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## Therapy for Cystic Fibrosis Caused by Nonsense Mutations

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Additional information is available at the end of the chapter

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### Abstract

Nonsense mutations cover about 10% of cystic fibrosis (CF) patients and generate premature termination codons (PTCs) leading to premature translational termination and causing the synthesis of truncated non-functional or partially functional CFTR (cystic fibrosis transmembrane conductance regulator) protein. The read-through approach is the suppression of translation terminations at PTCs and it has been developed as a therapeutic strategy to restore full-length protein using aminoglycoside antibiotics or PTC124. Phenotypic consequences of PTCs can be exacerbated by the nonsense-mediated mRNA decay (NMD) pathway, which detects and degrades mRNA containing PTC. Therefore, modulation of NMD is also of interest as a potential target for suppression therapy. Not all PTCs are susceptible to the read-through treatment alone, especially where the nonsense mutations are combined with other CFTR mutations. For example, many CF patients present the highly frequent F508del CF mutation, causing an alteration of the cell membrane positioning of the CFTR channel. Pharmacological correctors that rescue the trafficking of F508del CFTR may overcome this defect. A combined administration of correctors/potentiators, read-through molecules, and/or NMD inhibitors, depending on the genotype of the CF patients, could be the basis for the design of a personalized therapeutic approach.

**Keywords:** Cystic fibrosis, PTCs, read-through, NMD inhibitors, correctors, potentiators

1. Introduction

1.1. Classes of mutation in cystic fibrosis

The CFTR protein is a cAMP-regulated chloride channel that resides in the apical membrane of epithelial cells of different organs such as the intestines, lung, pancreas, and sweat glands. However, the main pathologic symptom of cystic fibrosis (CF) is respiratory disease with recurring infections, inflammation, and obstructions that induce progressive lung damage and possible respiratory difficulties [1].

More than 1,900 different CFTR mutations have been reported so far and can be generally categorized into five classes (Table 1). Approximately 40% of CFTR mutations belong to class I. Nonsense mutations cover 10% of CF patients, but in some populations specific nonsense mutations can occur in up to 50% of CF subjects. For example, G542X mutation, the most common CFTR nonsense mutation, has been found in 2% of Caucasian CF patients. The most frequent mutation of class II is the F508del mutation (a deletion of the phenylalanine residue at position 508) found in 75% of CF patients. While for class III, the G551D mutation has been found in approximately 4% of CF patients. Class I-III mutations determine an important defect in CFTR function and cause a severe CF phenotype, while mutations belonging to Classes IV-V permit a residual CFTR protein function and induce a milder CF phenotype. There is general consensus on the fact that in order to ameliorate the CF phenotype, the restoration of at least 5–35% of normal CFTR function is necessary [1-4].

Class	I	II	III	IV	V
Types of mutations	Nonsense; Frame shift	Missense	Missense	Missense	Missense
Defect on CFTR	No expression	No correct localization to cell membrane	No chloride channel function	Reduced chloride channel conductance	Reduced expression
Examples	G542, W1282X	F508del	G551D	R117H; R334W	A445E
Possible pharmacological therapy	Read-through	Correctors	Potentiators	Potentiators	Potentiators

Table 1. Principal classes of CF mutations and therapeutic intervention.

1.2. PTCs and NMD mechanism

Premature termination codons (PTCs) lead to premature translational termination resulting in absent functional proteins. Different inherited and acquired diseases can be attributable to PTCs, such as cystic fibrosis, Duchenne muscular dystrophy (DMD), mucopolysaccharidosis, spinal muscular atrophy (SMA) type I and type II, X-linked nephrogenic diabetes insipidus, rhinitis pigmentosa X-linked, nephropathic cystinosis, and  $\beta$ -thalassemia [5].

PTCs are responsible for about 10% of CF cases worldwide, but are particularly common in Ashkenazi Jews (40% of Israeli CF patients) [6]. The translation of PCT-containing mRNAs leads to the formation of truncated CFTR, in most cases lacking in its function [7].

