Turk J Pharm Sci 2020;17(6):686-697
DOI: 10.4274/tjps.galenos.2020.76401

REVIEW

New Therapeutic Approaches in Cystic Fibrosis

Kistik Fibroziste Yeni Terapötik Yaklaşımlar

Dolunay Merve FAKIOĞLU[1], Beril ALTUN[2]*

[1]Gazi University Faculty of Pharmacy, Department of Clinical Pharmacy, Ankara, Turkey
[2]Gazi University Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkey

ABSTRACT

Cystic fibrosis (CF) is a hereditary, multisystemic disease caused by different mutations in the *CFTR* gene encoding CF transmembrane conductance regulator. CF is mainly characterized by pulmonary dysfunction as a result of deterioration in the mucociliary clearance and anion transport of airways. Mortality is mostly caused by bronchiectasis, bronchiole obstruction, and progressive respiratory dysfunction in the early years of life. Over the last decade, new therapeutic strategies rather than symptomatic treatment have been proposed, such as the small molecule approach, ion channel therapy, and pulmonary gene therapy. Due to considerable progress in the treatment options, CF has become an adult disease rather than a pediatric disease in recent years. Pulmonary gene therapy has gained special attention due to its mutation type independent aspect, therefore being applicable to all CF patients. On the other hand, the major obstacle for CF treatment is to predict the drug response of patients due to genetic complexity and heterogeneity. The advancement of 3D culture systems has made it possible to extrapolate the disease modeling and individual drug response *in vitro* by producing mini adult organs called "organoids" obtained from rectal cell biopsies. In this review, we summarize the advances in the novel therapeutic approaches, clinical interventions, and precision medicine concept for CF.

Key words: Cystic fibrosis, gene therapy, gene modulators, rectal organoids

ÖZ

Kistik fibrozis (CF), CF transmembran iletkenlik düzenleyicisini kodlayan *CFTR* genindeki farklı mutasyonların neden olduğu kalıtsal, multisistemik bir hastalıktır. CF, esas olarak hava yollarındaki mukosiliyer klerensin ve anyon transportunun bozulması sonucu gelişen pulmoner disfonksiyon ile karakterizedir. Mortalite, genellikle bronşektazi, bronşiyollerin tıkanması ve erken dönemde progresif solunum fonksiyon bozukluğundan kaynaklanır. Son on yılda, küçük molekül yaklaşımı, iyon kanal tedavisi ve pulmoner gen tedavisi gibi semptomatik tedaviden ziyade hastalığı tedavi etmeye yönelik yeni stratejiler geliştirilmiştir. Tedavi seçeneklerindeki önemli ilerlemeler sayesinde, CF son yıllarda pediatrik bir hastalıktan ziyade yetişkin hastalığı haline gelmiştir. Pulmoner gen tedavisi, mutasyon tipinden bağımsız olması ve tüm CF hastalarına uygulanabilirliği nedeniyle özellikle dikkat çekmiştir. Diğer taraftan CF tedavisindeki en büyük sorun, hastalardaki genetik karmaşıklık ve heterojenite nedeniyle ilaç yanıtını öngörememektir. 3D hücre kültürü sistemlerindeki ilerlemeler, rektal hücre biyopsilerinden "organoidler" adı verilen kişiye özel mini organlar üreterek hastalığın modellenmesini ve bireysel ilaç yanıtını *in vitro* olarak tahmin etmeyi mümkün kılmıştır. Bu derlemede, CF için yeni terapötik yaklaşımlar, klinik girişimler ve hassas tıp konseptindeki ilerlemeler özetlenmektedir.

Anahtar kelimeler: Kistik fibrozis, gen terapisi, gen modülatörleri, rektal organoidler

INTRODUCTION

Cystic fibrosis (CF) is a hereditary, multifactorial, multisystemic disease characterized by obstruction of airways, microbial infection, digestive disorders, and other complications. CF is known as the most common autosomal recessive disease in Caucasians.[1]

Although the incidence of disease varies greatly throughout the world, the highest incidence rate is seen in Northern Europe and the United States with 1/3,000 in white Americans, 1/4,000-10,000 in Hispanics, and 1/15,000-20,000 in African Americans. In Turkey, the incidence rate was reported as 1/3,400, close to that of the regions with the highest incidence rates. Globally, around 70,000 to 100,000 people suffer from CF.[2]

CF is caused by different mutations in the *CFTR* gene encoding CF transmembrane conductance regulator (CFTR), which regulates the mucociliary clearance and anion transport in airways.[3] The *CFTR* gene is located on the long arm of chromosome 7 and the CFTR protein product is 1,480 amino acids in length. CFTR acts as a cAMP regulated chlorine channel in apical membranes, providing Na^+ and water transport from epithelial cells in many organs and glands.[4] CFTR dysfunction primarily affects epithelial cells and causes chronic microbial infection and subsequently airway inflammation. Mortality from CF is commonly caused by bronchiectasis, bronchiole obstruction, and progressive respiratory dysfunction.[5] The severity of the disease is directly

*Correspondence: E-mail: berilaltun@gmail.com, Phone: +90 506 820 12 82 ORCID-ID: orcid.org/0000-0003-3083-9854
Received: 14.02.2020, Accepted: 04.05.2020

proportional to the extent the lungs are affected and varies by person.[6]

The pathophysiology of CF cannot be explained by a single hypothesis. The most common theory is the excessive reabsorption of Na^+ and water from the airway surface, resulting in a more viscous and elastic state of the airway secretions. These changes in the secretions cause dehydration of the airway surface and the formation of mucus plugs; mucociliary clearance becomes difficult. In addition to these changes, low HCO_3^- further affects the microenvironment by making the pH more acidic. Since bacterial eradication in the airways is pH dependent, changes in pH disrupt the natural immunity by attenuating the effectiveness of endogenous peptides.[7-9] In addition to these changes, decreased HCO_3^- levels contribute to the increase in mucus intensity.[10] This leads to accumulation of secretions and obstruction of the airways starting from the bronchioles. Mucociliary clearance of inhaled microorganisms that are trapped in mucus becomes gradually more difficult.[11] In a typical infant with CF, *Haemophilus influenzae*, *Staphylococcus aureus*, or both rapidly colonize and *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Burkholderia cepacia* may all be present even in infants.[12] In a short time, *P. aeruginosa* becomes the most dominant microorganism in the airways. It is the main pathogen in CF patients and its prevalence is around 70% in adults with CF.[13] *P. aeruginosa* forms a polysaccharide film to protect itself from antimicrobial agents. Therefore, bacterial binding to the epithelial cells increases and bacterial clearance decreases with natural immune mechanisms.[14,15] The management of pulmonary infection is of great significance since it affects the time of survival.[16] The most important concern regarding CF treatment is the increasing bacterial resistance to standard antibiotics.

CF also affects various organs and systems such as the intestinal tract, biliary tract, pancreas, and genitourinary system. Co-morbidities are pancreatic malabsorption (malnutrition), biliary cirrhosis, and infertility. Pancreatic and bile duct epithelial cells are affected by CFTR dysfunction as well. Chronic obstructive pancreatitis is observed due to excessive mucus secretion. Severe pancreatic exocrine deficiency causes symptomatic fat malabsorption.[17] If the pancreatic insufficiency cannot be controlled, this may cause damage to islet cells and leads to insulin deficiency and CF related-diabetes mellitus (CF-DM). The vascular outcomes of diabetes are evidential in typical DM patients; however, in CF-DM patients, nutritional and pulmonary outcomes might be life-threatening. The first treatment option is insulin (i.m.) rather than oral antidiabetics in CF-DM patients after the endocrinologic consultation, unlike the typical type-2 DM patients.[18] The intravenous (iv) administration of aminoglycoside and CF-DM are the major causes of renal failure in CF patients.[19]

The main objective of treatment of CF is to remove excessive mucus from the lungs, to control pulmonary infection, and to reverse pancreatic insufficiency and malnutrition. This perspective has led to a significant increase in the life span and quality of CF patients in recent years. In this review, we aim to summarize the novel treatment options and innovative therapeutic approaches for CF.

Classification of CFTR mutations

To date, approximately 2,000 different types of mutations have been identified in the *CFTR* gene.[20] However 15% of those are not associated with CF.[21] The most common mutation, called ΔF508, is the 3 base deletion leading to loss of phenylalanine at position 508 in the CFTR protein.[22] The ΔF508 mutation accounts for two-thirds of all CF alleles.[23] Approximately 90% of CF patients carry at least one copy of the ΔF508 mutation.[24] Determination of the CFTR mutation type is of great importance, since the mutation type shows the disease phenotype and indicates the way for the treatment strategy. CF is classified according to the step in which the mutation takes place. The conventional classification system divides CFTR mutations into 6 categories according to CFTR synthesis, trafficking, or function. However, De Boeck and Amaral[20] grouped mutations into seven classes according to functional defects and separated the previous class I mutations into class I (stop-codon mutations) and a new class VII [no messenger RNA (mRNA) transcription] (Table 1).

