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## New horizons for cystic fibrosis treatment

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## ABSTRACT

Cystic fibrosis is an inherited multi-system disease associated with chronic lung infection, malabsorption, salt loss syndromes, male infertility and leading to numerous comorbidities. The landscape in cystic fibrosis care has changed markedly with currently more adult patients than children in many countries. Over 2000 different mutations in the *CFTR* gene have been reported and the majority are extremely rare. Understanding how *CFTR* mutations translate to disturbed synthesis or function of the CFTR protein has opened the way to 'personalized' treatments to correct the basic defect. The first 2 drugs have reached the clinic: a CFTR potentiator to augment CFTR channel function, and the combination of this potentiator with a corrector to increase CFTR expression at the cell membrane. To obtain robust correction of CFTR expression at the cell membrane, combinations of correctors with additive efficacy are under investigation. Other mutation type-specific treatments under clinical investigation are premature stop codon-read through drugs and antisense oligonucleotides that correct the basic defect at the mRNA level. Restoring the defective gene by gene editing can already be achieved ex vivo. Mutation agnostic treatments are explored as well: stabilizing CFTR expression at the cell membrane, circumventing the CFTR channel by blocking or activating other ion channels, and gene therapy. Combinations of these therapies can be anticipated. The pipeline of corrective strategies under clinical investigation is increasing continuously and a rising number of pharmaceutical companies are entering the field.

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## Contents

1. Introduction . . . . .	0
2. Clinical manifestations and current symptomatic treatments . . . . .	0
3. Basic defects in CF and theoretical approach to correct them . . . . .	0
4. Modulating the CFTR protein is possible: the potentiator ivacaftor . . . . .	0
5. Further improvements are needed in CFTR correction: more potent correctors or corrector combinations . . . . .	0
6. Other pharmacological strategies under investigation . . . . .	0
7. Strategies using oligonucleotides to treat at the <i>CFTR</i> gene or mRNA level . . . . .	0
8. Challenges to correct the basic defect in every patient with cystic fibrosis . . . . .	0
9. Conclusion . . . . .	0
Conflict of interest statement . . . . .	0
References . . . . .	0

## 1. Introduction

Cystic fibrosis (CF) is the most common life-shortening genetic disease in the caucasian population, affecting approximately 75,000 individuals worldwide (Farrell, 2008). It is an autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) (Riordan et al., 1989). The *CFTR* gene encodes the CFTR protein which is a chloride channel expressed in

Abbreviations: CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; MSD, membrane-spanning domain; NBD, nucleotide-binding domain; FEV1, forced expiratory volume in 1 s.

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many epithelial cells. CF is a multi-system disease affecting organs and tissues where CFTR is expressed. The most common clinical features are exocrine pancreatic insufficiency and bronchiectasis with chronic airway infection leading to respiratory failure and premature death. Current treatments are mainly symptomatic focusing on compensating for exocrine pancreatic insufficiency with pancreatic enzymes, and slowing lung disease progression with airway clearance techniques and antibiotic therapy (Cohen-Cymberknoh, Shoseyov, & Kerem, 2011). New therapies aiming at treating the downstream complications of CFTR dysfunction are in development including novel inhaled antibiotics, anti-inflammatory drugs, agents to enhance mucociliary clearance and nutritional/pancreatic replacement therapies. But since 2012, two new drugs called CFTR modulators with the aim of restoring CFTR protein function have become available, and several other CFTR modulators are in development. In this review, we will focus on these CFTR modulators that are likely to dramatically change CF care and prognosis over the coming decades. However, drugs allowing better restoration of CFTR function are still needed and development of these disease-modifying drugs for all patients with CF especially those with rare mutations is challenging.