During molecular evolution, a mechanism to eliminate these aberrant mRNAs containing PTCs has been developed, known as nonsense-mediated mRNA decay (NMD). This process of quality control is observed in all eukaryotic organisms and has an important role in the regulation of many cellular functions. Its physiological substrates act in a wide range of processes such as transcription, DNA repair, cell growth, intracellular transport, and NMD itself. Through this process, the mRNA levels containing PTCs are reduced but not completely eliminated, inducing a reduced level of proteins, which are synthesized in a truncated form [5].

In mammals the NMD process operates by the recognition of a premature stop codon. It is observed that the ability of PTCs in inducing NMD depends on its location with respect to the sequence downstream and associated proteins [8]. The NMD process often occurs in mammalian cells after the splicing of pre-mRNA and, in most cases, is controlled by the exon junction complex (EJC). This complex consists of at least 10 proteins binding to regions consisting of 20–24 nucleotides upstream of the exon-exon junction and including UPF2 and UPF3 proteins.

According to the currently proposed models of the NMD mechanism, for the majority of mRNAs, the stop codons that are localized more than 50–55 nucleotides upstream of an exon-exon junction are recognized as premature, since the EJC is found downstream to the stop codon. The EJC is recognized by UPF2 and UPF3 and ribosomes start to translate the mRNA until reaching the PTC. The termination of the translation is triggered by the recognition of PTCs by eRF1 and eRF3, release factors recruited by ribosomes. These proteins then recruit the NMD key factor UPF1, which in turn binds to the SMG1 kinase. These four proteins (eRF1, eRF3, UPF1, SMG1) constitute the SURF complex, responsible for ribosome blocking. When the PTC is upstream of this EJC, UPF1 at the termination site may interact with UPF2 (associated with EJC) causing the phosphorylation of UPF1 by SMG1 and the dissociation of release factors (eRF1 and eRF3). The phosphorylated UPF1 recruits additional factors (SMG5, SMG6, and SMG7) triggering NMD. Once the NMD is stimulated, target transcripts are degraded by 5' and 3' exonucleases [5].

## 2. PTCs and NMD mechanism as targets for CF therapy

### 2.1. The read-through approach in CF

With the aim of overcoming the effects of the presence of PTCs in CF, several drugs were studied, such as aminoglycoside antibiotics and PTC124 (PTC Therapeutics), and found to be able to incorporate a random amino acid at the PTC position of mRNAs. This induces a ribosomal read-through of the premature, but not of the natural termination codons, restoring CFTR function in CF patients [9-10]. These treatments may provide a means of restoring clinically relevant levels of protein function in patients carrying PTCs in the mutated gene,

and introduce new hope for the development of a pharmacologic approach for the cure of several important genetic diseases, including CF [9-10].

### 2.1.1. Aminoglycosides

Aminoglycoside antibiotics selectively bind to ribosomes (at decoding aminoacyl site, A-site) and cause the insertion of a near cognate amino-acyl tRNA into this ribosomal A site, allowing ribosome read-through at PTC and production of a full-length protein [11].

Previous studies have shown that a variety of aminoglycosides are able to suppress CFTR nonsense mutations and restore functional CFTR protein levels not only in mouse models, but also in human clinical trials [1,12-16].

Gentamicin and amikacin are able to suppress the common G542X nonsense mutation in a transgenic CF mouse model, restoring approximately 20–30% of full-length CFTR protein, suggesting these compounds as read-through molecules to treat CF patients carrying PTCs [1]. In particular, the CFTR nonsense transgenic mouse model carrying a human CFTR cDNA with G542X nonsense mutation was used to screen the read-through activity of different aminoglycosides [1,12]. Five to thirty-four mg/kg of gentamicin was administered by daily subcutaneous injections for 14 days and found to induce a 22% increase of the wild-type CFTR function. Similar results were observed with amikacin, used at 15–170 mg/kg, while tobramycin was much less efficient than these two aminoglycosides in rescuing CFTR function [1,12,13].

