ACTA SCIENTIFIC CLINICAL CASE REPORTS

Volume 4 Issue 12 December 2023

Research Article

New Integrated Approaches for Cystic Fibrosis

Amelia Morgillo[1,2]*

[1]*Department of Biological Science, University of Sannio, Benevento*

[2]*Department of Medicine and Surgery, University of Siena – SI– Street Mario Bracci 16, Italy*

***Corresponding Author:** Amelia Morgillo, Department of Medicine and Surgery, University of Siena – SI– Street Mario Bracci 16, Italy.

Received: October 19, 2023

Published: November 02, 2023

Abstract

Introduction: Cystic fibrosis, while remaining a rare disease, is the most common autosomal recessive genetic form in the Caucasian population and is a chronic developmental multisystem disease for which until a few years ago there were only supportive therapies, which did not however modify the long-term outcome, inevitably characterized by chronic lung damage, nutritional imbalances and, in severe cases, delayed physical development in affected children and endocrine-metabolic disorders. Today, new drugs are available that act directly on the CFTR protein, which is responsible for clinical signs and symptoms. In this article we wanted to deepen the pharmacology of these new molecules and the trials concerning gene therapy and new pharmacological approaches.

Objective and Materials and Methods: A computerized search was carried out for the articles to be included through the use of international databases such as pubmed, scopus, researchgate, google scholar, by typing in keywords such as: gene therapy for cystic fibrosis and the names of the new drugs and integration with literature data. In addition, data from paper documents such as books and articles have been integrated. The articles relating to the new therapies just approved or in the process of being approved and the related studies were selected.

Results: Although it remains a non-curable disease to date, the therapeutic possibilities for cystic fibrosis are expanding considerably and to date four modulating drugs (correctors and enhancers) CTFR are already available in clinical practice. Furthermore, gene therapy-based methodologies are being developed to directly correct the causal genetic defect, even if they are not yet directly applicable in clinical practice. Although gene therapy in lung diseases has not yet been fully realized, recent efforts in molecular virology aimed at the development of vectors will undoubtedly lead to great benefits in the next decade in this area. From the point of view of safety and efficacy, the current most promising gene transfer systems to the lung for inherited genetic diseases are adeno-associated vectors.

Conclusion: Novel approaches for purifying large amounts of vectors have also made widespread clinical applications more viable. With the help of the appropriate animal model for a given disease and efficient vectors for gene transfer, the field of gene therapy is likely to see great progress in the next decade.

Keywords: CF; CFTR; Viral Vector; Gene Therapy and ATMP

Introduction

Cystic fibrosis is a multisystemic disease with predominantly pulmonary involvement with a chronic progressive course and, despite being a rare disease, it is the most frequent hereditary genetic disease among populations of Caucasian origin (Europe and North America), with an average incidence in Italy of one affected newborn for every 2500-3000 live births [1]. It has an autosomal recessive mode of inheritance with variable penetrance: this means that only homozygous individuals (who have inherited two pathogenic mutations - one allele mutated by each parent) are phenotypically affected, but the severity of the disease varies with milder forms, without pancreatic insufficiency or with moderate respira-

tory insufficiency. The first medical descriptions of CF date back to the 1930s. Previously, it was even thought that the latter was caused by witchcraft [2].

The gene that causes the disease is the Cystic Fibrosis Transmembrane Regulator (CFTR) gene, which is found along the q31.2 locus in the arm of chromosome 7 (mainly expressed in cell membranes of the respiratory, digestive, sweat glands, and tract reproductive) [3]. The CFTR gene encodes a protein with ion channel function, involved in the regulation of chloride ion transport across the cell membrane. To date, over 2,000 mutations have been described, grouped into 6 classes, based on their functional defect [4].

- Class I: defect of protein synthesis
- Class II: protein folding defect with premature degradation
- Class III: "gating" mutations responsible for insufficient opening of the channel
- Class IV: decrease in chlorine permeability of the CFTR conductance channel
- Class V: decrease in the amount of CFTR protein synthesis
- Class VI: reduced stability of the CFTR protein in the cell membrane.

Materials and Methods

A computerized research was carried out for the articles to be included was conducted through the use of databases such as pubmed, scopus, researchgate, google scholar, by typing in keywords such as "multiple sclerosis therapies" the names of the new drugs and integrating with literature data. In addition, the data of paper documents such as books and articles have been integrated. The articles relating to the new therapies just approved or in the approval phase and the studies conducted were selected, also by comparing them with drugs already approved for some time.

Discussion

Although there is no definitive cure available for cystic fibrosis to date, the management of the disease has improved significantly over the past 70 years. A better understanding of the disease is imperative to continue improving the life expectancy of a CF patient [5]. The cornerstones of management are treating airway infections, encouraging good nutrition and an active lifestyle. The management of the condition must be protracted throughout the patient's life, and is aimed at maximizing the function of the organs and consequently the quality of life. The primary targets of therapy are the lungs, gastrointestinal tract (including supplemental therapy for pancreatic enzymes) and reproductive organs (including assisted reproductive techniques) [6].

The most consistent aspect of cystic fibrosis therapy is the limitation and treatment of lung damage caused by thick sputum and subsequent infections. Antibiotics are prescribed to treat chronic and acute infections [7]. Mechanical inhalation devices and medications are used to remove excess secreted mucus [8]. Prevention and treatment of respiratory infections can reduce the vicious circle of bronchiectasis [9]. Prevention of chronic Pseudomonas Aeruginosa infection is now a therapy goal for infants and very young children [10]. This strategy consists of frequent monitoring with sputum cultures, and treatment with appropriate antibiotics whenever *P. aeruginosa* is observed [11]. Vaccines intended to prevent *P. aeruginosa* infection and new antibiotics for treatment are in development [12]. Many new drugs that can help people with CF are being studied [13]. Experimental drugs that help improve salt transport between cells include denufosol, which activates the non-CFTR chloride ion channel, to increase airway surface fluid volume [14]. There are also other potential treatments for infections [15].

Among the most encouraging advances in CF drug therapies are new drugs that have been designed to correct the underlying defect in the CFTR protein [16]. The development and early clinical trials of these drugs have been complex, since different genetic mutations cause different problems in the production of proteins; therefore these drugs are specific for defined genetic mutations [17].

Some of these drugs have been shown to improve CFTR function, measured by evaluating sweat, chloride levels, and differences in nasal potential, a way to directly measure the transport of salt across the nasal membranes [18]. However, it must be said that there are therapies that can improve lung function and reduce infection [19]. These therapies have been designed to improve airway chloride secretion or increase airway mucus hydration or airway mucus hydration and can improve bronchial hygiene and preserve lung function and this obviously could translate into a better life expectancy [20].