## 2. Clinical manifestations and current symptomatic treatments

CF-related symptoms appear throughout life, with great overlap and variability of symptoms and timing from patient to patient. The main clinical features of CF reflect gastrointestinal and respiratory symptoms. Gastrointestinal symptoms are mainly due to pancreatic insufficiency. Typical signs are greasy stools and poor weight gain. Pancreatic insufficiency leads to steatorrhea, fat-soluble-vitamin deficiency and malnutrition (Gelfond & Borowitz, 2013). Seventy years ago, when CF was described, children died of malnutrition. When pancreatic replacement enzyme therapy became available in the 1950s, CF prognosis began to change. CF lung disease is now the major cause of morbidity and mortality. It is characterised by chronic airway infection and inflammation leading to bronchiectasis. Daily symptoms as disease progresses are cough and sputum production. Patients with CF develop bacterial infections which can be cleared initially with antibiotic therapy. Later, persistent bacterial infection of the airways occurs. One of the most frequent pathogens isolated in the CF airways is *Pseudomonas aeruginosa* and infection with *P. aeruginosa* is associated with a worse prognosis (Lund-Palau et al., 2016). Intermittent episodes of acute worsening of respiratory symptoms called pulmonary exacerbations are treated by intensification of daily therapies and antibiotics in order to restore the lung function commonly lost during an exacerbation. Other pathogens such as *Staphylococcus aureus* including Methicillin-Resistant *S. aureus*, Gram-negative bacterial species, non-tuberculous mycobacteria or *Aspergillus* species may also be isolated from the airways. Acute respiratory complications such as pneumothorax or hemoptysis may occur. Chronic lung infection and airway obstruction lead to a progressive decline in airway function and respiratory failure which is the main cause of death. CF is a multiorgan disease and many comorbidities may occur such as salt loss syndromes, diabetes, gall stones, cholangiectasis, cirrhosis, chronic sinusitis and nasal polyps, bone disease or infertility.

The main principles of CF treatment were established as early as the 1960s, and steadily evolved with the better understanding of the disease and the availability of new drugs. They are based on a holistic approach to care and intensive symptomatic treatment (Cohen-Cymberknoh et al., 2011). Specialised CF centers formed by a multidisciplinary team experienced in CF have become the model of care for patients with CF (Conway et al., 2014). The principles of symptomatic treatment are maintenance of good nutrition, enhancement of mucociliary clearance, prevention and aggressive treatment of pulmonary infection, treatment of airway inflammation, and early identification and treatment of complications. CF has benefited from the development of new and effective treatments such as antibiotics against *P. aeruginosa*, including inhaled antibiotics. As a result of this structured

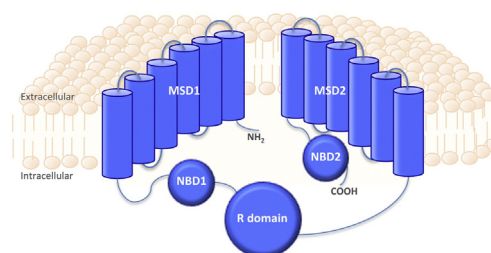
follow-up in dedicated centers and implementation of aggressive and complex treatments, projected life expectancy for patients with CF has increased from a matter of months to nearly 50 years (MacKenzie et al., 2014). Similarly, in several countries the number of adults with CF is currently larger than the number of children with CF (Burgel et al., 2015). However, the expected survival of a child born today with CF is still only 50 years and the current median age of death is around 30 years with respiratory failure being the common cause of death (ECFS Patient Registry Report 2013, available: <https://www.ecfs.eu/projects/ecfs-patient-registry/annual-reports>).

## 3. Basic defects in CF and theoretical approach to correct them

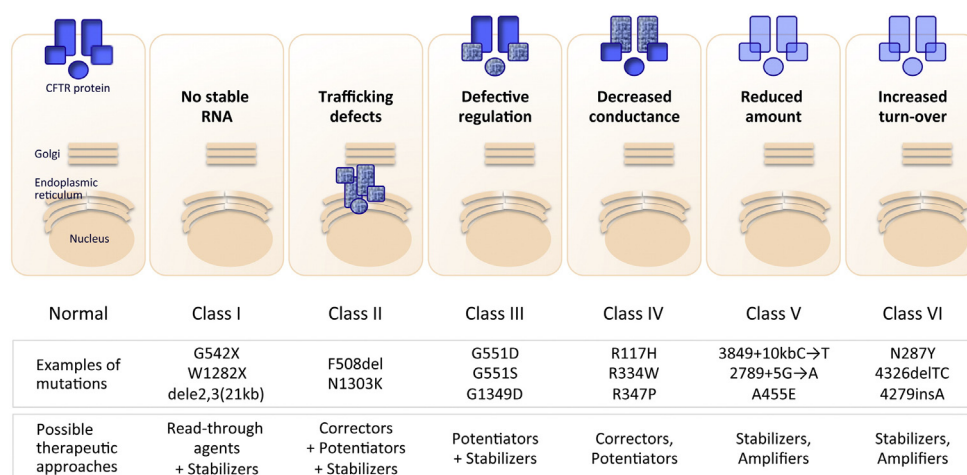
Cystic fibrosis is caused by mutations in the *CFTR* gene which was identified in 1989. It comprises 27 coding exons, spanning over 250 kb on chromosome 7 (Riordan et al., 1989). It encodes the CFTR protein which is an anion channel of primary importance for chloride and bicarbonate transport. The CFTR protein is expressed at the apical membrane of many epithelial cells with direct relationships between abnormal expression and CF pathology. When open or activated, it allows passive diffusion of chloride ions down their electrochemical gradient. It has also many other roles such as inhibition of sodium transport through the epithelial sodium channel and regulation of other chloride channels. It is also thought to interact with cellular pathways related to inflammation (Cohen & Prince, 2012; Stoltz, Meyerholz, & Welsh, 2015). The CFTR protein is a member of the ATP-binding cassette (ABC) protein superfamily. It is characterised by 2 membrane-spanning domains (MSD1 and MSD2) which anchor the protein in the plasma membrane. Each is conjoined to a nucleotide binding domain (NBD1 and NBD2) which binds and hydrolyses ATP. Unique to CFTR is a regulatory domain which has to be phosphorylated by the cAMP-dependent protein kinase for channel gating by ATP (Fig. 1) (for review: (Hwang & Kirk, 2013).

