

Combination therapy in Phe508del CFTR: how many will be enough?

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Cystic fibrosis (CF) is a complex inherited disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene¹. Around 2000 disease causing mutations are known for this gene, which encodes a Chloride (Cl⁻) channel expressed at the plasma membrane (PM) of epithelial cells^{1,2}. Clinically the disease affects mostly the respiratory tract, where obstruction of the airways by thick mucus leads to: bacterial infections, extensive lung damage, and eventually, respiratory failure. Other affected systems include the gastrointestinal and reproductive tracts and endocrine system. Thus, the severity of symptoms can differ widely within individuals depending on their mutations, environment and biometrical characteristics. These variables modify the clinical course of the disease and each patient response to therapy^{3,4}.

Current therapies are typically focused on treating CF multi-organ symptoms, as opposed to targeting the underlying defect specific to each mutation^{5,6}. This has been the goal of several CF drug discovery programs, either by companies or academic labs^{6,7}, including our own^{8,9}. This review aims to highlight the most recent therapies that target the molecular defect in CFTR, particularly the most common CFTR mutation worldwide, the deletion of phenylalanine 508 (Phe508del)^{10,11}.

It is estimated that approximately 85% of all CF patients have at least 1 copy of the Phe508del mutant^{10,11}. This mutation is characterized by defective protein processing, resulting in considerable endoplasmic reticulum (ER) retention and premature degradation, preventing the mutant protein from trafficking to the cell surface. The Phe508del-CFTR molecules that reach the cell surface present partial channel function and a highly decreased PM half-life, due to accelerated endocytosis and fast turnover^{5,12}. Hence, applying strategies to correct Phe508del-CFTR multiple functional defects is complex, as more than one type of CFTR-modulator drug has to be used^{13,14}.

At present, there are several CFTR-modulator drug combinations and exciting new next-generation CFTR modulators under study for the clinical treatment of CF patients with the Phe508del mutation^{7,15} (see Fig. 1). The most important will be addressed below.