While gene therapy has not yet achieved its initial promise, research is underway to develop a safe and efficient method of transferring a normal CFTR gene to the airways of patients with this disease [21]. Successful gene therapy could lead to a definite cure for CF. Drugs designed to improve the function of mutant CFTR, thereby correcting the problem of ion transport, are currently in phase 2 and 3 [22]. If these studies show both efficacy and safety, these drugs could lead to stabilization or improvement of the disease CF and allow for prolonged survival [23].

Some of these drugs have been shown to improve CFTR function, measured by evaluating sweat, chloride levels, and differences in nasal potential, a way to directly measure the transport of salt across the nasal membranes [24]. However, it must be said that there are therapies that can improve lung function and reduce infection. These therapies have been designed to improve airway chloride secretion or increase airway mucus hydration or airway mucus hydration and can improve bronchial hygiene and preserve lung function and this obviously could translate into a better life expectancy.

Inhalation biopharmaceutical and application in cystic fibrosis

Inhalation therapy is the most effective and safe therapeutic modality for the treatment of respiratory diseases, as it allows the drug used to act directly on the target organ, distributing itself in the airways, both intra- and extra-pulmonary and avoiding recourse to systemic administration and offering the opportunity to obtain the same therapeutic effect with a lower dosage than that required by oral or parenteral therapy and with greater rapidity of action [25]. Inhaled drugs are commonly referred to as aerosols, liquid droplet suspensions or solid particles in gaseous media. The main characteristics of aerosol particles are penetration, deposition, retention and clearance. Aerosol therapy refers to the administration of inhalation in the form of aerosol particles [26]. The most important factors that regulate aerosol deposition include: the drug formulation, the performance of the delivery device, the patient's inhalation technique and the physical characteristics of the airways. Depending on the size of the particles, the drugs will settle in different areas of the respiratory tract [27]. The smaller the size of the aerosol particles, the greater their penetrating capacity into the bronchial tree. Particles of 5 m size will settle mainly in the oropharynx. In fact, aerosol kinetics studies have shown that particles with a diameter between 0.5 and 5 m are able to reach the airways with a diameter of less than 2 mm (pulmonary alveoli) [28]. Some of these drugs have been shown to improve CFTR function, measured by evaluating sweat, chloride levels, and differences in nasal potential, a way to directly measure the transport of salt across the nasal membranes. However, it must be said that there are therapies that can improve lung function and reduce infection. These therapies have been designed to improve airway chloride secretion or increase airway mucus hydration or airway mucus hydration and can improve bronchial hygiene and preserve lung function and this obviously could translate into a better life expectancy [29].

Inhalation biopharmaceutical and application in cystic fibrosis

The recent technological innovations introduced in nebulizers together with a greater understanding of the phenomena of nebulization have significantly improved the efficiency in the delivery of inhaled drugs. The most important characteristic for evaluating the performance of a nebulizer is the respirable fraction, that is the proportion of particles delivered with a diameter between 1 and 5 microns. A fill volume of 4-5 mL is generally recommended and saline must be added to achieve this volume in the ampoule. Traditional aerosol devices are represented by pneumatic continuous flow nebulizers [30].

The administration of aerosol antibiotics in patients with chronic Pseudomonas aeruginosa infection has established itself as a cornerstone of cystic fibrosis therapy over the past twenty years. There are 3 clinical settings in which anti-PA inhalation therapy has been used in the management of CF:

- The first PA infection, with the aim of eradicating and preventing chronic colonization;
- Chronic PA infection, with the aim of reducing the progression of lung damage (maintenance therapy);
- Disease flare-ups.

In addition, various molecules are available as support and supplementation or inhaled steroids such as beclomethasone, flunisolide or fluticasone, which reduce inflammation in the airways, edema and symptoms such as cough and expectorant mucolytics such as acetylcysteine or ambroxol [31].

Current therapies approved for clinical use

Until recently, there were no treatments for the basic defect of CF, but only therapies aimed at counteracting the evolution of respiratory disease, correcting pancreatic insufficiency and maintaining good nutritional status. In particular, drug therapy was supportive, based on the administration of antibiotics, especially aerosolically, to control infections, and of mucoregulators to favor the clearance of secretions. Antibiotic therapy should only be prescribed based on the result of the microbiological examination of bronchial secretions obtained after coughing or by pharyngeal aspirate. This examination must be repeated every 2 or 3 months [32]. In particular, great attention must be paid to the treatment of Pseudomonas aeruginosa infection, to prevent it from becoming chronic. The first Pseudomonas aeruginosa infection must be dealt with promptly, to eliminate the germ and prevent it from becoming chronic.

In the treatment of chronic infection, however, it would be good to rotate the prescribed drugs in combination with high doses and

in combinations, to prevent the onset of resistance to the antibiotics themselves. In addition, pancreatic insufficiency is corrected by administering enzymes at each meal [33].

The quantity to be administered must be evaluated taking into account the severity of the pancreatic compromise and the eating habits of the patients, who must undergo a free, balanced, high-calorie diet. Caloric supplements are used only in special cases. The loss of salts can be avoided by taking sodium chloride and saline supplements, especially in the first years of life [34]. In patients with cholestasis it is useful to administer hydrophilic bile salts (ursodeoxycholic acid), to make the bile more fluid. Pharmacotherapy of the basic defect appears much more interesting in recent years; Since CFTR proteins, despite being misfolded, still exhibit normal conductance to chlorine ions, compounds that are capable of reducing degradation and increasing the trafficking of CFTR proteins to the membrane are considered as a potential treatment option. In 2012, the first drug belonging to this class, Ivacaftor, was approved, the first drug capable of acting effectively on the causes of the disease, improving the performance of the defective protein [35].

It is available as coated tablets in two strengths, either as monotherapy for patients 6 years of age or older with one of the following 'gating' mutations in the CFTR gene: G551D, G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N or S549 or in combination with tezacaftor/ivacaftor for the treatment of adults, adolescents and children aged 6 years and older with cystic fibrosis (CF) who are homozygous for the 3 F508del mutation or heterozygous for the F508del mutation and one of the following mutations in the CFTR gene: P67L, R117C, L206W, R352Q, A455E, D579G, 711 + 3A → G, S945L, S977F, R1070W, D1152H, 2789 + 5G → A, 3272-26A → G and 3849 + 10kbC → T o In a combination regimen with ivacaftor/tezacaftor/elexacaftor tablets for the treatment of adults and adolescents aged 12 years and older with cystic fibrosis (CF) who have at least one F508del mutation in the CFTR gene. The approval of ivacaftor is based on the results of phase 3 clinical trials conducted around the world, which evaluated the efficacy and safety profile of the drug and demonstrated its benefits in terms of better nutrition, weight gain, improvement of respiratory function, reduction of oxygen requirements, reduction of the frequency of respiratory exacerbations [36].

At the end of 2019 the European Commission approved the extension for Kalydeco to include treatment for children with cystic fibrosis aged between 6 and 12 months, weighing no less than 5 kg and with at least one of the following 9 mutations of the CFTR gene: G551D, G1244E, G1349D, G178R, G551S, S1255P, S549N, S549R [37].

