Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2022.02.2.026

December 2022
Volume 2
Issue 2

Cystic Fibrosis Treatment; Overview of Small Molecule Modulators and Genetic Therapy

Salma Elmallah[1] and Raafat Soliman[2]

[1]Department of Pharmaceutical Sciences (Pharmaceutical Chemistry), College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport, Abu Qir, Alexandria, Egypt
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Alexandria University, Alexandria 21521, Egypt

Email: salmamallah@egypt.aast.edu, salmamallah@hotmail.com, rafsoli41@yahoo.com

Received on, 27 April 2022 - Accepted on, 15 September 2022 - Published on, 23 October 2022

Abstract:

Cystic fibrosis, CF, is a life-shortening genetic disease affecting Caucasian population. It is a recessive genetic disease that is mainly caused by different types of mutations affecting the gene encoding for the cystic fibrosis transmembrane conductance regulator, CFTR, protein. A malfunctioning CFTR protein would lead to the accumulation of a thick viscous mucous layer blocking pancreatic ducts, intestines and airways which is the primary reason of death. Treatment of cystic fibrosis was mainly addressing the symptoms to overcome the complications of the disease. Since the early 2010s, the development of an actual therapy has reached great milestones including small molecule modulators and genetic therapy. Small molecule therapy depends on the development of small pharmacological agents that can, through different mechanisms, restore the function of the mutated CFTR protein. On the other hand, gene-editing techniques are evolving showing very promising results. Gene therapy entails the relocation of a proper copy of the CFTR gene in the aim of expressing a functional CFTR protein. Interesting advances in the development of small molecule and genetic therapies are discussed in this review article with a highlight on their benefits and limitations.

Key words: *cystic fibrosis, treatment, disease, drug, pulmonary, small molecule modulators, gene therapy*

INTRODUCTION

1. Background

Cystic fibrosis (CF) is one of the most common life-threatening genetic diseases among people of Caucasian origin. It affects over 90,000 individuals who are heterogeneously distributed worldwide where the United States of America contains the highest number of registered patients, (Figure 1).[1] It is an autosomal recessive disorder characterized by a widespread dysfunction of exocrine glands particularly in lungs and the gastrointestinal tract leading to severe complications and often early death.[2] CF was first recognized in 1938 by the pathologist Dorothy Andersen and since then the interest in understanding the pathophysiology and the underlying mechanism of the disease has been in continuous growth.[2] In the 1950's, it was noted by Kessler and Andersen that cystic fibrosis patients show an abnormal electrolyte composition of the sweat (excess sodium chloride, NaCl).[3] Further investigations suggested that the disturbance in fluids and electrolyte transport is associated with an abnormally low chloride ion permeability through epithelial membranes that leads to poor NaCl reabsorption in sweat ducts and consequently high concentration of NaCl in the sweat of cystic fibrosis patients.[4]

The underlying cellular defect of this abnormality remained unknown until the identification of the gene mutation responsible for this defect in 1989.[2] Molecular cloning experiments have allowed the isolation of DNA segments containing the cystic fibrosis gene locus. The CF gene is located on the long arm of chromosome 7, embracing 180000 base pairs.[5] In 1989, John Riordan et al. were able to fully characterize the product of the CF gene and it was given the name of CFTR (cystic fibrosis transmembrane conductance regulator) protein.[5]

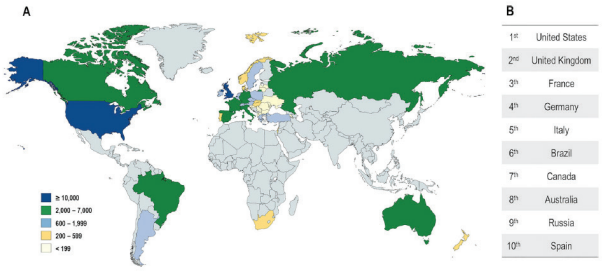

Figure 1: Demography of cystic fibrosis patients in different countries. (A) Distribution according to the total number of patients registered. (B) Top 10 countries with the highest number of patients registered. [1]

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2022.02.2.026

2. Structure of CFTR Protein

The CFTR glycoprotein is comprised of 1480 amino acids and is a member of the Adenosine TriPhosphate (ATP) Binding Cassette (ABC) superfamily; it is a family of active transporters that use the energy of ATP hydrolysis to translocate ions across epithelial membranes based on concentration gradient.[6, 7] Being a multi-domain membrane, CFTR protein consists of two membrane spanning domains (MSD1 and MSD2), two intracellular nucleotide binding domains (NBD1 and NBD2) and an intracellular regulatory domain (R) that connects both halves of the protein and regulates the gating of the CFTR channel, (Figure 2).[8, 9]

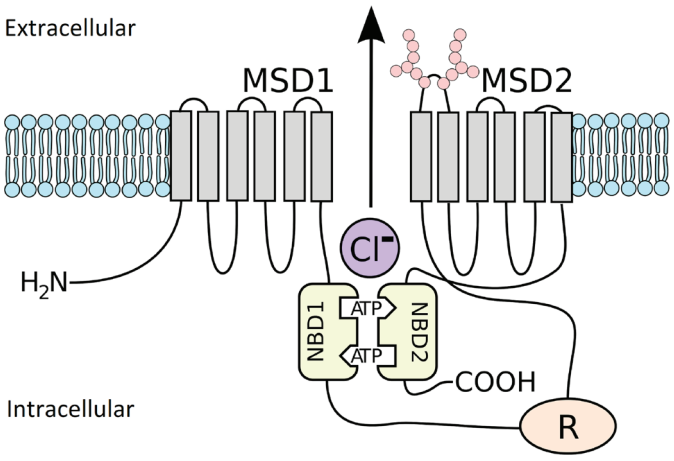

Figure 2: Predicted topology of CFTR protein. [7]

Each MSD subunit consists of six hydrophobic transmembrane domains that together form the channel pore of the protein to allow ion conductance.[8] Activation of the CFTR protein requires 2 main factors; it starts with the phosphorylation of the R domain, which is a highly charged sequence containing several phosphorylation sites, by various protein kinases such as PKA, PKC and AMP-activated protein kinase.[10] The second factor is the binding and hydrolysis of ATP molecules at the NBDs which stimulates the opening and closure of the chloride channel respectively.[11] CFTR is a multifunctional protein where besides being a chloride and bicarbonate channel, it can also regulate the transport of other ions by having either a positive or a negative effect on other channels and transporters. A working CFTR protein can help in the efflux of Cl^-, $(HCO_3)^-$ and K^+ ions through different apical channels.[12] Furthermore, it down regulates the reabsorption of Na^+ ions from epithelial Na^+ channels (ENaC).[13] Decreased secretion of Cl^- ions and increased reabsorption of Na^+ ions is the main biomarker of CF as patients would have salty sweat due to increased sodium and chloride levels.

