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MULTI-AUTHOR REVIEW

Cystic fibrosis: a clinical view

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Abstract Cystic fibrosis (CF), a monogenic disease caused by mutations in the CFTR gene on chromosome 7, is complex and greatly variable in clinical expression. Airways, pancreas, male genital system, intestine, liver, bone, and kidney are involved. The lack of CFTR or its impaired function causes fat malabsorption and chronic pulmonary infections leading to bronchiectasis and progressive lung damage. Previously considered lethal in infancy and childhood, CF has now attained median survivals of 50 years of age, mainly thanks to the early diagnosis through neonatal screening, recognition of mild forms, and an aggressive therapeutic attitude. Classical treatment includes pancreatic enzyme replacement, respiratory physiotherapy, mucolitics, and aggressive antibiotic therapy. A significant proportion of patients with severe symptoms still requires lung or, less frequently, liver transplantation. The great number of mutations and their diverse effects on the CFTR protein account only partially for CF clinical variability, and modifier genes have a role in modulating the clinical expression of the disease. Despite the increasing understanding of CFTR functioning, several aspects of CF need still to be clarified, e.g., the worse outcome in females, the risk of malignancies, the pathophysiology, and best treatment of comorbidities, such as CF-related diabetes or CF-related bone disorder. Research is focusing on new drugs restoring CFTR function, some already available and with good clinical impact, others showing promising preliminary results that need to be confirmed in phase III clinical trials.

Keywords Cystic fibrosis · CFTR · Genotype · Phenotype · Precision medicine

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Introduction

The history of cystic fibrosis (CF), the severest autosomal recessive disease in caucasians, can be considered a paradigm of the successful outcomes achievable by collaborative international efforts in the basic and clinical research. Since its recognition as a specific nosographic entity [1], at a time, when it was almost always considered lethal in the early childhood, the clinical management of CF has slowly but constantly improved and patients median predicted survival has increased over the decades until reaching in some areas the age of 50 years [2, 3]. In several countries, the majority of patients are represented by adults and this preponderance is expected to amplify in the next years [4]. Concurrently, CF has been increasingly emerging as a disease more complex than previously thought and the much pursued and welcomed improvement in the disease control has implied downsides of significant clinical relevance, such as the increased prevalence of malignancies and renal and bone metabolism complications [5].

The protein, whose deficiency is responsible for the disease, named CF transmembrane regulator (CFTR), is expressed in several organs, but its full tissue-specific role still needs clarification. In epithelial cell CFTR, an ABC protein [6] exhibits the properties of a chloride and anionic channel involved in a variety of physiological processes and is now seen as a hub modulating several functions [7].

In lower and upper airways, the intestine, pancreatic, and liver ducts, lack of functional CFTR is the major factor in determining the degree of disease expression, and eventually mortality. The protein plays a direct role in organs, such as the hypothalamus, kidney, and bone, where its malfunction may be implied in linear growth retardation [8], delayed pubertal onset [9], bone density modulation [10], and susceptibility to renal calculi [11], all of which may be present in CF and for many years have been considered only secondary to pulmonary or intestinal disease. Besides, CFTR is involved in several physiological mechanisms concerning natural immunity or immune response, which in turn play a role in the development of lung disease. As patients reach older ages, relatively high malignancy rates have led to hypothesize that impaired CFTR activity might also increase the risk of colonic cancer and leukemia [12, 13]. Finally, the malfunction of CFTR is not only causative of CF but seems also involved in very different disorders, such as secretory diarrhea and adult polycystic kidney disease, conditions, where the activity of the protein is higher than normal [14].

After reviewing the various clinical aspects of CF, this chapter will focus on the therapeutic approaches that may potentially impact or have shown effects on the clinical course of the disease.

From the gene to the disease

The identification of the CFTR gene in 1989 has opened a new era in the understanding of CF [15–17]. Since then, over 2000 mutations have been identified [18] and various structural defects in the protein elucidated. The steps forwarded in the comprehension of molecular mechanisms are leading to the development of compounds aimed at modifying the clinical course of CF and thus impacting even more substantially on long-term outcome. Moreover, we have learned that the forms of disease connected with CFTR are widely heterogeneous in severity, rate of progression, and body district involvement and that, among the organ specific manifestations of CF, lung disease is possibly the most variable in its expression. The large number of CFTR sequence variations, and, therefore, of genotypes, is the major but by no means the only cause of such clinical heterogeneity.