The update is based on safety data from the ongoing Phase 3 'Arrival' study (performed on children with cystic fibrosis less than 24 months of age and a GATING mutation) which showed a similar safety profile to that observed in previous Phase 3 studies in older children and adults and improvements in sweat chloride as a secondary endpoint [38]. Ivacaftor is an enhancer of the CFTR protein, i.e. it increases the gating of the CFTR channel *in vitro* to enhance chloride transport in specific gating mutations with reduced probability of channel opening compared to normal CFTR. Ivacaftor also enhances the channel opening probability of R117H-CFTR, which has both a low channel opening probability and a low amplitude of the current through the channel (conductance) [39]. The G970R mutation causes a splice defect, which results in a deficiency or absence of CFTR protein on the cell surface, which may explain the results observed in subjects with this mutation in study 5 (see data reported in Pharmacodynamic effects and Clinical efficacy). The *in vitro* responses observed in single-channel patch clamp experiments using membrane fragments from rodent cells expressing mutant CFTR forms do not necessarily correspond to the *in vivo* pharmacodynamic response (e.g. sweat chloride) or clinical benefit. The exact mechanism that induces ivacaftor to enhance the gating activity of normal forms and some mutant forms of CFTR in this system has not been fully elucidated [40].

Kaftrio (trikafta) is a combination of 3 active ingredients (ivacaftor/tezacaftor/elexacaftor) aimed at correcting changes in the CFTR protein produced by the F508del mutation, the most common of the mutations that cause cystic fibrosis [41]. In combination regimen with KALYDECO (ivacaftor) is a drug developed for the treatment of cystic fibrosis in patients 12 years of age and older who have two F508del (F/F) mutations or one F508del mutation and one mutation with minimum function (F/MF). elexacaftor and tezacaftor are CFTR correctors, which bind to different sites on the protein and have an additive effect in facilitating cellular processing and trafficking of F508del-CFTR, to increase the amount of protein brought to the cell surface, compared to each of the molecules alone. Ivacaftor increases the likelihood of channel opening (or gating) of the CFTR protein on the cell surface. The combined effect of ELX, TEZ and IVA is an increase in the quantity and function of F508del-CFTR on the cell surface, resulting in an increase in CFTR activity measured by CFTR-mediated chloride transport [42]. Regarding the non-F508del-CFTR variants on the second allele, it is not clear whether and to what extent the combination of these active substances also increases the amount of these CFTR variants

mutated on the cell surface and enhances its probability of opening of the channel. The current Marketing Authorization in Europe was supported by the positive results of two PHASE III studies published in November 2019 [43]. The two PHASE III clinical trials evaluated the efficacy and safety of this drug and have shown a positive impact on lung disease (respiratory function and risk of pulmonary exacerbations) and nutritional status. People with cystic fibrosis enrolled in the aforementioned trials had an age equal to or greater than 12 years and two F508del mutations or, an F508del mutation in one allele and a "minimal function" mutation in the other allele. "Minimal function" mutations are characterized by absent or less than 10% of normal CFTR protein synthesis. The evident reduction in sweat chlorine, recorded during therapy with Kaftrio, demonstrated its direct effect on the CFTR protein, also present in the sweat glands [44]. Together with the drug Kalydeco, already approved in Italy for the rare class III mutations and the R117H mutation in adults, Kaftrio stands out as a drug capable of substantially improving the clinical development of the disease, a result highly anticipated by people with CF, their families and the scientific community.

Simkevi is the third drug for the treatment of cystic fibrosis approved in the US by the Food and Drug Administration (FDA). It is a combination of ivacaftor and tezacaftor coated tablets. Approved and available in class C-NN, for patients aged 12 years and older who are homozygous for the F508del mutation. Also approved for patients 12 years of age and older with 1 copy of F508del associated with one of the following 10 CFTR residual function (RF) mutations: P67L, R117C, L206W, R502Q, A455E, D579G, S945L, S977F, R1070W, D1152H. Also approved for patients 12 years of age and older with 1 copy of F508del associated with one of the following 4 CFTR mutations with residual function and splice defect: 2789 + 5 G/A, 3272-26A/G, 3849 + 10KbC/T, 711 + 3A/G [45].

Orkambi (lumacaftor/ivacaftor) is instead the first drug capable of intervening on the defect in processing and transport of the CFTR protein in patients with cystic fibrosis aged 12 years or older with double copy of the F508del mutation, the most common genetic alteration. common, present in about 21.1% of Italian patients in homozygosity for the F508del mutation affected by the disease [46].

After having received the marketing authorization from the EMA, it was approved with a centralized procedure by AIFA. In Italy it can be prescribed by authorized Centers in patients aged over or equal to 2 years homozygous F508del, it is reimbursable by the NHS for patients aged over or equal to 12 years homozygous F508del. The F508del mutation affects the CFTR protein in various ways, mainly by causing a defect in cellular processing and trafficking, which reduces the amount of CFTR on the cell surface. The small amount of F508del-CFTR that reaches the cell surface has a low probability of the channel opening (defective channel gating). Lumacaftor is a CFTR corrector, which acts directly on F508del-CFTR to improve cellular processing and trafficking, thus increasing the amount of functional CFTR on the cell surface. Ivacaftor is a CFTR enhancer, which favors the increase of chloride transport by enhancing the probability of opening the channel (or gating) of the CFTR protein on the cell surface. The combined effect of lumacaftor and ivacaftor is an increase in the quantity and function of F508del-CFTR on the cell surface, with a consequent increase in the transport of chloride ions. The exact mechanisms by which lumacaftor improves the processing and cellular trafficking of F508del-CFTR and by which ivacaftor enhances F508del-CFTR are not known [47].

The approval of Orkambi was based on the full results of Phase III clinical trials (TRAFFIC and TRANSPORT), published in the New England Journal of Medicine (NEJM), which analyzed the efficacy and safety of the combination of lumacaftor and ivacaftor.

TRAFFIC and TRANSPORT were two randomized, controlled, double-blind, multicenter trials involving approximately 200 centers located in the United States, Europe and Australia. The trial involved a total of 1108 patients over the age of 12 carrying the double mutation F508del. Based on clinical trials, the efficacy of the drug in terms of recovery of respiratory function is lower than the results obtained by Ivacaftor in class III mutations. In terms of reducing infectious respiratory exacerbations and increasing the body mass index (BMI) it seems that Orkambi reduces these events which negatively affect the long-term prognosis of the disease.

However, its efficacy has not been demonstrated in patients with mutations other than homozygous F508del.

Gene Therapy

Gene therapy, as a treatment modality for lung disorders, has generated significant interest over the past decade. Since the beginning of the first clinical trials for CF associated lung disease, recombinant adenovirus has been used, but research has found numerous obstacles including inflammation and inefficient vector targeting and production [48].