2.1. Normal CFTR Protein Synthesis:

Since the characterization of the CFTR gene, over 1600 different gene mutations have been identified.[14] These mutations interfere with the normal pathway of CFTR from the nucleus to the plasma membrane leading to either no Cl^- channel or a malfunctioning one. The journey of the CFTR protein includes many cellular proteins within multiple compartments.[15] It first starts with the transcription of the DNA code, within the nucleus, into mRNA. The mRNA leaves the nucleus to be translated, mainly in the endoplasmic reticulum (ER), into a nascent polypeptide. Within the ER lipid bilayer, the nascent protein undergoes further protein maturation and a cotranslational folding process. [7] Any formed misfolded CFTR protein will be bound to a specific chaperone protein to carry it through an ER-associated degradation process, ubiquitination, followed by degradation in the ubiquitin proteasome system. The cytoplasmic transfer of the properly folded CFTR protein to Golgi apparatus is done through a variety of chaperone proteins, where it will undergo posttranslational modification, notably glycosylation. The CFTR processing involves its conversion from mannose-enriched to a mature oligosaccharide side chain attached to the aspargine residues located in the fourth loop of MSD2. [7] The mature CFTR will be shuttled, by clathrin-coated vesicles, from the Golgi apparatus and stationed in the plasma membrane. Once inserted into the plasma membrane, CFTR turns over at a rate of 10% per minute and has a half-life of ~ 12-24 hrs. Finally, the internalization of the mature protein proceeds through clathrin-coated endosomes. [7]

2.2. CFTR Mutations:

There are more than 2000 identified mutations that can affect the CFTR gene leading to a wide range of phenotypes since the discovery of the CFTR gene in 1989. They can be classified into six main classes according to their resulting defect in the CFTR production, (Figure 3).[16]

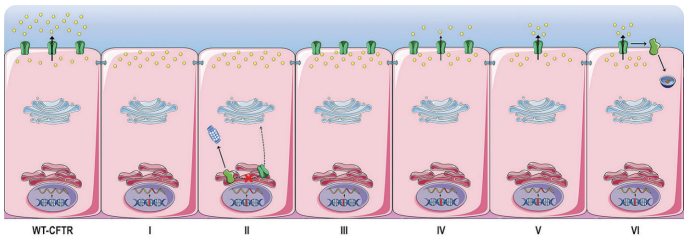

Figure 3: Schematic representation of the different classes of CFTR gene mutations.[1]

2.2.1. Class I Mutations, Defective Synthesis:

This class of mutations results in truncated or unstable mRNA leading to defective expression of CFTR gene and consequently defective synthesis of CFTR protein.[17] This will lead to complete absence of the protein at the cell surface and it affects ~ 7% of CF patients worldwide. [17] Class I mutation most common examples are; W1282X and G542X. [17]

2.2.2. Class II Mutations, Defective Processing:

This class of mutation results in defective protein processing. The formed misfolded CFTR protein will be retained at the endoplasmic reticulum leading to premature degradation and preventing the trafficking

of the protein to the plasma membrane.[17] The most common examples of this class are; F508del and N1303K.[17] This class includes F508del, also termed as ΔF508, mutation which is the most frequent CF mutation affecting ~90% of CF patients in at least one allele.[19] This mutation involves the deletion of phenylalanine amino acid located at position 508 at the NBD1 of the CFTR protein.[19]

2.2.3. Class III Mutations, Defective Gating:

In this type of mutation, the CFTR protein successfully inserted at the epithelial membrane will suffer from defective gating. This will lead to a closed channel or reduced open probability of the CFTR channel and consequently decreased chloride ion efflux.[18] The most common examples of this class are; G551D and S549R. [17] G551D is the third most common type of mutation affecting ~5% of CF patients worldwide.[20] It involves the replacement of the glycine amino acid located at position 551 of the NBD1 of the CFTR protein with aspartic acid and it leads to a reduced open probability of tenfold less than the normal CFTR channel.[21]

2.2.4. Class IV Mutations, Defective Conductance:

This class of mutation involves defects in the channel pore leading to decreased open probability to be around one third that of the normal protein. [7] This will consequently lead to lower permeation and conductance of chloride ions through the CFTR channel. It affects mainly the MSD of the CFTR protein.[22] The most frequent examples of this class are; R117H and R334W.

2.2.5. Class V Mutations, Defective mRNA Stability:

This class of mutation results in splicing errors which will lead to defective mRNA stability. The defective stability will lead to the formation of a mixture of both correct and aberrant protein and thus reduced synthesis of the normal CFTR channel. [7] As a result, a reduced total amount of CFTR protein is found at the plasma membrane leading to decreased chloride ion secretion. The most frequent examples of this class are; A455E and c.1680-886A>G. [17]

2.2.6. Class VI Mutations, Defective CFTR Stability:

This class of mutation will cause instability of the CFTR channel already localized at the plasma membrane leading to an accelerated turnover rate from the cell surface. The most frequent examples of this class are; 4326delTC and 4279insA. [17]
Broadly speaking, mutations of classes I, II and III are the most severe types as they are associated with few or no CFTR protein at the plasma membrane. While classes IV, V and VI have milder disease conditions as they involve only partial loss of the CFTR function activity. [17]

3. Symptoms of Cystic Fibrosis:

The lack of functioning CFTR at the proper location in the epithelial membrane, due to different types of mutations, leads to malfunctioning CFTR-dependent ion channels. This results in the disruption of ion transport homeostasis through epithelial membranes in different organs. [2] In the lungs, CFTR and ENaC are important for the regulation of the water volume and height of the airway surface liquid (ASL). [23] ASL is the layer covering the lung surface. It is composed of mucus and periciliary liquid and it is necessary for mucociliary clearance. [2] In CF, overexpression of ENaC leads to increased absorption of salt and water from airway surfaces and ASL depletion.[23] Dehydration of airway surface liquid leads to defective mucociliary clearance and reduced bacterial clearance.

Mucus accumulation provides a good environment for bacterial colonization and subsequently chronic airway diseases, chronic inflammation and respiratory failure. Pulmonary disease is the primary cause of morbidity in cystic fibrosis (>90%). [2, 24] Thick mucus can also accumulate in the pancreas and intestines, leading to the obstruction of pancreatic ducts and preventing the passage of digestive enzymes to small intestines. This leads to pancreatic insufficiency, maldigestion, poor growth and weight loss. Other symptoms of cystic fibrosis are liver cirrhosis, rectal prolapse, male infertility, diabetes, and a salty sweat.

4. Towards The Treatment of CF:

Previously, CF therapy was limited to symptomatic treatment. The main goal was to alleviate the symptoms; improving the quality of life of CF patients by treating downstream disease processes that are secondary to CFTR malfunction, without curing the disease itself.[25] Symptomatic treatment includes antibiotics and anti-inflammatory agents that help to eradicate chronic bacterial infections such as Pseudomonas Aeruginosa. Antibiotics used are mainly in the inhaled form such as; azithromycin, tobramycin, aztreonam and levofloxacin. Other antibiotics are also recommended such as; ciprofloxacin, cephalexin, amoxicillin and doxycycline according to sensitivity patterns.[27, 28] Secondary airway inflammation is controlled using NSAIDs, inhaled and systemic steroids and cromolyn.[29]

Mucolytic agents to break down mucus and clear up airways and pancreatic ducts are recommended along with the combination of inhaled β-agonists with humidified oxygen; a 3–6% hypertonic saline solution and dornase alfa to decrease viscoelasticity of mucus and dilating the airways.[30, 31] CF patients are also given pancreatic enzymes to help digestion; combinations of proteases, lipases and amylases, and supplementary vitamins to ensure good nutrition.[32] More agents for symptomatic improvements are in the pipeline.