Classification of mutations helps us to understand the CFTR defect; however, mutations might be more than just a feature, because they are the most important determinant of disease severity.[25] Class I, II, and III mutations are related to no CFTR function and severe phenotype. However, class IV, V, VI, and VII mutations involve residual functional CFTR protein and therefore moderate phenotype and pancreatic insufficiency.[5]

Mutations of class I include nonsense, frameshift, or mRNA splicing mutations leading to absence of CFTR expression, therefore resulting in a reduced number of CFTR channels. Class II mutations, including ΔF508, lead to faulty CFTR processing. Even if CFTR is properly synthesized, missense and in-frame deletion mutations interrupt CFTR folding and trafficking. Some class II mutations partially disrupt protein stability. In class III mutations, channel gating is defective due to diminished ATP binding to the channel and results in impaired chloride transport. In class IV mutations, chloride transport is disrupted due to the abnormal CFTR channel pore. Class IV mutations often result in a milder phenotype because of the partial CFTR function. A low amount of CFTR protein is available, but aberrant splicing defects lead to defective mRNA processing (no full length or stable mRNA). Class VI mutations are characterized by a functional but unstable CFTR protein, and premature degradation of CFTR results in high CFTR turnover at the cell surface. The last category, class VII mutations, consist of large deletions on the *CFTR* gene and therefore no mRNA transcription process.[20,21,26,27]

New treatment approaches

New options in the management of pulmonary infection

Ceftazidime/avibactam is a new cephalosporin-beta lactamase inhibitor combination that is effective for multiple drug resistant infections.[28] Although ceftazidime has been in clinical use for many years as an antipseudomonal, its efficacy is unclear due to decreased sensitivity in recent years. The ceftazidime/

Table 1. Classification of CFTR mutations[20,21]

Mutation class	Defect	Phenotype	Example	Treatment strategy
I	Reduced CFTR protein expression	No protein	Gly542X Trp1282X	Production correctors (ataluren)
II	Misfolded CFTR protein not transported to the cell surface	No traffic	Phe508del (ΔF508) Asn1303Lys Ala561Glu	Corrector + potentiator (lumacaftor + ivacaftor, VX-661+ ivacaftor)
III	Reduced/lack of CFTR channel opening	Impaired gating	Gly551Asp Ser549Arg Gly1349Asp	Potentiator (ivacaftor)
IV	Misshaped CFTR pore restricts Cl^- movement	Decreased conductance	Arg117His Arg334Trp Ala455Glu	Potentiator (ivacaftor)
V	Reduced CFTR protein production	Less protein	3849+10 kb C→T Ala455Glu 3272-26A → G	No data available
VI	High CFTR protein turnover at the cell surface	Less stable	120del23 rPhe508del	No data available
VII	No transcription due to large deletions on *CFTR* gene	No mRNA	dele2,3 (21kb) 1717-1G →A	Unrescuable (By pass therapies?)

Kb: Kilobases, CFTR: Cystic fibrosis transmembrane conductance regulator

avibactam combination offers a potential improvement for CF pulmonary infections involving *P. aeruginosa.*[29,30] Combination with avibactam increases the activity of ceftazidime against *Enterobacteriaceae* and *P. aeruginosa*, since avibactam inhibits serine β-lactamases including ESBL, AmpC, and KPC. On the other hand, avibactam does not increase ceftazidime activity against *Acinetobacter* spp., *Burkholderia* spp., or most anaerobic Gram (-) bacilli.[31] Co-administration of ceftazidime/avibactam and aztreonam gave successful results for extremely drug resistant *Burkholderia multivorans* infections.[32]

Ceftolozane/tazobactam is a novel β-lactam/β-lactamase inhibitor combination approved by the Food and Drug Administration (FDA) in 2014 for the treatment of complicated intraabdominal and urinary tract infections.[33] Ceftolozane/tazobactam is promising for the treatment of *P. aeruginosa* infection in CF patients, alone or in combination with tobramycin or amikacin. The efficacy of amikacin and the ceftolozane/tazobactam combination is higher than that of tobramycin-ceftolozane/tazobactam. This encouraging progress led to further clinical research on multidrug resistant *P. aeruginosa* infections in CF patients.[34] Neither the ceftazidime/avibactam nor the ceftolozane/tazobactam combination has been approved for patients under the age of 18 yet, but pediatric population studies are in progress and currently in phase 2.[35-37]

Inhaled antibiotics are another widely used treatment option alone or in conjunction with oral antibiotics to prevent pulmonary exacerbations. The use of inhaled antibiotics has advantages compared to iv administration or the oral route. Increased antibiotic concentration at the infection site through inhalation enhances bacterial eradication, and systemic side effects such as nephrotoxicity and ototoxicity can be avoided.[38]

The inhaled antibiotics used for CF are aztreonam lysine, tobramycin inhalation powder/solution, inhaled colistin, liposomal amikacin, liposomal ciprofloxacin, and inhaled levofloxacin.[39-42] Inhaled tobramycin and inhaled aztreonam are the two inhaled antibiotics with FDA approval. Liposomal amikacin was approved by the FDA in 2018.[43]

Inhaled colistin (colistimethate sodium) has been approved by the European Medicines Agency, but not the FDA yet. Other antibiotics, i.e. liposomal ciprofloxacin and inhaled levofloxacin, have not been approved for CF. These are under investigation in the earlier stages of development, in phase studies.[44]

Ion channel therapies: non-CFTR modulating therapies

Inhibition of Na^+absorption

Fluid hydration in the airway depends on Cl^--bicarbonate secretion by CFTR channels and sodium absorption mediated by epithelial sodium channels (ENaCs). Although the CFTR channel defect mainly affects the secretion of Cl^- and bicarbonate ions from epithelial cells, it also leads to deterioration in the secretions and absorption of electrolytes.[45] Increased Na^+ absorption (2-3 times higher than normal) is observed through the ENaCs, as well as impaired Cl^- secretion. Na^+ hyperabsorption leads to more dehydration of respiratory secretions and further deterioration of mucociliary clearance. Blockage of the epithelial Na^+ channel and prevention of Na^+ hyperabsorbion have been recommended as a treatment strategy.[46]

Amiloride is a first generation potassium-sparing ENaC antagonist, developed as a sodium channel inhibitor in the 1960s. Although intranasal administration of amiloride has reduced the pulmonary mortality rate, the risk of hyperkalemia

limited its use.[47] The study had to be terminated due to acute hyperkalemia caused by inhibition of ENaCs in the kidneys.[48]

AZD5634 is a new inhalable, second-generation amiloride derivate and it is well tolerated without considerable hyperkalemia risk.[49] ENaC antagonists QBW 276 and BI 443651 have undergone clinical investigation and demonstrated remarkable safety profiles in phase 1 trials. However, phase 2/ efficacy outcomes are still pending.[50,51]

SPX-101 is another inhalable ENaC inhibitor peptide that has undergone phase 2 trials.[52] SPX-101 showed positive and significant results without causing hyperkalemia. The aerosol administration of antisense oligonucleotides may provide an alternative approach.

Stimulation of Cl^- secretion

Luminal Cl^- secretion of epithelial cells is mediated by the CFTR and alternative chlorine channels. Increased activity of alternative chlorine channels like the calcium-activated chloride channel (CaCC) in the lower respiratory tract may compensate for decreased or absent CFTR function and improve the clinical status of CF patients.[53]

Activation of $P2Y_2$ nucleotide receptor activates the CaCCs by causing a rapid increase in cytosolic free calcium concentration. ATP and uridine 5'-triphosphate (UTP), endogenous $P2Y_2$ receptor ligands, increase ion and liquid secretion.[54] However, the short half-lives of extracellular ATP and UTP limit their clinical utility. To induce chlorine secretion by $P2Y_2$-mediated CaCC pathway, more stable inhaled $P2Y_2$ receptor agonists needed to be developed. Denufosol is an inhaled $P2Y_2$ receptor agonist that increases the Cl^- ion and fluid secretion in luminal clearance by $P2Y_2$-mediated CaCC stimulation. Although denufosol was found to be effective and well tolerated in mild CF patients,[55,56] it failed in the phase 3 step due to unsatisfactory results in terms of pulmonary function. Another reason for failure is its short half-life.[57]

Moli1901, also known as duramycin, a stable 19-residue-polycyclic peptide that is derived from *Streptomyces cinnamoneum*, interacts with phospholipids and thereby activates alternative chloride channels by elevated intracellular calcium levels.[58] Although Moli1901 showed promise as a chloride channel activator, it could not be further developed due to formulation problems.