In humans, Wilschanski et al. [1,14] reported a study where 0.9 mgs of gentamicin were administered daily via nasal drops for 14 days in nine CF patients. The data obtained demonstrated a significant improvement in chloride conductance by nasal potential difference (PD) measurements. Full-length CFTR protein was also detected in the nasal epithelia of treated CF patients. On the contrary, patients homozygous for the F508del mutation, used as a control group, did not show improvements in the CFTR function or restoration of CFTR protein [14].

Clancy et al. [15] reported a study where 2.5 mg/kg of gentamicin was administered intravenously every 8 hours for one week to 10 CF patients. They reported a three-fold increased incidence of nasal PD readings in the direction of chloride secretion in treated PTC subjects relative to CF controls. Airway chloride secretion was also stronger, with a high level of chloride secretion not detected in the control subjects, while sweat chloride measurements were not increased. In recent studies [1, 16], two different groups (11 CF patients with nonsense mutations and 18 CF patients without nonsense mutations) were treated with 3.6 mg of gentamicin or tobramycin daily by a nasal spray device for 28 days. The results indicated no improvement in chloride ion transport or CFTR localization using these two aminoglycosides. The authors underlined that nasally administered aminoglycosides did not produce detectable improvement in CFTR function because the CF subjects were heterozygous for different CFTR mutations [16]. This suggests that not all premature stop mutations are susceptible to the read-through treatment alone in order to ameliorate the phenotype of CF patients carrying nonsense mutations. When the read-through approach is combined with the use of potentiators/correctors of CFTR, however, the clinical outcome may be much more positive and this strategy could delineate a personalized therapeutic approach based on the CF genotype of patients [16].



A recent study using tobramycin was performed in yeast models with modulated NMD by UPF1 deletion [17]. Yeast strains were transformed with the Renilla/Firefly dual-luciferase reporter plasmids carrying either nonsense (UAG, UGA, UAA) or sense (CAG, CGA, CAA) codon between the luciferase genes. Tobramycin, a aminoglycoside antibiotic normally used to treat *Pseudomonas aeruginosa* pulmonary infection in CF patients, exhibited read-through ability on PTCs preferentially in the absence of NMD mechanism. These findings could be an explanation of the lower activity of tobramycin in restoring CFTR synthesis observed in mouse models and human clinical trials, while the data indicate possible combined treatment of tobramycin in the presence of NMD inhibitors.

Most of the novel aminoglycoside molecules have been synthesized with the major aim of optimizing their antibacterial activity, and not with the objective of obtaining the highest possible read-through action on PTCs. Current clinical studies investigating this class of molecules were performed using only commercially available aminoglycosides, such as gentamicin, amikacin, and tobramycin [14-18]. The general conclusion of these studies is that tobramycin presented a low efficiency as PTC suppressors, while gentamicin was efficient but toxic [14-18]. Alternative options to gentamicin could be amikacin [13] or paromomycin [19], two aminoglycosides with lower cellular toxicity, but not yet studied in human clinical trials as read-through molecules.

A future goal should be the design and synthesis of new aminoglycosides with high read-through activity and low toxicity. Baasov et al. [18] reported the synthesis of a series of new derivatives of paromomycin proposed as read-through molecules in vitro. In particular NB30, a pseudo-trisaccharide derivative, had higher read-through activity compared to paromomycin and gentamicin and reduced toxicity [20, 21]. Another derivative, NB124, presented a more efficient read-through activity than gentamicin. It restored full-length CFTR expression and chloride transport in Fischer rat thyroid cells stably transduced with a CFTR-G542XcDNA transgene and 7% of wild-type CFTR function in primary human bronchial epithelial CF cells with G542X/delF508 genotype [22].

These studies suggest that the reduction of the efficiency of translation termination, such as in the case of induction of ribosomal read-through, is a potential strategy for developing treatments of genetic diseases caused by PTCs. It is known that aminoglycosides cause toxic side effects that do not seem directly associated with their ability to suppress PTC. Monitoring the read-through efficiency of aminoglycosides with low toxic side effects is required and the synthesis of novel aminoglycoside derivatives exhibiting better parameters of administration to the patient, as well as availability and lower toxicity in vivo, is of interest to the research community.