CFTR mutations are currently grouped into six categories, based on their mechanisms of dysfunction and effects on the protein (Table 1) [19–21]. Despite a few difficulties in fitting some mutations into this classification, these classes have proved useful in functional studies and to test new treatments supposed to act on specific protein defects. Their utility is more limited in interpreting the clinical liability of specific mutations [18, 22]. Large cohorts of patients carrying mutations which allow some residual protein activity have been shown to benefit from milder disease, while patients with class 1–3 mutations on both alleles tend to have a more rapid deterioration of respiratory function and more severe lung disease. However, there is considerable phenotypic overlap among classes and it is not possible to predict individual outcome based on CFTR genotype (Fig. 1).

Table 1 Functional classification of *CFTR* mutations

Mutation class	Mechanism of dysfunction	Representative mutations	Notes
1	Premature termination codon in mRNA → formation of a truncated, unstable protein that is rapidly degraded → no functional protein in the apical cell membrane	G542X R553X W1282X	Usually associated to more severe phenotypes
2	Synthesis of a protein that is not properly processed to a mature glycosylated form → only a small quantity of partially functioning protein is transported to the apical membrane	F508del	F508del is the most common mutation worldwide
3	A normal amount of CFTR protein that is correctly folded and trafficked to the apical membrane, but the channel opening time is greatly reduced	G551D	These so-called gating mutations have been the first targeted by a specific drug, Ivacaftor, currently used in the treatment of patients
4	Reduced conductivity of the channel	R117H R334W R347P	Usually connected with pancreatic sufficiency and milder phenotypes
5	Partially aberrant splicing or inefficient trafficking → reduced synthesis of fully active CFTR	3849–10kbC>T A455E	Usually connected with pancreatic sufficiency and milder phenotypes
6	Instability of an otherwise fully processed and functional protein	Q1412X 4326delTC 4279insA	Usually nonsense or frameshift mutations Generally associated with a severe clinical presentation

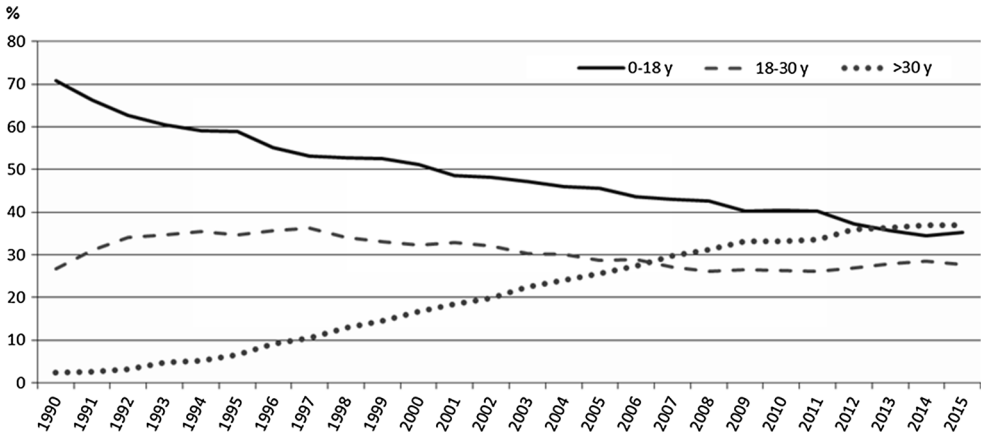

Fig. 1 Time-related distribution of age groups of CF patients in a large clinical Centre. Data refer to the Verona CF Centre, Italy. *y*-axis, percent of patients; *x*-axis, year