Osmotic therapy

Airway surface fluid (ASL) is a thin layer of fluid that covers the lumen surface of the airway epithelium and maintains mucociliary clearance, ciliary function, and antimicrobial features of the airway, a key regulator of airway homeostasis.[59] ASL depletion is a significant factor in the pathogenesis of cystic fibrosis, so it has been shown that osmotic water withdrawal to the airway surface may improve the damaged mucociliary transport.[60,61] The main building block of CF treatment is actually correcting mucociliary clearance; a small molecule approach like CFTR modulators is able to do this by correcting dysfunctional CFTR as is an approach to targeting ion channels in airway epithelial cells pharmacologically. Hypertonic saline and dry mannitol powder, which directly correct mucociliary transport, produce an osmotic gradient by drawing water from the aquaporins of epithelial cells.[60,62] Hypertonic saline, usually used as a 7% solution, induces the release of inflammatory mediators such as prostaglandin E2, altering the rheology of the mucus and increasing mucociliary clearance.[63] Mannitol is a nonionic osmotic agent. The larger size of mannitol is a disadvantage over hypertonic saline, and it is difficult to accumulate in small airways; however, it is easier to administer via a metered dose inhaler (compared to nebulizer hypertonic saline).[64]

Small molecule approach: CFTR modulating therapies

CFTR modulators were described first by Verkmen in 2003. They are novel therapeutics that correct CFTR protein production, defective CFTR protein itself, and/or its intracellular function. CFTR modulators play a significant role in CF treatment since they provide a fundamentally therapeutic approach rather than symptomatic therapy by targeting the production or function of CFTR protein.[65-67]

The first group, called CFTR potentiators, increase the function of the expressed CFTR channels and ameliorate class III or IV defects even when CFTR reaches the cell surface but is nonfunctional. The second group, called CFTR correctors, are drugs that can act to improve the intracellular processing of proteins, thereby providing CFTR proteins to move to the appropriate site on the cell surface. Finally, the third group, CFTR production correctors, induce more CFTR protein production.[68]

The first small molecule defined as a CFTR potentiator (potential enhancer) is ivacaftor, which was developed as VX-770 at first.[69] Ivacaftor facilitates the transport of chloride by enhancing the channel opening of the CFTR protein on the cell surface. Ivacaftor is approved by the FDA for all class III mutations involving G1244E, G1349D, G178R, G551S, G1370D, S1251N, S1255P, S549N, S549R, and particularly G551D mutations for patients over 12 months of age.[70]

Ivacaftor has been shown to improve lung function and nutritional status and diminish the mortality rate associated with lung dysfunction.[71]

In *in vitro* studies, ivacaftor improves not only class III mutations, but also some mutant proteins of IV and V classes.[72] A class IV mutation, Arg117His, that leads to impairment of CFTR conductivity is seen in approximately 3% of patients with CF.[73]

Novel CFTR potentiator drugs are currently undergoing clinical trials. QBW251 is in the phase 2 stage of a randomized controlled trial involving 153 patients. Other candidates such as GLPG1837 and CTP-656 are also in phase 2.[74,75]

Despite the fact that ivacaftor improves channel opening time and chloride conductivity, it is not effective in patients who are homozygous for the ΔF508 mutation. The primary problem in the ΔF508 mutation is inaccurate folding of the protein and inability to reach the cell surface.[76] Therefore, co-administration of potentiators and correctors is recommended for patients homozygous for ΔF508. It has been shown that the combination

of corrective and potentiator therapies has been more effective than single regimens.[77] The FDA has approved the lumacaftor/ivacaftor combination for patients homozygous for the ΔF508 mutation who are 2 years old or older.[78] Lumacaftor, also known as VX-809, improves the conformational stability of the ΔF508-CFTR, thereby enhancing the processing of CFTR and its transfer to the cell surface.

Tezacaftor (VX-661) enhances the processing and transfer of CFTR proteins, including both normal and mutant ones (including ΔF508-CFTR), and thus increases the amount of protein reaching the cell surface. The tezacaftor/ivacaftor combination was approved by the FDA in 2018. It is indicated for the treatment of CF in patients at the age of 12 or older who are homozygous for the ΔF508 mutation.

The combination of tezacaftor/ivacaftor exhibits fewer side effects than the combination of lumacaftor/ivacaftor especially in terms of increased respiratory symptoms at the beginning of treatment. However, there is no therapeutic advantage of tezacaftor/ivacaftor when compared to lumacaftor/ivacaftor combination therapy.

To date, no combination therapy has been approved for patients who have heterozygous ΔF508 mutations (ΔF508 mutation in one allele + another mutation in another allele=ΔF508-MF) on the *CFTR* gene and minimal functional CFTR. Patients who carry two copies of the ΔF508 CFTR mutation (homozygous) are typically treated with a corrective and a potentiator, but this is not successful in heterozygotes.

The new generation CFTR correctors VX-659 and VX-440 are small molecule drugs that are expected to emerge as part of the triple combination regimen and phase 3 studies are in progress.[79]

VX-659 and VX-440 have different structures and different mechanisms of action.[80] Thus, the use of two distinct correctors in triple combination therapy acting via different mechanisms has come up. These drugs were developed for use in combination with tezacaftor and ivacaftor (VX-659/tezacaftor/ivacaftor or VX440/tezacaftor/ivacaftor) to restore the function of the ΔF508 CFTR protein of patients who have heterozygous ΔF508 CFTR (ΔF508-MF genotypes) and minimal CFTR function or homozygous ΔF508 mutations. Undoubtedly, the most important outcome of triple combination therapy is the success in treating the heterozygous ΔF508 mutation, for which CFTR modulator treatment is not available currently.[81]

Ataluren (PTC124): potential treatment for class I mutations

Stop codon mutations account for 10-12% of all CFTR mutations.[80] This mutation truncates CFTR protein production by introducing a premature stop in the mRNA and leads to unfinished protein formation. Ataluren is a novel oral drug that allows ribosomal reading of premature stop codons selectively. Ataluren activity for nonsense mutations has been shown *in vitro*, but its efficiency remains unclear due to inconsistent results in clinical trials.[82-86] The reason may be the suppression of ataluren activity by aminoglycosides.[85] Ivacaftor may increase the efficacy of ataluren by activating a specific protein. A recently completed study at the University of Alabama at Birmingham aimed to evaluate the effectiveness of ivacaftor with ataluren in a patient after one year of treatment.[87]

Personalized treatment and pulmonary gene therapy

The concept of precision medicine, which functions via the notion that "there is no disease, there is a patient", is defined as the planning of appropriate treatment by taking into account the patient's genetic background. Undoubtedly, gene therapy is one of the cornerstones of precision medicine and it gave direction to CF studies. Human gene therapy aims to alter, manipulate, or change the expression of a gene or the biological properties of living cells for therapeutic use.[88]

Gene therapy involves the correction of a defective *CFTR* gene by inserting an extra copy of a non-defective intact *CFTR* gene into the cell, which is called gene replacement, or using specially designed enzymes called nucleases, which also function as molecular scissors, which is called gene editing. The major obstacle for gene replacement/editing is gene delivery, which is hindered by the mucociliary barrier.

Gene editing uses the cell's own DNA repair machinery to correct the mutation in the DNA. Hence, a specific gene repair system should be designed for each type of mutation. Recently, the use of *CRISPR/Cas9* gene editing technology is on the rise due to its success. *CRISPR/Cas9* gene editing includes a "guide" that locates the mutated sequence in the *CFTR* gene and "scissors" that break the patient's DNA at the site of the mutation. This DNA damage gets the attention of the cell's DNA repair machinery, which will then fix the DNA breakage. This continuously corrects the mutation in the cell; therefore, its great advantage is that the effect is permanent. However, gene editing tools should be designed specifically for each type of CFTR mutation. This creates an obstacle since there are so many types of mutations in CF (approximately 2000 mutations). Moreover, gene editing tools can break the DNA in the wrong place (off-target) and cause an error resulting in new mutations in other genes. This might lead to unintended consequences, such as an increased risk of cancer.[89]

Although recent technological advances in gene editing (homologous recombination, zinc finger nucleases, transcriptional activator-like effector nuclease, CRISPR/Cas9) are promising, this option have been pushed into the background since there are many types of CF mutations and partially insufficient results.[90] However, the repair of a defective gene with the *CRISPR/Cas9* tool has huge advantages over gene replacement therapy. First of all, the corrected gene remains under the control of its endogenous promoter and therefore engages with life-long expression by the native regulation in the cell. Moreover, gene replacement has the potential to involve foreign DNA, thus increasing the risk of insertional mutations. *CRISPR/Cas9* gene editing technology is still being improved; promising results were obtained in CF tissue and animal models.[91] CF models, generated in 5 animal species (mice, rats, ferrets, pigs, and rabbits), clearly reflect the mechanisms of disease pathogenesis and CFTR function.[92] Recently, the sheep model has been proposed due to the similarity of lung anatomy between the two species.[93]

Some researchers have focused on gene replacement therapy for CF, which includes presentation of the nondefective *CFTR* gene (wild-type) into the lung cells. The entrance of functional CFTR DNA or RNA into the nucleus of lung epithelial cells through a vector and providing the expression of the functional *CFTR* gene instead of the mutant one are the main goals of the treatment.[94]