#### 2.1.2. PTC124

PTC124 (3-(5-(2-fluorophenyl)-1,2,4-oxadiazol-3-yl)-benzoic acid), also known as Ataluren, is a small non-aminoglycoside molecule that has been suggested to allow PTC read-through even though its target has yet to be identified [23]. It is an orally available and non-toxic small molecule, identified by HTS and is the first drug in its class. PTC124 appears to allow cellular machinery to read-through premature stop codons in mRNA, enabling the translation process

to produce full-length, functional proteins [24]. In a transgenic mouse model carrying CFTR with nonsense mutations, 0.9 mg/ml of PTC124 was administered orally for 14–21 days. The results reported a partial CFTR expression at the apical surface of intestinal tissues and a restoration of normal CFTR function up to 24% with dose given in a liquid diet or 29% with at dose of 15–60 mg/kg daily by subcutaneous injections [1,25].

PTC124 was studied in two different Phase II human clinical trials with modest results in terms of efficacy [26–27]. In one study [26], PTC124 was orally administered to 23 CF patients carrying nonsense mutations. They were treated with 16 mg/kg daily of PTC124 for 14 days, then after 14 days without treatment, with a dose of 40 mg/kg daily for further 14 days. An improvement of chloride conductance was observed by nasal potential difference measurements after the two treatments, where approximately half of the patients showed measurements within the normal range [1,26]. Another Phase II trial was conducted with a similar treatment schedule for 12 weeks in 19 CF patients carrying nonsense mutations [27]. An increased nasal chloride transport in approximately 60% of treated patients was reported. CFTR function was restored more efficiently with increased time of treatment [1, 27]. PTC124 has also been examined in 30 pediatric CF patients with nonsense mutations [1, 28]. PTC124 was administered in two 14-day cycles as described earlier. Approximately 50% of the treated patients partially restored CFTR-mediated nasal epithelial chloride transport and full-length CFTR protein at the apical membrane of nasal epithelial cells, with only mild or no adverse effects.

Despite these successes in Phase II trials [11,27,28], PTC124 did not significantly improve the primary end-point in large Phase III clinical trials. A study [29], enrolling patients from 36 sites in 11 countries in North America and Europe, showed no improvement in the primary endpoint (forced expiratory volume in the first second, FEV<sub>1</sub>%) as predicted. It did however demonstrate conservation of lung function compared to placebo in a predefined subset of subjects who were not treated with the inhaled antibiotic tobramycin, known to influence the efficacy of PTC124 [29,30].

The development of novel small molecules having efficient read-through activity on PTCs and restoring CFTR function with low toxicity should be considered as a major issue in CF research. The CF read-through therapy clinical trials indicate that both gentamicin and PTC124 are able to restore CFTR protein in a fraction of CF patients with PTCs. Longer-term studies will be required to determine whether the therapeutic effects found in patients upon short-term treatment can be realized, and if sufficient CFTR can be rescued to reduce pathological respiratory aspects of CF [1]. The NMD inhibitors studied may lead to the discovery of biomolecules to be employed in combination with read-through correctors, in order to achieve clinically relevant results.

## 2.2. NMD inhibitors in CF

PTCs located >50–55 nucleotides upstream of an exon-exon junction can be minimized by NMD in order to detect and degrade mRNA containing PTC and minimize the negative consequences of nonsense mutations [5]. This should be carefully considered in the context of the development of read-through approach for restoring CFTR function. NMD is a cellular mechanism, aimed to detect and degrade PTC containing mRNA [31–34] and as part of an

mRNA surveillance system, it has a crucial role in preventing accumulation of aberrant transcripts and their translation [32,34]. Down-modulation of NMD is an important therapeutic goal, in order to (a) maintain mRNA levels suitable for read-through correction and (b) restore sufficient full-length protein expression levels. The major molecular consequences of nonsense mutations are the promotion of premature translational termination and NMD. These two features are strictly associated.

