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Abstract

Cystic fibrosis (CF) is the most common life-threatening recessive genetic disease in the Caucasian population. This multi-organ disease is caused by mutations in the gene encoding the Cystic Fibrosis Transmembrane conductance Regulator (*CFTR)* protein, a chloride channel recognized as regulating several apical ion channels. The gene mutations result either in the lack of the protein at the apical surface, or in an improperly functioning protein. Morbidity and mortality due to the mutation of *CFTR* are mainly attributable to lung disease resulting from chronic infection/inflammation. Since its discovery as the causative gene in 1989, much progress has been achieved not only in clinical genetics but also in basic science studies. Recently, combinations of these efforts have been successfully translated into development and availability for patients of new therapies targeting specific *CFTR* mutations to correct the CFTR at the protein level. Current technologies such as Next Gene Sequencing (NGS) and novel genomic editing tools may offer new strategies to identify new *CFTR* variants and modifier genes, and to correct *CFTR* to pursue personalized medicine, which is already developed in some patient subsets. Personalized medicine, or P4 medicine for *P*ersonalized, ''*P*redictive'', ''*P*reventive'' and ''*P*articipatory'', is currently booming for

cystic fibrosis. The various current and future challenges of personalized medicine as they

apply to the issues faced in cystic fibrosis are discussed in this review.

Introduction

Cystic fibrosis (CF) is the most common life-threatening recessive genetic disease in the Caucasian population, affecting approximately 70,000 individuals worldwide. This genetic disorder, caused by a mutation of the Cystic Fibrosis Transmembrane conductance Regulator (*CFTR*) gene, is mostly characterized by recurrent lower respiratory infections, exocrine pancreatic insufficiency (85% of patients), and increased electrolyte concentration in sweat. A subset of patients also presents with a variety of symptoms which less commonly result in a CF diagnosis, such as meconium ileus, liver disease and pancreatitis (1). Additional CF complications may also be observed in these patients, such as CF related diabetes. When CF was initially described in the 1930's, the patients only survived for a few months or years. While still associated with fatality, the median predicted life expectancy in CF has dramatically increased to around 40 years of age. However, analysis of demographic data of the European CF population highlights disease outcome disparities within Europe (2). This improvement of CF survival within specific European populations is multifactorial, including better diagnosis, phenotype characterization and treatment (e.g. antibiotics). To extend the survival and quality of life for CF patients, specific therapies targeting *CFTR* have been developed highlighting the translational research pipeline from basic science to clinical trials (3).

These specific therapies have directly affected the current progress in cystic fibrosis of the use of P4 medicine (for ''Personalized'', ''Predictive'', ''Preventive'' and ''Participatory''). Medicine is *per se* ''Personalized'', *i.e.* individualized. Indeed, the best healthcare for each patient is chosen depending on his or her pathology and overall environment (allergic, psychological, socioeconomic). The concept of personalized medicine was developed in the 1990's by the pharmaceutical company Roche®, in regards to the fact

that one specific drug can induce various reactions in different individuals; and, for one individual, some drugs are highly effective whereas others are ineffective. Thus, anticipating which drug will be effective in one patient *versus* another is the basis for personalized medicine. This concept has been extended to the ''4P medicine'' which takes into account the patient as a whole (4) with:

- ''*P*ersonalized medicine'', founded on how the individual genetic background could guide each patient's healthcare.

- ''*P*redictive medicine'', that allows the physician to assess the risk for one person to develop a disease or a disease's complication, leading to consider an individualized strategy to avoid disease appearance and/or progression.

- ''*P*reventive medicine'', that promotes a proactive approach, allowing to switch from a curative approach to a method based on the overall state of health and well-being.

- ''*P*articipative medicine'', that allows the patient to take informed decisions and to be responsible for his own health.

This concept, well known and described in cancer care, is relevant to almost all other diseases, and particularly for genetic diseases, for which large scale genomics studies have been performed, such as CF (5).