Isolation of CFTR protein and understanding of molecular mechanisms behind the clinical manifestations of the disease has helped in the development of new treatments tackling the main cause of cystic fibrosis. At present, different drug approaches are being developed to overcome the underlying genetic defect causing the disease and help to halt or reduce the progression of the disease. Agents focusing on correcting structural and

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2022.02.2.026

functional abnormalities of CFTR protein can be divided mainly into; small molecule modulators and gene therapy. Figure 4 summarizes the main abnormalities and treatment approaches in CF patients.[33]

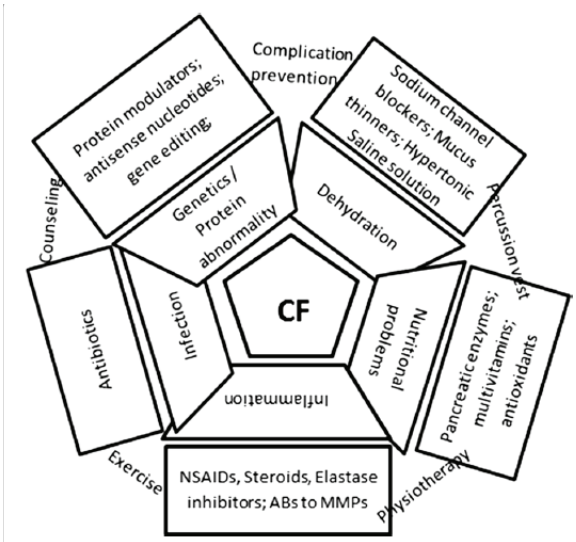

Figure 4: The main physiological dysfunctions and treatment approaches for CF patients. Inner trapezoid boxes depict the physiological abnormalities and outer rectangular boxes depict the main treatments. The texts connecting the outer boxes show nonpharmacological management. [33]

4.1. Small Molecule Modulators:

The discovery of small molecules, drug candidates, which can interact with the malfunctioning CFTR protein, has evolved over the years. This therapy has the advantage over gene therapy, as it avoids the potential treatment of wrong cells and/or losing the physiological CFTR regulation.[19] Small molecule therapy mainly involves the development of new compounds that can overcome the underlying CFTR defects and help in the restoration of its function. These defects can be mainly summarized into 2 points:

1. Aberrant CFTR folding and premature degradation,
2. Defective channel gating.

Development of such pharmacological agents is usually done through high throughput screening. High throughput screening involves testing a large collection of small molecules, either natural or synthetic drug-like compounds, using an automated assay designed to recognize active compounds with high efficiency and reliability.[34] The active candidates in the initial primary screen are further evaluated and the lead compounds are then optimized and intensively screened as potential new drugs.[34] Small molecule modulators can be classified according to their mechanism of activity into four main classes; correctors , potentiators, amplifiers and stabilizers.[1]

4.1.1. Potentiators:

Potentiators are small molecules that can repair the gating defect of CFTR ion channel by interacting with mutant CFTR protein, localized at the cell surface. They potentiate the channel activity of mutant CFTR and increase Cl^- transport across the membrane.[35] VX-770 is the first potentiator that was able to pass in vitro and clinical trials to be the first CF drug approved by US FDA on January 31, 2012 after an unusually fast 3 months review, Figure 5.[36] It was developed from quinolinone-3-carboxamide moeity and was given the generic name of Ivacaftor, then was released in the market under the name of Kalydeco™.[37] Ivacaftor was discovered via high throughput on Fisher rat thyroid (FRT) cells.[17] Using fluorescence based assays, changes in the membrane potential after applying Kalydeco due to CFTR-mediated chloride efflux were measured.[37]

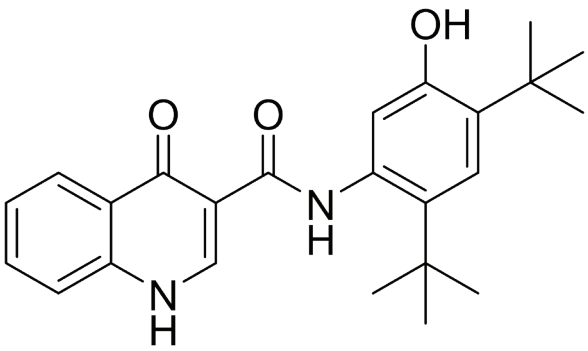

Figure 5: Chemical structure of VX-770, ivacaftor.

Kalydeco was able to increase the transepithelial current (Isc) four to six folds by increasing both the frequency and the duration of the channel openings. [17] It showed excellent activity and pharmacokinetic properties which qualified it to be the first human therapeutic agent that actually targets the main cause of the disease. Ivacaftor is a potentiator where it binds to the NBD1 of the CFTR protein already inserted into the plasma membrane to solve the gating problem by increasing the channel open probability (P_o), Figure 6.[38]

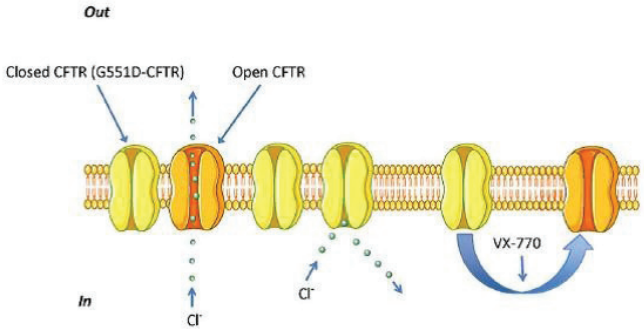

Figure 6: Effect of ivacaftor, VX-770, on G551D mutated CFTR protein. VX-770 allows CFTR channels to remain open, thereby, restoring chloride transport function. [38]

Children aged 6 years and older carrying class III mutation were able to benefit from ivacaftor by taking one tablet, 150 mg, every 12 hrs. Patients reported significant improvement in lung function, weight gain, markedly reduced sweat salt concentrations and overall improved lifestyle quality.[39]

Being in the market for over 7 years, patients treated with ivacaftor showed good resistance to secondary infection with Pseudomonas Aeruginosa and other pathogens, improved body mass index and quality of life.[40] With the aim of extending the target mutations of ivacaftor, a series of experiments and clinical trials were done. Ivacaftor is now an approved drug for 38 other gating mutations increasing the number of patients who may benefit from the drug.[1]

Being a successful example, ivacaftor analogs are being developed with the aim of acquiring improved pharmacokinetic properties and a better metabolic profile with low toxic byproducts. VX-561, formerly known as CTP-656, is a deuterated ivacaftor analog that is now in phase II clinical trials showing enhanced stability in vitro and healthy volunteers compared to ivacaftor, figure 7.[41] Having improved clinical profile, VX-561 is planned to be taken once daily rather than twice in the case of ivacaftor offering better patient compliance. CF therapy based on multiple potentiators has also been tested.

A combination of ivacaftor with natural food components like genistein and curcumin was tested on CF patients carrying gating mutations. The double or triple combination was found to successfully synergize restoring CFTR-dependent fluid secretion.[43] The inspiring story of ivacaftor being the first CF treatment has opened the way for the development of more small molecule modulators acting either as potentiators or having different mechanisms of action.

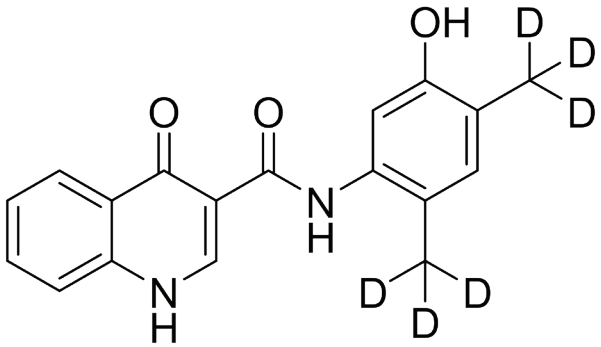

Figure 7: Chemical structure of VX-561, Deutivacaftor.