To date, around 2000 *CFTR* mutations have been described. However, a molecular alteration in the DNA sequence does not equal a potential defect in expression or function of the protein product. Around 250 variants have evidence supporting a disease-causing effect (Castellani et al., 2008; Sosnay et al., 2013). Mutations in the *CFTR* gene have been grouped into six classes according to their effects on the maturation and function of the CFTR protein (Welsh & Smith, 1993; Zielenski & Tsui, 1995) (Fig. 2):

- I. Class I mutations group mutations that result in no protein production. They comprise premature stop codon mutations (e.g. G542X), frameshift mutations or large deletions. These mutations are present in around 10% of patients worldwide.
- II. Class II mutations cause protein trafficking defects which may result in premature degradation of CFTR. The most common mutation, F508del, belongs to this class. It is present on at least one allele in 70% of patients with CF.



**Fig. 1.** Diagram of the CFTR protein structure. The two transmembrane spanning domains (MSD1 and MSD2) form the channel pore. Opening of the pore and anion flow through it is powered by cycles of ATP binding and hydrolysis at the two ATP-binding sites located on the intracytoplasmic nucleotide-binding domains (NBD1 and NBD2). Phosphorylation of the intracellular regulatory domain (R domain) stimulates CFTR function by enhancing ATP-dependent channel gating at the NBDs.



**Fig. 2.** Classes of *CFTR* mutations. Some examples of mutations are shown for each class as well as some therapeutic approaches.

- III. Class III mutations are gating mutations, allowing trafficking of CFTR to the apical membrane but causing defective regulation of the chloride channel. They result in a very poorly functional CFTR protein. These class III mutations are present in 4–5% of patients worldwide. The most common class III mutation is G551D and is observed in around 3% of patients worldwide (around 2000 patients).
- IV. Class IV mutations, such as R117H, lead to a CFTR protein present at the apical membrane but with decreased conductance.
- V. Class V mutations lead to the production of a normal CFTR protein but in a reduced quantity because of aberrant splicing (e.g. 3849 + 10kbC → T) or moderately decreased trafficking (A455E).
- VI. Class VI mutations (e.g. 120del23) lead to a high turnover of CFTR at the apical surface.

Recently a class VII has been proposed, regrouping mutations such as large deletions anticipated not to be amenable by therapy with small molecules (De Boeck & Amaral, 2016).

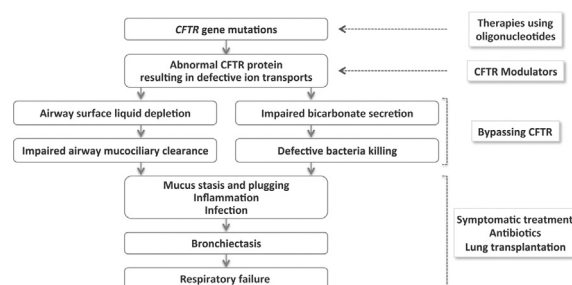
Class I, II and III mutations are commonly associated with pancreatic insufficiency and severe disease, whereas class IV, V and VI mutations are frequently associated with pancreatic sufficiency and a milder phenotype (Castellani et al., 2008). Only ~20 mutations occur at a worldwide frequency above 0.1% in CF patients, and determining the disease liability of very rare mutations is often difficult (Ferec & Cutting, 2012). The relative frequency of mutation classes in European patients with CF has been evaluated (De Boeck, Zolin, Cuppens, Olesen, & Viviani, 2014). Some mutations can reach high prevalence in selected populations, due to a founder effect in religious, ethnic or geographical isolates.

