New Therapeutic Approaches in Cystic Fibrosis

Kistik Fibroziste Yeni Terapötik Yaklaşımlar

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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 chloride 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

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, CFTR protein ürünün 1,480 amino asit uzunluğunda, 3D hücre kültür sistemi ile in vitro/organoit tercih edilmiştir.

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

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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 HCO3− 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.7,8 In addition to these changes, decreased HCO3− levels contribute to the increase in mucus intensity.9 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.10 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.11 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.12 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 evident 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 (i.v) 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 ∆F508, is the 3 base deletion leading to loss of phenylalanine at position 508 in the CFTR protein.22 The ∆F508 mutation accounts for two-thirds of all CF alleles.23 Approximately 90% of CF patients carry at least one copy of the ∆F508 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 Amaral20 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.3

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 ∆F508, 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/
avibactam combination offers a potential improvement for CF pulmonary infections involving *P. aeruginosa*. 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. Co-administration of ceftazidime/avibactam and aztreonam gave successful results for extremely drug resistant *Burkholderia multivorans* infections. 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. 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 *Burkholderia multivorans* infections.

**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. 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⁺ hyperabsorption have been recommended as a treatment strategy.

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 trials 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 chloride channels. Increased activity of alternative chloride 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 P2Y₂ nucleotide receptor activates the CaCCs by causing a rapid increase in cytosolic free calcium concentration. ATP and uridine 5′-triphosphate (UTP), endogenous P2Y₂ receptor ligands, increase ion and liquid secretion.⁵⁴ However, the short half-lives of extracellular ATP and UTP limit their clinical utility. To induce chloride secretion by P2Y₂-mediated CaCC pathway, more stable inhaled P2Y₂ receptor agonists needed to be developed. Denufosol is an inhaled P2Y₂ receptor agonist that increases the Cl⁻ ion and fluid secretion in luminal clearance by P2Y₂-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 Moli901, 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 Moli901 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.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 Verkman 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. The FDA has approved the lumacaftor/ivacaftor combination for patients homozygous for the ΔF508 mutation who are 2 years old or older. 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.

VX-659 and VX-440 have different structures and different mechanisms of action. 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 VX-440/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.

**Ataluren (PTC124): potential treatment for class I mutations**

Stop codon mutations account for 10-12% of all CFTR mutations. 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. The reason may be the suppression of ataluren activity by aminoglycosides. 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.

**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.

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.

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. 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. CF models, generated in 5 animal species (mice, rats, ferrets, pigs, and rabbits), clearly reflect the mechanisms of disease pathogenesis and CFTR function. Recently, the sheep model has been proposed due to the similarity of lung anatomy between the two species.
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.26

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.91 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 (adenov-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.95

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. They are used to study heterogeneous medical conditions such as CF and cancer where genetics can influence disease severity, prognosis, and drug efficacy. 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 individual’s functional expressions of their own genomes. 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. The efficacy of Geneticin, ataluren, ivacaftor, and lumacaftor in combination therapy has been tested by FIS method in intestinal organoids with rare mutations.

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. 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. 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. 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.

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.

**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 potentiatior 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.

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