4.1.2. Correctors:

Correctors are pharmacological agents that overcome the defective processing of mutant CFTR. They can interact with either chaperone proteins to block CFTR premature degradation, or with the CFTR protein itself to help its proper folding and trafficking to the cell membrane, thus rescuing the cell surface expression of the mutant CFTR.[43] They have been classified according to their molecular targets into 3 main classes; class 1 correctors which can stabilize NBD1-TMD1 and/or NBD1-TMD2 interfaces, class 2 correctors having the ability to stabilize NBD2 and its interfaces with other CFTR domains and class 3 correctors which directly stabilize NBD1.[44] This classification can be useful to evaluate corrector combinations from different classes to have a complementary effect.

The great need to discover an effective therapy that can treat CF patients carrying the most common mutation, F508del, has fueled the innovation of a plethora of correctors. Lumacaftor, VX-809, is the fruit of research advances, Figure 8. It is developed by Vertex pharmaceuticals through high throughput screening.

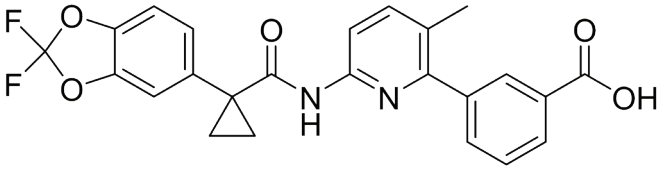

Figure 8: Chemical structure of VX-809, lumacaftor.

Lumacaftor is a corrector that can bind to the F508del mutated CFTR promoting proper folding of the protein, its trafficking to the plasma membrane escaping the endoplasmic reticulum premature degradation and maintaining its stability at the cell surface.[45] It has been proposed that the binding site of lumacaftor in the pocket created by the deletion of phenylalanine at position 508 found at the interface between NBD1 and the fourth transmembrane domain of MSD2.[46] It was found that lumacaftor can rescue 30% of CFTR protein from degradation.[47] Lumacaftor has no effect on the open probability of the CFTR chloride channel, gating, and the efflux of ions. Therefore, a combination of a lumacaftor and ivacaftor is needed in order to solve both trafficking and gating problems associated with F508del mutation.

Orkambi® is the first combination medicine of "lumacaftor and ivacaftor" to target CF patients carrying the most frequent mutation, F508del. Orkambi represents a breakthrough in CF therapy and it was approved by the US FDA in July, 2015. [47] It exerts its effect through a synergistic mechanism between a corrector and a potentiator.

It combines a chemical corrector, lumacaftor, that helps proper folding and trafficking of the protein and therefore increasing the total amount of channels at the cell membrane and a potentiator, ivacaftor, to increase the frequency and duration of the channel opening which in turn will increase the total movement of chloride across the membrane, Figure 9.[48]

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2022.02.2.026

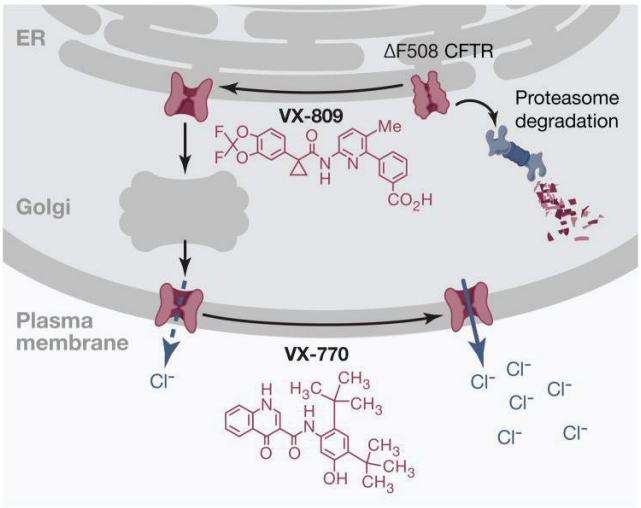

Figure 9: Orkambi's dual effect as corrector and potentiator. Lumacaftor rescues CFTR mutant from premature degradation while ivacaftor increases the channel open probability. [48]

Clinical trials proved that Orkambi has a significant but modest effect on lung function in patients carrying F508del mutation with a 2.6 to 4% increase in FEV1.[49] FEV1 or forced expiratory volume in one second is a standard test for lung function that measures the amount of air that can be exhaled in one second.[49] Orkambi is administered as two orally bioavailable combination tablets consisting of 200 mg lumacaftor and 125 mg ivacaftor to be taken every 12 hrs with a good safety profile in the short and long term.[46] Due to the lipophilicity of ivacaftor and lumacaftor, taking Orkambi with high fat-containing food highly increased their absorption. It was found that co-administration with high fat meal improved the absorption of lumacaftor with 2 fold and that of ivacaftor was 3 fold higher.[16]

Albeit patients treated with Orkambi have experienced an improvement in their quality of life, the drug-induced improvement in lung function was modest. This limited effect is maybe due to an inhibitory action exerted by ivacaftor on lumacaftor.[50] Further in vitro studies were conducted in order to identify the main cause behind Orkambi's reduced efficacy. These studies revealed that the reduced efficacy was related to the negative impact of ivacaftor, VX770, on the stability of the lumacaftor rescued F508del CFTR protein.[51] It determined that ivacaftor has a non-specific destabilizing effect on the lipid bilayer of the plasma membrane as it increased its fluidity or lipid packing and reorganized the membrane.[51] This membrane disruption causes a decreased abundance of the corrected protein leading to an inhibitory effect on the lumacaftor mediated correction of the CFTR protein. The negative effect of ivacaftor was correlated to its predicted lipophilicity as less lipophilic ivacaftor derivatives had milder inhibitory effect.[51]

Looking for other agents having better synergistic effect with ivacaftor, other correctors were developed. Tezacaftor, VX-661, was developed based on the structure of lumacaftor and it showed very good clinical results, Figure 10. The combination of tezacaftor and ivacaftor was approved by the FDA in 2018 under the name of Symdeko® but it failed to have any better results than orkambi.[1]

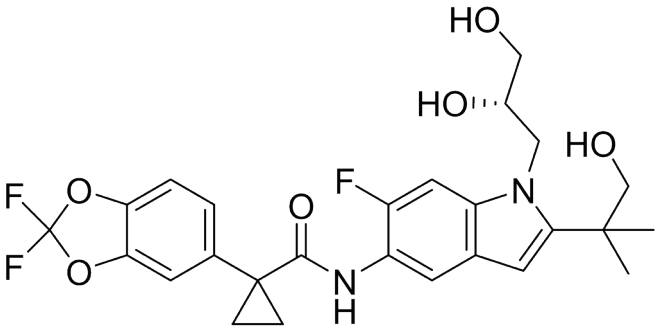

Figure 10: Chemical structure of VX-661, tezacaftor.

In the hope of having better synergistic results, a triple combination of lumacaftor-ivacaftor with new correctors; such as elexacaftor and bamocaftor, were developed, Figure 11. The combination of lumacaftor-ivacaftor-elexacaftor is recently approved by FDA under the name of Trikafta®.[1] The long term effects of this combination are still ongoing. Recently, another corrector-potentiator triple therapy of elexacaftor-tezacaftor-ivacaftor, kaftrio, is being tested showing very promising results.[67] The clinical trials of kaftrio showed improved lung function and increased body mass index with decreased sweat chloride levels.[68] More and more correctors and combinations are being in the loop offering big hopes to CF patients carrying the most frequent mutation.