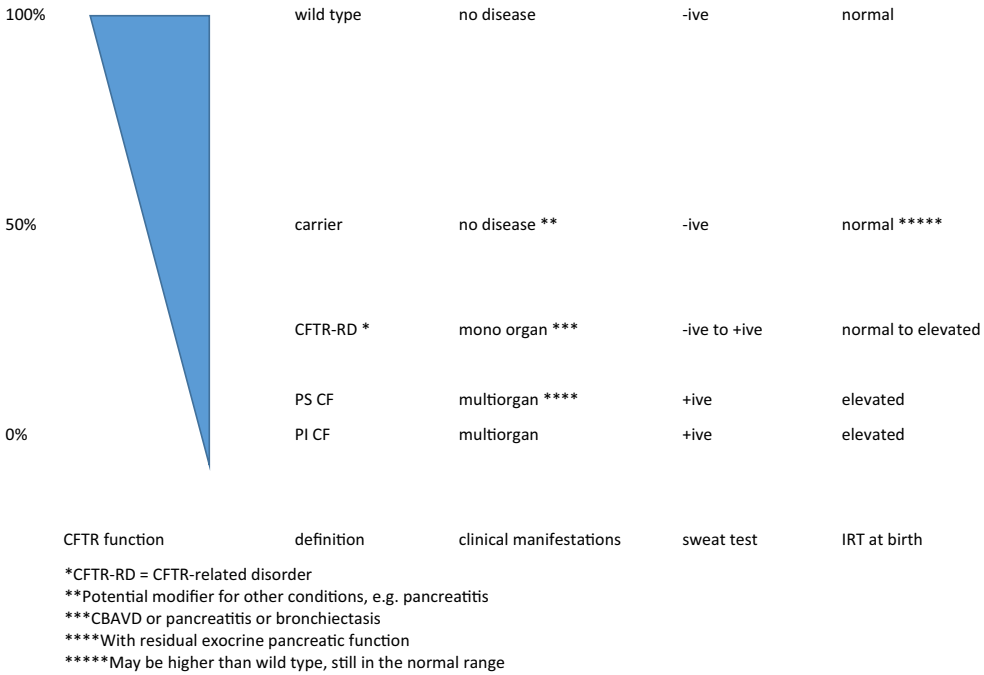

Fig. 2 Correlation among CFTR function, clinical manifestations, sweat test, and IRT at birth

The CF clinical spectrum

The correlation between genotype and expression of disease is influenced by various factors that make phenotype variability extend along a wide spectrum (Fig. 2). The classical clinical picture of CF is mainly characterized by fat maldigestion due to pancreatic insufficiency and chronic obstructive airway disease with bacterial colonization predominantly by microorganisms, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This was already known when Dorothy Andersen reported the first series of patients, whom she subdivided into three groups: patients with congenital intestinal obstruction, i.e., meconium ileus; patients with onset respiratory symptoms in the first months of life; and patients who developed chronic cough after 6 months of age [23].

At the other end, the spectrum is CFTR-related disorder. These are conditions determined by mutations in the CFTR gene but not giving rise to the usual CF picture. They have been defined "clinical entities associated with CFTR dysfunction that do not fulfil diagnostic criteria for CF". Clinical manifestations are limited to a single district and include episodes of recurrent pancreatitis or isolated bilateral bronchiectasis. Males can manifest bilateral agenesia of the vas deferens (CBAVD) with no digestive or respiratory involvement [24].

The wide variability of clinical expression, particularly in lung disease, suggests that non-CFTR factors play an important role in the development of individual clinical histories [25–27]. This is not unexpected given the complexity of the mechanisms involved in the natural defense of the airways, which include resident macrophages,

epithelial lining fluid pH, mucins and antibacterial proteins, and a network of cytokines regulating the inflammatory response to infectious agents. Studies have investigated the role of specific candidate proteins, and a number of modifier genes which can influence the course of the respiratory disease have been identified [28, 29], such as mannose binding lectin [30], interleukin-8 [31], and pentraxin [32]. Conclusions have not always been consistent, probably influenced by different methodological approaches and by selection bias, such as the age of the population under study and the number of patients examined. Genome-wide association [33] and twin and siblings studies [34] are moving this research field forward, but further analyses are needed before reaching more definite conclusion.

