cystic fibrosis? From treating symptoms to correction of the basic defect. *Eur. J. Pediatr.* 167(8), 839–849 (2008).

▪▪ **Overview of most aspects of cystic fibrosis.**

2 Buzzetti R, Salvatore D, Baldo E *et al.* An overview of international literature from cystic fibrosis registries: 1. Mortality and survival studies in cystic fibrosis. *J. Cyst. Fibros.* 8(4), 229–237 (2009).

3 Bobadilla JL, Macek M Jr, Fine JP, Farrell PM. Cystic fibrosis: a worldwide analysis of CFTR mutations-correlation with incidence data and application to screening. *Hum. Mutat.* 19(6), 575–606 (2002).

4 Munck A, Dhondt JL, Sahler C, Roussey M. Implementation of the French nationwide cystic fibrosis newborn screening program. *J. Pediatr.* 153(2), 228e1–233e1 (2008).

5 De Boeck K, Wilschanski M, Castellani C *et al.* Cystic fibrosis: terminology and diagnostic algorithms. *Thorax* 61(7), 627–635 (2006).

6 Borowitz D, Robinson KA, Rosenfeld M *et al.* Cystic fibrosis foundation evidence-based guidelines for management of infants with cystic fibrosis. *J. Pediatr.* 155(Suppl. 6), S73–S93 (2009).

7 Goss CH, Burns JL. Exacerbations in cystic fibrosis. 1: epidemiology and pathogenesis. *Thorax* 62(4), 360–367 (2007).

8 Goubau C, Wilschanski M, Skalicka V *et al.* Phenotypic characterisation of patients with intermediate sweat chloride values: towards validation of the European diagnostic algorithm for cystic fibrosis. *Thorax* 64(8), 683–691 (2009).

9 Kerem BS, Rommens JM, Buchanan JA *et al.* Identification of the cystic fibrosis gene: genetic analysis. *Science* 245, 1073–1080 (1989).

10 Bombieri C, Claustres M, De Boeck K *et al.* Recommendations for the classification of diseases as CFTR-related disorders. *J. Cyst. Fibros.* 10(Suppl. 2), S86–S102 (2011).

11 Collaco JM, Blackman SM, McGready J, Naughton KM, Cutting GR. Quantification of the relative contribution of environmental and genetic factors to variation in cystic fibrosis lung function. *J. Pediatr.* 157(5), 802–807 e1–e3 (2010).

12 Collaco JM, Vanscoy L, Bremer L *et al.* Interactions between secondhand smoke and genes that affect cystic fibrosis lung disease. *JAMA* 299(4), 417–424 (2008).

13 Riordan JR, Rommens JM, Kerem BS *et al.* Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 245, 1066–1073 (1989).

14 Mall M, Bleich M, Greger R, Schreiber R, Kunzelmann K. The amiloride-inhibitable Na^+ conductance is reduced by the cystic fibrosis transmembrane conductance regulator in normal but not in cystic fibrosis airways. *J. Clin. Invest.* 102(1), 15–21 (1998).

15 De Boeck K, Derichs N, Fajac I *et al*. New clinical diagnostic procedures for cystic fibrosis in Europe. *J. Cyst. Fibros.* 10(Suppl. 2), S53–S66 (2011).

16 Chen JH, Stoltz DA, Karp PH *et al.* Loss of anion transport without increased sodium absorption characterizes newborn porcine cystic fibrosis airway epithelia. *Cell* 143(6), 911–923 (2010).

17 Moskwa P, Lorentzen D, Excoffon KJ *et al.* A novel host defense system of airways is defective in cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 175(2), 174–183 (2007).

▪ **An inactive oxidative antimicrobial system is described as an important cellular and molecular basis for defective cystic fibrosis (CF) airway immunity.**

18 Jacquot J, Tabary O, Le Rouzic P, Clement A. Airway epithelial cell inflammatory signalling in cystic fibrosis. *Int. J. Biochem. Cell Biol.* 40(9), 1703–1715 (2008).

19 Hwang TC, Sheppard DN. Gating of the CFTR Cl^- channel by ATP-driven nucleotide-binding domain dimerisation. *J. Physiol.* 587(Pt 10), 2151–2161 (2009).

20 Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 73, 1251–1254 (1993).