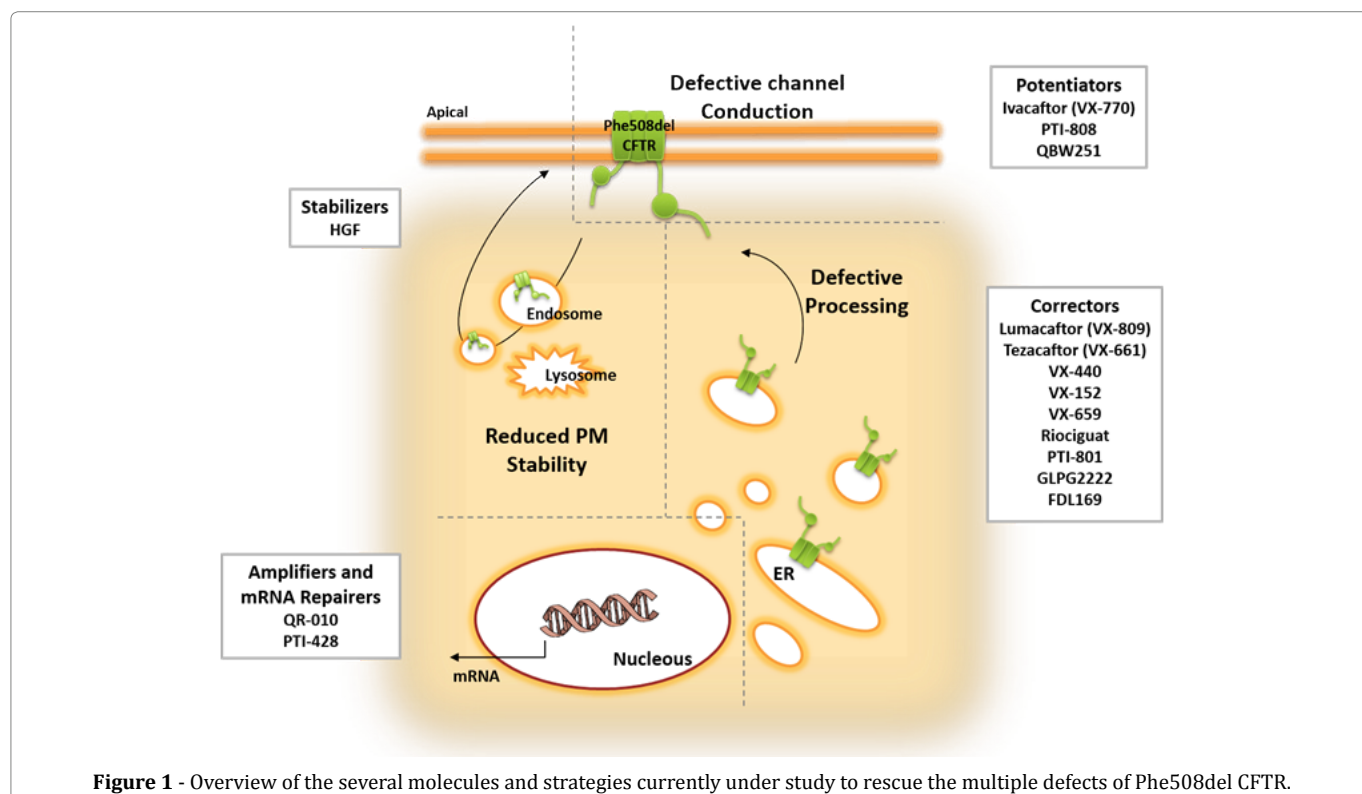


Figure 1 - Overview of the several molecules and strategies currently under study to rescue the multiple defects of Phe508del CFTR.

Combination therapy

Lumacaftor plus Ivacaftor (Orkambi®)

Ivacaftor, also known as VX-770, is labeled as a CFTR potentiator, as it increases the time that activated CFTR channels remain open at the cell surface¹⁶. It was first introduced for the treatment of the Gly551Asp CFTR mutation, where it increased predicted mean forced expiratory volume in one second (FEV_1 ¹⁷, which in patients decreases from ~90% in infancy to ~50% by the age of 40 years-old¹⁸) by 10%, and was associated with less risk of pulmonary exacerbations and weight gain¹⁶. Since any Phe508del-CFTR protein reaching the cell surface also presents reduced channel function, the efficacy of Ivacaftor was assessed in subjects homozygous for the Phe508del mutation. In these phase II studies, the difference in the change of FEV_1 % and other spirometric parameters did not reach statistical significance, thus, indicating that a CFTR potentiator alone is insufficient for the treatment of patients who are homozygous for the Phe508del genotype^{19,20}.

Lumacaftor, also known as VX-809, is an established CFTR corrector drug that has been extensively characterized. Although the correction mechanism of Lumacaftor is not fully understood, there is evidence that it promotes the proper folding of Phe508del-CFTR during its biogenesis and processing in the ER, allowing it to exit the ER and traffic to the cell surface²¹⁻²⁴. Improvement of CFTR function to clinically meaningful levels was proposed

to require a combination of Lumacaftor, to deliver CFTR channels to the PM, and Ivacaftor, to increase the proportion of time those channels are open²⁵. Based on this knowledge, Lumacaftor advanced into clinical trials in patients homozygous and heterozygous for the Phe508del mutation, with the aim of evaluating its safety and efficacy alone and in combination with Ivacaftor. As assessed by a phase II study, administration of Lumacaftor alone did not provide a significant therapeutic benefit, as predicted FEV_1 % was similar between the studied groups²⁶. Subsequently, the combination of Ivacaftor and Lumacaftor was explored in a series of clinical studies. In a phase II study, combination of Lumacaftor and Ivacaftor (in the higher doses administrated) significantly improved FEV_1 by a mean of 6% for patients homozygous for Phe508del CFTR, decreased sweat chloride concentration by a mean of 8.9 to 10.3 mmol/L, and decreased pulmonary exacerbations in the treatment groups²⁷. Phe508del CFTR heterozygous patients did not have a significant improvement in FEV_1 or any other parameters²⁷. Data from two phase III trials in patients homozygous for Phe508del showed that there were significant improvements in FEV_1 , ranged from a mean of 2.6 to 4.0%, and that the rate of pulmonary exacerbations was 30 to 39% lower, since hospitalization or the use of intravenous antibiotics was reduced in the treatment groups²⁸. While significant, these results fell below initial expectations and experimental evidence emerged to, at least partially, explain the limited improvements observed in patients. It was shown that chronic administration of Ivacaftor, as well as most other potentiators, results in a