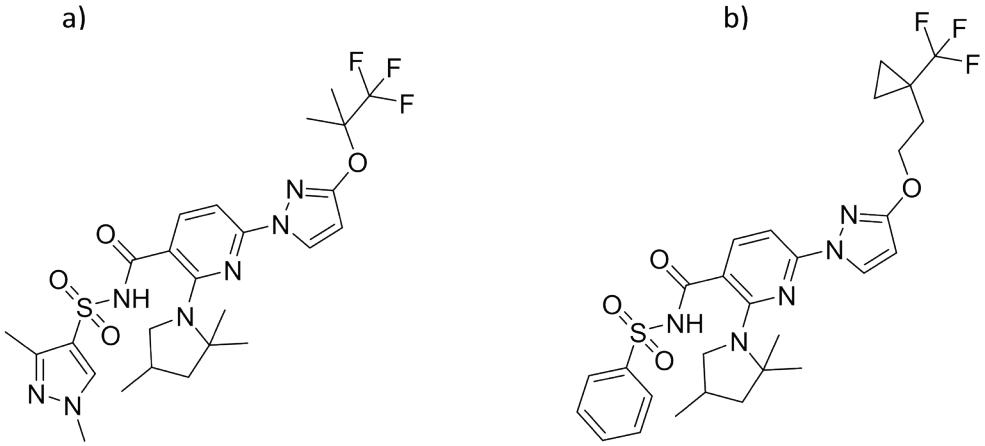

Figure 11: Chemical structure of; a) elexacaftor, b) bamocaftor.

4.1.3. Stabilizers:

Stabilizers are agents that anchor the CFTR protein at the cell surface. They are mainly useful in class VI mutation preventing CFTR removal and degradation by lysosomes.[1] They can be used alone or in combination with other classes of modulators to rectify the intrinsic stability of the CFTR protein and increase its residence time at the cell surface.[52] One of the most common stabilizers is the hepatocyte growth factor (HGF). It has the ability to enhance the CFTR stabilization at the plasma membrane by activating Rac1 GTPase signaling.[53] HGF can be combined with correctors such as lumacaftor in the treatment of F508del mutation to further enhance CFTR maturation and anchoring at the cell surface.[54] Prolonged HGF treatment showed a beneficial effect on ivacaftor/lumacaftor combination by preventing ivacaftor mediated destabilization of lumacaftor-rescued CFTR in F508del-expressing cells.[55]

Other stabilizers have been developed such as vasoactive intestinal peptide (VIP) and Cavosonstat. VIP enhances the phosphorylation of the actin-binding complex ezrin/radixin/moesin (ERM) which interacts with the scaffolding protein Na^+/H^+ exchange factor1 (NHERF-1) and CFTR to prevent CFTR endocytosis and lysosomal degradation.[56] Cavosonstat (N91115) is the first stabilizer to reach clinical trials. It can inhibit S-nitrosoglutathione (GSNO) reductase increasing cellular GSNO and stabilizing cell surface expression of F508del CFTR.[57] Unfortunately, cavosonstat failed to show promising clinical results in terms of lung function and sweat chloride concentration even when combined with modulators such as ivacaftor or lumacaftor reaching a dead end in clinical development.[58]

4.1.4. Amplifiers:

Amplifiers are pharmacological agents that amplify the expression of CFTR mRNA and, consequently, the biosynthesis of the CFTR protein.[59] The strategy is to augment the abundance of the CFTR protein which can then be folded properly and have a corrected function with the help of correctors and potentiators. The first developed amplifier nesolicaftor (PTI-428) was able to pass phase II clinical trials. Albeit, clinical testing of nesolicaftor combined with lumcafor-ivacaftor therapy showed promising results in terms of lung function and reduction of sweat chloride concentration levels, further clinical studies are now discontinued.[57]

4.2. Gene Therapy:

Being a monogenic disease, treatment of CF with gene therapy sounds a very promising approach having the advantage of targeting all CF patients regardless of the CFTR mutation present.[60] Gene therapy involves the relocation of proper copies of CFTR gene into the epithelial cell layer of the affected organs mainly the airways.[61] The gene translocation is done through vectors either viral, *e.g.* adenovirus vector, or nonviral which will be cationic molecules able to form a complex with the negatively charged DNA.[62] Gene therapy has been subjected to clinical trials but several challenges were faced such as the low delivery of functional genes by most available vectors, short half-life of therapeutic gene expression leading to low efficacy, and undesirable inflammatory responses.[63] To overcome the limited effect of gene therapy, the development of efficient vectors to deliver the cDNA to the airway cells is under progress.

4.2.1. Gene editing:

Gene editing is a genetic approach that implies the permanent correction of the mutated CFTR gene.[62] CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 approach is a gene editing strategy built on the same idea as bacterial defense mechanisms against foreign DNA, Figure 12.[61] It is currently the simplest, most versatile and precise method of genetic manipulation where the corrective DNA containing multiple small pieces is incorporated into a locus consisting of short palindromic repeats, called CRISPR. Cas9 is an enzyme acting as a pair of molecular scissors that can cut the two strands of DNA at a specific location in the genome so that introduced DNA can be added.[64]

This technique has been in *in vitro* clinical trials since 2013 showing very promising results.[65] Gene editing strategy has the advantage of allowing life-long expression and natural adjustment in the cell but no clinical data have been reported yet.[62]

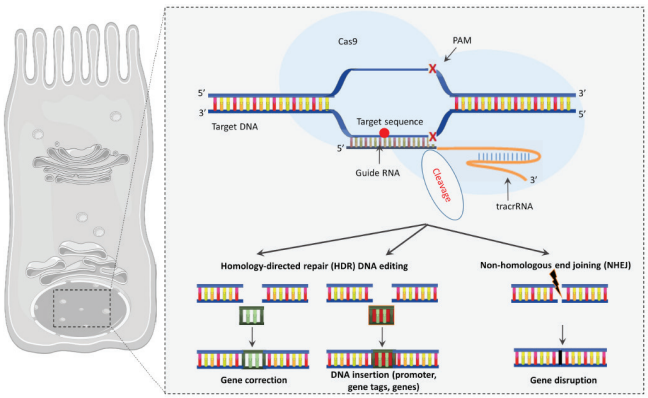

Figure 12: The CRISPR/Cas9 gene editing technique. Cas9 endonuclease binds to the target site using a guide RNA to precisely cut DNA allowing genome editing. CRISPR/Cas9 may drive to gene correction or DNA insertion. [61]

4.2.2. mRNA repair:

mRNA repair technique implies repairing the abnormal CFTR mRNA via the insertion of the missing bases. Eluforsen, formerly known as QR010, is an antisense RNA-based oligonucleotide sequence that is formulated as an aqueous solution to be administered by inhalation.[66]

It is designed to mainly target the abnormal mRNA in patients with the F508del mutation. Eluforsen has the ability to bind to the mRNA region around the F508-encoding deletion and restore the CFTR protein function in the airway epithelium.[66] The drug is still in clinical trials showing promising results in terms of safety and efficacy.

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2022.02.2.026

CONCLUSION:

Cystic Fibrosis is a recessive genetic disease caused by a plethora of CFTR gene mutations. The treatment of CF remained symptomatic until the development of new therapies that tackle the main root of the disease. Since the structure of the CFTR protein is fully elucidated, small molecule modulators that have the ability to bind to the mutated CFTR protein and restore its function were developed. Small molecule modulators can be classified into; correctors, potentiators, stabilizers and amplifiers.

They are personalized to specific types of mutations and it is a therapy that should be taken for a lifetime. Several modulators have been successfully approved by the FDA and EMA and released in the market increasing the life expectancy of CF patients from less than 20 years to over 50. Small molecule modulators have the advantage of improving the life quality of CF patients but being an expensive lifetime therapy that should be taken once or twice daily fueled the urge to the development of a permanent treatment.