CFTR

CFTR (OMIM: 602421) was identified in 1989 as the abnormal gene for CF (6-8). It is located on chromosome 7 (7q31.2), composed of 27 exons, and encodes for a protein, an ion channel that belongs to the ATP-binding cassette (ABC) transporter family of proteins. Consistent with the ABC transporters, the CFTR structure has 2 transmembrane domains (TMDs), and 2 nucleotide-binding domains (NBDs), but uniquely possesses a regulatory

domain (R) (9). CFTR has several functions acting primarily as a chloride channel and regulating several other apical ion channels.

To date, almost 2,000 mutations have been identified and collected from the international CF genetics research community (http://www.genet.sickkids.on.ca/app; http://www.cftr2.org) (10). *CFTR* mutations have been found mostly in European-derived populations, and, with much less frequency, in African and East Asian populations (11). Missense mutations are the most prevalent (40%), compared to frame shift (16%), Splicing (12%) and non-sense mutations (8%) (11, 12). The most common mutation (70% of patients) is a three base deletion (c.1521_1523delCTT; Refseq transcript ID: NM_000492.3) that induces a deletion of a phenylalanine in position 508 (p.phe508del (p.F508del); Refseq protein ID NP_000483.3).

CFTR mutations are classified into five classes (sometimes six), according to their resulting damaging effect on the protein (reviewed in (13)) (Table 1). These classes are not exclusive, given that one mutation could have both a defective channel regulation ("class III") and a defective channel conduction ("class IV"), such as the c.350G>A/p.Arg117His (formerly R117H) mutation.

I Defective protein production	II Defective protein processing	III Defective Channel regulation	IV Defective Channel conduction	V Reduced protein synthesis and function
c.1624G>T p.Gly542X (G542X) **2.6%**	c.1521_1523del p.Phe508del (F508del) **70%**	c.1652G>A p.Gly551Asp (G551D) **2%**	c.350G>A p.Arg117His (R117H) **1.14%**	c.2657+5G>A - (2789+5G>A) **0.76%**
c.3846G>A p.Trp1282X (W1282X) **1.49%**	c.3909C>G p.Asn1303Lys (N1303K) **1.75%**	c.1679G>C p.Arg560Thr (R560T) **0.28%**	c.1040G>C p.Arg347Pro (R347P) **0.33%**	c.3717+12191C>T - (3849+10kbC>T) **0.74%**
c.489+1G>T - (621+1G<T) **1.15%**	c.1519_1521del pIle507del (I507del) **0.45%**		c.1000C>T p.Arg334Trp (R334W) **0.24%**	c.2988+1G>A - (3120+1G>A) **0.37%**
c.1657C>T p.Arg553X (R553X) **0.91%**	c.254G>A p.Gly85Glu (G85E) **0.44%**			c.1364C>A p.Ala455Glu (A455E) **0.3%**
c.1585-1G<A - (1717-1G<A) **0.89%**	c.2052delA p.Lys684AsnfsX38 (2184delA) **0.16%**			
c.3484C>T p.Arg1162X (R1162X) **0.48%**	c.3528delC p.Lys1177serfsX15 (3659delC) **0.035%**			
c.579+1G>T - (711+1G<T) **0.23%**				
c.1766+1G>A - (1898+1G>A) **0.34%**				

Table 1. *CFTR* mutation classes. Mutations are the classical American College Medical Genetics (ACMG) recommended panel of 23 classic CF-causing mutations for diagnosis. Names of variants followed HGVS nomenclature (c. denotes cDNA position, p., protein position) using reference transcript/protein (NM_000492.3/ NP_000483.3) sequence for *CFTR* respectively. Legacy names are in bracket. % is the frequency of the mutation based on the CFTR2 database (February 2015).