dose-dependent reversal of Lumacaftor- and Tezacaftor (another investigational corrector, see below)-mediated CFTR correction in Phe508del homozygous primary airway cell cultures^{20,29}. This was due to protein destabilization and increase turnover rate, resulting in its reduced functional expression at the cell surface. A posterior study confirmed that exposure to high concentrations (>1 μ M) of ivacaftor did inhibit lumacaftor's rescue of Phe508del-CFTR but reported that chronic exposure to low (\leq 100 nM), clinically relevant concentrations of the potentiator did not³⁰. Thus, since combination therapy presumes an improvement of beneficial effects over the use of each drug alone, the inhibitory effect of ivacaftor on lumacaftor efficacy requires further evaluation, perhaps in post-treatment patient samples. In addition, it was also observed that *P. aeruginosa* reduces Phe508del-CFTR function in cells treated either with Lumacaftor alone or the with the Ivacaftor/Lumacaftor combination^{31,32}. Since 85% of adult CF patients are colonized with *P. aeruginosa*, this data suggests that infection with these bacteria may also partially account for a reduction in the therapeutic efficacy of these drugs in Phe508del-homozygous patients.

Nevertheless, despite its modest efficacy, the combination therapy clinical trial data supported an apparent benefit for patients. Accordingly, Ivacaftor plus Lumacaftor (commercial name: Orkambi®³³) was approved for the clinical treatment of CF patients homozygous for the Phe508del mutation by the Food and Drug Administration (FDA) and European Medicines Agency (EMA)³⁴. This drug combination has now been used in patients since 2015, and several consequent studies of its long-term usage indicate that it does benefit CF patients, although several cases of off-target side-effects have been reported, including dyspnea (14%), diarrhea (11%), and nausea (10%) as well as serious adverse hepatobiliary reactions occurring in at least 0.5% of patients³⁵⁻³⁷.

Tezacaftor plus Ivacaftor

Tezacaftor, also known as VX-661, is an investigational CFTR corrector, structurally similar to Lumacaftor, which also improves Phe508del CFTR folding and traffic to the cell surface³⁸. Tezacaftor was introduced in clinical trials as an alternative to Lumacaftor, with the advantage that it is not an inducer of CYP3A4 enzymes and, therefore, does not interfere with other medications that are frequently used in CF, particularly Ivacaftor^{33,39}. The safety and efficacy of Tezacaftor monotherapy, and Tezacaftor plus Ivacaftor combination therapy was evaluated in a phase II trial in patients homozygous for Phe508del or heterozygous for Phe508del and a second Gly551Asp CFTR mutation³⁹. Administration of the combined therapy resulted in a significantly decrease in sweat chloride around 6.04 mmol/L and predicted FEV₁ of 3.75% for Phe508del homozygous subjects, and 7.02 mmol/L in sweat chloride

and 4.6% predicted FEV₁ for heterozygous³⁹. These results supported continued clinical studies of this drug combination, since the improvements in lung function are comparable to those observed in patients treated with lumacaftor plus Ivacaftor²⁸. Results from phase III studies were similar, with values of 4.0% for predicted FEV₁ in homozygous patients, with a 35% reduction on the rate of pulmonary exacerbation in the treatment group and 6.8% for predicted FEV₁ in heterozygous patients^{40,41}. In both phase II and III studies the treatment had less respiratory adverse events^{40,41} when compared with previous reports from Lumacaftor trials³⁵⁻³⁷, revealing itself to be an appealing alternative to the approved therapy.

