Ref: Ro J Pediatr. 2021;70(1)
DOI: 10.37897/RJP.2021.1.3

INNOVATIVE THERAPIES IN GENETIC DISEASES: CYSTIC FIBROSIS

Elena-Silvia Shelby[1], Florina Mihaela Nedelea[2,3], Tanser Huseyinoglu[2], Relu Cocos[3], Mihaela Badina[1], Corina Sporea[1], Liliana Padure[1,4], Andrada Mirea[1,4]

[1] „Dr. Nicolae Robănescu" National Clinical Center for Children's Neurorecovery, Bucharest, Romania

[2] Medical Genetics Department, „Filantropia" Clinical Hospital of Obstetrics and Gynaecology, Bucharest, Romania

[3] Genetics Chair, „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

[4] Faculty of Midwifery and Nursing, „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

ABSTRACT

Cystic fibrosis, also named mucoviscidosis, is the most frequent hereditary pulmonary disease and is produced by mutations in the CFTR gene, encoding an anionic channel for chloride and bicarbonate involved in the regulation of salt and bicarbonate metabolisms.

Currently, about half of the patients with cystic fibrosis can benefit personalized therapy consisting in modulators, drugs which restore or improve the functionality and stability of CFTR. Moreover, presently, other therapies, such as gene therapy using the CRISP/CAS-9, modified antisense oligonucleotides or the insertion of the wild-type gene using nanolipidic particles or viral vectors, are being developed. This article aims to take stock of the principal types of cystic fibrosis therapies which have been approved or are in clinical trials.

Keywords: cystic fibrosis, modulators, gene therapy

INTRODUCTION

Cystic fibrosis, also named mucoviscidosis, is an autosomal recessive genetic disease (1), with a prevalence of 1 in 2,500 newborns and a carrier frequency of 1 in 25 in the Caucasian population (2). It is the most frequent hereditary pulmonary disease (3) and it is produced by mutations in the CFTR gene, or ABCC7 (OMIM # 602421) situated in the 7q31.2 locus (4), which encodes an anionic channel for chloride and bicarbonate (5) localized in the apical membrane of the epithelial cells of more exocrine organs (6). CFTR is involved in regulating the metabolism of salt and bicarbonate (6,7).

Currently over 2,000 pathogenic mutations in the CFTR gene are known (6), approximately 70% of them consisting in the deletion of an aminoacid in the 508 position of the protein (the mutation being called F508del or, using the old adnotation, ΔF508), which leads to the erroneous packaging of the protein, with its subsequent destruction (8).

Pathogenic mutations in the CFTR gene lead to the production of sweat with an increased content of salt and viscous mucus, which accumulates in the gastrointestinal tract, impairing the pancreatic function (through the obstruction of the pancreatic duct which leades to the inability to secrete pancreatic enzymes and pancreatic hormones, which consequently leads to the appearance of diabetes mellitus) or the respiratory system (the accumulation of viscous mucus leading to obstruction and inflammation of the respiratory tract, this terrain favorizing the bacterial colonization and progressive lung degradation) (6,9). The fetuses with cystic fibrosis present intrauterine

Corresponding author:
Elena-Silvia Shelby
E-mail: silviajdx@yahoo.com

growth retardation, and, in the male fetuses, the congenital bilateral absence of the vas deferens can sometimes be present (8). The mean age of survival of the patients with cystic fibrosis which receive medical care is, according to The CF Foundation Patient Registry, 47.7 years (6,10). The main cause of mortality and morbidity of these patients is pulmonary impairment (8,11).

The carriers of CFTR mutations have a natural resistance to the cholera toxin and typhoid fever, as *Salmonella typhi* uses the CFTR channel to enter the cell. This phenomenon could explain the increased frequency of the carriers in the general population (8).

THE STRUCTURE OF THE CFTR PROTEIN AND TYPES OF MUTATIONS THAT CAN OCCUR IN THE CFTR GENE

The CFTR channel is a glycoprotein composed of a lasso amino terminal domain (12), several transmembrane units which form two MSD domains (membrane-spanning domains), namely MSD1 and MSD2 (which assemble the walls of the channel, their conformational changes leading to the channel's closing and opening) (12,13), an R domain, which, when phosphorilated, determins the activation of the CFTR channel (12-15), as well as two NBD domains (nucleotide binding domain), namely NBD1 and NBD2, which hydrolize ATP, controlling the closing and opening of the CFTR channel (12,13).