Mutation type is important for the small molecule approach, but not for gene replacement therapy. Since there is no need to identify the mutation type of the patient, gene replacement is suitable for all CF patients.[95] Pulmonary gene therapy is important since it is a non- symptomatic and mutation agnostic treatment, especially when compared with the other treatment strategies such as the potentiator and corrector regimens, which are limited by genotype. With the discovery of the *CFTR* gene in 1989, studies on gene therapy in CF have gained momentum.[24] Initially, viral and nonviral approaches were developed to deliver the *CFTR* gene (adeno-associated viruses, adenoviruses, plasmids formulated in cationic liposomes, and lentiviral and retroviral vectors). However, the lung, which has strong intracellular and extracellular barriers to protect itself from foreign particles, is a complex and difficult target organ.[95] Since gene transfer vectors can be deactivated by the immune system or inflammation products, this complicates pulmonary gene therapy. The vector carrying the gene reaches the cell surface but the receptors responsible for its uptake into the cell may be inadequate, which means inefficient gene transfer. CF is a lifelong disease and the life cycle of airway epithelial cells requires repetitive administration of the *CFTR* gene. All of these can account for the challenges of pulmonary gene therapy.[96,97] In general, viral vectors are more effective than nonviral alternatives. However, nonviral vectors are safer, cheaper, and easier to produce.[90]

In vitro studies have shown that the expression of the complementary DNA of the whole *CFTR* gene in the cell improves the anion channel activity. The most important question in this respect concerns at least how many cells must be corrected in order to benefit therapeutically. Studies showed that at least 6-10% of airway epithelial cells should be able to express functional CFTR for wild-type anion transport.[98] In 1992, with the production of animal models with CF, there was an increase in the number of gene therapy studies. In parallel with *in vitro* studies, transduction of up to 5% of the airway cells with the CFTR expressing vector has reached 50% of Cl^- transport levels in non-CF subjects in animal models.[99]

Clinical studies involving CF gene therapy were first performed in 1993 using viral and nonviral gene transfer agents from the nasal and bronchial epithelium. Adenoviruses were found to be safe in repetitive applications and did not trigger any immune response in animal experiments.[100] However, they caused immune response in clinical studies.[100,101] Despite vector modifications afterwards (such as the removal of all adenoviral genes in gutless vectors) to reduce immunodeficiency, there is little interest for the development of pulmonary gene therapy with adenoviruses.

The other promising application is recombinant adeno-associated viruses. Adeno-associated vectors are DNA based and lack some viral genes, such as gutless vectors (also called co-dependent vectors) that require assistance from a helper virus for replication. AAV2 is the first serotype to be clinically evaluated in CF patients but it has created frustration in repetitive applications due to changes in lung function.[96]

Lentiviruses are RNA-based vectors that belong to the family *Retroviridae*. Once lentiviruses enter the cell, they are reverse transcribed into DNA and the transcribed DNA is integrated into the genome of the host cell. The advantage of genomic integration is the transfer of undamaged *CFTR* gene into the daughter cells after the cell division. Therefore, it provides long-term expression. Recombinant lentiviral vectors can be modified to enhance their effectiveness by adding new surface proteins. Question marks remain as to whether the genomic integration of lentiviral vectors is safe.[90,96,102]

The failure of viral vectors has led to studies on the development of nonviral alternatives. The main objective in the development of nonviral (synthetic) vectors is to minimize the risk of immunogenicity. Nonviral vectors are circular, plasmid DNA (pDNA) molecules that are complexed with a series of cationic lipids and polymers called "lipoplexes" and "polyplexes".[96] However, nonviral vectors have no specific components required for cell entry. Nevertheless, delivery of the pDNA complicated by cationic liposomes to the lung epithelial by the aerosol system resulted in a 25% correction of the CFTR ion transport defect.[103]

In a randomized, double-blind, phase 2 trial, nonviral gene therapy pGM169/GL67A was administered for 1 year and pre- and posttreatment FEV1% values of 114 patients were calculated. The FEV1 results showed a modest but significant improvement in lung function compared with the placebo.[104]

To date, the presence of bacterial infection in the lungs has been ignored in terms of the efficacy of pulmonary gene therapy. In fact, the presence of infection can greatly affect the success of gene delivery. In recent years, several studies have focused on developing multifunctional models that will provide both antibacterial effects and gene distribution.[105] This method provides better protection of DNA during the delivery of the gene and better transfection into the bronchial epithelium, as well as contributing to bacterial eradication in the airways.

Organoids

As CF is a genetically heterogeneous disease, currently available treatment options do not cover all CFTR mutations. Many of the known CFTR mutations are associated with a variety of disease expression and this complicates the estimation of individual disease phenotypes. Moreover, phenotypic variations can be seen even in patients having identical CF mutations. CFTR genotype-based stratification for medication is challenging for many patients with rare CFTR mutations who are not included in clinical trials due to the low prevalence of the mutations ("orphan" mutations frequency <0.1%).[106,107] Due to genetic heterogeneity, there is great variability in drug responses such as to ivacaftor, lumacaftor, or their combination among

CF patients, from no clinical benefit to complete recovery. Therefore, there is an urgent need to elucidate the individualistic drug response from patients who have different types of CFTR mutations. *In vitro* organoid-based functional assays have been developed for this purpose. Organoids are a useful tool to predict the pharmacogenomics of diverse CFTR mutations and particularly CF drug response.

Organoids, also called mini-organs, are organ-specific 3D cell cultures derived from adult organs or pluripotent stem cells that reflect the features of the parental organ where they originated.[108] They are used to study heterogeneous medical conditions such as CF and cancer where genetics can influence disease severity, prognosis, and drug efficacy.[109,110] Organoids can be used to test drug efficacy and compare different combination treatments. Furthermore, patient-derived organoids represent an important tool of personalized medicine allowing the prediction of clinical disease phenotype and how a patient will respond to a drug (e.g., CFTR modulating drugs), since they have individuals' functional expressions of their own genomes.[111,112] Drug testing in patient-derived stem cells gathered by rectal biopsy offers an opportunity to select appropriate treatment on an individual basis. Scientists have demonstrated that CFTR function can be readily measured in colorectal organoids by a forskolin-induced swelling (FIS) assay.[107,113,114] The efficacy of Geneticin, ataluren, ivacaftor, and lumacaftor in combination therapy has been tested by FIS method in intestinal organoids with rare mutations.[86]

The first study to measure the correlation between *in vivo* and *in vitro* drug response in stem cell culture derived from CF patients was performed by Berkers et al.[111] in 2019. They showed a high correlation between the *in vitro* and *in vivo* effects of CFTR modulating drugs and demonstrated that organoids play an ideal role in CF modeling in a cost-effective and patient-friendly manner.

Immunotherapy for CF

Immunotherapy aims to improve how the immune system works. Chronic elevation in TNF-α, Interleukin-6 (IL-6), and IL-8, as well as IL-17, IL-13, and IL-5 levels in CF has shown to be important for disease exacerbation. IL-17 levels are found to be high in patients with *P. aeruginosa* infection.[115] IL-17, IL-5, and IL-13 levels increase with disease exacerbation and IL-17 was shown to be negatively correlated with FEV1 results. It is stated that the IL-17 increase was similar in CD4 + Th17 cells and lymph nodes.[116,117] Another study showed that tryptophan metabolism affects IL-17 levels and the RAR-related orphan receptor c (Rorc) expression. Reduction in tryptophan/kynurenine metabolism due to defective indoleamine 2,3-dioxygenase (IDO) causes susceptibility to *Aspergillus* infections and murine CF sensitivity due to type 17 helper T-cell/regulatory T-cell (Th17/Treg) imbalance. The importance of immunomodulation in CF through Th17-cell activation and IDO agonist is emphasized.[118]

The first clinical trial for immunomodulator therapy in CF is based on anti-pseudomonas aeruginosa IgY. Twenty patients with CF were involved in a phase 2 study. "Anti-pseudomonas IgY" was obtained from chicken eggs vaccinated with *Pseudomonas aeruginosa*. The preliminary results showed that it takes much longer to get a new infection and treated patients get fewer infections than controls. In addition, patients had no new opportunistic bacterial or fungal infections (*B. cepacia*, *S. maltophilia*, *A. xylosoxidans*, atypical *Mycobacteria*, *Aspergillus fumigatus*), antibiotic use was greatly diminished, and lung functions and nutritional status were stable.[119]

CONCLUSION

Over the last decade, CF has become one of the most studied hereditary diseases with novel treatment options. Since the availability to access new treatments, life quality of CF patients have increased and their survival has been prolonged. CF has become an adult disease rather than a pediatric disease in countries which devote more financial resources to health expenditures. However, the average life expectancy may still be in the 20s in low-income economies.