Inflammation and the immune system

The CF airway inflammatory response is characterized by neutrophilic infiltration, excessive pro-inflammatory cytokine production, and presence of free neutrophil elastase, and has been reported even in the absence of bacterial infection, suggesting that it may be at least partially unrelated to bacterial infection [35, 36]. The increased inflammatory response, inefficient bacterial phagocytosis, and unbalanced oxidative stress in the CF respiratory tract have been extensively studied, and different mechanisms have been suggested to explain the link between CFTR malfunction and these events. Several studies have focused on the possible role of CFTR in bacterial adhesion and local inflammatory responses in bronchial epithelial cells. The role of the innate immune response and of macrophages and neutrophils in the lung has also been considered [37–39]. Increased inflammation is not only a major cause of the progression of respiratory morbidity, but also a significant determinant of CF intestinal disease [40].

Diagnosis

Diagnoses of CF are usually straightforward, but occasionally, they may prove difficult to make. This has led to the implementation of guidelines for diagnosis [41, 42] and to the development of assays, testing CFTR function in vivo [43] and ex vivo [44]. Notwithstanding such diagnostic aids, a few diagnoses remain problematic and controversial.

Prenatal and early diagnosis

Prenatal and preimplantation genetic diagnosis is possible whenever parents are known heterozygotes and their mutations have been detected. During pregnancy, increased echogenicity of the fetal intestine is occasionally detected in the course of routine ultrasound examination, but hyperechogenic bowel is neither sensitive nor specific, as it is detected only in a minority of CF affected fetuses and it very often have causes other than CF [45]. At birth meconium ileus, a neonatal emergency strongly suggests the diagnosis of CF. CF neonatal screening (NBS) programs are based on blood trypsinogen (IRT) measurement in the first days of life followed in infants with raised IRT by various combinations of genetic analysis, measurement of the pancreatitis associated protein, and IRT retesting by 1 month of age. CF NBS, when properly designed and managed, has high sensitivity and specificity, and has been proved to be cost effective and to ameliorate prognosis [46].

Diagnosis in pediatric age

Fully expressed CF can be easily suspected on clinical grounds, since it is one of the few causes of pancreatic insufficiency, bronchiectasis, and extra renal loss of sodium in childhood. Evocative manifestations include chronic productive cough, typical CF pathogens in bronchial secretions, oily stools, wasting, stunting, and pseudo-Bartter syndrome. The involvement of the sweat gland has been recognized in the 1950s when some affected children developed an extrarenal salt loosing syndrome. NaCl loss through the sweat gland is a hallmark of cystic fibrosis and has led to the development of the sweat test that quantifies sweat chloride content under standardized conditions and is the gold standard to diagnose CF [41, 47].

Diagnosis in adolescence and adulthood

A diagnosis of CF can also be formulated in adolescents and adults, occasionally, because a classic clinical picture had not been previously correctly interpreted, more frequently, because of milder or incomplete phenotype. The latter may lack signs of maldigestion and malnutrition, prevented by residual exocrine pancreatic function. Sweat chloride concentrations in the borderline range and mutations not unquestionably associated with CF are not uncommon in these situations.

The respiratory disease

Chronic pulmonary infection leading to respiratory failure is the main cause of death and the main determinant of the burden of the disease on quality of life.

The lung

The detection of inflammatory markers from bronchoalveolar lavage [48, 49] and the evidence from High

Resolution Chest Tomography of structural lung damage in asymptomatic infants [50, 51] attests that the lung is affected very early. Structural damage is in turn probably responsible for lung ventilation inhomogeneity found by washout techniques in CF infants [52].

Different hypotheses have been suggested to illustrate the pathogenesis of lung disease in CF. According to the most accredited theory, the lack of CFTR or its impaired function results in lower water content in the periciliary fluid and thus in abnormally dense mucous. Besides, CFTR downregulates the sodium channel (ENac) on the apical side of bronchial epithelial cells and the consequent hyperreabsorption of sodium reduces the hydration and increases the density of bronchial mucus. The hyperviscose secretions sequent to this complex process end up hindering ciliary activity and their clearance mechanism [53]. HCO3 transmembrane traffic is probably involved too. CFTR is an HCO_3 channel, and in the knockout, pig-deficient bicarbonate excretion has been connected to reduce bacterial killing capacity [54].