Numerous recently identified serotypes of adeno-associated viruses have shown great potential in animal models which are

likely to be reflected in clinical studies. Furthermore, a greater understanding of vector biology has also led to the development of new technologies to improve the efficiency and selectivity of gene transfer to the lung. Although the promise of gene therapy in lung diseases has not yet been realized, recent efforts in molecular virology aimed at vector development will undoubtedly lead to great benefits in the next decade in the treatment of lung diseases. Gene therapy, by definition, is part of the ATMP (medicinal products for advanced therapies) and uses nucleic acids (DNA or RNA) with a therapeutic function to facilitate the expression of therapeutic proteins, in order to correct a specific underlying monogenic defect which causes pathology. In this it differs from oligonucleotides which, being purely chemically synthesized molecules, are not considered by regulatory bodies as ATMP. Gene therapy approaches can broadly be classified into three mechanically distinct categories:

- Gene substitution
- Gene reprogramming
- Gene repair

The first, at the moment, is the most commonly used approach and also the only clinically tested method for gene therapy; tries to add a copy of a corrected gene to overcome an inherited genetic mutation. The advantages of adding genes for the treatment of hereditary disorders include its relative simplicity, while the disadvantages often include a lack of controlled gene expression. Obviously this therapeutic approach is the most effective as it is aimed at the direct etiology of the disease but it is difficult to apply directly to clinical practice as, for biopharmaceutical reasons, it requires in the meantime parenteral infusional administrations and also methods of cellular delivery of nucleic acids based, in general, on the use of viral or bio-inspired vectors (such as liposomes, lipid nanoparticles and the like) which involve problems of chemical-physical stability in storage and high immunogenic power, with the risk of development of anti-drug antibodies and loss of efficacy or induction of dysimmune pathologies in the patient. For these reasons, to date, this therapy remains of an experimental type and is not directly applicable to the patient, but biotechnological advances probably in the coming years will make this model more applicable *in vivo*.

Expression of CFTR

The challenges that accompany gene therapies for CF lung disease are not limited to the development of efficient and safe vectors for gene transfer, but must also reconcile the complexity of the cell sites in the lung, which express the CFTR protein and the multiple functions that CFTR plays in lung physiology. In fact, the expression of CFTR is highly regulated in the lung; hair cells appear to express very low levels of functional CFTR, for example.

In contrast, submucosal glands and a subpopulation of non-ciliated cells with unknown function express extremely high levels of the CFTR protein and related mRNA. In addition to acting as a chloride channel, it has been shown that CFTR regulates the activity of other epithelial ion channels such as ENaC and ORCC. A defective regulation of the ENaC has been proposed at the basis of the altered transport of fluids through the airways typical of CF, but this notion still remains too controversial.

It is currently unclear whether the highly regulated nature of CFTR expression in various types of airway cells is an important aspect required for normal lung physiology, such as the movement of ions and fluids through the airways and the maintenance of defense. innate pulmonary. If proper regulation of a CFTR transgene is required to restore normal physiology and innate immunity overall. The use of the CFTR promoter in gene transfer vectors or in direct RNA reprogramming may be a potential solution if a regulated expression of CFTR is required to restore a normal phenotype in the airways of CF patients.

Structural mechanisms of the pharmacological therapy of CFTR

The cloning of the CFTR gene has made it possible to develop expression systems for studying the function of CFTR at the molecular level, allowing the identification of fundamental defects associated with pathogenic mutations. In particular, using high-speed drug screening techniques, small molecules involved in the modulation of CFTR function have been identified. These studies are particularly important because mutations associated with loss of CF function cause secretory diarrhea, resulting from CFTR hyperactivity, as a result of the presence of bacterial toxins. Great strides have been made in recent decades in the development of CFTR modulators. Some of the CFTR modulators have been used with success in clinics (eg Ivacftor and lumacaftor), but the availability of high resolution CFTR facilities has ushered in a new era for drug design based on structural studies.

CFTR correctors

Many mutations associated with CFTR disease, including most of the ∂f508 mutation, cause conformational defects, leading to a reduction in functional CFTR proteins expressed on the cell surface. Compounds that increase the mature transport of CFTR to the membrane are known as CFTR correctors. A different binding site for VX-809 has recently been proposed. Using dynamic molecular

simulation and surface plasmon resonance, it has been shown that three residues in NBD1, Y577, V580 and E655, bind VX-809 and interact with each other through hydrogen bonds.

In addition, many experimental VX-809 CFTR correctors have been developed, and it has been shown that combinations of different correctors could have additive or synergistic effects.

This finding is not surprising, given that the ∂f508 mutation causes multiple biochemical defects, including the misfolding of NBD1, where F508 is located, but also the interruption of the interaction between NBD1 and ICL4 which subsequently alters the domain assembly of CFTR. A combination of different correctors with different action mechanisms is therefore required.

CFTR blockers and inhibitors

There is a general principle that hydrophobic blockers are more effective than hydrophilic ones. The structure-activity relationship for internal blockers also provides insights into their interactions with the CFTR protein. For example, extensive studies on hypoglycemic agents other than sulfonylurea, of glibenclamide interact with two different sites in CFTR, respectively. For example, extensive studies of hypoglycemic agents other than sulfonylurea such as mitiglinide and meglitinide suggest that the sulfonylurea and benzamide groups of glibenclamide interact with two different sites in CFTR, respectively [34]. The anchoring simulation also supports the idea that K95 is a critical and common binding site for several internal blockers, including glibenclamide. However, these positively charged amino acids (i.e. K95 and R303) located in a large vestibule are likely well protected by the water molecules, or the negatively charged amino acids (e.g. E92) allow to avoid chloride entrapment in the permeation path. The internal vestibule is hypothesized to influence the local electropositive energy profile and subsequently prevent the movement of anionic blockers in the pore. These positively charged amino acids do not necessarily have to be the binding sites.

Similarly, mutations on TM6 and TM12 are also expected to affect the electrostatic profile of charged amino acids along the permeation pathway and thus affect the movement of internal blockers. Just as blocking the CFTR passage of large organic anions exhibits a high voltage dependence, these blockers are likely to transiently pass through the internal vestibule and subsequently lodge deeper than the pore [37]. Contrary to the internal blockers described above, the external entry of the CFTR pore appears to be quite resistant to blocking by large anions 38, GlyH-101 is the only blocker that appears to occlude the pore from the extracellular side. A possible binding site for GlyH-101 has been proposed at the outer end of the pore, where the GlyH-101 charged head is located near air residues F337 and T338 with its hydrophobic tail, inserted into the pore. SCAM studies have, in fact, shown that both F337 and T338 are involved in the open conformation of CFTR4. On the contrary, in all three cryo-EM structures, the proposed binding site for GlyH-101 consists of the side chains of surrounding amino acids. Therefore, a rearrangement of the TMDs must take place upon opening the gate, so that the binding site for GlyH-101 is exposed to the anion on the permeation pathway.