Recent advancements led to a paradigm shift from symptomatic treatment to therapeutic approach which targeted the mutant *CFTR* gene. The first one is to use small molecules, which covers a limited number of CF patients, and another one is pulmonary gene therapy, which represents an important tool for full recovery. Despite many efforts, there is no FDA-approved pulmonary gene therapy for CF. The major obstacle is the immune surveillance mechanisms of the lung, which hinder repeated administration of viral vectors.

CF treatment usually depends on the identification of the underlying genetic defect. Although the clinical outcome is mostly similar, CF patients differ from each other in terms of mutation type and disease progress. Thus, mutation-specific treatment and personalized therapy was an achievable goal for CF. CFTR modulators have become a remarkable step in terms of personalized treatment in CF. The CFTR potentiator ivacaftor and other correctors such as lumacaftor and tezacaftor, have been approved by the FDA for different types of mutations, such as the homozygous ΔF508 allele in CF. However, the treatment gap for the heterozygous ΔF508 allele still remains. New generation CFTR modulators have potential to fix the heterozygous ΔF508 allele, by improving CFTR folding and trafficking. The development of new generation modulator drugs (e.g., triple combinations) offers an alternative for a much larger CF population, including the patients having the heterozygous ΔF508 allele.

Significant efforts have been made to improve the treatment of patients with CF using various strategies targeting the underlying genetic defect and its subsequent results. However, the determination of CF drug efficacy is challenging because of the great heterogeneity of CFTR mutations, as well as other unknown factors that contribute to individual drug efficacy. The advancement in 3D culture systems made it possible to extrapolate the disease modeling and individual drug response *in vitro* by producing mini adult organs, which have been termed "organoids". Further studies are needed to confirm the

correlation between *in vitro* organoid-based functional assays and *in vivo* clinical phenotype and drug efficacy.

Over the 20 years following the cloning of the *CFTR* gene, the gene therapy for CF has evolved in two distinct areas: gene editing and gene replacement. Gene editing and gene replacement have advantages and disadvantages over each other. The repaired *CFTR* gene by gene editing technologies remains under the control of its endogenous promoter, and therefore a definitive and long-lasting treatment is guaranteed. However, the huge number of *CFTR* gene mutations is a major obstacle for gene editing tools. On the other hand, gene replacement requires repeated administration of the wild-type *CFTR* gene throughout the lifetime.

The full restoration of CFTR protein functionality was achieved by using *CRISPR/Cas9* gene editing technology in cultured intestinal stem cells (organoids) obtained from pediatric CF patients. An *ex vivo* repaired *CFTR* gene by *CRISPR/Cas9* in cultured organoids can be reinserted into the host successfully; this might be the beginning of a new era. In the near future, it may be possible to obtain lung stem cells from CF patients, engineering them with CRISPR/Cas9 to fix the CFTR mutation, and engraft them into lungs where stem cells find their suitable microenvironment to reconstruct the patients' airway.

In conclusion, gene therapies will continue to be an important strategy for CF as well as other genetic diseases, and organoid-based regenerative medicine designed with gene engineering technologies can provide an enormous innovation for CF therapy in the next years.

Conflicts of interest: No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of this article.

REFERENCES

1. Robert Kliegman RB, Hal Jenson, Bonita Stanton. Nelson Textbook of Pediatrics. Philadelphia, United States: Elsevier - Health Sciences Division; 2018.
2. Hangül M, Pekcan S, Köse M, Acıcan D, Şahlar TE, Erdoğan M, Kendirici M, Güney D, Demir O, Göçlü F, Öznavruz H, Ercan Ö. The Incidence of Cystic Fibrosis in the Central Region of Anatolia in Turkey Between 2015 and 2016. Balkan Med J. 2019;36:179-183.
3. Rudolp CD RA, Lıster GE, Fırst LR, Gershon AA. Rudolph's Pediatrics. New York: McGraw-Hill Education; 2011.
4. Anderson MP, Gregory RJ, Thompson S, Souza DW, Paul S, Mulligan RC, Smith AE, Welsh MJ. Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. Science. 1991;253:202-205.
5. Elborn JS. Cystic fibrosis. Lancet. 2016;388:2519-2531.
6. WHO Human Genetics Programme. (2004). The molecular genetic epidemiology of cystic fibrosis: report of a joint meeting of WHO/IECFTN/ICF(M)A/ECFS, Genoa, Italy, 19 June 2002. World Health Organization. Available from: https://apps.who.int/iris/handle/10665/68702
7. Smith JJ, Travis SM, Greenberg EP, Welsh MJ. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. Cell. 1996;85:229-236.
8. Shah AV, McColley SA, Weil D, Zheng XT. Trichosporon mycotoxinivorans Infection in Patients with Cystic Fibrosis. J Clin Microbiol. 2014;52:2242-2244.
9. Berkebile AR, McCray PB. Effects of airway surface liquid pH on host defense in cystic fibrosis. Int J Biochem Cell B. 2014;52:124-129.
10. Elborn JS. CFTR Modulators: Deciding What Is Best for Individuals in an Era of Precision Medicine. Ann Am Thorac Soc. 2018;15:298-300.
11. Massip-Copiz MM, Santa-Coloma TA. Extracellular pH and lung infections in cystic fibrosis. Eur J Cell Biol. 2018;97:402-410.
12. Steinkamp G, Wiedemann B, Rietschel E, Krahl A, Gielen J, Barmeier H, Ratjen F, Emerging Bacteria Study Group. Prospective evaluation of emerging bacteria in cystic fibrosis. J Cyst Fibros. 2005;4:41-48.
13. Bensman TJ, Wang J, Jayne J, Fukushima L, Rao AP, D'Argenio DZ, Beringer PM. Pharmacokinetic-pharmacodynamic target attainment analyses to determine optimal dosing of ceftazidime-avibactam for the treatment of acute pulmonary exacerbations in patients with cystic fibrosis. Antimicrobial agents and chemotherapy. 2017;61:e00988-17.
14. Imudo L, Barasch J, Prince A, Alawqati Q. Cystic fibrosis epithelial-cells have a receptor for pathogenic bacteria on their apical surface. P Natl Acad Sci USA. 1995;92:3019-3023.
15. Matsui H, Wagner VE, Hill DB, Schwab UE, Rogers TD, Button B, Taylor 2nd RM, Superfine R, Rubinstein M, Iglewski BH, Boucher RC. A physical linkage between cystic fibrosis airway surface dehydration and Pseudomonas aeruginosa biofilms. P Natl Acad Sci USA. 2006;103:18131-18136.
16. Parkins MD, Somayaji R, Waters VJ. Epidemiology, Biology, and Impact of Clonal Pseudomonas aeruginosa Infections in Cystic Fibrosis. Clinical microbiology reviews. 2018;31:e00018-e00019.
17. Somayaji R, Ramos KJ, Kapnadak SG, Aitken ML, Goss CH. Common clinical features of CF (respiratory disease and exocrine pancreatic insufficiency). Presse Med. 2017;46:e109-e124.
18. Moran A, Brunzell C, Cohen RC, Katz M, Marshall BC, Onady G, Robinson KA, Sabadosa KA, Stecenko A, Slovis B, CFRD Guidelines Committee. Clinical care guidelines for cystic fibrosis-related diabetes: a position statement of the American Diabetes Association and a clinical practice guideline of the Cystic Fibrosis Foundation, endorsed by the Pediatric Endocrine Society. Diabetes Care. 2010;33:2697-2708.
19. Andersen HU, Lanng S, Pressler T, Laugesen CS, Mathiesen ER. Cystic fibrosis-related diabetes - The presence of microvascular diabetes complications. Diabetes Care. 2006;29:2660-2663.
20. De Boeck K, Amaral MD. Progress in therapies for cystic fibrosis. Lancet Resp Med. 2016;4:662-674.
21. Brodlie M, Haq IJ, Roberts K, Elborn JS. Targeted therapies to improve CFTR function in cystic fibrosis. Genome Med. 2015;7:101.
22. O'Riordan TG, Donn KH, Hodsman P, Ansede JH, Newcomb T, Lewis SA, Flitter WD, White VS, Johnson MR, Montgomery AB, Warnock DG, Boucher RC. Acute Hyperkalemia Associated with Inhalation of a Potent ENaC Antagonist: Phase 1 Trial of GS-9411. J Aerosol Med Pulm D. 2014;27:200-208.
23. Dell'Edera D, Benedetto M, Gadaleta G, Carone D, Salvatore D, Angione A, Gallo M, Milo M, Pisaturo ML, Pierro GD, Mazzone E, Epifina AA. Analysis of cystic fibrosis gene mutations in children with cystic fibrosis and in 964 infertile couples within the region of Basilicata, Italy: a research study. J Med Case Rep. 2014;8:339.