After its synthesis, the CFTR protein is packed, glycosilated at the level of the MSD2 domain and transported on the surface of the cell (6,16).

According to the produced effect, the mutations of the CFTR gene can be divided in six classes, adnotated, using Roman numerals, from 1 to 6 (17). Thus, class I mutations, in which no functional CFTR protein is created (22% of the cases), are represented by nonsense mutations, splice sites abnormalities or deletions. The mRNA produced by the genes with these mutations is unstable and immediately degraded; an example of class I mutations are the G542X, W1282X or R553X mutations (6,16,17).

The transcription of genes with class II mutations allows for the creation of a complete CFTR protein, but due to errors in the packaging process, the CFTR protein cannot reach the cell surface (88% of the cases). Examples of class II mutations are the F508del, N1303K, I507del and R1066C mutations (6,16,17).

Class III mutations allow for the synthesis of a complete CFTR protein which reaches the cell surface, but the channel doesn't open/ doesn't open correctly (6% of the cases) – "gating mutations"; examples of such mutations are the G551D, G178R and G551S mutations (6,16,17).

Class IV mutations also allow for the synthesis of a complete CFTR protein which reaches the cell surface, but the channel doesn't function correctly (6% of the cases), and the quantity of transported chloride is insufficient; examples of such mutations are R334W, R347P and R1070W (6,17).

Class V mutations lead, as well, to the creation of a functional, complete CFTR protein, which reaches the cell surface, but it is produced in an insufficient amount (5% of the cases). Examples of such mutations are 3849+10kbC->T, 3272-26A>G (6,16,17).

Class VI mutations allow for the production of a complete, correctly localized CFTR protein, but the protein has reduced stability and is rapidly degraded. Examples of class VI mutations are 1811+1.6 kb A>G, 4326del and 4279insTC (6,17).

MODULATORS USED IN THE TREATMENT OF CYSTIC FIBROSIS

Currently, according to the type and position of the mutation in the CFTR gene, approximately half of the cystic fibrosis patients can benefit personalized therapy (6,18), with the aid of modulators, substances which restore or improve the functionality and stability of CFTR (17). Based on their effect in modulating the expression and function of the CFTR protein, the modulators are divided in 5 classes, namely: read-through agents, potentiators, correctors, stabilizers and amplifiers (17). The read-through agents promote the continuation of the translation in spite of a stop-codon, with the subsequent production of a complete CFTR protein; the potentiators restore or preserve the conductibility of the CFTR channel, maintaining it open to allow for the transport of chloride, while the correctors restore the correct packaging and processing, as well as the proper transportation of CFTR towards the cell membrane (17).

The stabilizers have a role in correct anchoring of CFTR in the plasma membrane, preventing its degradation by the lysosomes, while the amplifiers increase the expression of messenger RNA for CFTR, thus increasing the quantity of produced protein (17).

Thus, for class I mutations – which lead to a significant decrease in the quantity of produced CFTR protein (6), for nonsense mutations which lead to the apparition of premature stop codons and the coding of a trunked protein, read through agents are used, which supress the termination of the translation (preventing the stoppage of the coding once the stop-codon is reached), together with inhibitors for the nonsense-mediated decay (NMD) pathway, which has

the role to detect and degrade messenger RNA containing premature stop codons (6,9). In this regard, aminoglycosides (such as, for example, gentamicin, amikacin and, to a lesser degreee, tobramicin) which, following the attachment to the A ribosomal situs, lead to the inclusion of an alternative transfer RNA, with the subsequent insertion of a random aminoacid corresponding to the premature stop codon and, consequently, the production of a complete and functional CFTR protein (9). The only disadvantage of the aminoglycosides consists in the ototoxic and renal side effects (11).

Another compound with the same mechanism of action as the aminoglycosides is ataluren, with the commercial name Translarna (9). Ataluren has not proved the same benefic effects as in Duchenne muscular dystrophy, therefore studies regarding its use as a potential therapy have been stopped (11).

For the treatment of class I mutations, the combination ivacaftor (Kalydeco)/ataluren has been tested, but according to a study from 2020 (19), it has not proved to be efficient. Ivacaftor is a potentiator which increases the length of the opening of the CFTR channel in the cell membrane (20,21), thus correcting the transport of chloride ions at this level (24). Nevertheless, ivacaftor is used in patients with cystic fibrosis with the age over four months which have one of the 97 mutations presented in the list from the reference (22).