CFTR sequence variants are usually named using two systems, either *traditional*, or *recommended* by the Human Genome Variation Society System (HGVS, www.hgvs.org/mutnomen/). One particular study highlighted the urgent need to move away from the traditional and historical naming towards the newer recommended HGVS nomenclature in order to standardize *CFTR* mutation screening results. The HGVS nomenclature (alone or in combination with the traditional one) for the common mutation c.1521_1523delCTT was only used by 20% of laboratories (14). Thus, it is now proposed that

CFTR genetic reports should include both nomenclatures. A new database called ''CFTR2'' (Clinical and Functional Translation of CFTR; www.cftr2.org) has been recently created. This website is designed to provide information about specific CF mutations to patients and their family, clinicians, researchers, and the general public. It imparts information about the disease liability of a specific mutation when combined with another CF-causing mutation, and, furthermore, offers information regarding the sweat chloride levels, lung function, pancreatic status, and *Pseudomonas aeruginosa* infection rates in patients carrying these mutations and reported in the CFTR2 database.

New high throughput sequencing approaches will generate many genetic variations both in CF patients and in the general population. For CF and other monogenic diseases, annotating variants (*i.e.* assessing the disease liability of these rare variants) is a considerable challenge for the future (15). For *CFTR*, the disease liability has been validated and established for only a few dozen variants, in comparison to the almost 2,000 reported. In this context, a phenotypic-driven approach was used in the CFTR2 project to assess the disease liability of *CFTR* variants (10). This project has succeeded thanks to the cooperation of geneticists, clinicians and basic scientists. This strategy includes phenotypic and genomic data analyses, as well as biochemical measurements (Mean Sweat Chloride) and molecular biology studies (CFTR RNA and protein expression). Starting with 39,696 subjects, 159 variants were selected (seen at ≥0.01% in CFTR2). Finally, 127 variants have been assigned as "CF causing", 20 as "indeterminate", and 12 as "non-CF causing". Annotating these *CFTR* variants will benefit both clinical diagnosis and personalized therapies. In fact, the disease liability of the 23 mutations included in the AMCG testing panel (16, 17) (table 1) has been established for many years. However, the 85% sensitivity of this North-American panel left resulted in almost 30% of newborns with insufficient evidence to confirm the diagnosis of CF. Indeed, sensitivity of the mutation tests varies between populations. In non-Caucasian groups

and Hispanic, the sensitivity is lower compared to Caucasians (18). In Europe, extensive heterogeneity in the distribution of *CFTR* is seen across the different European countries. Thus, best practices guidelines for CF molecular genetic diagnosis have been established, especially to take into account the regional disparities. Inclusion of the 127 disease-causing variants from CFTR2 increased the sensitivity for detection of CF alleles in white European individuals up to 95 % (10). The non-Caucasian inventory of *CFTR* variants will be achieved due to the future CFTR2 recruitment that will include affected individuals from East Asia, Middle East, Africa and South-America (15).

The biological consequences (direct or indirect) of *CFTR* mutations are numerous and have been extensively studied, particularly for the p.F508del mutation. Not all the relevant publications can be included here. Briefly, the defective ion transport due to *CFTR* mutations results in reduced apical airway surface liquid (ASL), which leads to impaired mucociliary clearance that progressively induces obstruction of the airways with thickened mucus. This in turn creates a favorable environment for bacterial contamination and colonization. Interestingly, recent observations using a piglet model of CF suggest that mucociliary transport is a primary defect in CF (19). Patients with CF have an impaired host immune response especially against *Pseudomonas aeruginosa*, which is characterized by an exaggerated and ineffective airway inflammatory response. There is evidence suggesting that CFTR dysfunction affects innate immunity pathways, notably those associated with Toll Like Receptors (TLRs) (20) and that the initial predisposition to infection in infants with CF may represent a primary defect in local mucosal immunity (21). The temporal and causal interplay between infection and inflammation (chicken or egg) is still in debate. However, it has recently been shown that *cftr*$^{-/-}$ newborn piglets, a model which well reflects human CF lung disease, have an impaired host response without inflammation, suggesting that bacterial infection may initiate inflammation (22).