The efficiency of restoration of correct translation using read-through molecules, with respect to modulation of NMD is still an unresolved problem. Differences in NMD efficiency of CFTR transcripts carrying the W1282X mutation among different epithelial cell lines have been reported [35], and in some CF cells the NMD of all transcripts was efficient and in others NMD was less efficient. Down regulation of NMD in cells carrying the W1282X mutation was reported to increase the level of CFTR nonsense transcripts and enhanced the CFTR chloride channel activity in response to gentamicin [9].

Data published to date indicate that the efficiency of PTC read-through is low [10], probably due to degradation of PTC-mRNA by NMD mechanism, depleting substrates available for read-through correction. Modulation of NMD, aimed to increase CFTR mRNA available to PTC read-through, could be another important therapeutic goal [9]. A possible strategy to modulate NMD mechanism may be based on novel molecules acting as NMD inhibitors. These molecules should not affect physiological substrates of NMD mechanism.

Recently Wang et al. [36] have demonstrated that a modest depletion (80% of the activity of untreated cells) of UPF1 or UPF2 through shRNA could suppress NMD activity without affecting the proliferation or survival of cells. This suggests that pharmacologic NMD inhibition could be proposed as a therapeutic approach with limited toxicity or side effects. In particular, the same group identified NMD inhibitors (called NMDIs) acting against targeting SMG7-UPF1 complex obtained from a virtual library screening, with various chemical backbones and acting at nanomolar concentration [37]. It was demonstrated that the combination of a read-through drug and NMDIs increased protein expression and relative biological functions of a PTC-mutated p53. These novel data are promising, as previous pharmacologic inhibitors, useful in investigating the NMD mechanism, have been found to be toxic, did not synergize with read-through molecules and had an unknown mechanism of action [38,39].

Amlexanox, a drug used for over 30 years orally or topically to treat asthma and aphthous ulcers [40,41], has also been identified [42] as a potent NMD inhibitor. Amlexanox is not cytotoxic, does not inhibit general translation, and does not affect natural NMD substrate expression. This drug presents a dual activity of interest in CF research, that is, induction of an increased level of PTC mRNA, and induction of efficient expression of full-length and functional CFTR protein. The novelty of amlexanox is the ability to both inhibit NMD and facilitate PTC read-through, in contrast to other read-through molecules that present only read-through activity strongly influenced by the identity of the PTC and its nucleotide environment [43]. Amlexanox in combination with PTC124 induces an increase of CFTR channel activity in cells with heterozygous genotype for nonsense mutations [43].



The clinical use of these molecules may be possible after further studies relative to the short- and long-term effects of potent NMD inhibition as the combination of a read-through approach with pharmacologic NMD inhibition is an appealing therapeutic strategy for CF patients carrying nonsense mutations.

### 2.3. Treatment of CF heterozygous patients for nonsense mutations

Clancy's group [16] nasally administered aminoglycosides did not produce detectable changes in CFTR function in subjects heterozygous for a variety of stop codon mutations within CFTR, suggesting the need to perform a combined therapy based on the most potent suppressors of nonsense mutations co-delivered with CFTR potentiators and/or correctors [16]. Many CF patients heterozygous for premature stop mutations carry a genotype with F508del. Initial results of a Phase II trial of VX-809, a lead F508del CFTR corrector developed by Vertex Pharmaceuticals (Cambridge, Massachusetts, USA), established that systemic administration of the compound for 4 weeks modestly improved sweat chloride at the highest dose tested compared with placebo [44]. VX-809 is reported to normalize the gating of corrected F508del-CFTR but has no direct potentiating action suggesting that one straightforward approach could be the co-administration with a potentiator of CFTR channel gating [11,44,45]. If CF patients have nonsense mutations together with F508del, a cocktail of potentiators of CFTR channel gating, CFTR correctors, and read-through molecules could be proposed as a therapeutic strategy.