21 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* 372(9640), 719–727 (2008).

22 Kerem B, Rommens JM, Buchanan JA *et al.* Identification of the cystic fibrosis gene: genetic analysis. *Science* 245(4922), 1073–1080 (1989).

23 McKone EF, Emerson SS, Edwards KL, Aitken ML. Effect of genotype on phenotype and mortality in cystic fibrosis: a retrospective cohort study. *Lancet* 361(9370), 1671–1676 (2003).

24 Thauvin-Robinet C, Munck A, Huet F *et al.* The very low penetrance of cystic fibrosis for the R117H mutation: a reappraisal for genetic counselling and newborn screening. *J. Med. Genet.* 46(11), 752–758 (2009).

25 Belcher CN, Vij N. Protein processing and inflammatory signaling in cystic fibrosis: challenges and therapeutic strategies. *Curr. Mol. Med.* 10(1), 82–94 (2010).

26 Thomas PJ, Pedersen PL. Effects of the δ F508 mutation on the structure, function, and folding of the first nucleotide-binding domain of CFTR. *J. Bioenerg. Biomembr.* 25(1), 11–19 (1993).

27 Kalid O, Mense M, Fischman S *et al.* Small molecule correctors of F508del-CFTR discovered by structure-based virtual screening. *J. Comput. Aided Mol. Des.* 24(12), 971–991 (2010).

28 Wang Y, Loo TW, Bartlett MC, Clarke DM. Modulating the folding of P-glycoprotein and cystic fibrosis transmembrane conductance regulator truncation mutants with pharmacological chaperones. *Mol. Pharmacol.* 71(3), 751–758 (2007).

▪ **Shows that the pharmacological chaperone VRT-325 enhances proper folding of F508del-CFTR by promoting proper insertion of the transmembrane domains, and not by promoting dimerization between the two nucleotide binding domains.**

29 Hutt DM, Herman D, Rodrigues AP *et al.* Reduced histone deacetylase 7 activity restores function to misfolded CFTR in cystic fibrosis. *Nat. Chem. Biol.* 6(1), 25–33 (2010).

30 Amaral MD, Kunzelmann K. Molecular targeting of CFTR as a therapeutic approach to cystic fibrosis. *Trends Pharmacol. Sci.* 28(7), 334–341 (2007).

▪▪ **Comprehensive information on cystic fibrosis transmembrane conductance regulator correctors and potentiators.**

31 Van Goor F, Hadida S, Grootenhuis PDJ *et al.* Rescue of the protein folding defect in cystic fibrosis *in vitro* by the investigational small molecule, VX-809. *J. Cys. Fibros.* 9(Suppl. 1), S14–S14 (2010).

32 Clancy JP, Spencer-Green G. Clinical evaluation of VX-809, a novel investigational oral F508del-CFTR corrector, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation. *J. Cys. Fibros.* 9(Suppl. 1), S20–S20 (2010).

33 Benito JM, Garcia Fernandez JM, Ortiz Mellet C. Pharmacological chaperone therapy for Gaucher disease: a patent review. *Expert Opin. Ther. Pat.* 21(6), 885–903 (2011).

34 Noel S, Wilke M, Bot AG, De Jonge HR, Becq F. Parallel improvement of sodium and chloride transport defects by miglustat (*N*-butyldeoxynojyrimicin) in cystic fibrosis epithelial cells. *J. Pharmacol. Exp. Ther.* 325(3), 1016–1023 (2008).

▪ **This study concludes that miglustat corrects both defective Cl^- and Na^+ transports in CF patients and therefore shows the potential for a miglustat-based CF treatment.**

35 Van Goor F, Yu H, Burton B *et al.* Investigational compound VX-770 potentiated multiple CFTR channel gating mutants in vitro. *J. Cys. Fibros.* 10(Suppl. 1), S16–S16 (2011).

36 Accurso FJ, Rowe SM, Clancy JP *et al.* Effect of VX-770 in persons with cystic fibrosis and the G551D-CFTR mutation. *N. Engl. J. Med.* 363(21), 1991–2003 (2010).

▪▪ **Exceptional results obtained with VX-770 in Phase II trial.**

37 Lubamba B, Lebacq J, Reychler G *et al.* Inhaled phosphodiesterase type 5 inhibitors restore chloride transport in cystic fibrosis mice. *Eur. Respir. J.* 37(1), 72–78 (2011).