For class II mutations, which consist in protein folding defects, correctors (drugs that stimulate the correct folding of CFTR so that it reaches the surface of the cell) are being used, alone or in combination with other modulators (9,16,18,19).

Thus, for the most common mutation responsible of cystic fibrosis, namely the F508del mutation, a combination of corrector and potentiator was successfully used (11). The combination Lumacaftor/ivacaftor, known as Orkambi, known under the commercial name of Orkambi, was approved by FDA for patients with ages over two years who are homozygous for the F508del mutation (approximately 44% of the patients with cystic fibrosis) (23). Lumacaftor is a corrector that increases the quantity of CFTR in the cell membrane (23). Orkambi significantly increases the lung function and decreases the number of pulmonary exacerbations (23).

Also for the treatment of biallelic F508del mutations as well as another 154 mutations – presented in the list from the reference (24), for patients over 6 years old, a combination of tezacaftor and ivacaftor, called Symdeko, is being used. Symdeko improves lung function and has the advantage of presenting less drug interactions than Orkambi (23,24).

In October 2019, FDA has approved Trikafta, a triple combination of elexacaftor, ivacaftor and tezacaftor, to treat patients with the F508del mutation, this therapy being produced by Vertex Pharmaceuticals (25,26). The drug is meant for persons over the age of 12 who have the F508del mutation in a homozygous or heterozygous state, as well as patients having one of the117 mutations responsive to this drug, included in the list from the reference (27) (25,27).

It has been shown that Trikafta significantly improves the lung function and body mass index, improves the level of chloride secreted in the sweat and decreases the number of pulmonary exacerbations, while a the same time having minor side effects, such as erithema, an increase in the level of transaminases and bilirubin, or flu-like symptoms – headache, diarrhea or rinitis (25). There is also a risk to develop cataracts (25).

Elexacaftor is a new generation corrector of CFTR, while tezacaftor, approved by FDA in February 2018, is a first generation corrector of the structure of this protein (21,28). Elexacaftor facilitates the transport of the protein towards the cell surface, with the increase of the protein expression at this level (28), while tezacaftor improves the cell processing of the CFTR protein, modulaing its position in the plasmalemma (29). Thus, althogh they have different mechanisms of action (28), elexacaftor and tezacaftor act synergically to increase the expression of CFTR in the cell membrane (20). Taken separately, each of these correctors improves the pulmonary function, nutritional status and quality of life (28). It has been shown that elexacaftor, tezacaftor and ivacaftor have synergic effects, their combination being stronger than other previously tested for cystic fibrosis treatment corrector-potentiator combinations (30).

For class III (in which the protein reaches the cell membrane, but an opening defect decreases its functionality) and IV (in which the protein reaches the surface of the cell, but a conduction defect leads to impaired opening and a reduction of the functionality) mutations, potentiators – drugs which support the opening of the CFTR channel and, thus, the increase of the chloride transportation – are being used (16,18,19).

Moreover, the modulation of other chloride (SLC6A14, SLC6A9, ANO-1, TMEM16A) and sodium (ENaC, SLC9A3), hydrogen and potasium (ATP12A) channels is being considered. This could restore the volume of the fluid on the surface of the respiratory tract, which is decreased in patients with cystic fibrosis, thus impairing the clearance of respiratory secretions (12).

Ivacaftor (Kalydeco) was approved by FDA (Food and Drug Administration) in January 2012 (22) to correct the G551D mutation (a class III mutation), present in about 5% of the patients with cystic fibrosis, with the restitution of the functionality of CFTR channel (11,22). This potentiator has proved an increase with 10.5% of the FEVS (forced expiratory volume in one second), a decrease of 55% of the pulmonary exacerbations and an increase of the patients' quality of life (11).

Furthermore, Ivacaftor was also approved for usage in patients with class IV mutations (ex.: Arg117His), ensuring the maintainance of the residual functionality of CFTR (11,22). Currently, Kalydeco was approved for the treatment of 38 mutations, in patients over 6 months (22).

Moreover, the combination of tezacaftor (corrector) and ivacaftor (potentiator), called Symdeko, was successfully used for the improvement of lung function in these patients (11). Nevertheless, its worth mentioning that not all patients with elligible mutations are responsive to the treatment (11).

So far, no therapy has been approved yet for patients with class V and VI mutations (18).