This complex classification is useful but quite theoretical: some mutations occur rarely and their effect on CFTR function, and thus their class, is unknown. Similarly, most mutations induce several CFTR protein defects belonging to different classes (Brodie, Haq, Roberts, & Elborn, 2015). For example, the most frequent F508del mutation belongs to class II because it leads to a CFTR protein with trafficking defects. But it also belongs to class III and class VI because it presents gating defect and a high turnover when expressed at the apical cell membrane.

In the airways, the CFTR protein plays a major role in determining the airway surface liquid volume (Knowles & Boucher, 2002), and the hydration and expansion of mucus (Quinton, 2008). Thus, it plays a major role in airway mucociliary clearance which is a primary innate defense mechanism: when the airway surface liquid volume is normal, it promotes normal ciliary beat and effective clearance of the overlying mucus gel layer in which bacteria are trapped. When the CFTR protein is

defective, there is an imbalance between CFTR-dependent chloride secretion and ENaC-mediated sodium absorption. It leads to low volume and dehydration of airway surface liquid, therefore impairing airway mucociliary clearance (Knowles & Boucher, 2002). CFTR dysfunction also causes impaired bicarbonate secretion resulting in retention of mucus and reduced pH of the airway surface liquid, thus interfering with the innate immune system's ability to kill bacteria (Pezzulo et al., 2012). These defects in the airways' innate defense trigger a chain of events including mucus stasis and plugging, airway obstruction, infection and inflammation (Mall & Boucher, 2014; Stoltz et al., 2015) (Fig. 3).

Since the cloning of the *CFTR* gene in 1989 and the subsequent growing knowledge about the CFTR protein's maturation, structure and function, development of drugs correcting the basic defect in CF has been a major goal. Such a therapy would possibly slow or halt the development of respiratory disease or, provided such drugs could be given very early in life, they could even prevent lung disease. There are mainly 2 approaches to correct the basic defect in CF: the first approach is to use oligonucleotides to intervene at the gene or mRNA level by delivering the normal *CFTR* cDNA which is the gene therapy approach, or by repairing the abnormal *CFTR* gene or mRNA. This would produce a normal CFTR protein. Despite extensive research in this area and some promising findings, this approach has not been successful yet. The second approach is CFTR pharmacotherapy which aims at identifying small molecules able to correct the abnormal CFTR protein. Since 2012, two such CFTR modulators have been marketed showing that this pharmacological modulation of CFTR is doable. Even with those two CFTR modulators, CFTR function is still suboptimal but the goal of developing disease-modifying drugs for CF appears within reach.



**Fig. 3.** Pathophysiology of CF lung disease and main targets of current and emerging therapies.

#### 4. Modulating the CFTR protein is possible: the potentiator ivacaftor

Ivacaftor (VX-770; Kalydeco® from Vertex Pharmaceuticals) is the first drug aiming at correcting the basic defect in CF that was approved for marketing. It was identified by high-throughput screening and shown *in vitro* to enhance chloride transport and increase airway surface liquid height and ciliary beat frequency in airway epithelial cells expressing the G551D class III CFTR mutation (Van Goor et al., 2009). Ivacaftor is thought to bind directly to the CFTR protein, most plausibly to the transmembrane domains (Eckford, Li, Ramjeesingh, & Bear, 2012), and increases the open probability of the CFTR protein. This is the main defect of class III mutations. But ivacaftor was also shown to increase the open probability of normal CFTR, as well as CFTR resulting from several class IV and V mutations. These are associated with a CFTR protein present at the apical membrane which is either dysfunctional or in a low amount (Van Goor, Yu, Burton, & Hoffman, 2014; Yu et al., 2012).