Lung modifiers

Due to the intensive work on *CFTR*, the diversity of the clinical phenotype of lung disease among patients harboring the same genotype (e.g p.F508del homozygous) suggests that other environmental and genetic factors influence the clinical phenotype. Interestingly, discordant phenotypes are observed in CF siblings carrying the same *CFTR* mutations and sharing a similar environment, thusly supporting the need to search for CF modifier genes (23-29). To date the most studied respiratory phenotypes are lung function (FEV$_1$) decline and the susceptibility to *Pseudomonas aeruginosa* infection. This pathogen is known to be associated with a more rapid decline of the lung function and with increased mortality. Various strategies have been employed in order to discover these modifiers such as Genome Wide Association Studies (GWAS), candidate gene approaches, and, more recently, exome sequencing. These different strategies have identified many potential modifiers (reviewed in (30, 31)). Briefly, the genes that have been mostly studied (replicated, large cohorts of patients) are the transforming growth factor beta 1 (*TGFB1*), mannose binding lectin 2 (*MBL2*), and the ancestral haplotype (AH) 8.1. TGFβ is a multifunctional protein involved in the regulation and the proliferation of numerous cell types. The most studied variants are in the promoter region (-509) and in codon 10 (2500 patients). Although some discrepancies exist between the various studies, an association has been observed with the variants inducing an increased expression of the protein (-509TT and codon 10CC) and the severity of the lung phenotype in CF patients (32, 33). MBL is an innate protein produced by the liver. During the acute inflammatory process, it accumulates in the lung and has a pivotal role in infectious diseases, specifically due to its binding properties to CF pathogens such as *P. aeruginosa, S. aureus and B. cepacia.* The haplotype in the first exon (A/O), known to modulate the serum level of MBL, has been investigated by several independent teams. A meta-analysis has shown that this haplotype, corresponding to a MBL deficiency, is associated with the severity

of CF lung disease with an early acquisition of *P. aeruginosa*, more frequent airway colonization by *B. cepacia*, a decreased lung function in adults, more frequent transplantation needs and an increased mortality (34). The haplotype 8.1AH is called *ancestral* as it is highly conserved. It is frequent in Caucasians (25% in the general population) and is localized on the short arm of chromosome 6, in the coding region of the MHC (major histocompatibility complex). It includes major genes of the inflammatory response such as lymphotoxin a, Tumor Necrosis Factor (TNF), Heat Shock Protein A1B (HSPA1B) and RAGE (Receptor for Advanced Glycation Endproducts) (35). This haplotype has been shown to be associated with the severity of the lung disease in 404 CF European patients; the 8.1AH carriers having a faster FEV_1 decline (36). Finally, a GWAS performed in a large CF cohort, of more than 3000 patients (37), has identified 7 regions associated with the respiratory phenotype near the genes *APIP/EHF, AGTR2, HLA-DRA, EEA1, SLC8A3, AHRR* and *CDH8*. The region around the *EHF* and *APIP* genes, involved in apoptosis, was highly significant. Moreover, a linkage analysis in related patients showed another interesting region on chromosome 20q13.2, that remains to be fully studied.

Other phenotypes have been studied inorder to discover modifier genes and have been extensively reviewed elsewhere (30, 31).

Identification and characterization of these modifiers would allow a ''*predictive*'' and ''*preventive*'' medicine. Predictive medicine underlines the concept of the physician possessing the knowledge of the patient's whole genome in order to predict the onset of complications before they start. Preventive medicine may then be applied, and therapies may be provided prior to the onset of these complications. This would be a new area for the patients care, "proactive" rather than the current "reactive" care.