Xue X et al. [22] reported a novel synthetic aminoglycoside based on NB124 that efficiently restored CFTR function in primary human bronchial epithelial (HBE) CF cells carrying PTC (G542X/delF508). The efficacy of NB124 was further enhanced by addition of the oral CFTR potentiator Ivacaftor (VX-770) to airway cells expressing CFTR PTCs [22]. Ivacaftor exhibited excellent activity and pharmacokinetic properties, providing the opportunity to treat the underlying cause of CF in combination with CFTR correctors and read-through molecules, depending on the CFTR mutations [46].

Many new compounds are expected to be proposed in the future for the potentiation and correction of CFTR. One example is 4,6,4'-trimethylangelicin (TMA), a psoralen-related compound, which obtained the orphan drug designation from the EMA and is already in clinical use for psoriasis. Tamanini et al. [47] demonstrated that TMA, at nanomolar concentrations, inhibited the expression of the IL-8 gene in bronchial epithelial cells in which the inflammatory response has been challenged with *P. aeruginosa*. The acute addition (15 minutes treatment) of 250 nM TMA potentiated FSK-stimulated chloride secretion in airway cell monolayers expressing wild type CFTR or in CF cells in which F508del CFTR was already rescued to the apical membrane by overexpressing the interacting protein NHERF1. More recently, the same group [48] found that long pre-incubation with nanomolar concentrations of TMA was able to effectively restore both F508del CFTR-dependent chloride secretion and F508del CFTR cell surface expression in both primary or secondary airway cell monolayers homozygous for F508del mutation. These results indicate that TMA, besides its anti-inflammatory and potentiator activities, also displays corrector properties, suggesting that this

compound (or more effective analogues) could be a potential candidate for combination therapies with read-through molecules.

Other CFTR potentiator and/or corrector molecules with different mechanisms of action have been recently reported in literature [11]. These include the cyanoquinoline CoPo-22 [49], the inhibitor Hsp90 co-chaperone Aha1 [50], phosphodiesterase type V (PDE5) inhibitors [51-53], the isoflavones genistein, and curcumin [54]. Additional studies concerning their mechanism of action and toxicity are required before clinical testing, and combination with read-through molecules can be considered.

### 3. Conclusions

More than 1,900 different CFTR mutations have been reported in CF patients, 10% being nonsense mutations and 75% represented by the most frequent CF F508del mutation [1,11]. In order to ameliorate the CF phenotype, restoration of at least 5–35% of normal CFTR function is required [2-4]. Several CFTR modulator therapies have been investigated in vitro and in late phase clinical trials, including CFTR potentiators, correctors, and PTC read-through molecules [1,2-5,9-16,21-30,37-39,42,45]. To achieve substantial clinical benefit in CF, a combined administration of two correctors with synergistic action [55,56] or a corrector with a potentiator that counteracts distinct conformational defects in trafficking and activation of F508del CFTR [57, 58] are required.

Current open clinical trials, excluding studies with unknown status, are being conducted on Ivacaftor and PTC124 (Table 2).

Ivacaftor alone is not sufficient to efficiently alter CFTR activity in patients with CF homozygous for F508del [59]. The difficulty in achieving higher levels of correction is due to the fact that the F508del mutation induces multiple conformational defects in the mutant protein.

In current clinical trials (Table 2) Ivacaftor is being tested in combination with CFTR correctors such as Lumacaftor (VX-809, Vertex Pharmaceuticals, Cambridge, Massachusetts, USA) and VX-661 (Vertex Pharmaceuticals) in order to correct the protein misfolding and increase the CFTR localization to the cell surface.

VX-809 is selective for F508del CFTR and enhances chloride secretion to 15% of that found in non-CF human bronchial cells [45,48,55]. Preclinical research [45,60] and known effects of the F508 deletion on gating in addition to cellular processing [56-58] support the combination strategy of VX-809 (or VX-661, an alternate CFTR corrector to VX-809) with Ivacaftor in current human clinical trials (Table 2).