Interestingly, this scenario also suggests a state-dependent constraint of GlyH-101 and CFR that has yet to be demonstrated. Unlike pore blockers, there are other small molecules that can reduce CFTR currents by interfering with the CFTR gate.

For example, (R) - benzopyrimido-pyrrole-oxazine-dione inhibits CFTR gating by competing with ATP in NBD42. On the other hand, CPTRinh-17243, a thiazolidine and dionic derivative exerts its inhibitory effect on CFTR, which crosses an unknown binding site **. Although site-directed mutagenesis studies identified the conserved residue R347 in TM6, as an important residue for the action of CFTRinh-17245, comparing the effect of CFTRinh-172 on CFIR orthologs that respond differently to CFTR in h-172, Stahl., *et al*. [4] hypothesize that the binding site is more likely located elsewhere in the CFTR.

Kinetic studies provide evidence that CFTRinh-172 can bind to both open and closed states.

In particular, the binding of CFTR in h-172 does not inhibit CFTR, an observation which excludes a direct mechanism of direct pore blocking; instead, a further step (conformational changes) after binding leads to inhibition. Perhaps the most unique feature of CFTR in h-172 is that the dissociation of CFTRinh-172 is dramatically slowed when the two NBDs of CFTR are locked in a dimeric configuration, suggesting that CFTR in h-172 actually binds more tightly in one state. closed, with an NBD48 dimer.

CFTR enhancers

For obvious reasons, enormous efforts have been devoted to finding small molecules capable of enhancing CFTR function, ever since the responsible gene has been identified. A powerful and effective reagent, VX-770 has been discovered through high throughput screening and a series of structural optimizations or first use of VX-770 has been approved to treat a broad spectrum of mutations that cause CFTR gating defects. Although the mechanism of action

for VX-770 on CFTR gating has been characterized, the exact binding site for VX-770 remains unknown. Recent studies exclude the R and NBD2 domains as targets for VX-77053 and suggest that the binding site for VX-770 resides at the interface between membrane lipids and CFTR $ 4 TMDs, based on its hydrophobic property, and enhancement effects, similar by intracellular and extracellular application. X-ray diffraction investigations show that VX-770 can penetrate the lipid bilayer and accumulate mainly in the inner layer5. Although there is still limited information on binding sites for a CFTR enhancer, it is interesting to note that many CFR enhancers possess limited structural similarities. However, the newly developed CFTR enhancers, GLPG1837 and VX-770, despite their structural differences, share the same mechanism of action, probably by binding to the same site in CFTR57, but GLPG1837, compared to VX-770, possesses greater efficacy, but less potency. This latest study also reveals a state-dependent bond of GLPG1837: stronger bond in the open state, compared to the closed one. A thermodynamic analysis of the state-dependent binding of aLPC1837, using a classical allosteric modulation scheme, argues that the potency of a CPTR enhancer is determined by the absolute binding affinity of the drug to the CFR protein, while efficacy is determined by the Alfierence in binding affinities between closed and open states. With the high-resolution structures of CFR's TMDs obtained by cro-EM, the next step will be to use this powerful technique to identify binding sites for these clinically useful drugs. The resulting structural and functional insights can help design better drugs for the treatment of CF. Not all enhancers share the same mechanism of action; there are enhancers whose chemical structures are very different from VX-770 and GLPG1837.

For example, ATP analogs that bind to normal ATP binding sites in NBD have been shown to exhibit greater affinity to CFTR than to ATP, the natural ligand. Genistein is the first identified enhancer that binds directly to the CFTR protein 'Although the exact mechanism of action of genistein is unclear, different approaches have been used to localize the binding sites at the NBD dimer interface. In contrast, Lansdell., *et al*. have proposed that genisteinase also binds within the CFTR pore. NPPB, which also pore blocks CFTR63, is another enhancer, which has a dual effect (i.e., pore blocking and gate modulation) on CFTR. Several studies have demonstrated the effects of NPPB on CPTpc garing, never its mechanisms of action remain debated. Although Csanádye and Tordesik "Shanno suggested that the NPPB modulates the state transition of CPTR, Lin., *et al*. argue that the NPPB facilitates the dimerization of NBD, stabilizing the NBD dimer. Successes in this area of research will lay the groundwork for design of drugs, based on the structure of CFTR enhancers, which exhibit pharmacological synergism for the improvement of gating.

Goals and trials with airway stem cells for gene therapy

The development of gene transfer approaches to target airway stem cells will likely be required to achieve long-lasting therapeutic effects in the lung. Two approaches for gene transfer into airway stem cells can be envisaged. First, gene transfer into airway stem cells in utero has advantages for correcting defects in those regions of the lung not accessible from the lumen of the adult airway (i.e. submucosal glands in CP). Second, gene transfer, which uses integrative vectors for adult airway stem cells, could substantially improve the duration of transgene expression in the adult lung. To date, AAVs, retroviruses and lentiviruses are the most promising vectors because they possess the ability to integrate into the host genome. In part, the difficulties in identifying and characterizing human airway epithelial progenitor and stem cells have been amplified by differences in lung biology between rodent and human airways. However, human bronchial xenograft models have been useful in studying relationships progenitor-progeny in human airways. In the mouse airways, the predominant cell types include Clara cells and hair cells, with a lower abundance of basal cells in the proximal airways. Until recently, the mouse airway stem cell was thought to be a subset of Clara. However, more recent studies have suggested that the mouse proximal airways may also contain a subset of basal-type stem cells that appear to reside in the submucosal gland ducts in the proximal trachea and in the neuroepithelial bodies of the bronchial airways. Contrary to the airways of the mouse, airway epithelial cell types, predominant in human proximal airways, include basal, intermediate, calyx and ciliated cells. However, the more distal airway bronchioles of humans have a cellular composition similar to that of mice with Clara cells and hair cells as the predominant cell types. Analysis of retroviral derivation in adult human bronchial xenografts identified a diversity of progenitor cells with limited or pluripotent capacity for differentiation into superficial airway epithelial phenotypes. Interestingly, one of the progenitor cell populations represented from a phenotype similar to a basal cell it has the differentiation capacity typical of the state of pluripotency, being able to generate epithelial cells both of the superficial airways and of the submucosal glands. The recent identification of retention cells by BraU at submucosal gland duct sites in mouse airways suggests that stem/progenitor cell populations in adult proximal airways may reside in selective niches protected from the external environment. If this is true, then gene transfer to these progenitor cells can be difficult as they are located below the surface of the airways. An alternative strategy to target these populations involves gene transfer in utero before the lung architecture is fully developed. Such strategies, at least in principle, have been tested using tracheal xenografts of the newborn ferret. In these studies, retroviral vectors were able to successfully