Cystic fibrosis therapy

New personalized therapies are under development, targeting either the dysfunctional gene or protein. This new era allows hoping for curative strategies in a very next future, whereas current treatments mostly focus on end-stage disease of the affected organs.

Gene therapy

Gene therapy would be the hoped for gold standard treatment for CF patients. Compared to CF protein therapy, gene therapy is expected to be broadly prescribable to CF patients, without restriction to a specific class of mutations. However, this strategy faces important challenges. The concept of gene therapy in CF consists of adding a correct sequence of *CFTR* in a way that it is incorporated into the patients' cells and is able to circumvent the *CFTR* mutation. An important step in creating this correct version of the gene is choosing a transporter. Two types of vectors are currently available, either synthetic or viral. The viruses studied for gene transfer are adenovirus, adeno associated virus (AAV), lentivirus and retrovirus. Inconveniently, these vectors induced an excessive inflammatory response or showed poor efficacy in CF patients (38). Thus, synthetic vectors that do not or weakly induce immunogenicity would be an interesting alternative. Currently, a phase III trial is ongoing in the UK with a synthetic lipidic vector. It will include 130 patients older than 12 years old, who will be given the normal gene by inhalation once a month for one year (39). Effects will be evaluated by recording the respiratory symptoms, the lung function (FEV_1), blood markers (inflammation and immunity) and sputum characteristics.

It is important to highlight that so far gene therapy agents have been delivered only to the lung, *i.e.* by inhalation, and would have no benefit for other organs. Moreover, another challenging issue is access to the site of delivery, as the thick respiratory mucus may prevent a good bioavailability. Chronic administration may also be problematic even with a low immunogenic system.

New genome editing tools including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and RNA guide engineered nucleases derived from the bacterial clustered regulatory interspaced short palindromic repeat (CRISPR)-cas (CRISPR-associated) system associated with stem cell therapy offer attractive alternatives. Briefly, these nucleases cleave DNA at a specific site of interest and allow genome modifications of the gene (40). Compared to TALENs and ZNFs, the CRISPR/Cas9 system which requires short programmable gRNA for DNA targeting is cheap and easy to design and produce. However much work remains to be done, in particular enhancing the specificity to avoid deleterious off target effects and to further develop the delivery methods (41). In primary adult stem cells, intestinal cell organoids of CF patients, the CRISPR/Cas9 system was used to restore the *CFTR* locus and successfully restore CFTR function (organoid swelling) (42). Using an iPS (induced pluripotent stem cell) cell line homozygous for the p.F508del mutation, candidate TALENs were generated to correct the *CFTR* defect. Generation of mature airway epithelia with a functional CFTR from human embryonic stem cells have been recently realized (43). Also, in this study, CF-induced pluripotent stem cells (iPSCs)-derived from epithelial cells treated by the CFTR corrector compound C18, showed an increased amount of CFTR in the plasma membrane. This is promising for the future of a "personalized" regenerative medicine. In this context, iPSC-derived epithelial cells may be also used to test therapeutics in a patient-specific manner, taking into account the whole genome of a patient, especially the *CFTR* allelic heterogeneity and the modifier genes.

Protein personalized Therapy

Protein therapies are aimed at correcting the dysfunction of CFTR. As mentioned previously, many *CFTR* mutations alter the structure/function of the protein. In this context, various therapies have been developed targeting specific mutation classes, in order to

personalize the treatment. Here, the different classes will be explained and the current strategies to correct the mutations within those classes will be discussed.