Gene therapy involves the relocation of proper copies of CFTR gene into the epithelial cell layer. It is a permanent therapy targeting all different classes of mutations. Different techniques for gene editing are being developed and some of them have reached clinical trials with promising results. Gene therapy has the advantage of being a permanent treatment with a broad spectrum regardless of the type of mutation although its development is challenging.

REFERENCES

[1] Lopes-Pacheco, M. CFTR modulators: the changing face of cystic fibrosis in the era of precision medicine. Front. Pharmacol. 2020 Feb 21; 10, 1662.

[2] Simmonds, N.J. Cystic fibrosis and survival beyond 40 years. Ann. Respir. Med. 2011 Jan ; 2 (1), 55-63.

[3] Kessler, W.R.; Andersen, D.H. Heat prostration in fibrocystic disease of the pancreas and other conditions. Pediatrics. 1951 Nov; 8 (5), 648-656.

[4] Quinton, P.M. Chloride impermeability in cystic fibrosis. Nature. 1983 Feb 3. 301 (5899), 421-422.

[5] Riordan, J.R.; Rommens, J.M.; Kerem, B.S.; Alon, N.; Rozmahel, R.; Grzelczak, Z.; Zielenski, J.; Lok, S.; Plavsic, N.; Chou, J.L.; Drumm, M.L.; Iannuzzi, M.C.; Collins, F.S.; Tsui, L.C. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science. 1989 Sep 8; 245 (4922), 1066-1072.

[6] Licht, A.; Schneider, E. ATP binding cassette systems: structures, mechanisms, and functions. Cent. Eur. J. Biol. 2011 Sep 2; 6 (5), 785-801.

[7] Rogan, M.P.; Stoltz, D.A.; Hornick, D.B. Cystic fibrosis transmembrane conductance regulator intracellular processing, trafficking, and opportunities for mutation-specific treatment. CHEST. 2011 Jun 1; 10 (139), 1480-1490.

[8] Schmidt, B.Z.; Haaf, J.B.; Leal, T.; Noel, S. Cystic fibrosis transmembrane conductance regulator modulators in cystic fibrosis: current perspectives. Clin. Pharmacol. 2016 Sep 21; 8, 127–140.

[9] Eckford, P.D.; Ramjeesingh, M.; Molinski, S.; Pasyk, S.; Dekkers, J.F.; Li, C.; Ahmadi, S.; Ip, W.; Chung, T.E.; Du, K.; Yeger, H.; Beekman, J.; Gonska, T.; Bear, C.E. VX-809 and related corrector compounds exhibit secondary activity stabilizing active F508del-CFTR after its partial rescue to the cell surface. Chem. Biol. 2014 May 22; 21 (5), 666–678.

[10] Chiaw, P.K.; Eckford, P.D.W.; Bear, C.E. Insights into the mechanisms underlying CFTR channel activity, the molecular basis for cystic fibrosis and strategies for therapy. Essays Biochem. 2011 Sep 7; 50 (1), 233-248.

[11] Wellhauser, L.; Chiaw, P.K.; Pasyk, S.; Li, C.; Ramjeesingh, M.; Bear, C.E. A Small-Molecule Modulator Interacts Directly with ΔPhe508-CFTR to Modify Its ATPase Activity and Conformational Stability. Mol. Pharmacol. 2009 Jun; 75 (6), 1430-1438.

[12] Schwiebert, E.M.; Benos, D.J.; Egan, M.E.; Stutts, M.J.; Guggino, W.B. CFTR is a Conductance Regulator as well as a Chloride Channel. Physiological Reviews. 1999 Jan 1; 79 (suppl.1), S145-S166.

[13] Conese, M; Ascenzionic, F.; Boyd, A.C.; Coutelle, C.; De Fino, I.; Smedt, S.; Rejman, J.; Rosenecker, J.; Schindelhauer, D.; Scholte, B.J. Gene and cell therapy for cystic fibrosis: From bench to bedside. J. Cyst. Fibros. 2011 Jun; 10 (suppl.2), S114-S128.

[14] Di Saint'Agnese, P.A.; Darling R.C.; Perera, G.A.; Shea, E. Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas. Pediatrics. 1953 Nov; 12 (5), 549-563.

[15] McKone, E.F.; Aitken, M.L. Cystic fibrosis: disease mechanisms and therapeutic targets. Drug Discovery Today: Disease Mechanisms. 2004 Oct; 1 (1), 137-143.

[16] Schneider, E.K.; Reyes-Ortega, F.; Li, J.; Velkov, T. Can cystic fibrosis patients finally catch a breath with Lumacaftor/Ivacaftor? Clin. Pharmacol. Ther. 2017 Jan; 101 (1), 130-141.

[17] Harutyunyan, M.; Huang, Y.; Mun, K.S.; Yang, F.; Arora, K.; Naren, A.P. Personalized medicine in CF: from modulator development to therapy for cystic fibrosis patients with rare CFTR mutations. Am. J. Physiol. Lung Cell Mol. Physiol. 2018 Apr 1; 314, L529–L543.

[18] Quint, A.; Lerer, I.; Sagi, M.; Abeliovich, D. Mutation spectrum in Jewish cystic fibrosis patients in Israel: implication to carrier screening. Am. J. Med. Genet. A. 2005 Jul 30; 136A (3), 246–248.

[19] Pedemonte, N.; Sonawane, N.D.; Taddei, A.; Hu, J.; Moran, O.Z.; Suen, Y.F.; Robins, L.I.; Dicus, C.W.; Willenbring, D.; Nantz, M.H.; Kurth, M.J.; Galietta, L.J.V.; Verkman, A.S. Phenylglycine and Sulfonamide Correctors of Defective ΔF508 and G551D Cystic Fibrosis Transmembrane Conductance Regulator Chloride-Channel Gating. Mol. Pharmacol. 2005 May; 67 (5), 1797-1807.

[20] Yu, Y.C.; Miki, H.; Nakamura, Y.; Hanyuda, A.; Matsuzaki, Y.; Abe, Y.; Yasui, M.; Tanaka, K.; Hwang, T.C.; Bompadre, S.G.; Sohma, Y. Curcumin and genistein additively potentiate G551D-CFTR. J. Cyst. Fibros. 2011 Mar 26; 10 (4), 243-252.

[21] Dérand, R.; Pignoux, L.B.; Becq, F. The Cystic Fibrosis Mutation G551D Alters the Non-Michaelis-Menten Behavior of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Channel and Abolishes the Inhibitory Genistein Binding Site. J. Biol. Chem. 2002 Jul 17; 277 (39), 35999-36004.

[22] Welsh, M.J.; Smith, A.E. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell. 1993 Jul 2; 73 (7), 1251-1254.

[23] Shoseyov, D.; Cymberknoh, M.C.; Kerem, E. Could you please pass the salt? Am. J. Respir. Crit. Care Med. 2011 Jun 1; 183 (11), 1444-1446.

[24] Narasimhan, M.; Cohen, R. New and investigational treatments in cystic fibrosis. Ther. Adv. Respir. Dis. 2011 Mar; 5 (4), 275-282.

[25] Van Goor, F.; Hadida, S.; Grootenhuis, P.D.J.; Burton, B.; Cao, D.; Neuberger, T.; Turnbull, A.; Singh, A.; Joubran, J.; Hazlewood, A.; Zhou, J.; McCartney, J.; Arumugam, V.; Decker, C.; Yang, J.; Young, C.; Olson, E.R.; Wine, J.J.; Frizzell, R.A.; Ashlock, M.; Negulescu, P. Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. Proc. Natl. Acad. Sci.USA. 2009 Nov 3; 106 (44), 18825-18830.