target stem/progenitor cells capable of generating epithelial phenotypes of the superficial airways and submucosal glands. Similar approaches have also been tested in sheep using retroviral vectors targeting the lung. However, the feasibility of an in utero pulmonary gene transfer approach for humans is difficult to imagine with current vectors. The first phase I clinical trial for CF was initiated in 1993 using a recombinant CFR-expressing adenovirus administered to the human nasal epithelium of CF patients. In this study, and in numerous other studies, partial transient corrections of chloride transport defects were observed, assessed using measurements of the potential difference in the nasal epithelium. However, subsequent efforts to evaluate adenovirus-mediated recombinant CFTR transfer to the nasal epithelium of CF patients failed to reproduce the functional correction, leaving investigators to speculate that the transfer method in previous studies may have harmed. the epithelium leading to higher levels of translation. It was more difficult to assess functional correction in Phase I studies of adenovirus-mediated transfer of CFTR into the lung airways; However, expression analyzes indicate that the efficiency of gene transfer is low (<1%) and at high doses it is associated with immunological responses to the vector. A recent study by Harvey and colleagues, using repeated localized nebulization of recombinant adenovirus, demonstrated CFTR mRNA expression at levels theoretically sufficient to correct CFTR chloride transport (- 5%) after two administrations. However, complete loss of expression was observed after a third repeat administration due to a humoral immune response. A more recent Phase I clinical study, using aerosol administration of recombinant CFR adenovirus, demonstrated by in situ hybridization that nuclear-localized vector DNA was not readily detected in small epithelial samples. However, high levels of infection have occurred in mononuclear cells and squamous metaplastic epithelial cells. In summary, clinical studies conducted so far with adenoviral vector-mediated recombinant CFTR transfer have demonstrated low levels of translation, possibly because the apical surface of the airway epithelium has no CAR receptors. Due to the inflammatory responses observed in the initial clinical trials based on adenoviruses, less immunogenic vectors such as liposome/DNA complexes were also tested as an alternative [47]. In 1995, the first clinical study with liposomes demonstrated 20% restoration of the pp ransepithelial in response to low chloride at 3 days after administration of cationic liposomes/DNA complexes to the nasal epithelium. Subsequent studies, which mitigated different lipid compositions and different promoters to stimulate CFTR expression, demonstrated comparable results to the initial study in CF patients when administered to the nasal and pulmonary airway epithelium. Despite the apparent reduced toxicity of liposome/DNA complexes compared to adenovirus studies, a more recent study that delivered liposome/DNA aerosol complexes to the lung showed that four out of eight CF patients developed adverse reactions. Fever, myalgia and arthralgia in response to vector administration have been associated with increased IL-6 expression. These adverse effects are believed to be the result of unmethylated pG nucleotides in bacterial plasmid DNA. The consensus regarding the usefulness of liposome/DNA complexes in clinical trials for CF is that the corrective effect is extremely transient. The most recent vector type tested in clinical trials for CF includes rAA V type 2. The first clinical study rAAV expressing CFTR was performed in the maxillary sinuses in 1998. The results of this study in 10 patients demonstrated a dose-response dependent on the accumulation of vector genomes in the sinus epithelium, with little or no immunological consequences. In the follow-up of this study, a second dose escalation study demonstrated partial correction of chloride transport abnormalities by measuring PD in the maxillary sinus with a maximum transfer of 0.1-1 rAAV genome/cell copies [48]. The expression of the CPTR transgene was detected for 41-70 days in 2 out of 10 silent ones. The results of this study demonstrated no output without a higher dose-related immune response (10 13 DNA particles/lung vector genomes were quantified at 0.6 and 1 copy/cell at 14 and 30 days, respectively. and decreased to undetectable levels 90 days after infection. Disappointingly, no vector-derived mRNA could be detected at any of the times and doses tested by RT PCR. These results are reminiscent of *in vitro* studies of pathway epithelia. human airways demonstrating effective uptake of rAAV-2 and prolonged DNA persistence in the absence of gene expression. In addition, as indicated earlier in this review, the rAAV vector tested in these clinical trials used IR as a weak promoter for drive CFTR expression due to the size constraints of the vector system. This probably also contributed to the undetectable expression levels in these clinical trials. With a better understanding of the translation biology of AAV and novel AAV serotypes, very promising prospects for rAAV-mediated gene therapy of lung diseases are expected.

Conclusion

The severity of cystic fibrosis has prompted researchers to investigate its underlying cause since the past. Thanks to the increasing knowledge in the genetic field, it has been possible to identify the gene responsible for CF and studies have been carried out on the protein encoded by this gene, the CFTR chlorine channel. Thus, the structure and function of this channel was gradually delineated, allowing the identification of possible targets and therapeutic strategies. New cryo-EM structures have been determined, molecular dynamics models and simulations have been built, as well as domain-level studies exploring channel gating regulation, drug

interactions, and mutations that cause CF. However, many doubts remain to be clarified and the field requires further significant insights, including mechanisms of modulation of channel activity, for the development of effective therapeutic strategies.

Considering the 3D structures and their respective dynamic behaviors, significant results were nevertheless achieved for the identification of potential targets that can facilitate the design and optimization.

drugs. Broadly speaking, gene therapy may offer an opportunity for CF treatment by repairing the defective CFTR gene. The treatments that exist today, or those under development, can only slow down the progression of the disease, and show variable effects, depending on the type of mutation present in the patient: gene therapy, on the other hand, has the potential advantage of being able to be used, in the presence of any defect in the FTR gene, representing a virtual option of universal treatment.

The idea behind gene therapy is to provide a functional copy of the gene to cells, and program them to synthesize perfectly functional proteins. There are numerous approaches to this type of therapy, but the most common strategy is to insert the therapeutic gene into a virus, known as a vector, for its transport into cells. Viruses have evolved over millions of years to escape the immune system, attack cells, reprogram them and replicate their own genome, therefore, a virus is an optimal candidate, for the transport and integration of exogenous genetic material into the cellular genome. For gene therapy, the virus is modified to be harmless and to insert the desired gene into the viral genome. It must be said that several obstacles still need to be overcome in order to obtain a better efficiency of gene transfer: among these, the host immune response, the mucus barrier, the point of attachment to the apical surface of the respiratory cells and the transport of the vector and/or or exogenous DNA within the cell to reach the nucleus, where genes are transcribed. The host's immune response is directly proportional to the dose of the viral vectors and also limits the possibility of subsequent administration. Furthermore, the antibody response to viral proteins can neutralize the vector before it infects the cells, while the T lymphocyte response tends to remove the transfected cells. In recent times, the use of a virus, associated adenovirus (AAV), a single-stranded DNA parvovirus, naturally unable to replicate and not responsible for any disease in humans, but which integrates with chromosomal DNA, has found more success. Some recent studies have shown good efficiency and even one certain safety in the use of AAV, as repeated doses were well tolerated by the patients.