Phase II and III placebo-controlled clinical trials showed that ivacaftor was well-tolerated and effective in both children ( $\geq 6$  years) and adult patients with CF who had at least one G551D (class III) mutation in the CFTR gene (Accurso et al., 2010; Davies, et al., 2013; McKone, et al., 2014; Ramsey, et al., 2011). The main evidence for clinical benefit was the sustained and robust improvement in respiratory function (mean increase of 10% predicted forced expiratory volume in 1 s (FEV<sub>1</sub>)), in weight, and in the rate of pulmonary exacerbations upon oral administration of ivacaftor for up to 144 weeks. Patients with CF receiving ivacaftor also had reduced sweat chloride concentrations showing an improvement of CFTR function in the sweat glands. These results led to the approval in 2012 in the US and EU of ivacaftor for patients with CF aged 6 years and above bearing at least one copy of the G551D mutation. The clinical improvement was also observed in real life after the drug was marketed and long-term effects are becoming apparent such as a slower rate of lung function decline in patients treated with ivacaftor (Sawicki et al., 2015).

Further studies then broadened the efficacy of ivacaftor to patients bearing at least one copy of the other CFTR gating mutations (De Boeck et al., 2014) or the class IV R117H CFTR channel conductance mutation (Moss, et al., 2015). In an open-label study, ivacaftor was shown to be well tolerated in children with CF aged 2 to 5 years and bearing one class III mutation (Davies, et al., 2016). Thus, ivacaftor is currently approved in the US, EU, Canada and Australia for patients with CF aged  $\geq 2$  years (or  $\geq 6$  or  $\geq 18$  years, depending on the country and the mutation) bearing at least one copy of specific class III mutations or the R117H class IV mutation.

Ivacaftor is the first personalized or genomically-guided therapy for CF. Around 3400 patients with CF are eligible for treatment with ivacaftor worldwide and it represents only around 5% of the total CF population. However, ivacaftor's development and approval was a major breakthrough for CF treatment since it was the proof-of-concept that small molecule therapy may improve CFTR function. It was demonstrated that ivacaftor achieves a CFTR activity equivalent to approximately 35–40% of normal activity (Accurso et al., 2014). Clinical trials and subsequent studies showed that even in adults with a long history of respiratory disease and abnormal lung function, restoring CFTR function can significantly improve lung function and slow the course of the disease. In very young patients with normal lung function, beginning ivacaftor treatment early in life may well be truly disease-modifying: if the treatment is well tolerated in the long term, it may help preserve lung function and improve expected survival. It should be noted however that ivacaftor is currently a very high cost treatment (around 294,000\$ per year), which is a significant issue for a lifelong therapy. It limits access to the treatment in several lower income countries. The benefits of ivacaftor on health and possibilities of active life, and the expected decrease in symptomatic treatment burden and hospitalisation (and their related costs) will need to be thoroughly assessed (Barrett, Alagely, & Topol, 2012). Several new potentiators

are currently being evaluated in clinical trials, such as QBW251 from Novartis (NCT02190604), GLPG1837 from Galapagos (NCT02707562 and NCT02690519) or CTP-656 from Concert Pharmaceuticals (NCT02599792) (Fig. 4).

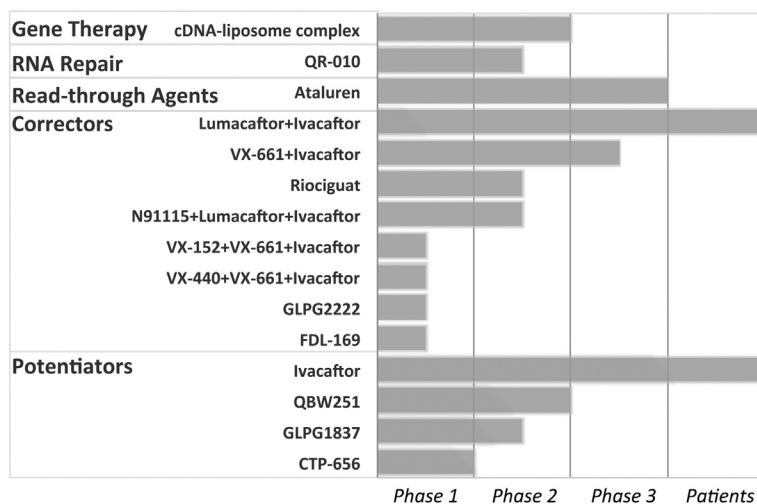
#### 5. Further improvements are needed in CFTR correction: more potent correctors or corrector combinations