[26] Kaiser, J. New Cystic Fibrosis Drug Offers Hope, at a Price. Science. 2012 Feb 10; 335 (6069), 645.

[27] Moss R.B. Long-term benefits of inhaled tobramycin in adolescent patients with cystic fibrosis. Chest. 2002 Jan; 121 (1), 55-63.

[28] Konstan, M.W.; Flume, P.A.; Kappler, M.; Chiron, R.; Higgins, M.; Brockhaus, F.; Zhang, J.; Angyalosi, G.; He, E.; Geller, D.E. Safety, efficacy and convenience of tobramycin inhalation powder in cystic fibrosis patients: the EAGER trial. J. Cyst. Fibros. 2011 Jan; 10 (1), 54-61.

[29] Flume, PA; O'Sullivan, B; Robinson KA; Goss, C.H.; Mogayzel, P.J.Jr, Willey-Courand, D.B.; Bujan, J.; Finder, J.; Lester, M.; Quittell, L.; Rosenblatt, R.; Vender, R.L.; Hazle, L.; Sabadosa, K.; Marshall, B. Cystic fibrosis pulmonary guidelines: chronic medications for maintenance of lung health. Am J. Respir. Crit. Care Med. 2007 Nov 15; 176 (10), 957-69.

[30] Salvatore, D.; d'Andria, M. Effects of salmeterol on arterial oxyhemoglobin saturations in patients with cystic fibrosis. Pediatr. Pulmonol. 2002 Jul; 34 (1), 11-5.

[31] Quan, J.M.; Tiddens, H.A.; Sy, J.P.; McKenzie, S.G.; Montgomery, M.D.; Robinson, P.J.; Wohl, M.E.; Konstan, M.W.; Pulmozyme Early Intervention Trial Study Group. A two-year randomized, placebo-controlled trial of dornase alfa in young patients with cystic fibrosis with mild lung function abnormalities. J. Pediatr. 2001 Jul 31; 139 (3), 813-20.

[32] Stern, R.C.; Eisenberg, J.D.; Wagener, J.S.; Ahrens, R.; Rock, M.; doPico, G.; Orenstein, D.M. A comparison of the efficacy and tolerance of pancrelipase and placebo in the treatment of steatorrhea in cystic fibrosis patients with clinical exocrine pancreatic insufficiency. Am. J. Gastroenterol. 2000 Aug; 95 (8), 1932–8.

[33] Misbahuddin, M.R.; Hussam, A.S.M.; Cystic fibrosis: current therapeutic targets and future approaches. Transl. Med. 2017 Apr 27; 15 (1), 84.

[34] Verkman, A.S.; Haggie, P.M.; Galietta, L.J.V. Application of green fluorescent protein-based chloride indicators for drug discovery by high-throughput screening. Rev. Fluor. 2004. 1, 85-98.

[35] Wang, W.; Okeyo, G.O.; Tao, B.; Hong, J.S.; Kirk, K.L. Thermally unstable gating of the most common cystic fibrosis mutant channel (ΔF508). J. Biol. Chem. 2011 Dec 9; 286 (49), 41937-41948.

[36] U.S. Food and Drug Administration: Drugs@FDA, FDA Approved Drug Products. http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/203188s007lbl.pdf.

[37] Hadida, S.; Van Goor, F.; Zhou, J.; Arumugam, V.; McCartney, J.; Hazlewood, A.; Decker, C.; Negulescu, P.; Grootenhuis, P.D.J. Discovery of N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (VX-770, Ivacaftor), a potent and orally bioavailable CFTR potentiator. J. Med. Chem. 2014 Dec 11; 57 (23), 9776-9795.

[38] Erlinger, S. Molecular repair of a defective CFTR protein in cystic fibrosis. Clin. Res. Hepatol. Gastroenterol. 2011 Apr; 35 (4), 254-256.

[39] Davies, J.C.; Wainwright, C.E.; Canny, G.J.; Chilvers, M.A.; Howenstine, M.S.; Munck, A.; Mainz, J.G.; Rodriguez, S.; Li, H.; Yen, K.; Ordon, C.L.; Ahrens, R.; on behalf of the VX08-770-103 (ENVISION) Study Group. Efficacy and safety of Ivacaftor in patients aged 6 to 11 years with cystic fibrosis with a G551D Mutation. Am. J. Respir. Crit. Care Med. 2013 Jun 1; 187 (11), 1219-1225.

[40] Heltshe, S.L.; Mayer-Hamblett, N.; Burns, J.L.; Khan, U.; Baines, A.; Ramsey, B.W.; Rowe, S. Pseudomonas aeruginosa in cystic fibrosis with G551D-CFTR treated with ivacaftor. Clin. Infect. Dis. 2015 Mar 1; 60 (5), 703–712.

[41] Harbeson, S.L.; Morgan, A.J.; Liu, J.F.; Aslanian, A.M.; Nguyen, S.; Bridson, G.W.; Brummel, C.L.; Wu, L.; Tung, R.D.; Pilja, L.; Braman, V; Uttamsingh, V. Altering metabolic profiles of drugs by precision deuteration 2: discovery of a deuterated analog of ivacaftor with differentiated pharmacokinetics for clinical development. J. Pharmacol. Exp. Ther. 2017 Aug; 362 (2), 359–367.

[42] Becq, F.; Mall, M.A.; Sheppard, D.N.; Conese, M.; Moran, O.Z. Pharmacological therapy for cystic fibrosis: From bench to bedside. J. Cyst. Fibros. 2011 Jun; 10 (Suppl 2), S129–S145.

[43] Dekkers, J.F.; Mourik, P.V.; Vonk, A.M.; Kruisselbrink, E; Berkers, G.; de Winter-de Groot, K.M.; Janssens, H.M.; Bronsveld, I.; Van der Ent, C.K.; de Jonge, H.R.; Beekman, J.M. Potentiator synergy in rectal organoids carrying S1251N, G551D, or F508del CFTR mutations. J. Cyst. Fibros. 2016 Sep; 15 (5), 568–578.

[44] Okiyoneda, T.; Veit, G.; Dekkers, J.F.; Bagdany, M.; Soya, N.; Roldan, A.; Verkman, A.S.; Kurth, M.; Simon, A.; Hegedus, T.; Beekman, J.M.; Lukacs, G.L. Mechanism-based corrector

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2022.02.2.026

combination restores DF508-CFTR folding and function. Nat. Chem. Biol. 2013 Jul; 9 (7), 444–454.

[45] Hutt, D.M.; Loguercio,S. ; Campos, A.R.; Balch, W.E. A proteomic variant approach (ProVarA) for personalized medicine of inherited and somatic disease. J. Mol. Biol. 2018 Sep 14; 430(18 Pt A), 2951-2973.

[46] Bulloch, M. N.; Hanna, C; Giovane, R. Lumacaftor/ivacaftor, a novel agent for the treatment of cystic fibrosis patients who are homozygous for the F580del CFTR mutation. Expert Rev. Clin. Pharmacol. 2017 Oct; 10 (10), 1055–1072.

[47] DeFrancesco, L. Drug pipeline: 3Q15. Nat. Biotechnol. 2015 Nov; 33 (11), 1126.

[48] Brodsky, J.L.; Frizzell, R.A. A combination therapy for cystic fibrosis. Cell. 2015 Sep 24; 163 (1), 17.