Non-viral vectors are represented by liposomes that do not induce an immune response, but have a poor efficacy due to the difficulty of penetration into the nucleus. Ultimately, the prospects seem promising, and are linked to obtaining greater knowledge on the structure and function of CFTR, on the physiological and pathological mechanisms of regulation related to the CF mutant genes, however, experiments have been attempted to overcome these obstacles. such as coating the carrier with polyethylene glycol or other means. In addition, the difficulty in reaching the target cell must be considered. First of all, it is necessary to overcome the thick mucus that can block and harness the vector before it reaches the cell surface; to cope with this, alpha-dornase can be used. Recent studies have shown that the virus delivery technique inside the liposome has proved to be very efficient for the release of viruses in the lungs. The treatment was administered through a nebulizer and resulted in a 3.7% improvement in lung function, compared to placebo. The modest improvement is seen as a very encouraging sign. An ongoing study at University College Cork is looking into whether the defective CFTR gene can be repaired, rather than replaced as in conventional gene therapy. In conventional gene therapy, an additional copy of the entire gene is transferred to the cells. However, the malfunctioning gene is still present and therefore the 'correction' from gene therapy is inherently partial. The CFTR gene repair strategy eliminates a portion of the gene containing the six most common mutations, which lead to cystic fibrosis (accounting for approximately 80% of all cases), and replaces the entire region with a normal sequence. Recently, some studies have been published that have shown the possibility of hematopoietic or stromal stem cells to migrate into the lungs of recipient animals and become respiratory epithelium.

However, some promising results have already been obtained *in vitro* with cells from the bone marrow of CF patients. Gene editing is a technique closely related to gene therapy. While in gene therapy a new functional gene is transferred into cells to replace a defective gene, gene editing works by repairing the defective gene at the DNA level. Operating at a different level, CFTR gene repair, i.e. gene editing, aims to "rewrite" the mutated sequence directly within the original gene, similar to editing a misspelled sentence. Gene editing technology is still in its infancy, although studies in other diseases such as cancer and HIV have yielded promising results, and will soon be tested in clinical trials. The technique could even more develop with the advent of many promising technologies and improve the prospects for gene therapy for inherited and acquired lung diseases. Many of these advances have resulted from a comprehensive analysis of vector transduction mechanisms and host-vector cell interactions. Since currently viral vectors are clearly the

most efficient gene transfer systems, aspects relating to the immunology of these vectors in the host will continue to be at the forefront of safety precautions. Equally important to the gene transfer vehicle is a concrete understanding of the pathophysiology of the disease and the cell biology of the target organ. The identification of relevant pathophysiological cellular targets and the understanding of the function of the target gene remain fundamental for the development of appropriate strategies for gene therapy and the prediction of the efficacy of such approaches. Cystic fibrosis is an excellent example where an interaction between gene therapy and cell biology research has improved understanding of pathological processes in relation to the function of CFTR. The development of appropriate animal models for human disease is also crucial for the further development of gene therapy applications in the clinic. In the case of CF, the lack of animal models that mirror the pathogenesis of human lung disease will continue to hamper the field of gene therapy for this.

CFTR knockout mice, while extremely useful for studying CFR function in the gut, have been disappointing when addressing aspects of lung disease. With the advent of new transgenic technologies based on nuclear cloning of somatic cells, it is possible to envisage the use of larger animal models for those genetic diseases that cannot be modeled in mice. From the point of view of safety and efficacy, the current most promising gene transfer systems to the lung for inherited genetic diseases are rAAV vectors. Novel approaches for purification of large amounts of vectors have also made widespread clinical applications more viable. With the help of the appropriate animal model for a given disease and efficient vectors for gene transfer, the field of gene therapy is likely to see great progress in the next decade.

Conflict of Interest Statement

The author had no conflicts of interest to declare. For the purposes of compliance with the provisions of art. 6-bis of Law no. 241/1990 and of the art. 7 of the Code of Conduct for public employees, issued with Presidential Decree no. 62/2013; - aware of the penal sanctions resulting from untruthful declarations and/or falsehoods in acts; A.M. DECLARE: not to find themselves in situations of incompatibility or in conditions of conflict of interest also potential.

Funding Sources

A.M. did not receive any funding for this manuscript.

Author Contributions

We worked in an integrated way on the development of the article, both contributing to the drafting of all the paragraphs and to the complete bibliography and website research. A.M. had the idea of writing the article. Then, she started writing the article, which among other things was the subject of her bachelor's thesis in biological sciences, and she started researching the sources and developing the theory. A.M. verified the methods, investigating the specific aspect and A. supervised the results of this work. All authors discussed the results and contributed to the final manuscript.

Bibliography

1. Accurso FJ., *et al*. "Effect of VX-770 in persons with cystic fibrosis and the G551D-CFTR mutation". *The New England Journal of Medicine* 363 (2010): 1991-2003.

2. Akabas MH., *et al*. "Acetylcholine receptor channel structure probed in cysteine-substitution mutants". *Science* (1992).

3. Al-Awqati Q. "Alternative treatment for secretory diarrhea revealed in a new class of CFTR inhibitors". *Journal of Clinical Investigation* 110 (2002): 1599-1601.

4. Aleksandrov AA., *et al*. "Nucleoside triphosphate pentose ring impact on CFTR gating and hydrolysis". *FEBS Letter* 518 (2002a): 183-188.

5. Aleksandrov L., *et al*. "The First Nucleotide Binding Domain of Cystic Fibrosis Transmembrane Conductance Regulator Is a Site of Stable Nucleotide Interaction, whereas the Second Is a Site of Rapid Turnover". *Journal of Biological Chemistry* 277 (2002b): 15419-15425.

6. Alexander C., *et al*. "Cystic fibrosis transmembrane conductance regulator: using differential reactivity toward channel-permeant and channel- impermeant thiol-reactive probes to test a molecular model for the pore". *Biochemistry* 48 (2009): 10078-10088.

7. Aller SG., *et al*. "Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding". *Science* 323 (2009): 1718-1722.

8. Amaral MD and K Kunzelmann. "Molecular targeting of CFTR as a therapeutic approach to cystic fibrosis". *Trends in Pharmacological Sciences* 28 (2007): 334-341.

9. Anderson MP and MJ Welsh. "Regulation by ATP and ADP of CFTR chloride channels that contain mutant nucleotide-binding domains". *Science* 257 (1992): 1701-1704.

10. Anderson MP., *et al*. "Nucleoside triphosphates are required to open the CFTR chloride channel". *Cell* 67 (1991): 775-784.

11. Angelow S and AS Yu. "Structure-function studies of claudin extracellular do mains by cysteine-scanning mutagenesis". *Journal of Biological Chemistry* 284 (2009): 29205-29217.

12. Astiley L Cooney., *et al*. "Cystie Fibrosis Gene Therapy: Looking Back, Looking Forward". *Genes (Basel)* (2018).

13. AtWell S., *et al*. "Structures of a minimal human CFTR first nucleotide-binding domain as a monomer, head-totail homodimer, and pathogenic mutant". *Protein Engineering Design and Selection* 23 (2010): 375-384.