38 Mall M, Kunzelmann K. Correction of the CF defect by curcumin: hypes and disappointments. *BioEssays* 27(1), 9–13 (2005).

39 Singh OV, Pollard HB, Zeitlin PL. Chemical rescue of d F508-CFTR mimics genetic repair in cystic fibrosis bronchial epithelial cells. *Mol. Cell Proteomics* 7(6), 1099–1110 (2008).

40 Yu YC, Miki H, Nakamura Y *et al.* Curcumin and genistein additively potentiate G551D-CFTR. *J. Cyst. Fibros.* 10(4), 243–252 (2011).

41 Fischer H, Schwarzer C, Illek B. Vitamin C controls the cystic fibrosis transmembrane conductance regulator chloride channel. *Proc. Natl Acad. Sci. USA* 101(10), 3691–3696 (2004).

42 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.* 182(10), 1262–1272 (2010).

▪ **Exceptional results obtained with ataluren with respect to activity, safety and pharmacokinetics for treatment of CFTR nonsense mutations.**

43 Wilschanski M, Miller LL, Shoseyov D *et al.* Chronic ataluren (PTC124) treatment of nonsense mutation cystic fibrosis. *Eur. Respir. J.* 38(1), 59–69 (2011).

▪ **Exceptional results obtained with ataluren with respect to activity, safety and pharmacokinetics for treatment of CFTR nonsense mutations.**

44 Knowles MR, Clarke LL, Boucher RC. Activation by extracellular nucleotides of chloride secretion in the airway epithelia of patients with cystic fibrosis. *N. Engl.J. Med.* 325(8), 533–538 (1991).

45 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.* 183(5), 627–634 (2011).

46 Oliynyk I, Varelogianni G, Roomans GM, Johannesson M. Effect of duramycin on chloride transport and intracellular calcium

concentration in cystic fibrosis and non-cystic fibrosis epithelia. *APMIS* 118(12), 982–990 (2010).

47 Yang YD, Cho H, Koo JY *et al.* TMEM16A confers receptor-activated calcium-dependent chloride conductance. *Nature* 455(7217), 1210–1215 (2008).

48 Rock JR, O'Neal WK, Gabriel SE *et al.* Transmembrane protein 16A (TMEM16A) is a Ca^{2+} regulated Cl^- secretory channel in mouse airways. *J. Biol. Chem.* 284(22), 14875–14880 (2009).

49 Anderson MP, Sheppard DN, Berger HA, Welsh MJ. Chloride channels in the apical membrane of normal and cystic fibrosis airway and intestinal epithelia. *Am. J. Physiol.* 263(Pt 1), L1–L14 (1992).

50 Lacy BE, Campbell Levy L. Lubiprostone: a chloride channel activator. *J. Clin. Gastroenterol.* 41(4), 345–351 (2007).

51 Schwiebert EM, Cid-Soto LP, Stafford D *et al.* Analysis of ClC-2 channels as an alternative pathway for chloride conduction in cystic fibrosis airway cells. *Proc. Natl Acad. Sci. USA* 95(7), 3879–3884 (1998).

52 MacDonald KD, McKenzie KR, Henderson MJ, Hawkins CE, Vij N, Zeitlin PL. Lubiprostone activates non-CFTR-dependent respiratory epithelial chloride secretion in cystic fibrosis mice. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 295(5), L933-L940 (2008).

53 Mall MA. Role of cilia, mucus, and airway surface liquid in mucociliary dysfunction: lessons from mouse models. *J. Aerosol Med. Pulm. Drug Deliv.* 21(1), 13–24 (2008).

54 Kerem E, Bistritzer T, Hanukoglu A *et al.* Pulmonary epithelial sodium-channel dysfunction and excess airway liquid in pseudohypoaldosteronism. *N. Engl. J. Med.* 341(3), 156–162 (1999).

55 Mall M, Grubb BR, Harkema JR, O'Neal WK, Boucher RC. Increased airway epithelial Na^+ absorption produces cystic fibrosis-like lung disease in mice. *Nat. Med.* 10(5), 487–493 (2004).