Triple combinations

Last year Vertex announced the first results for the triple combination studies with Tezacaftor/Ivacaftor plus VX-440, VX-152, or VX-659, three investigational drugs that are next-generation correctors of the defective Phe508del-CFTR protein⁴²⁻⁴⁵. Data from the Phase II studies showed values of mean predicted FEV₁ of 9.7% and 12.0% for the triple combination regimens with VX-152 or VX-440 respectively, in patients heterozygous for Phe508del and one minimal function mutation. In the same category of CF patients, initial data from a phase I study for the triple combination regimen of VX-659 showed an improvement in predicted FEV₁ of 9.6%. Initial data with VX-152 or VX-440 in patients homozygous for Phe508del, who were already receiving Tezacaftor and Ivacaftor, also showed an improvement in mean predicted FEV₁ of 7.3% and 9.5%. Furthermore, it was reported that all triple combinations were generally well tolerated and the majority of adverse events were mild to moderate⁴².

Plasma membrane stabilizers

Although preliminary results for the triple combination therapy in patients with the Phe508del mutation look promising, there is still an unmet target for truly effective new therapies. Given the complexity of protein defects presented in Phe508del CFTR, part of the incomplete effectiveness of the described combined therapies may derive from an inability to retain sufficient CFTR levels at the apical surface of epithelial cells. Therefore, there is a real need for molecular strategies achieving the PM retention of corrected rescued Phe508del CFTR. Our group has investigated the peripheral protein quality control (PPQC) checkpoint in lung epithelial cells in Phe508del CFTR exposed to VX-809. We found that the conformation of the scaffold protein NHERF1 (Na(+)/H(+)) exchange regulatory factor 1) determined whether or not the PPQC recognized rescued Phe508del CFTR at the PM⁹. Moreover, we showed that activation of the cytoskeletal regulator Rac1 promoted an interaction between the actin-binding adaptor protein ezrin and NHERF1 in a way that triggered

exposure of the second PDZ domain of NHERF1, which interacted with rescued Phe508del CFTR. Because binding of Phe508del CFTR to the second PDZ of NHERF1 precluded the recruitment of ubiquitin ligase CHIP, the co-exposure of airway cells to the Rac1 activator HGF (hepatocyte growth factor) nearly tripled the functional rescue of Phe508del CFTR by VX-809, retaining the rescued channels at the PM⁸. Since HGF signaling is determinant for lung tissue repair after acute lung injury⁴⁶, these findings open new areas of investigation worth pursuing in the development of small-molecule drugs for CF treatment.

mRNA repairers

ProQR Therapeutics is initiating clinical trials for a novel mRNA-based strategy for correction of Phe508del CFTR mutation⁷ after obtaining extremely promising *in vitro* and *in vivo* preclinical data⁴⁷. Their innovative compound, QR-010, is an investigational 33mer chemically modified, antisense oligoribonucleotide (AON) complementary to wt-CFTR mRNA, that was designed to repair Phe508del CFTR transcripts. QR-010 mechanism of action is not yet fully characterized but it was postulated to involve RNase H-mediated degradation of the mutant transcript, followed by RNA repair⁴⁸. Treatment with inhaled QR-010 brought near complete restoration of chloride transport across the nasal mucosa of Phe508del homozygous mice. Moreover, two doses of QR-010 were able to improve CFTR-mediated saliva secretion up to 80% of the wild-type levels⁴⁷.

Proteostasis regulators

Proteostasis regulators are a novel class of CFTR correctors that act indirectly on CFTR by modulating components of the channel's interactome. One of the most promising was Cavosonstat⁷, developed by Nivalis Therapeutics, Inc. Cavosonstat is a S-nitrosoglutathione reductase (GSNOR) inhibitor that increases GSNO and NO levels, which are lower in CF tissues⁴⁹. This was postulated to promote a chaperone-dependent increase in CFTR abundance, stability and function. Unfortunately, in a February 2017 press-release, the company announced that the drug did not meet primary endpoints in a Phase II trial⁵⁰. Nonetheless, it highlighted NO signaling as a new research venue for CFTR modulators.

Another NO-related proteostasis regulator is Riociguat, the active ingredient of Adempas, an oral drug marketed by Bayer for the treatment of pulmonary hypertension. Riociguat increases the sensitivity of soluble guanylate cyclase (sGC) to NO, increasing cyclic guanosine monophosphate (cGMP) production. In addition to decreasing blood pressure, some studies report that the sGC-NO-cGMP pathway may regulate CFTR channel conductivity⁵¹. A Phase 2 study is now underway in CF patients homozygous for the Phe508del mutation⁷.