Alternatively, dual-acting molecules (such as TMA [47,48], aminoarylthiazole [61], and cyanoquinoline [49,62] derivatives) that both correct F508del CFTR and potentiate the F508del CFTR-dependent chloride permeability has been proposed, avoiding multiple combined administration.

Molecule	Title of Study	ClinicalTrials.gov Identifier
Ivacaftor(VX770; Kalydeco)	Ivacaftor (Kalydeco) and Insulin in Cystic Fibrosis (CF)	NCT02039986
	Short Term Effects of Ivacaftor in Non-G551D Cystic Fibrosis Patients	NCT01784419
	Ivacaftor in French Patients with Cystic Fibrosis and a G551D Mutation (IVACFTOR1)	NCT02194881
	A Phase 3 Study to Evaluate the Efficacy and Safety of Ivacaftor and VX-661 in Combination with Ivacaftor in Subjects Aged 12 Years and Older with Cystic Fibrosis, Heterozygous for the F508del-CFTR Mutation	NCT02392234
	A Phase 3 Study of VX-661 in Combination with Ivacaftor in Subjects Aged 12 Years and Older with Cystic Fibrosis, Who Have One F508del-CFTR Mutation and a Second Mutation that Has Been Demonstrated to Be Clinically Responsive to Ivacaftor	NCT02412111
	Study to Evaluate Lumacaftor and Ivacaftor Combination Therapy in Subjects 12 Years and Older with Advanced Lung Disease (Phase III)	NCT02390219
	Airway Infection, Inflammatory Markers and Exercise Capacity in Patients with Cystic Fibrosis and at Least One G551D Mutation Taking VX-770 (Ivacaftor) (Phase IV)	NCT01937325
	A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study to Evaluate the Efficacy and Safety of VX-661 in Combination with Ivacaftor (Phase III)	NCT02347657
	(Study: Vertex IIS) Does Ivacaftor Alter Wild Type CFTR-Open Probability in the Sweat Gland Secretory Coil?	NCT02310789
Ataluren(PTC124)	Study of Ataluren in Nonsense Mutation Cystic Fibrosis (ACT CF), Phase III	NCT02139306
N91115	Study of N91115 in Patients with Cystic Fibrosis Homozygous F508del-CFTR Mutation (SNO4) (Phase I)	NCT02275936
Glycerol Phenylbutyrate	Glycerol Phenylbutyrate Corrector Therapy For CF (GPBA)	NCT02323100
FDL169	FTIH – Single and Repeat Oral Doses of FDL169 in Healthy Volunteers (Phase I)	NCT02359357

**Table 2.** Current open clinical trials on CFTR modulators (source ClinicalTrials.gov).

Other ongoing human clinical trials are investigating novel CFTR modulators on F508del such as N91115, glycerol phenylbutyrate, FDL169 (Table 2). N91115 is currently in Phase I. In next Phase II this molecule will be studied in addition to Lumacaftor/Ivacaftor.

The combination of CFTR potentiators and correctors could result in effective therapies in a large proportion of patients with CF (Table 2).

Other CFTR modulator classes include molecules with read-through activity, such as PTC124 currently in Phase III clinical study. Based on efficacy in vitro and in animal models [10,25,63], PTC124 was studied in a series of conflicting Phase II trials [26-28,63]. A long-term study has demonstrated no improvement in FEV1 % predicted, the primary endpoint. Conversely a small effect on lung function was demonstrated in a predefined subset of individuals untreated with inhaled antibiotics that can modify the efficiency of PTC124 [29,63]. So further clinical studies for this compound should be necessary to demonstrate its activity in CF as read-through therapeutic approach also in combination with NMD inhibitors or potentiators/correctors as reported in studies in vitro [22,43].

Results to date suggest, in the case of nonsense mutations, efficient and therapeutic restoration of a functional CFTR channel may be possible drugs administered in combination.

In conclusion, it is essential to stratify CF patients with the aim of creating a personalized therapy based on the use of a combination of drugs targeted at specific classes of mutations. This approach represents an appealing therapeutic strategy and should be the subject of further investigation.

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