14. Aubin CN and P Linsdell. "Positive charges at the intracellular mouth of the pore regulate anion conduction in the FIR chloride channel". *The Journal of General Physiology* 128 (2006): 535-545.

15. Bai Y., *et al*. "Dual roles of the sixth transmembrane segment of the CFTR chloride channel in gating and permeation". *The Journal of General Physiology* 136 (2010): 293-309.

16. Bai Y., *et al*. "Structural basis for the channel function of a degraded ABC transporter, CFIR (ABCCT)". *The Journal of General Physiology* 138 (2011): 495-507.

17. Baker JM., *et al*. "CFTR regulatory region interacts with NBD1predominantly via multiple transient helices". *Nature Structural and Molecular Biology* 14 (2007): 738-745.

18. Baroni D., *et al*. "Direct interaction of a CFTR potentiator and a CFR corrector with phospholipid bilayers". *European Biophysics Journal* 43 (2014): 341-346.

19. Barreto-Ojeda E., *et al*. "Coarse grained molecular dynamics simulations reveal lipid access pathways in P-glycoprotein". *The Journal of General Physiology* (2018).

20. Barrett KE and SJ Keely. "Chloride secretion by the intestinal epithelium: molecular basis and regulatory aspects". *Annual Review of Physiology* 62 (2000): 535-572.

21. Basso C., *et al*. "Prolonged nonhydrolytic interaction of nucleotide with CFTR's NH2-terminal nucleotide binding domain and its role in channel gating". *The Journal of General Physiology* 122 (2003): 333-348.

22. Beck EJ., *et al*. "Conformational changes in a pore-lining helix coupled to cystic fibrosis transmembrane conductance regulator channel gating". *Journal of Biological Chemistry* 283 (2008): 4957-4966.

23. Bozoky Z., *et al*. "Regulatory R region of the CFTR chloride channel is a dynamic integrator of phospho- dependent intra- and intermolecular interactions". *Proceedings of the National Academy of Sciences of the United States of America* 110 (2013): E4427-E4436.

24. Bridges RJ., *et al*. "Capitalizing on corrector mechanistic differences to achieve synergy in F508del-CFTR expression". *Pediatric Pulmonology* 45 (2010).

25. Cai Z., *et al*. "Inhibition of heterologously expressed cystic fibrosi transmembrane conductance regulator Cl channels by non- sulphonylurea hypoglycaemic agents". *British Journal of Pharmacology* 128 (1999): 108-118.

26. Cai Z., *et al*. "Differential sensitivity of the cystic fibrosis (CF)-associated mutants G551D and G1349D to potentiators of the cystic fibrosis transmembrane conductance regulator (CFTR) C1- channel". *Journal of Biological Chemistry* 281 (2006): 1970-1977.

27. Cai ZW., *et al*. "Targeting F508del-CFTR to develop rational new therapies for cystic fibrosis". *Acta Pharmacologica Sinica* 32 (2011): 693-701.

28. Cai., *et al*. "Impact of the F508del mutation on ovine CFTR, a CI- channel with enhanced conductance and ATP. dependent gating". *Journal of Physiology* 593 (2015): 2427-2446.

29. Chaves LA and DiC Gadsby. "Cysteine accessibility probes liming and Putent of NBD separation along the dimer interface in galing CPTR channels". *Genetic Physiology* 145 (2015): 261-283.

30. Chen TY and TC Hwang. "CLC-0 and CPTR: chloride channels evolved from transporters". *Physiology Review* 88 (2008): 351-387.

31. Chen J., *et al*. "A tweezers-like motion of the ATP-binding cassette dimer in an ABC transport cycle". *Molecular Cell* 12 (2003): 651-661.

32. Chin S., *et al*. "Attenuation of Phosphorylation-dependent Activation of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) by Disease-causing Mutations at the Transmission Interface". *Journal of Biological Chemistry* 292 (2017): 1988-1999.

33. Corradi V., *et al*. "Cystic Fibrosis Transmembrane Conductance Regulator (CFTR): CLO SED AND OPEN STA TE CHA NNEL MOD ELS". *Journal of Biological Chemistry* 290 (2015): 22891-22906.

34. Cotten JF and MJ Welsh. "Covalent modification of the nucleotide binding domains of cystic fibrosis transmembrane conductance regulator". *Journal of Biological Chemistry* 273 (1998): 31873-31879.

35. Cotten JF and MJ Welsh. "Cystic fibrosis-associated mutations at arginine 347 alter the pore architecture of CFTR. Evidence for disruption of a salt bridge". *Journal of Biological Chemistry* 274 (1999): 5429-5435.

36. Csanády L and B Töröcsik. "Catalyst-like modulation of transition states for CFTR channel opening and closing: New stimulation strategy exploits nonequilibrium gating". *The Journal of General Physiology* 143 (2014a): 269-287.

37. Coanady L., *et al*. "Preferential phosphorylation of R-domain serine 768 dampens activation of CPTR channels by PKA". *The Journal of General Physiology* 125 (2005): 171.

38. Csanády L., *et al*. "Strict coupling between CFTR's catalytic cycle and gating of its CI- ion pore revealed by distributions of open channel burst durations". *Proceedings of the National Academy of Sciences of the United States of America* 107 (2010): 1241-1246.

39. Cystic Fibrosis Trust, Gene Therapy and Gene Editing Cui G., *et al*. "Mutations at arginine 352 alter the pore architecture of CFTR". *The Journal of Membrane Biology* 222 (2008): 91-106.

40. Cui G., *et al*. "Differential contribution of TM6 and TM12 to the pore of CFTR identified by three sulfonylurea- based blockers". *Pflugers Arch* 463 (2012): 405-418.

41. Cui G., *et al*. "Three charged amino acids in extracellular loop 1 are involved in maintaining the outer pore architecture of CFTR". *The Journal of General Physiology* 144 (2014): 159-179.

42. Cui L., *et al*. "Domain interdependence the biosynthetic assembly of CFTR". *Journal of Molecular Biology* 365 (2007): 981-994.

43. Dalton J., *et al*. "New model of cystic fibrosis transmembrane conductance regulator proposes active channel-like conformation" (2012).

44. Das J., *et al*. "Transmembrane helical interactions in the CFTR channel pore" (2017).

45. Doyle DA., *et al*. "The structure of the potassium molecular basis of K+ conduction and selectivity". *Science* 280 (1998): 69-77.

46. Du K and GL Lukacs. "Cooperative assembly and misfolding of CFTR domains in vivo". *Molecular Biology of the Cell* 20 (2009): 1903-1915.

47. Du K., *et al*. "The DeltaF508 cystic fibrosis mutation impairs domain-domain interactions and arrests post-translational folding of CFTR". *Nature Structural and Molecular Biology* (2005).

48. Dyson HJ and PE Wright. "Intrinsically unstructured proteins and their functions". *Nature Reviews Molecular Cell Biology* 6 (2005): 197-208.