Amplifiers

Amplifiers are compounds designed to increase CFTR expression and thus increase ER protein load. In that sense, to support Phe508del CFTR correction, they must be used in combination therapy. The starting point for the development of this CF drug class was the finding that the small-molecule HDAC7 inhibitor, SAHA1, significantly amplified Phe508del CFTR quantity and surface expression in human bronchial epithelial cells, possibly by interfering with CFTR's proteostasis network⁵². However, the clinical applicability of HDAC inhibitors in CF treatment remains controversial. In a recent study using air-liquid interface cultures of differentiated nasal epithelia cells from CF patients, SAHA1 failed to increase CFTR transcript levels but rather inhibited mucine expression and goblet epithelial cell differentiation⁵³. Nonetheless, those initial findings opened a new field in CF modulator drugs' research. Proteostasis Therapeutics, Inc., for instance, has developed a specific CFTR amplifier, PTI-428, that increases the CFTR mRNA pool, feasibly by improving mRNA stability and/or events surrounding CFTR translation. A phase II study evaluating the efficacy and safety of PTI-428 in CF patients receiving Orkambi[®]³³ demonstrated a mean absolute improvement in predicted FEV₁ of 5.2% in the tested group, with no significant adverse effects⁵⁴.

Additional small-molecule compounds

Proteostasis Therapeutics, Inc., developed two additional investigational compounds selectively targeting Phe508del CFTR: PTI-801, a new-generation CFTR corrector, and PTI-808, a CFTR potentiator⁷. The company just finished the first Phase I and II studies for these compounds, which showed promising results, and therefore, is now preparing new clinical studies and cohorts for the triple combination of PTI-428, PTI-801, and PTI-808, called PTI-NC-733⁵⁴.

Galapagos/AbbVie has also developed four novel correctors currently under clinical trials⁷. Correctors GLPG222 and GLPG2851 (C1) are additive to GLPG2737 and GLPG3221 (C2) and may be combined in therapy. GLPG222 shares structural similarities with Lumacaftor and Tezacaftor, but was reported to produce a more efficient rescue of Phe508del CFTR *in vitro*⁵⁵.

FDL169, a drug being developed by Flatley Discovery Lab, has been shown to increase Phe508del CFTR cell-surface abundance with similar potency and efficacy as VX-809. However, FDL169 activity is not additive to VX-809, suggesting a similar mode of action⁵⁶. Notwithstanding, FDL169 exhibited a higher free fraction in human serum and improved distribution in mice lungs⁵⁷, which makes it a promising alternative to Lumacaftor.

QBW251, a compound developed by Novartis Pharmaceuticals, is currently in phase II clinical trials⁷.

It is described as a CFTR potentiator, similar to Ivacaftor. Preclinical data indicated that, when combined with Lumacaftor, QBW251 was more efficacious than Ivacaftor in sustaining Phe508del CFTR membrane expression and function⁵⁸. In an initial study in CF patients homozygous and compound-heterozygous for the Phe508del mutation, QBW251 proved to be safe and well tolerated. It promoted a significant decrease in sweat chloride and improved lung function in heterozygous patients, but, as Ivacaftor, showed no efficacy in Phe508del homozygous individuals⁵⁸. Nevertheless, these initial results suggest QBW251 may constitute an attractive alternative to Ivacaftor in combination therapies.