Another category of modulators are the stabilizers, substances which increase the lifespan of CFTR by anchoring it to the cell membrane, preventing the accelerated endocytosis and rapid distruction of CFTR in the lysosomes (17). In this regard, studies on cell lines with knock-out for CFTR have proven that the administration of HGF (hepatocyte growth factor) stabilizes CFTR in the cell membrane, HGF's effect being significantly amplified when in combination with lumacaftor (17).

Another stabilizer which seemed promising for patients with biallelic F508del mutations was cavosonstat, which promoted, in vitro, the stability of CFTR in the cell membrane, by inhibiting S-nitrosoglutathione. Nevertheless, in class II clinical trials cavosonstat has not proved its efficiency in combination with lumacaftor/ivacaftor or just ivacaftor, and the clinical studies of this stabilizer in cystic fibrosis have been stopped (17).

Regarding the amplifiers, currently nesolicaftor is being studied clinically, as it has proven its capacity to increase the quantity of CFTR with certain mutations, as well as, in combination with lumacaftor/ivacaftor, to restaurate the necessary quantity of CFTR in the cell membrane in cell lines with knock-out for F508del (17).

Furthermore, combined with posenacaftor (corrector) and dirocaftor (potentiator), nesolicaftor increases, on cell lines with knockout for F508del, the chloride secretion up to a level which is very close to normal levels. Currently, this combination is in phase I/II clinical studies (17).

GENE THERAPY FOR THE TREATMENT OF CYSTIC FIBROSIS

Therapies involving splice sites are currently being developed, the target being decreasing the efficiency of a donor or acceptor splice site, or creating a new splice site in an intron using antisense oligonucleotides (11).

Furthermore, genetic editing of certain mutations using the CRISPR/Cas9 system has been successfully attempted, both in the respiratory tract cells as well as in certain cryptic splice sites at the intronic level (11). In this regard, the effect of antisense oligonucleotides is being studied (11). For example, studies on cell lines and mice with knockout for F508del have proven that eluforsen, a modified antisense oligonucleotide, has the capacity to restore CFTR functionality (17). A study on a small number of patients with cystic fibrosis caused by biallelic mutations F508del has shown that this compound was well tolerated and has improved these patients' quality of life. In spite of the significant side effects, currently the clinical trials for this compound have been stopped (17).

Gene therapy using a cDNA for CFTR transgene included in cationic lipid and viral vectors, among which the adenovirus, administered in the respiratory tract, has not proven to be efficient so far, either because the benefits of the therapy were below expectations, because of the transient effect of the insertion of the gene in the respiratory tract, using adeno-associated viruses, or because of the lack of succes in the administration of the gene in the respiratory tract, using other viruses (11,16). The errors which appear in the mRNA are corrected either by direct targeting of mRNA, or by supplying mRNA at the cell level (31).

CONCLUSIONS

If in the last century, the prognosis for patients with cystic fibrosis was a somber one, currently about half of these patients can benefit personalized therapy according to the type and position of the mutation they have in CFTR gene. The numerous studies in the works will bring new therapies and offer, at the same time, the hope that the diagnosis of cystic fibrosis will no longer represent a sentence for these patients.