Lack of CFTR function in the bronchial epithelia is also involved in increased inflammatory response and reduced activity of natural defense mechanisms that ultimately result in facilitating the infection and chronic bacterial colonization of the lung [35]. Microorganisms typically involved in pulmonary chronic infection are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, and *Burkholderia cepacia*. More adequate collections of sputum, samples from bronchoalveolar lavage, increased sensitivity of cultures, and the use of non cultural methods are showing that the microbiome of CF lung is far more complex and that other bacteria, including anaerobes, might affect the progression of pulmonary disease [55]. Furthermore, bacteria are not the only microbiological agents that have to do with the development of lung disease. Viral infection may trigger pulmonary exacerbations and CF patients are more prone to fungal infections, particularly to *Aspergillus fumigatus* and *Scedosporium Apiospermium*. An increased rate of allergic reactions to *Aspergillus* (Allergic Bronchopulmonary Aspergillosis) is the characteristic of CF.

Segregation of infected patients, frequent sputum sampling, and aggressive protocols of eradication have succeeded in reducing or delaying chronic bacterial colonization of the lower airways. Aggressive antibiotic treatment of pulmonary exacerbations is the standard of care in CF. Unfortunately, in spite of a very proactive therapeutic conduct, a relatively large proportion of patients still become chronically infected and suffer from frequent pulmonary exacerbations, which greatly contribute to the decay of lung function, poor quality of life and may eventually end up in transplantation or end-stage disease [56–58].

Upper airways

Upper airways are almost invariably affected by CF, with patients exhibiting a variety of conditions, such as sinusitis, nasal polyposis, and mucocele, and often requiring nose or sinusal surgery. The relationship between upper and lower airway colonizations by *Pseudomona aeruginosa* as not yet been completely understood, and it has been suggested that upper airway disease sustains lower infection [59, 60]. Aggressive treatment of nasal or paranasal infection/colonization by bacteria, such as *Pseudomonas aeruginosa* or *Staphylococcus aureus*, is offered by many CF clinics [61].

The gastrointestinal disease

CFTR is expressed along the GI tract and pancreatic and intestinal involvement due to loss of CFTR function begins as early as fetal life [62].

The pancreas

CFTR is expressed in ductal epithelial cells. Lack of CFTR function results in reduced water content of the pancreatic secretions and decreased pH. Increased viscosity of the luminal content and the presence of pancreatic enzymes cause obstruction and progressive destruction of the acini, inflammation, formation of cysts, and fibrosis. Hence, the complete name of the disease: cystic fibrosis of the pancreas.

Circulating trypsinogen is elevated in the CF neonate independently of the degree of pancreatic exocrine involvement, which makes IRT measurement in dry blood spots the mainstay of neonatal screening. Pancreatic exocrine insufficiency severe enough to cause symptomatic fat malabsorption is detectable at birth in 60–80 % of affected infants and causes malnutrition and poor growth [63]. The measurement of fecal pancreatic enzymes is used for diagnosing exocrine pancreatic insufficiency, usually measuring fecal elastase-1 [64]. Ductal plugging in patients with partially preserved pancreatic function can cause recurrent episodes of pancreatitis and, in the long term, the total loss of enzyme secretion. Endocrine pancreatic function is usually preserved in infancy, but in older ages, parenchymal progressive destruction leads to the so-called CF-related diabetes.

The intestine

CFTR-mediated bicarbonate secretion is essential to buffer gastric acidity and to allow expansion and hydration of the intestine mucus. Meconium ileus affects approximately 20 % of CF neonates and is more frequently associated with severe mutations. Its occurrence is influenced by modifier genes [29, 65]. Older patients may exhibit

constipation or obstipation and may develop a subocclusive or fully occlusive manifestation called distal intestinal obstructive syndrome. Signs of inflammation have been observed in intestinal biopsies [66] as well as altered microbiome composition [67] and are consistent with experimental findings in the knockout mouse [68].