56 Groman JD, Meyer ME, Wilmott RW, Zeitlin PL, Cutting GR. Variant cystic fibrosis phenotypes in the absence of CFTR mutations. *N. Engl. J. Med.* 347(6), 401–407 (2002).

57 Azad AK, Rauh R, Vermeulen F *et al.* Mutations in the amiloride-sensitive epithelial sodium channel in patients with cystic fibrosis-like disease. *Hum. Mutat.* 30(7), 1093–1103 (2009).

58 Pons G, Marchand MC, d'Athis P *et al.* French multicenter randomized double-blind placebo-controlled trial on nebulized amiloride in cystic fibrosis patients. The amiloride-AFLM collaborative study group. *Pediatr. Pulmonol.* 30(1), 25–31 (2000).

59 Hirsh AJ, Zhang J, Zamurs A *et al.* Pharmacological properties of *N*-(3,5-diamino-6-chloropyrazine-2-carbonyl)-*N′*-4-[4-(2,3-dihydroxypropoxy) phenyl] butyl-guanidine methanesulfonate (552–502), a novel epithelial sodium channel blocker with potential clinical efficacy for cystic fibrosis lung disease. *J. Pharmacol. Exp. Ther.* 325(1), 77–88 (2008).

60 Sobczak K, Bangel-Ruland N, Semmler J, Lindemann H, Heermann R, Weber WM. Antisense oligonucleotides for therapy of cystic fibrosis. Inhibition of sodium absorption mediated by ENaC in nasal epithelial cells. *HNO* 57(11), 1106–1112 (2009).

61 Okiyoneda T, Barriere H, Bagdany M *et al.* Peripheral protein quality control removes unfolded CFTR from the plasma membrane. *Science* 329(5993), 805–810 (2010).

62 Belcher CN, Vij N. Protein processing and inflammatory signaling in cystic fibrosis: challenges and therapeutic strategies. *Curr. Mol. Med.* 10(1), 82–94 (2011).

63 Phuan PW, Yang B, Knapp JM *et al.* Cyanoquinolines with independent corrector and potentiator activities restore dphe508-cystic fibrosis transmembrane conductance regulator chloride channel function in cystic fibrosis. *Mol. Pharmacol.* 80(4), 683–693 (2011).

64 Konstan MW, Wagener JS, Yegin A, Millar SJ, Pasta DJ, VanDevanter DR. Design and powering of cystic fibrosis clinical trials using rate of FEV_1 decline as an efficacy endpoint. *J. Cyst. Fibros.* 9(5), 332–338 (2010).

65 Zhou Z, Treis D, Schubert SC *et al.* Preventive but not late amiloride therapy reduces morbidity and mortality of lung disease in β-ENaC-overexpressing mice. *Am. J. Respir. Crit. Care Med.* 178(12), 1245–1256 (2008).

66 Elborn S. Oral communication ECFS conference June 2011, Late brake science. (2011).

67 Fischer D, Tessari M, Roseboom M *et al.* Identification and validation of novel drug targets to treat cystic fibrosis. *Pediatr. Pulmonol.* 43(S31), 213 (2008).

68 Carlile GW, Robert R, Zhang D *et al.* Correctors of protein trafficking defects identified by a novel high-throughput screening assay. *Chembiochem.* 8(9), 1012–1020 (2007).

Websites

101 The WHO. The molecular genetic epidemiology of cystic fibrosis. Report of a joint meeting of WHO/ECFTN/ICF(M)A/ECFS www.who.int/genomics/publications/en/ & WHO Geneva, Human Genetics Programme WHO/HGN/CF/WG/04.02

102 Cystic fibrosis mutation database www.genet.sickkids.on.ca/GenomicDnaSequencePage.html

103 Clinical trials registry www.clinicaltrials.gov

104 Vertex pharmaceuticals PR. Interim Phase II data showed a combination of VX-770 and VX-809 improved function of the defective protein that causes cystic fibrosis in people with the most common form of the disease http://investors.vrtx.com/releasedetail.cfm?releaseid=583683

105 Inspire Pharmaceuticals Inc PR. Inspire announces corporate restructuring and strategic focus on eye care business www.faqs.org/sec-filings/110217/INSPIRE-PHARMACEUTICALS-INC_8-K/dex992.htm#ixzz1SBPxYnXV

106 EMA. Guideline on clinical trials in small populations www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003615.pdf