The most common CFTR mutation is the F508del class II mutation with around 40–45% of patients being homozygous for this mutation. The F508del mutation leads to an absence of phenylalanine at position 508 of the protein. This results in an abnormal NBD1 domain which fails to mediate appropriate interactions with the carboxy terminal portion of the protein (for review: (Molinski et al., 2012)). This produces a misfolded F508del protein with impaired trafficking from the endoplasmic reticulum to the golgi. The majority F508del CFTR protein is degraded by cellular quality control mechanisms. The small amount that reaches the cell surface has gating defect and a short-half life. Several compounds called correctors were shown *in vitro* to improve the intracellular processing of the F508del CFTR protein and allow more protein to reach the cell surface. The addition of potentiators was required to help correct the gating defects. Lumacaftor (VX-809 from Vertex Pharmaceuticals) is a corrector thought to act early in CFTR biogenesis by interacting directly with MSD1. Thus, it suppresses folding defects by enhancing interactions among the NBD1, MSD1 and MSD2 domains (Ren et al., 2013). *In vitro* studies have shown that lumacaftor improves CFTR processing and chloride secretion in bronchial epithelial cells homozygous for the F508del mutation (Van Goor et al., 2011). Lumacaftor restored F508del-CFTR function to approximately 14% of normal airway cells. In phase II and phase III placebo-controlled trials in CF patients bearing two copies of the F508del CFTR mutation, significant improvement in respiratory function and a lower rate of pulmonary exacerbations were shown upon lumacaftor combined with ivacaftor treatment (Boyle, et al., 2014; Wainwright, et al., 2015). However, improvement in respiratory function was much less (mean increase of 3% predicted FEV<sub>1</sub>) than observed with ivacaftor in patients with a class III mutation (mean increase of 10% predicted FEV<sub>1</sub>). These successful clinical trials led to marketing approval of lumacaftor (VX-809) combined with ivacaftor (Orkambi®) in the US and EU in 2015 for patients with CF aged 12 and older homozygous for the F508del mutation. Orkambi®'s approval has been a new milestone in the treatment of CF as it showed that small molecule therapy is also a valid approach to correct the dysfunctional F508del CFTR protein. However, the efficacy of Orkambi® in restoring F508del-CFTR function is low and more robust correctors are needed. Correctors with a different mechanism of action than lumacaftor could be added to Orkambi® and increase its efficacy, as shown *in vitro* (Okiyonedo et al., 2013). But combining drugs increases possible drug interactions and risks of adverse events. Other correctors are currently in clinical development, such as tezacaftor (VX-661) from Vertex Pharmaceuticals (NCT02508207), riociguat from Bayer (NCT02170025), combinations of correctors such as VX-152 or VX-440 combined with tezacaftor (VX-661) from Vertex Pharmaceuticals, GLPG2222 from Galapagos (NCT02788721) or FDL-169 from Flatley Discovery Lab (NCT02767297) (Fig. 4).

#### 6. Other pharmacological strategies under investigation

##### 6.1. Agents increasing the amount of CFTR or stabilizing CFTR at the cell membrane

Novel compounds are being developed that could be combined with correctors and potentiators. They aim at increasing the amount of CFTR in the cell, so that more CFTR would be available for interaction with correctors and potentiators, or at stabilizing the modulated CFTR at the cell membrane. The amplifier PTI-428 from Proteostasis Therapeutics (NCT02718495) is thought to amplify the amount of CFTR in the



**Fig. 4.** Clinical development pipeline of CFTR modulators (from: <https://clinicaltrials.gov> accessed July 2016). Other strategies under investigation are agents amplifying the intracellular CFTR protein, stabilizing CFTR at the cell membrane or bypassing the CFTR protein by inhibiting the epithelial sodium channel ENaC or stimulating other chloride channels. Some of these strategies could be combined with correctors and potentiators.

cell. The stabilizer cavosonstat (N91115) from Nivalis aims at stabilizing the CFTR protein at the cell membrane. It is thought to inhibit S-nitrosogluthione reductase that is increased in CF human bronchial epithelial cells (Zaman et al., 2016). Restoring intracellular S-nitrosogluthione to normal levels would decrease CFTR degradation and improve CFTR stability at the cell surface. Phase 2 studies of N91115 combined with Orkambi® and Kalydeco® are underway (NCT02589236 and NCT02724527) (Fig. 4).