Bowel cancer

CFTR deficiency has been associated with raised oncological risks. A prospective 20 years study on more than 40,000 patients in the US Patient Registry resulted in a diagnosis of bowel cancer in 31 cases, a significantly raised frequency, either in the colon (26 observed vs 4.2 expected) or small bowel (five observed vs 0.4 expected). The frequency was not age-related and higher in males and in patients with mutations associated with pancreatic insufficiency. Increased cancer rates were also reported in the biliary tract, in the esophagus, and in the stomach [12]. In a single-center study, colonoscopic screening in CF patients (mean age 47 years) identified a high incidence of adenomatous polyps, again higher males. The authors concluded that this evidence warrants earlier colon screening in the CF adult population [13]. After transplantation, pharmacological immunosuppression increases cancer risk.

The liver

CFTR is expressed in epithelial cells of the biliary duct and regulates bile acid independent bile flow. Ispissated bile may cause obstructive liver disease progressing to multilobar biliary cirrhosis and portal hypertension. These complications occur in a minority of patients, but are not rare. The actual frequency of liver disease manifestations is difficult to determine, because designs and populations of studies included different definitions, such as neonatal cholestasis, abnormal aminotransferases, fibrosis, steatosis, focal biliary cirrhosis, and multilobular cirrhosis, with or without portal hypertension. The prevalence of severe liver disease peaks in adolescence and about 5 % of patients may require liver transplantation [69]. Progression of liver disease is influenced by the genetic background [70–72], and a strong association has been found with the Z-allele of the a1-antiprotease (SERPINA1) gene [72].

Endocrine comorbidities

CF-related diabetes

Diabetes is the most common endocrine complication of CF and is due to progressive pancreatic fibrosis gradually damaging the insulae. CF-related diabetes has distinctive peculiarities that make it different from type 1 and type 2: it originates from reduced secretion of insulin, but is also partially due to insulin resistance, particularly during acute pulmonary exacerbations.

Prevalence begins to rise after the age of 10 and reaches 40–50 % in older patients. Its insurgence is associated with worsening of the respiratory disease, and conversely, good control of hyperglycemia reduces the number of respiratory exacerbation and slows down pulmonary disease progression. CF-related diabetes is associated with increased mortality. Conflicting data exist on the role of gender and on the supposedly worse severity and higher mortality in CF diabetic female patients. Annual screening with oral glucose tolerance tests is recommended, since the age of 10 to identify diabetes or prediabetic conditions. Insulin treatment is recommended. Many patients with normal or borderline glycemic profiles develop diabetes after lung transplantation [73].

Bone disease

CF-related bone disease has been emerging in parallel with the progressive increase of survival. Between 10 and 15 % of patients, rising to 50 % in the late stage disease, show low bone mineral density at dual-energy X-ray absorptiometry (DEXA) and are at risk of osteopenia, osteoporosis, and vertebral fractures [74]. The risk of bone disease is related to malnutrition, low BMI, severity of lung disease, and a variety of other factors, such as poor mobility, reduced absorption of vitamin D, low levels of vitamin K, use of steroids, circulating inflammatory cytokines, and increased bone turnover. CFTR is expressed in bone cells, and a direct role of the protein on bone metabolism cannot be excluded [75, 76].

Male infertility

Congenital Bilateral Absence of Vas Deferens (CBAVD) is detected in up to 90 % of CF males [77] and is also found as an isolated clinical feature in CFTR-related disorders [24]. The most frequent genotype in mono organ conditions is the *in trans* combination of a CF causing mutation and the IVS8-5T polymorphism [24, 78].

Growth

Pubertal spurt retardation reduced growth velocity and diminished GH secretion after appropriate stimuli have been reported in CF children [79]. Although short stature is partly due to malnutrition and disease severity, low insulin-like growth factor 1 (IGF1) levels in CF pigs and in patients suggest a role of CFTR in the pituitary secretion of growth hormone [80].