24. Cooney AL, McCray PB, Jr., Sinn PL. Cystic Fibrosis Gene Therapy: Looking Back, Looking Forward. Genes (Basel). 2018;9:538.
25. Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell. 1993;73:1251-1254.
26. Ratjen F, Bell SC, Rowe SM, Goss CH, Quittner AL, Bush A. Cystic fibrosis. Nat Rev Dis Primers. 2015;1:15010.
27. Kerem E. Cystic fibrosis: priorities and progress for future therapies. Paediatr Respir Rev. 2017;24:14-16.
28. Farfour E, Trochu E, Devin C, Cardot Martin E, Limousin L, Roux A, Picard C, Jolly E, Vasse M, Lesprit P. Trends in ceftazidime-avibactam activity against multidrug-resistant organisms recovered from respiratory samples of cystic fibrosis patients. Transpl Infect Dis. 2018;20:e12955.
29. Pitart C, Marco F, Keating TA, Nichols WW, Vila J. Activity of ceftazidime-avibactam against fluoroquinolone-resistant Enterobacteriaceae and Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2015;59:3059-3065.
30. Rodriguez-Nunez O, Ripa M, Morata L, de la Calle C, Cardozo C, Feher C, Pellicé M, Valcarel A, Puerta-Alcalde P, Marco F, Garcia-Vidal C, Rio AD, Soriano A, Martinez-Martinez JA. Evaluation of ceftazidime/avibactam for serious infections due to multidrug-resistant and extensively drug-resistant Pseudomonas aeruginosa. J Glob Antimicrob Resist. 2018;15:136-139.
31. Lagace-Wiens P, Walkty A, Karlowsky JA. Ceftazidime-avibactam: an evidence-based review of its pharmacology and potential use in the treatment of Gram-negative bacterial infections. Core Evid. 2014;9:13-25.
32. Barlow G, Morice A. Successful treatment of resistant Burkholderia multivorans infection in a patient with cystic fibrosis using ceftazidime/avibactam plus aztreonam. J Antimicrob Chemoth. 2018;73:2270-2271.
33. Stokem K, Zuckerman JB, Nicolau DP, Wungwattana M, Sears EH. Use of ceftolozane-tazobactam in a cystic fibrosis patient with multidrug-resistant pseudomonas infection and renal insufficiency. Respir Med Case Rep. 2018;23:8-9.
34. Dassner AM, Sutherland C, Girotto J, Nicolau DP. In vitro activity of ceftolozane/tazobactam alone or with an aminoglycoside against multi-drug-resistant pseudomonas aeruginosa from pediatric cystic fibrosis patients. Infect Dis Ther. 2017;6:129-136.
35. Food and Drug Administration (FDA). These highlights do not include all the information needed to use ZERBAXA™ safely and effectively. See full prescribing information for ZERBAXA. 2014. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/206829lbl.pdf
36. ClinicalTrials.gov. MK-7625A Versus Meropenem in Pediatric Participants With Complicated Urinary Tract Infection (cUTI) (MK-7625A-034) Jully 26, 2017 [updated April 17, 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT03230838?term=ceftolozane+tazobactam&age=0&draw=2&rank=4
37. ClinicalTrials.gov. Evaluation of Pharmacokinetics, Safety, and Tolerability of Ceftazidime-avibactam in Neonates and Infants. (NOOR) October 14, 2019 [updated April 3, 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT04126031?term=ceftazidim+avibactam&age=0&draw=2&rank=2
38. Hewer SL. Inhaled antibiotics in cystic fibrosis: what's new? J R Soc Med. 2012;105(2):S19-24.
39. Zeitler K, Salvas B, Stevens V, Brown J. Aztreonam lysine for inhalation: new formulation of an old antibiotic. Am J Health Syst Pharm. 2012;69:107-115.
40. Vazquez-Espinosa E, Marcos C, Alonso T, Giron RM, Gomez-Punter RM, Garcia-Castillo E, Zamora E, Cisneros C, Garcia J, Valenzula C, Ancochea J. Tobramycin inhalation powder (TOBI Podhaler (R)) for the treatment of lung infection in patients with cystic fibrosis. Expert Rev Anti Infect Ther. 2016;14:9-17.
41. Antoniu SA, Cojocaru I. Inhaled colistin for lower respiratory tract infections. Expert Opin Drug Del. 2012;9:333-342.
42. Kirkby S, Novak K, McCoy K. Aztreonam (for inhalation solution) for the treatment of chronic lung infections in patients with cystic fibrosis: an evidence-based review. Core Evid. 2011;6:59-66.
43. Food and Drug Administration (FDA). These highlights do not include all the information needed to use ARIKAYCE safely and effectively 2018. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/207356s000lbl.pdf
44. ClinicalTrials.gov. MP-376 (Aeroquin™, Levofloxacin for Inhalation) in Patients With Cystic Fibrosis August 12, 2010. Last Accessed Date: 19.01.2018. Available from: https://clinicaltrials.gov/ct2/show/NCT01180634
45. Hobbs CA, Tan CD, Tarran R. Does epithelial sodium channel hyperactivity contribute to cystic fibrosis lung disease? J Physiol-London. 2013;591:4377-4387.
46. Shei RJ, Peabody JE, Kaza N, Rowe SM. The epithelial sodium channel (ENaC) as a therapeutic target for cystic fibrosis. Curr Opin Pharmacol. 2018;43:152-165.
47. Hirsh AJ, Zhang J, Zamurs A, Fleegle J, Thelin WR, Caldwell RA, Sabater JR, Abraham WM, Donowitz M, Cha B, Johnson KB, St. George JA, Johnson MR, Boucher RC. Pharmacological properties of N-(3,5-diamino-6-chloropyrazine-2-carbonyl)-N'-4-[4-(2,3-dihydroxypropoxy) phenyl] butyl-guanidine methanesulfonate (552-02), a novel epithelial sodium channel blocker with potential clinical efficacy for cystic fibrosis lung disease. J Pharmacol Exp Ther. 2008;325:77-88.
48. Moore PJ, Tarran R. The epithelial sodium channel (ENaC) as a therapeutic target for cystic fibrosis lung disease. Expert Opin Ther Tar. 2018;22:687-701.
49. ClinicalTrials.gov. To Assess the Safety, Tolerability and Pharmacokinetics of AZD5634 Following Inhaled and Intravenous (IV)Dose Administration February 10, 2016 . Last Accessed Date: 05.11.2018. Available from: https://clinicaltrials.gov/ct2/show/NCT02679729?term=NCT02679729&draw=2&rank=1
50. ClinicalTrials.gov. Safety, Pharmacokinetics and Pharmacodynamics Study of Inhaled QBW276 in Patients With Cystic Fibrosis October 1, 2015. Last Accessed Date: 17.07.2019. Available from: https://clinicaltrials.gov/ct2/show/NCT02566044?term=QBW+276&draw=2&rank=1
51. ClinicalTrials.gov. To Assess Safety, Tolerability and Pharmacokinetics of BI 443651 in Healthy Male Volunteers March 11, 2016. Last Accessed Date: 02.01.2020. Available from: https://clinicaltrials.gov/ct2/show/NCT02706925?term=BI+443651&draw=2&rank=3
52. Caldwell RA, Boucher RC, Stutts MJ. Neutrophil elastase activates near-silent epithelial Na+ channels and increases airway epithelial Na+ transport. Am J Physiol Lung Cell Mol Physiol. 2005;288:L813-L819.
53. Guilbault C, Saeed Z, Downey GP, Radzioch D. Cystic fibrosis mouse models. Am J Respir Cell Mol Biol. 2007;36:1-7.
54. Knowles MR, Clarke LL, Boucher RC. Activation by Extracellular Nucleotides of Chloride Secretion in the Airway Epithelia of Patients with Cystic-Fibrosis. New Engl J Med. 1991;325:533-538.

55. Deterding R, Retsch-Bogart G, Milgram L, Gibson R, Daines C, Zeitlin PL, Milla C, Marshall B, LaVange L, Engels J, Mathews D, Gorden JA, Schaberg A, Williams, Ramsey B. Safety and tolerability of denufosol tetrasodium inhalation solution, a novel P2Y2 receptor agonist: results of a phase 1/phase 2 multicenter study in mild to moderate cystic fibrosis. Pediatr Pulmonol. 2005;39:339-348.

56. Deterding RR, Lavange LM, Engels JM, Mathews DW, Coquillette SJ, Brody AS, Millard SP, Ramsey BW, Cystic Fibrosis Therapeutics Development Network and the Inspire 08-103 Working Group. Phase 2 randomized safety and efficacy trial of nebulized denufosol tetrasodium in cystic fibrosis. Am J Respir Crit Care Med. 2007;176:362-369.

57. Ratjen F, Durham T, Navratil T, Schaberg A, Accurso FJ, Wainwright C, Barnes M, Moss RB, The TIGER-2 Study Investigator Group. Long term effects of denufosol tetrasodium in patients with cystic fibrosis. J Cyst Fibros. 2012;11:539-549.

58. Grasemann H, Stehling F, Brunar H, Widmann R, Laliberte TW, Molina L, Döring G, Ratjen F. Inhalation of Moli1901 in patients with cystic fibrosis. Chest. 2007;131:1461-1466.