## 6.2. Overreading the premature stop codons

Class I nonsense mutations leading to premature termination of CFTR translation are present in around 10% of patients worldwide. During translation of mRNA, ataluren (PTC124), similar to the effect of high concentrations of aminoglycoside antibiotics, induces ribosomal read-through of premature but not normal termination codons, resulting theoretically in the formation of a functional protein (Welch et al., 2007). Phase II open-label studies with oral administration of ataluren in patients with CF and at least one CFTR nonsense mutation improved CFTR function as measured by nasal epithelial chloride transport (Kerem et al., 2008; Sermet-Gaudelus et al., 2010). But in a double-blind phase III clinical trial, this could not be confirmed and improvement in respiratory function was only observed in patients not using chronic inhaled tobramycin (Kerem et al., 2008; Kerem et al., 2014). It has been hypothesised that tobramycin interferes with the mechanism of action of ataluren. A new phase III trial in patients with CF bearing a nonsense mutation and not receiving inhaled tobramycin is ongoing (NCT02139306) (Fig. 4).

## 6.3. Bypassing the CFTR channel

The CFTR channel interacts with other ion channels at the cell membrane, an important function being the inhibition of the epithelial sodium channel ENaC. In CF, the overactivity of ENaC contributes to airway surface liquid dehydration (Knowles & Boucher, 2002). Hence ENaC blocking agents could improve airway hydration. Although development of inhaled ENaC inhibitors has been a long-lasting endeavour, their clinical development has been hampered so far by too low efficacy or deleterious effects due to systemic absorption and inhibition of ENaC in the kidney (Althaus, 2013). New promising compounds, such as VX-371 (formerly P-1037) from Vertex Pharmaceuticals and Parion (NCT02709109) are being evaluated. Stimulating chloride channels other than CFTR (alternate chloride channels like the Calcium-

activated Chloride Channel or TMEM16A or anoctamin-1) to compensate for the loss of function of CFTR is another therapeutic strategy. One candidate drug called denufosol could activate P2Y2 receptors and thus stimulate transepithelial chloride secretion; but the phase III program was not successful (Moss, 2013). Recently, significant preclinical progress has been made with identification of alternative chloride channels that should facilitate the development of specific activators (Mall & Galletta, 2015).

## 7. Strategies using oligonucleotides to treat at the CFTR gene or mRNA level

Another strategy is to develop a treatment intervening at the gene or mRNA level by delivering normal CFTR cDNA, or re-editing abnormal CFTR gene or correcting abnormal mRNA by using oligonucleotides. Since oligonucleotides are poorly suited for oral or systemic delivery, these strategies are likely to remain restricted to treatment via aerosol.

### 7.1. Gene therapy

Delivering normal CFTR cDNA is called gene therapy although its aim is not to repair abnormal CFTR gene DNA but to deliver functional CFTR cDNA that would lead to functional CFTR protein synthesis even though abnormal CFTR protein would still be expressed. The CFTR cDNA is a large molecule which needs to be compacted by molecules called vectors to enter the cells. These gene transfer agents are either of viral or nonviral, i.e. biochemical, origin. In CF, most of the vectors that have been studied are cationic nonviral molecules able to form a complex with the negatively charged DNA. There was great enthusiasm for gene therapy after the CFTR gene was identified. Indeed, such an approach has the potential to cure all patients with CF, regardless of the mutations present. The proof-of-concept of gene therapy in CF epithelial cells was published very soon after the gene's identification and to date, >25 clinical trials have been completed (Alton et al., 2016). Treatments were usually well tolerated, but the enthusiasm was quickly dampened by many challenges that were identified, including the low delivery of functional genes by most available viral and nonviral vectors, short half-life of therapeutic gene expression leading to low efficacy, and unwanted inflammatory responses (Griesenbach, Pytel, & Alton, 2015; Lee & Southern, 2013). A recent randomised, double-blind, placebo-controlled, multi-centre phase IIb trial included 140 patients with CF who received a monthly inhalation of a plasmid DNA encoding the CFTR cDNA complexed with a liposomal vector for 1 year. The treatment

was well tolerated and there was a stabilization of lung function as compared with the placebo group (Alton et al., 2015). However, concerns were raised concerning the placebo group because patients in that group received 0.9% saline which was not exactly the matching placebo for the active treatment, and the decline in their respiratory function over the study was higher than the one usually observed in placebo groups in similar studies. In any case, the treatment effect was limited and the crucial challenge for CF gene therapy is the need for more efficient vectors to deliver the cDNA to the airway cells.