Notably, several other compounds were reported to potentiate CFTR function, including the natural food components genistein, an isoflavonoid found in high concentrations in soy⁵⁹, and curcumin, a major constituent of turmeric⁶⁰. Curcumin is able to activate CFTR channels in both adenosine triphosphate (ATP) dependent and independent ways⁶⁰⁻⁶², whereas genistein acts through ATP-dependent CFTR gating⁶³. Patch clamp studies showed additive effects of curcumin and genistein on the gating of Gly551Asp CFTR channels⁶⁴ and, importantly, a recent study showed that genistein and curcumin also enhanced forskolin-induced swelling of ivacaftor/lumacaftor treated intestinal organoids derived from biopsies of Phe508del homozygous patients⁶⁵. Curcumin PKA-dependent, but ATP-independent, potentiation results from its binding to the stimulatory ICL1/ICL4-R interface to stabilize the channel open state, while PKA/ATP-dependent potentiation results from removal of inhibitory Fe³⁺ at the ICL3-R interface, which promotes dimerization of NBD1 and NBD2^{62,66-68}. These data suggest that ivacaftor, genistein, and curcumin, in double or triple combinations, can synergize to restore CFTR-mediated fluid secretion in primary CF cells, thus supporting a possible benefit if multiple potentiators are used for treatment of CF. Still on this note, the very hydrophobic nature and low stability in water of compounds, such as curcumin, is a major drawback in their clinical application. In a very recent work, Gonçalves et al.⁶⁹ showed that the neutral amphiphilic triblock copolymer MeOx6-THF19-MeOx6 (TBCP2) can solubilize curcumin and facilitate its penetration in Phe508del CFTR human airway epithelial cells, enhancing curcumin potentiation of CFTR mediate Cl⁻ selective currents in these cells. These data suggests that TBCP2 may constitute a helpful tool for the delivery of this and other highly insoluble therapeutic drugs to CF patients.

Gene Therapy

Gene therapy is a controversial subject that became a possibility for CF since the cloning of the CFTR gene¹. In the case of CF, direct administration of the agents to the lungs via aerosols is an attractive option for gene therapy as

pulmonary epithelium has a relative easy accessibility and reduces the risks associated with systemic delivery. Non-viral vectors seem to be the safest and most efficient gene transfer agents for this kind of therapy and, in the most promising trials, the plasmid DNA pGM169 (carrying the CFTR cDNA) combined with the cationic liposome GL67A, was the chosen formula for lung delivery^{70,71}. Monthly application of the pGM169/GL67A complex in CF patients was evaluated in a phase IIb trial, where a 3,7% increase in predicted FEV₁ and stabilization of lung function was observed in the treatment group⁷¹. Despite the modest results, the trial offered proof of concept that non-viral gene therapy can benefit the lung function of CF patients and creates the opportunity for follow-up studies, namely combining gene therapy with the co-administration of CFTR modulator drugs.

Concluding Remarks

In the field of CF personalized medicine it is imperative to always consider the multidimensional nature of the disease. Even for CF patients caring the Phe508del mutation, a universal treatment is not likely to become a reality, given the complexity of phenotypes observed among the several possible compound-genotypes and even among homozygous patients. The contribution of lung tissue integrity, modifier genes, environmental factors, etc., has to be accounted, while tuning drug combination therapies to each individual patient. This is even more relevant if one considers therapies to fit the plethora of CFTR mutations displaying distinct functional defects. Nonetheless, the data presented in this review support a direct association between targeting the various defects of Phe508del-CFTR with combinations of multiple pharmaceutical agents and a consistent gain in lung function, body weight, and reduced disease exacerbations in patients. Thus, these recent achievements with small-molecule therapies, coupled to the growing amount of novel compounds and innovative strategies, give the scientific, clinical and patient communities new hope of finding highly efficient therapeutically solutions for CF.

Conflict of interest statement

The authors declare no conflict of interest.

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Abbreviations

AON :Oligoribonucleotide; ATP : Adenosine

Triphosphate; CF : Cystic Fibrosis; CFTR : Cystic Fibrosis Transmembrane Conductance Regulator; cGMP : cyclic guanosine monophosphate; Chloride : Cl⁻; EMA : European Medicines Agency; ER : Endoplasmic Reticulum; FDA : Food and Drug Administration; FEV1 : Forced Expiratory Volume in One Second; GSNOR : S-nitrosoglutathione reductase; HGF : Hepatocyte Growth Factor; ICL : Intracellular Loop; NHERF1 : Na(+)/H(+) Exchange Regulatory Factor 1; Phe508del : Deletion of phenylalanine 508; PM : Plasma Membrane; PPQC : Peripheral Protein Quality Control; R : Regulatory; sGC : Soluble Guanylate Cyclase; TBPC2 : MeOx6-THF19-MeOx6.

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