59. Haq IJ, Gray MA, Garnett JP, Ward C, Brodlie M. Airway surface liquid homeostasis in cystic fibrosis: pathophysiology and therapeutic targets. Thorax. 2016;71:284-287.

60. Robinson M, Hemming AL, Regnis JA, Wong AG, Bailey DL, Bautovich GJ, King M, Bye PT. Effect of increasing doses of hypertonic saline on mucociliary clearance in patients with cystic fibrosis. Thorax. 1997;52:900-903.

61. Williams HD, Behrends V, Bundy JG, Ryall B, Zlosnik JE. Hypertonic saline therapy in cystic fibrosis: do population shifts caused by the osmotic sensitivity of infecting bacteria explain the effectiveness of this treatment? Front Microbiol. 2010;1:120.

62. Boucher R. Human airway ion transport. Part two. Am J Respir Crit Care Med. 1994;150:581-593.

63. Assouline G, Leibson V, Danon A. Stimulation of prostaglandin output from rat stomach by hypertonic solutions. Eur J Pharmacol. 1977;44:271-273.

64. Tildy BE, Rogers DF. Therapeutic options for hydrating airway mucus in cystic fibrosis. Pharmacol. 2015;95:117-132.

65. Springsteel MF, Galietta LJ, Ma T, By K, Berger GO, Yang H, Dicus CW, Choung W, Quan C, Shelat AA, Guy RK, Verkman AS, Kurth MJ, Nantz MH. Benzoflavone activators of the cystic fibrosis transmembrane conductance regulator: towards a pharmacophore model for the nucleotide-binding domain. Bioorg Med Chem. 2003;11:4113-4120.

66. Suen YF, Robins L, Yang B, Verkman AS, Nantz MH, Kurth MJ. Sulfamoyl-4-oxoquinoline-3-carboxamides: novel potentiators of defective DeltaF508-cystic fibrosis transmembrane conductance regulator chloride channel gating. Bioorg Med Chem Lett. 2006;16:537-540.

67. Barry PJ, Ronan N, Plant BJ. Cystic Fibrosis Transmembrane Conductance Regulator Modulators: The End of the Beginning. Semin Resp Crit Care. 2015;36:287-298.

68. Rubin BK. Unmet needs in cystic fibrosis. Expert Opin Biol Th. 2018;18:49-52.

69. Van Goor F, Hadida S, Grootenhuis PDJ, 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 ER, Wine JJ, Frizzell RA, Ashlock M, Negulescu P. Rescue of CF airway epithelial cell function *in vitro* by a CFTR potentiator, VX-770. P Natl Acad Sci USA. 2009;106:18825-18830.

70. Food and Drug Administration (FDA). Orphan Drug Designations and Approvals. Last Accessed Date: 21.02.2014. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=316&showFR=1

71. Bessonova L, Volkova N, Higgins M, Bengtsson L, Tian S, Simard C, Konstan MW, Sawicki GS, Sewall A, Nyangoma S, Elbert A, Marshall BC, Bilton D. Data from the US and UK cystic fibrosis registries support disease modification by CFTR modulation with ivacaftor. Thorax. 2018;73:731-740.

72. Van Goor F, Yu HH, Burton B, Hoffman BJ. Effect of ivacaftor on CFTR forms with missense mutations associated with defects in protein processing or function. Journal of Cystic Fibrosis. 2014;13:29-36.

73. Moss RB, Flume PA, Elborn JS, Cooke J, Rowe SM, McColley SA, Rubenstein RC, Higgins M, VX11-770-110 (KONDUCT) Study Group. Efficacy and safety of ivacaftor in patients with cystic fibrosis who have an Arg117His-CFTR mutation: a double-blind, randomised controlled trial. Lancet Resp Med. 2015;3:524-533.

74. ClinicalTrials.gov. Study of GLPG1837 in Subjects With Cystic Fibrosis (G551D Mutation) (SAPHIRA1) March 14, 2016. Last Accessed Date: 07.12.2016. Available from: https://clinicaltrials.gov/ct2/show/NCT02707562?term=NCT02707562&draw=2&rank=1

75. ClinicalTrials.gov. Study to Evaluate the Safety and Efficacy of CTP-656 in Patients With Cystic Fibrosis With CFTR Gating Mutations November 23, 2016. Last Accessed Date: 15.08.2018. Available from: https://clinicaltrials.gov/ct2/show/NCT02971839?term=NCT02971839&draw=2&rank=1

76. Boyle MP, De Boeck K. A new era in the treatment of cystic fibrosis: correction of the underlying CFTR defect. Lancet Resp Med. 2013;1:158-163.

77. Van Goor F, Hadida S, Grootenhuis PDJ, Burton B, Stack JH, Straley KS, Elborn JS, Melotti P, Bronsveld I, Fajac I, Malfroot A, Rosenbluth DB, Walker PA, McColley SA, Knoop C, Quattrucci S, Rietschel R, Zeitlin PL, Barth J, Elfring GL, Welch EM, Branstrom A, Spiegel RJ, Peltz SW, Ajayi T, Rowe SM, Cystic Fibrosis Ataluren Study Group. Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. P Natl Acad Sci USA. 2011;108:18843-18848.

78. Food and Drug Administration (FDA). ORKAMBI July 2, 2015. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/211358s000lbl.pdf

79. Relations I. Vertex Selects Two Next-Generation Correctors, VX-659 and VX-445, to Advance into Phase 3 Development as Part of Two Different Triple Combination Regimens for People with Cystic Fibrosis Feb 1, 2018. Available from: https://investors.vrtx.com/news-releases/news-release-details/vertex-selects-two-next-generation-correctors-vx-659-and-vx-445

80. Doull I. Cystic fibrosis papers of the year 2017. Paediatr Respir Rev. 2018;27:2-5.

81. Davies JC, Moskowitz SM, Brown C, Horsley A, Mall MA, McKone EF, Plant BJ, Prais D, Ramsey BW, Taylor-Cousar JL, Tullis E, Uluer A, McKee CM, Robertson S, Shilling RA, Simard C, van Goor F. Waltz D, Xuan F, Young T,Rowe SM, VX16-659-101 Study Group. VX-659-Tezacaftor-Ivacaftor in Patients with Cystic Fibrosis and One or Two Phe508del Alleles. N Engl J Med. 2018;379:1599-1611.

82. Du M, Liu XL, Welch EM, Hirawat S, Peltz SW, Bedwell DM. PTC124 is an orally bioavailable compound that promotes suppression of the human CFTR-G542X nonsense allele in a CF mouse model. P Natl Acad Sci USA. 2008;105:2064-2069.

83. Sermet-Gaudelus I, Boeck KD, Casimir GJ, Vermeulen F, Leal T, Mogenet A, Roussel D, Fritsch J, Hanssens L, Hirawat S, Miller NL, Constantine S, Reha A, Ajayi T, Elfring GL, Miller LL. Ataluren (PTC124) induces cystic fibrosis transmembrane conductance regulator protein expression and activity in children with nonsense mutation cystic fibrosis. Am J Respir Crit Care Med. 2010;182:1262-1272.

84. Kerem E, Hirawat S, Armoni S, Yaakov Y, Shoseyov D, Cohen M, Nissim-Rafinia M, Blau H, Rivlin J, Aviram M, Elfring GL, Northcutt VJ, Miller LL, Kerem B, Wilschanski M. Effectiveness of PTC124 treatment of cystic fibrosis caused by nonsense mutations: a prospective phase II trial. Lancet. 2008;372:719-727.

85. Kerem E, Konstan MW, De Boeck K, Accurso FJ, Sermet-Gaudelus I, Wilschanski M, Elborn JS, Melotti P, Bronsveld I, Fajac I, Malfroot A, Rosenbluth DB, Walker PA, McColley SA, Knoop C, Quattrucci S, Rietschel E, Zeitlin PL, Barth J, Elfring GL, Welch EM, Branstrom A, Spiegel RJ, Peltz SW, Ajayi T, Rowe SM, Cystic Fibrosis Ataluren Study Group. Ataluren for the treatment of nonsense-mutation cystic fibrosis: a randomised, double-blind, placebo-controlled phase 3 trial. Lancet Resp Med. 2014;2:539-547.

86. Zomer-van Ommen DD, Vijftigschild LA, Kruisselbrink E, Vonk AM, Dekkers JF, Janssens HM, de Winter_de Groott KM, van der Ent CK, Beekman JM. Limited premature termination codon suppression by read-through agents in cystic fibrosis intestinal organoids. J Cyst Fibros. 2016;15:158-162.

87. ClinicalTrials.gov. PTC Study to Evaluate Ataluren in Combination With Ivacaftor. 2018. Available from: https://clinicaltrials.gov/ct2/show/NCT03256968

88. Food and Drug Administration (FDA). Human Gene Therapy for Rare Diseases. 2018. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-gene-therapy-rare-diseases

89. Hodges CA, Conlon RA. Delivering on the promise of gene editing for cystic fibrosis. Genes Dis. 2019;6:97-108.

90. Villate-Beitia I, Zarate J, Puras G, Pedraz JL. Gene delivery to the lungs: pulmonary gene therapy for cystic fibrosis. Drug Dev Ind Pharm. 2017;43:1071-1081.