[49] Molinski, S.V.; Ahmadi, S.; Ip, W.; Ouyang, H.; Villella, A.; Miller, J.P.; Lee, P.S.; Kulleperuma, K.; Du, K.; Di Paola, M.; Eckford, P.D.W.; Laselva, O.; Huan, L.J.; Wellhauser, L.; Li, E.; Ray, P.N.; Pomès, R.; Moraes, T. J.; Gonska, T.; Ratjen, F.; Bear, C.E. Orkambi® and amplifier co-therapy improves function from a rare CFTR mutation in gene-edited cells and patient tissue. EMBO Mol. Med. 2017 Sep; 9 (9), 1224–1243.

[50] Marengo, B.; Speciale, A.; Senatore, L.; Garibaldi, S.; Musumeci, F.; Nieddu, E.; Pollarolo, B.; Pronzato, M.A.; Schenone, S.; Mazzei, M.; Domenicotti, C. Matrine in association with FD-2 stimulates F508del-cystic fibrosis transmembrane conductance regulator activity in the presence of corrector VX809. Mol. Med. Rep. 2017 Dec; 16 (6), 8849-8853.

[51] Chin, S.; Hung, M.; Won, A.; Wu, Y.-S.; Ahmadi, S.; Yang, D.; Elmallah, S.; Toutah, K.; Hamilton, C.M.; Young, R.N.; Viirre, R.D.; Yip, C.M.; Bear, C.E. Lipophilicity of the cystic fibrosis drug, Ivacaftor (VX-770), and its destabilizing effect on the major CF-causing mutation: F508del. Mol. Pharmacol. 2018 Dec; 94 (2), 917–925.

[52] Fukuda, R.; Okiyoneda, T. Peripheral protein quality control as a novel drug target for CFTR stabilizer. Front. Pharmacol. 2018 Sep 27; 9, 1100.

[53] Moniz, S.; Sousa, M.; Moraes, B.J.; Mendes, A.I.; Palma, M.; Barreto, C.; Fragata, J.I.; Amaral, M.D.; Matos, P. HGF Stimulation of Rac1 signaling enhances pharmacological correction of the most prevalent cystic fibrosis mutant F508del-CFTR. ACS Chem. Biol. 2012 Feb 15; 8 (2), 432–442.

[54] Loureiro, C.A.; Matos, A.M.; Dias-Alvez, Â.; Pereira, J.F.; Uliyakina, I.; Barros, P.; Amaral, M.D.; Matos, P.M. A molecular switch in the scaffold NHERF1 enables misfolded CFTR to evade the peripheral quality control checkpoint. Sci. Signal. 2015 May 19; 8 (377), ra48-ra84

[55] Matos, A.M.; Gomes-Duarte, A.; Faria, M.; Barros, P.; Jordan, P.; Amaral, M.D.; Matos, P. Prolonged co-treatment with HGF sustains epithelial integrity and improves pharmacological rescue of phe508del-CFTR. Sci. Rep. 2018 Aug 29; 8 (1), 13026.

[56] Alshafie, W.; Chappe, F.G.; Li, M.; Anini, Y.; Chappe, V.M. Vip Regulates Cftr Membrane Expression and Function in Calu-3 Cells by Increasing Its Interaction with Nherf1 and P-Erm in a Vpac1- and Pkcepsilon-Dependent Manner. Am. J. Physiol. Cell Physiol. 2014 Jul 1; 307 (1), C107–C119.

[57] Marozkina, N.V.; Yemen, S.; Borowitz, M.; Liu, L.; Plapp, M.; Sun, F.; Islam, R.; Erdmann-Gilmore, P.; Townsend, R.R.; Lichti, C.F.; Mantri, M.; Clapp, P.W.; Randell, S.H.; Gaston, B.; Zaman, K. Hsp 70/Hsp 90 organizing protein as a nitrosylation target in cystic fibrosis therapy. Proc. Natl. Acad. Sci. USA 2010 Jun 22; 107 (25), 11393–11398.

[58] Bergeron, C.; Cantin, A.M. New therapies to correct the cystic fibrosis basic defect. Int. J. Mol. Sci. 2021 Jun 8; 22 (12), 6193.

[59] Giuliano, K.A.; Wachi, S.; Drew, L.; Dukovski, D.; Green, O.; Bastos, C.; Cullen, M.D.; Hauck, S.; Tait, B.D.; Munoz, B.; Lee, P.; Miller, J.P. Use of a high-throughput phenotypic screening strategy to identify amplifiers, a novel pharmacological class of small molecules that exhibit functional synergy with potentiators and correctors. SLAS Discovery. 2018 Feb; 23 (2), 111–121.

[60] Shteinberg, M.; Haq, I.J.; Polineni, D.; Davies, J.C. Cystic fibrosis. Lancet. 2021 Jun 5; 397(10290), 2195-2211.

[61] Pranke, I.; Golec, A.; Hinzpeter, A.; Edelman, A.; Sermet-Gaudelus, I. Emerging therapeutic approaches for cystic fibrosis. From gene editing to personalized medicine. Front. Pharmacol. 2019 Feb 27; 10, 121.

[62] Fajak, I.; Boeck, K.D. New horizons for cystic fibrosis treatment. Pharmacol. Ther. 2017 Feb; 170, 205-211.

[63] Griesenbach, U.; Pytel, K.M.; Alton, E.W. Cystic fibrosis Gene therapy in the UK and elsewhere. Human Gene Therapy. 2015 May; 26 (5), 266–275.

[64] Cheng, H.; Zhang, F.; Ding, Y. CRISPR/Cas9 Delivery system engineering for genome editing in therapeutic applications. Pharmaceutics. 2021 Oct 9; 13 (10), 1649.

[65] Schwank, G.; Koo, B.K.; Sasselli, V.; Dekkers, J.F.; Heo, I.; Demircan, T.; Sasaki, N.; Boymans, S.; Cuppen, E.; Van der Ent, C.K.; Nieuwenhuis, E.E.; Beekman, J.M.; Clevers, H. Functional repair of CFTR by CRISPR/Cas9 in intestinal stemcell organoids of cystic fibrosispatients. Cell Stem Cell. 2013 Dec 5; 13 (6), 653–658.

[66] Drevinek, P.; Pressler, T.; Cipolli, Z.M.; De Boeck, K.; Schwarz, C.; Bouisset, F.; Boff, M.; Henig, N.; Paquette-Lamontagne, N.; Montgomery, S.; Perquin, J.; Tomkinson, J.; den Hollander, W.; Elborn, J.S. Antisense oligonucleotide eluforsen is safe and improves respiratory symptoms in F508DEL cystic fibrosis. J. Cyst. Fibros. 2020 Jan; 19 (1), 99-107.

[67] King, J.A.; Nichols, A.L.; Bentley, S.; Carr, S.B.; Davies, J.C. An update on CFTR modulators as new therapies for cystic fibrosis. Paediatr. Drugs. 2022 Jul; 24 (4), 321–333.

[68] Nichols, D.P.; Paynter, A.C.; Heltshe, S.L.; Donaldson, S.H.; Frederick, C.A.; Freedman, S.D.; Gelfond, D.; Hoffman, L.R.; Kelly, A.; Narkewicz, M.R.; Pittman, J.E.; Ratjen, F.; Rosenfeld, M.; Sagel, S.D.; Schwarzenberg, S.J.; Singh, P.K.; Solomon, J.M.; Stalvey, M.S.; Clancy, J.P.; Kirby, S.; Van Dalfsen, J.M.; Kloster, M.H.; Rowe, S.M.; the PROMISE Study Group. Clinical effectiveness of Elexacaftor/Tezacaftor/Ivacaftor in people with cystic fibrosis. A clinical trial. Am. J. Respir. Crit. Care Med. 2022 Mar 1; 205(5), 529-539.