Therapy

The treatment of CF is multidisciplinary and has to be provided in specialized centers having access to all the necessary subdisciplines. This multiprofessional approach has been quite successful and greatly contributed to increased life expectancy, better lung function, and reduction in the prevalence of chronic infections [81].

Pulmonary therapy

The backbone of lung disease treatment consists of removal of ispissated secretions by means of airway clearance techniques and of nebulizations that diminish mucus viscosity or increase its water content. Prevention and treatment of airway infection represent the main therapeutic challenge in CF [81]. Various strategies have been suggested to avoid increased exposure to nosocomial strains and interpatients transmission of *Pseudomonas aeruginosa*, *Meticillin Resistant Staphilococcus aureus,* and *Burkholderia cepacia*. Other microorganisms may produce lower airway damage, and guidelines are periodically updated to face old and new pathogens [57]. Nebulized antibiotics are widely used to eradicate and control chronic infection by *Pseudomonas aeruginosa*. Notwithstanding aggressive preventive measures and treatment, the colonization of the lower airways remains a most significant clinical problem, leading to progressive lung damage and chronic or frequent antibiotic treatment, both nebulized and systemic.

Gastrointestinal therapy

Malabsorption and hypoproteinemia are usually managed with the administration of pancreatic enzymes and the addition of lipid soluble vitamins. Hypercaloric diets are recommended and have been proved to improve survival [82]. Specific nutrients (i.e., essential fatty acids, polyunsaturated fatty acids, and docosahesaenoic acid) have sometimes been used, based on CF specific abnormalities in lipid metabolism [83–87].

Surgery

A substantial subpopulation of patients develop respiratory insufficiency and are listed for double lung transplantation. This is rare nowadays in childhood but not in adults and the median age of the procedure is in the third decade of life [88, 89]. A small proportion of patients (2–3 %) will develop portal hypertension and undergo specific surgical procedures, including porto-systemic shunts and liver transplantation [90]. ENT surgery is frequently needed for nasal polyposis, mucocele, and chronic sinusitis [61, 91].

Anti-inflammatory therapies

Corticosteroids, ibuprofen, and macrolides have been shown to slow down the progression of lung disease. Whereas prolonged use of systemic steroids is limited by their considerable side effects, ibuprofen and macrolides are widely used [92–94].

Gene therapy

Shortly, after the identification of the CFTR gene, gene therapy has been experimented by various research groups. Although in vitro studies had been successful in reaching high levels of gene expression, clinical results were impaired by low efficiency of the vectors and inflammatory reactions [95, 96]. More recently, a 1 year study administering monthly treatments of a nebulized gene/liposome complex showed an increase in FEV1 % of 3.7 %, statistically significant but of modest clinical meaning [97].

Therapies under study Pharmacological therapy has gained interest in the last decade. Compounds, such as phenylbutyrate [98], glutathione [99], and amitryptiline [100–103], have been tested in clinical trials, but have not yet been definitely proved to produce clinically significant benefits. Natural compounds, such as genistein, curcumin, and resveratrol, have also been considered as potential treatment for cystic fibrosis [104]. Denufosol, an inhibitor of purinergic receptors, after initial promising results in a phase 3 clinical trial failed to reach the primary endpoint in a second large study [105]. Roskovitine is an inhibitor of kinases currently in clinical trial phase II for the treatment of a number of diseases. A phase II clinical trial is undergoing, and, pending on its results, a phase III trial could be activated in the next years [106].

Personalized medicine for CF

Since its recognition CF has been treated symptomatically, and until recently, no therapy directed to the restoration of CFTR function had been available. This is changing and compounds targeting CFTR are becoming available or are positioned in the therapeutic development pipeline.

The evaluation of the efficacy of drugs modifying disease

Evaluating the actual efficacy of treatments for CF is a major challenge. Survival, the more rational outcome measure in a life-shortening disease, such as CF, is inapplicable due to the great increase in life expectancy. Other endpoints are needed and have been employed in clinical trials as surrogate outcomes The most widely used is the

spirometric parameter forced expiratory volume in one second (FEV1), often in association with time to first pulmonary exacerbation, number of exacerbations, and measurements of quality of life.