91. Mention K, Santos L, Harrison PT. Gene and base editing as a therapeutic option for cystic fibrosis-learning from other diseases. Genes. 2019;10:387.

92. Marangi M, Pistritto G. Innovative therapeutic strategies for cystic fibrosis: moving forward to CRISPR technique. Frontiers Pharmacol. 2018;9:396.

93. Rosen BH, Chanson M, Gawenis LR, Liu J, Sofoluwe A, Zoso A, Engelhardt JF. Animal and model systems for studying cystic fibrosis. Journal of Cystic Fibrosis. 2018;17:S28-S34.

94. Martiniano SL, Sagel SD, Zemanick ET. Cystic fibrosis: a model system for precision medicine. Curr Opin Pediatr. 2016;28:312-317.

95. Griesenbach U, Pytel KM, Alton EW. Cystic Fibrosis Gene Therapy in the UK and Elsewhere. Hum Gene Ther. 2015;26:266-275.

96. Gill DR, Hyde SC. Delivery of genes into the CF airway. Thorax. 2014;69:962-964.

97. Burney TJ, Davies JC. Gene therapy for the treatment of cystic fibrosis. Appl Clin Genet. 2012;5:29-36.

98. Johnson LG, Olsen JC, Sarkadi B, Moore KL, Swanstrom R, Boucher RC. Efficiency of Gene-Transfer for Restoration of Normal Airway Epithelial Function in Cystic-Fibrosis. Nat Genet. 1992;2:21-25.

99. Dorin JR, Farley R, Webb S, Smith SN, Farini E, Delaney SJ, Wainwright BJ, Alton EW, Porteous DJ. A demonstration using mouse models that successful gene therapy for cystic fibrosis requires only partial gene correction. Gene Ther. 1996;3:797-801.

100. Zabner J, Petersen DM, Puga AP, Graham SM, Couture LA, Keyes LD, Lukason MJ, St George JA, Gregory RJ, Smith AE, et al. Safety and efficacy of repetitive adenovirus-mediated transfer of CFTR cDNA to airway epithelia of primates and cotton rats. Nat Genet. 1994;6:75-83.

101. Hay JG, McElvaney NG, Herena J, Crystal RG. Modification of nasal epithelial potential differences of individuals with cystic fibrosis consequent to local administration of a normal CFTR cDNA adenovirus gene transfer vector. Hum Gene Ther. 1995;6:1487-1496.

102. Valkama AJ, Leinonen HM, Lipponen EM, Turkki V, Malinen J, Heikura T, Ylä-Herttuala S, Lesch HP. Optimization of lentiviral vector production for scale-up in fixed-bed bioreactor. Gene Ther. 2018;25:39-46.

103. Prickett M, Jain M. Gene therapy in cystic fibrosis. Transl Res. 2013;161:255-264.

104. Alton EW, Boyd AC, Porteous DJ, Davies G, Davies JC, Griesenbach U, Higgins TE, Gill DR, Hyde SC, Innes JA, UK Cystic Fibrosis Gene Therapy Consortium. A Phase I/IIa Safety and Efficacy Study of Nebulized Liposome-mediated Gene Therapy for Cystic Fibrosis Supports a Multidose Trial. Am J Respir Crit Care Med. 2015;192:1389-1392.

105. Mottais A, Berchel M, Sibiril Y, Laurent V, Gill D, Hyde S, Jaffres PA, Montier T, Le Gall T. Antibacterial effect and DNA delivery using a combination of an arsonium-containing lipophosphoramide with an N-heterocyclic carbene-silver complex - Potential benefits for cystic fibrosis lung gene therapy. Int J Pharmaceut. 2018;536:29-41.

106. Rogan MP, Stoltz DA, Hornick DB. Cystic fibrosis transmembrane conductance regulator intracellular processing, trafficking, and opportunities for mutation-specific treatment. Chest. 2011;139:1480-1490.

107. Boj SF, Vonk AM, Statia M, Su J, Vries RR, Beekman JM, Clevers H. Forskolin-induced Swelling in Intestinal Organoids: An In Vitro Assay for Assessing Drug Response in Cystic Fibrosis Patients. J Vis Exp. 2017:55159.

108. van Mourik P, Beekman JM, van der Ent CK. Intestinal organoids to model cystic fibrosis. Eur Respir J. 2019;54:1802379.

109. Sachs N, de Ligt J, Kopper O, Gogola E, Bounova G, Weeber F, Balgobind AV, Wind K, Gracanin A, Begthel H, Korving J, van Boxtel R, Duarte AA, Lelieveld D, van Hoeck A, Ernst RF, Blokzijl F, Nijman IJ, Hoogstraat M, van de Ven M, Egan DA, Zinzalla V, Moll J, Boj SF, Voest EE, Wessels L, van Diest PJ, Rottenberg S, Vries RGJ, Cuppen E, Clevers H. A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity. Cell. 2018;172:373-386 e10.

110. Broutier L, Mastrogiovanni G, Verstegen MM, Francies HE, Gavarro LM, Bradshaw CR, Allen GE, Arnes-Benito R, Sidorova O, Gaspersz MP, Georgakopoulos N, Koo BK, Dietmann S, Davies SE, Praseedom RK, Lieshout R, Ijzermans JNM, Wigmore SJ, Saeb-Pars K, Garnett MJ, van der Laan LJ, Huch M. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. Nat Med. 2017;23:1424-1435.

111. Berkers G, van Mourik P, Vonk AM, Kruisselbrink E, Dekkers JF, de Winter-de Groot KM, Arets HGM, Marck-van der Wilt REP, Dijkema JS, Vanderschuen MM, Houwen RHJ, Heijerman HGM, van de Graaf EA, Elias SG, Majoor CJ, Koppelman GH, Roukema J, Bakker M, Janssens HM, van der Meer R, Vries RGJ, Clevers HC, de Jonge HR, Beekman

JM, van der Ent C. Rectal Organoids Enable Personalized Treatment of Cystic Fibrosis. Cell Rep. 2019;26:1701-1708 e3.

112. Noordhoek J, Gulmans V, van der Ent K, Beekman JM. Intestinal organoids and personalized medicine in cystic fibrosis: a successful patient-oriented research collaboration. Curr Opin Pulm Med. 2016;22:610-616.

113. Dekkers JF, van der Ent CK, Beekman JM. Novel opportunities for CFTR-targeting drug development using organoids. Rare Dis. 2013;1:e27112.

114. Dekkers JF, Wiegerinck CL, de Jonge HR, Bronsveld I, Janssens HM, de Winter-de Groot KM, Brandsma AM, de Jong NWM, Bijvelds MJC, Scholte BJ, Nieuwenhuis EES, van den Brink S, Clevers H, van der Ent CK, Middendorp S, Beekman JM. A functional CFTR assay using primary cystic fibrosis intestinal organoids. Nat Med. 2013;19:939-945.

115. McAllister F, Henry A, Kreindler JL, Dubin PJ, Ulrich L, Steele C, Finder JD, Pilewski JM, Carreno BM, Goldman SJ, Pirhonen J, Kolls JK. Role of IL-17A, IL-17F, and the IL-17 receptor in regulating growth-related oncogene-α and granulocyte colony-stimulating factor in bronchial epithelium: implications for airway inflammation in cystic fibrosis. J Immunol 2005;175:404-412.

116. Tan HL, Regamey N, Brown S, Bush A, Lloyd CM, Davies JC. The Th17 pathway in cystic fibrosis lung disease. Am J Respir Crit Care Med. 2011;184:252-258.

117. Chan YR, Chen K, Duncan SR, Lathrop KL, Latoche JD, Logar AJ, Pociask DA, Wahlberg BJ, Ray P, Ray A, Pilewski JM, Kolls JK. Patients with cystic fibrosis have inducible IL-17+ IL-22+ memory cells in lung draining lymph nodes. Journal of allergy and clinical immunology. 2013;131:1117-1129. e5.

118. Iannitti RG, Carvalho A, Cunha C, De Luca A, Giovannini G, Casagrande A, Zelante T, Vacca C, Fallarino F, Pucceyyi P, Massi-Benedetti C, Defilippi G, Russo M, Porcaro L, Colombo C, Ratclif L, De Benedicts FM, Romani L. Th17/Treg imbalance in murine cystic fibrosis is linked to indoleamine 2, 3-dioxygenase deficiency but corrected by kynurenines. Am J Respir Crit Care Med. 2013;187:609-620.

119. ClinicalTrials.gov. Anti-pseudomonas IgY to Prevent Infections in Cystic Fibrosis (PseudIgY) March 11, 2008 [updated September 1, 2016]. Available from: https://clinicaltrials.gov/ct2/show/NCT00633191?term=immunotherapy&cond=Cystic+Fibrosis&draw=2&rank=1