REFERENCES

1. OMIM # 219700. Cystic fibrosis; CF. Available at: https://www.omim.org/entry/219700.
2. Genetics Home Reference. Cystic fibrosis. Available at: https://ghr.nlm.nih.gov/condition/cystic-fibrosis#statistics.
3. KD Wenstrom, ML Russo. Cystic fibrosis: carrier screening. Available at: https://www.uptodate.com/contents/cystic-fibrosis-carrier-screening.
4. Mall MA, Hartl D. CFTR: cystic fibrosis and beyond. *European Respiratory Journal.* 2014;44:1042-1054.
5. OMIM # 602421. Cystic fibrosis transmembrane conductance regulator; CFTR. Available at: https://www.omim.org/entry/602421.
6. Pranke I, Golec A, Hinzpeter A, Edelman A, et al. Emerging Therapeutic Approaches for Cystic Fibrosis. From to Personalized Medicine. *Front Pharmacol.* 2019;10:121.
7. Linsdell P. Functional architecture of the CFTR chloride channel. *Mol Membr Biol.* 2014 Feb;31(1):1-16.
8. The Embryo Project Encyclopedia. Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene. Available at: https://embryo.asu.edu/pages/cystic-fibrosis-transmembrane-conductance-regulator-cftr-gene.
9. Gambari R, Breveglieri G, Salvatori F, Finotti A, et al. Therapy for Cystic Fibrosis Caused by Nonsense Mutations, Cystic Fibrosis in the Light of New Research. 2015. Dennis Wat, IntechOpen. Available at: https://www.intechopen.com/books/cystic-fibrosis-in-the-light-of-new-research/therapy-for-cystic-fibrosis-caused-by-nonsense-mutation.
10. What Is the Life Expectancy for People with Cystic Fibrosis?. Available at: https://www.healthline.com/health/cystic-fibrosis-life-expectancy#life-expectancy.
11. Strug LJ, Stephenson AL, Panjwani N, Harris A. Recent advances in developing therapeutics for cystic fibrosis. *Hum Mol Genet.* 2018 Aug 1;27(R2):R173-R186.
12. Zhang Z, Liu F, Chen J. Molecular structure of the ATP-bound, phosphorylated human CFTR. PNAS. 2018;115(50):12757-12762.
13. Sheppard DN, Welsh MJ. Structure and function of the CFTR chloride channel. *Physiol Rev.* 1999;79(1 Suppl):S23-S45.
14. Morales MM, Capella MAM, Lopes AG. Structure and function of the cystic fibrosis transmembrane conductance regulator. Brazilian Journal of Medical and Biological Research. 1999;32:1021-1028.
15. Riordan JR. CFTR function and prospects for therapy. *Annu Rev Biochem.* 2008;77:701-26.
16. Know your CFTR mutations. A CFTR Mutation Fact Sheet. Available at: https://www.cff.org/Care/Clinician-Resources/Network-News/August-2017/Know-Your-CFTR-Mutations.pdf.
17. Lopes-Pacheco M. CFTR Modulators: Shedding Light on Precision Medicine for Cystic Fibrosis. Front Pharmacol. 2016 Sep 5;7:275.
18. Ong T, Ramsey BW. New Therapeutic Approaches to Modulate and Correct Cystic Fibrosis Transmembrane Conductance Regulator. *Pediatr Clin North Am.* 2016;63(4):751-764.
19. Cystic Fibrosis Foundation. CFTR Modulator Types. Available at: https://www.cff.org/Research/Developing-New-Treatments/CFTR-Modulator-Types/.
20. Patient Brochure - Trikafta. Available at: https://www.trikafta.com/sites/default/files/patient-brochure.pdf.
21. CFTR Modulator Types. Available at: https://www.cff.org/Research/Developing-New-Treatments/CFTR-Modulator-Types/#:~:text=Drugs%20that%20target%20the%20underlying,potentiators%2C%20correctors%2C%20and%20amplifiers.
22. Cystic Fibrosis Foundation. List of CFTR gene mutations that are responsive to Kalydeco (ivacaftor). Available at: https://www.cff.org/Life-With-CF/Treatments-and-Therapies/Medications/Kalydeco-Approved-Mutations.pdf.
23. Dickinson K, Paranjape S. Triple combination therapy for cystic fibrosis is here! *Contemporary PEDS Journal.* 2020;37(2):17-19.
24. List of CFTR gene mutations that are responsive to Symdeko. Available at: https://www.cff.org/Life-With-CF/Treatments-and-Therapies/Medications/Symdeko-Approved-Mutations.pdf.
25. FDA approves new breakthrough therapy for cystic fibrosis. U.S. Food & Drug Administration. Available at: https://www.fda.gov/news-events/press-announcements/fda-approves-new-breakthrough-therapy-cystic-fibrosis.
26. Khamsi R. Cystic fibrosis outlook. Nature. 2020;583:S12-S14.
27. Cystic Fibrosis Foundation. List of CFTR gene mutations that are responsive to Trikafta. Available at: https://www.cff.org/Life-With-CF/Treatments-and-Therapies/Medications/Trikafta-Approved-Mutations.pdf.
28. Elexacaftor. Drug Bank. Available at: https://www.drugbank.ca/drugs/DB15444.
29. Tezacaftor. Drug Bank. Available at: https://www.drugbank.ca/drugs/DB11712.
30. Condren ME, Bradshaw MD. Ivacaftor: a novel gene-based therapeutic approach for cystic fibrosis. *J Pediatr Pharmacol Ther.* 2013;18(1):8-13.
31. Cystic Fibrosis: Investigational Therapies. Available at: https://www.uptodate.com/contents/cystic-fibrosis-investigational-therapies.