Inflammatory markers, particularly from bronchoalveolar lavage, have been used as clinical endpoints in studies on pathophysiology and in clinical trials of nebulized antibiotics and recombinant human (rh)DNase. Nonetheless, the lack of adequate standard operating procedures limits their use to monitor disease progression or response to treatment [107].

The sweat test, nasal potential difference (NPD) [108–110], and intestinal current measurements (ICM) have been employed as biomarkers of the activity of drugs targeting CFTR [111].

A crucial point in individualized therapy in CF is the screening of potentially useful drugs in patients carrying rare mutations. To this end, organoids permit to study in individual patients the effect of new compounds. They are generated using intestinal adult stem cell cultures from rectal biopsies and have been proven epigenetically stable and useful to store and biobank cells [112–115].

PTC 124 (Ataluren®)

This compound is meant to overcome the gene translation stoppage caused by nonsense mutations. It is not specifically designed for CF and has already been approved for the treatment of Duchenne muscular dystrophy. A phase III clinical trial has demonstrated some efficacy in patients non-treated with nebulized tobramycin, which is a frequent therapeutic choice in CF for its elevated anti-pseudomonas activity. It has been speculated that aminoglycosides, which also show activity in preventing incomplete translation, could antagonize Ataluren® [116].

Modulators of CFTR activity

CFTR modulators include correctors and potentiators. Correctors are small molecules designed to increase the availability of full length and physiologically active CFTR protein at the apical membrane level of epithelial cells. Potentiators are intended to improve the channel activity of CFTR proteins which reach the apical membrane but have reduced function. Modulators are mutation specific, i.e., they have effect only on mutations producing a particular protein defect.

The first clear evidence of success of a CFTR potentiator was published in 2011 and concerned patients bearing the G551D "gating" mutation. The molecule, known as Ivacaftor and commercially available as Kalydeco®, has produced unprecedented results in respiratory function gain and pulmonary exacerbations reductions, plus several other achievements, such as better control of diabetes and improvement of pancreatic function, infection, and nutrition. Ivacaftor efficacy in these patients appears to be sustained in treatments prolonged up to 4 years [117, 118]. Shortly, after this historical breakthrough, the use of Ivacaftor has been extended to other gating mutations and to patients bearing R117H, a residual function mutation [119, 120].

The rescue of proteins originated by CFTR genes harboring other types of mutations is proving more complex and laborious. Research efforts have concentrated on the F508del mutation, the most widely represented worldwide. A combination of two molecules, a corrector and a potentiator (ivacaftor + lumacaftor), tested in a large phase III international clinical trial involving one thousand F508del homozygotes, showed significant clinical improvements, but to a lower extent than Ivacaftor alone in patients carrying a gating mutation [121].

New compounds are under current investigation, both preclinically and in human trials, and some of them explore new avenues to the pharmacological treatment of the basic defect in CF. The website of the US CF Foundation contains an excellent illustration of the drug development pipeline [122].

Alternative approaches

Acting on the cellular environment instead of directly targeting mutant CFTR represents an alternative way to permit F508del-CFTR to traffic to and reside at the plasma membrane level. Autophagic flux is defective in CF epithelial cells because of overactivation of the pleiotropic enzyme Tissue Transglutaminase (TG2). Following preclinical evidence in CF mice and in primary nasal cells from CF patients, two phase 2 open-label clinical trials tested the combination of the TG2 inhibitor cysteamine, a repurposed drug already used for the treatment of cystinosis, together with the over-the-counter green tea flavonoid epigallocatechin-gallate (EGCG). The latter inhibits the master kinase CK2, the main responsible for CFTR fragmentation, and decreased stability. The combination decreased sweat chloride concentrations, increased chloride efflux in nasal cells, restored autophagy, decreased inflammatory cytokines in patient's sputum, and increased FEV1 of 4 % points. These results were observed in F508del homozygotes and in patients bearing F508del or other class II mutations *in trans* with a class I [123, 124].

A considerable progress has been made in the understanding of CF pathophysiology and in conceiving traditional and new approaches to treatment. Much has still to be done, but evidence is now available that targeted therapies for CF are within reach [125, 126].

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