## 7.2. Gene editing

A true gene therapy approach with permanent correction at the genome level is called gene editing. Accurate genome engineering is still in early stages with no clinical data for CF so far but it remains a promising approach. It relies on programmable nucleases allowing defined alterations in the genome with ease-of-use, efficiency, and specificity. Some *in vitro* data in intestinal organoids showed repair of the F508del mutation by gene editing (Schwank et al., 2013).

## 7.3. mRNA repair

Repairing the abnormal *CFTR* mRNA is another therapeutic approach and partial transcriptional repair of F508del-*CFTR* mRNA has been shown as early as 2004 (Zamecnik, Raychowdhury, Tabatadze, & Cantiello, 2004). The drug QR-010 is a single-stranded antisense RNA-based oligonucleotide sequence containing the missing bases and acting as a guide of base sequences to repair the targeted abnormal mRNA in patients with the F508del mutation. A phase I proof-of-concept study is ongoing in CF patients homozygous for the F508del mutation (NCT02532764) (Fig. 4).

## 8. Challenges to correct the basic defect in every patient with cystic fibrosis

The recent availability of *CFTR* modulators for patients with CF is a milestone in the history of CF and CF treatment. Indeed, some studies already show that the potentiator ivacaftor slows the rate of lung function decline (Sawicki et al., 2015). However, developing *CFTR* modulators for all patients and developing more potent *CFTR* modulators will be the main challenges for the coming years.

As mentioned above, hundreds of *CFTR* mutations have been described. Most of them are very rare and their effect on *CFTR* function, and thus their class, is unknown. These *CFTR* mutations each form a sort of very rare disease for which too few patients will be available to support adequately sized clinical trials. New ways of studying drug efficacy need to be developed such as patient-derived assays. Nasal cell-based assays or the recently described *ex vivo* assay using rectal biopsies to establish primary intestinal organoids to study *CFTR* function might help screen drugs for specific *CFTR* mutations (Dekkers et al., 2013). Then, selected drugs could be studied in N-of-1 trials in a multiple cross-over design in single individuals (Vohra, 2016). Such a drug development approach would need to be discussed with regulatory agencies. It could lead to conditional approvals that would require subsequent post-marketing studies to bridge the knowledge gap on their efficacy and safety.

More potent *CFTR* modulators need to be developed. But conducting clinical trials will be difficult as part of the target population may already be on treatment with an approved *CFTR* modulator. Comparison with a placebo control may be judged unethical and implementing active-comparator trials requires an increased sample size which may prove unfeasible. Moreover, the cost incurred for acquisition of the current approved *CFTR* modulators for use in a trial may well be excessive for companies. New clinical trial designs are being discussed such as one month withdrawal of an approved modulator to assess whether the candidate

modulator sustains the gain in lung function (Mayer-Hamblett, Boyle, & VanDevanter, 2016).

## 9. Conclusion

Despite CF being a rare disease, the deep commitment of developers, scientists, clinicians and patients has led to the recent marketing approval of two *CFTR* modulators. In theory nearly 40–45% of patients with CF could already benefit from these new drugs. Too high a drug cost however impedes access to treatment in many countries. Because of the limited activity of current *CFTR* correctors, more potent *CFTR* modulators need to be developed for all patients with CF. This will require innovative, feasible and regulatory-approved strategies to evaluate drug safety and efficacy. Nevertheless, a new era of genomically-guided medicine has begun for CF and it is foreseeable that it will lead to dramatic improvements in CF clinical disease and survival in the next decades.

## Conflict of interest statement

IF is or was principal investigator of clinical trials sponsored by Bayer, Insmmed, Novartis, PTC Therapeutics and Vertex Pharmaceuticals. She received compensation for consultant services, lectures or travel expenses from Actelion, Bayer, Gilead, Insmmed, Novartis, Pfizer, PTC Therapeutics, Vertex Pharmaceuticals and Zambon.

KDB is or was principal investigator of clinical trials sponsored by Boehringer, Galapagos, Gilead, PTC Therapeutics and Vertex Pharmaceuticals. She received compensation for consultant services, lectures or travel expenses from Ablynx, Aptalis, Bayer, Gilead, Insmmed, Novartis, Pfizer, PTC Therapeutics, Protalix, Raptor Pharmaceuticals and Vertex Pharmaceuticals.

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