Class I mutations are nonsense, frameshift and splice mutations resulting in the absence or little expression of CFTR (Figure 1.). Nonsense mutations induce a premature termination codon (PTC) in the mRNA, which results in a premature stop of translation, and the absence of the protein. To correct this type of mutation, molecules that would promote readthrough of the PTCs and, thus, allow the production of functional CFTR, are needed. The first studies have tested antibiotics of the aminoglycoside family, as gentamycin (44, 45). The several clinical trials with gentamycin have indeed shown benefits in the CF patients, with a normalization of the chloride transport in the nasal epithelium (46-48). However, due to adverse drug-effects of long-term use of aminosides (nephrotoxicity and ototoxicity), a molecule without antibiotic properties was identified and developed by PTC Therapeutics® known as PTC124 or Ataluren (49, 50). Clinical trials with this molecule have shown better transport of chloride in the nasal epithelium, but no real clinical benefit (51-53). Additionally, the efficiency is variable among patients, mostly due to the causal CFTR mutation. In fact, patients with the p.Trp1282X (p.W1282X) were better responders than those carrying other class I mutations (53). In the last international phase III clinical trial, 238 patients older than 6 years of age were enrolled. Patients received Ataluren or placebo for 48 weeks (54). No significant respiratory benefits were observed for the whole cohort, either for the evolution of the percent-predicted FEV_1, or for the numbers of exacerbations. However, in the subgroup of patients not chronically treated with inhaled aminosides, an increase of the percent-predicted FEV_1 (+5,7%), and a reduction in the number of exacerbations were observed. In the Ataluren group, a significant increase of the creatinine was detected in comparison to patients treated with the placebo, without other adverse effects. The clinical benefit seems modest, and will be important to confirm in larger cohorts before approving this drug as a standard of care. Thus,

an additional phase III international trial is underway, that excludes patients receiving chronic inhaled aminoglycosides (ClinicalTrials.gov, NCT02139306).

In class III mutations, CFTR is expressed at the cell surface but is non-functional. To correct this class of mutations, work is ongoing to identify molecules able to restore the CFTR chloride transport function at least partially, known as ''potentiators.'' In this field, Vertex$^®$ Laboratories were pioneers. Highthrough-put screening methodology investigating more than 200,000 molecules identified a potentiator named Vertex 770 (VX-770, Ivacaftor). VX-770 was indeed able to potentiate the mutated CFTR protein, to increase the open probability of the chloride channel, to decrease the excessive absorption of sodium, and to restore the ciliary beat. This was initially demonstrated in the CFTR p.Gly551Asp (p.G551D) mutation, which is the most frequent class III mutation, occuring in 2% (heterozygous) of CF patients worldwide (55). The clinical trial leading to commercialization was a phase III study (Strive$^®$ study) including 84 patients treated, against 83 with placebo, older than 12 years old and carrying at least one p.G551D *CFTR* allele (56). Significant clinical benefits have been shown both for respiratory status (10 % of increase for the percent-predicted FEV$_1$ with a decrease of the number of exacerbations), and for the general status (gain of weight, quality of life). A significant decrease in the sweat chloride levels was also demonstrated. The extended clinical study (Envision$^®$, children between 6-11 years old) showed similar improvements (57). Consequently, in 2012, Ivacaftor was approved in the US and in Europe by the Food and Drug Administration (FDA) and the European Medical Agency (EMA) respectively, and commercialized under the name of Kalydeco$^®$ for patients older than 6 years old, and with at least one p.G551D *CFTR* allele. Further, Kalydeco$^®$ has been tested in patients carrying other class III mutations, or targeted class IV and V mutations (sharing functional similarities with the class III) (58). The new trials led to an extension of the FDA and EMA approval to 8 additional gating mutations: p.Gly178Arg (p.G178R), p.Ser549Asn (p.S549N), p.Ser549Arg

(p.S549R), p.Gly551Ser (p.G551S), p.Gly1244Glu (p.G1244E), p.Ser1251Asn (p.S1251N), p.Ser1255Pro (pS1255P) and p.Gly1349Asp (p.G1349D) (59). Recently, Ivacaftor has also been shown to benefit patients carrying the c.350G>A/p.Arg117His (formerly R117H) mutation, that is relatively common in the CF population, and leads to both defective channel regulation (sharing similarities with the class III mutations) and conduction. Thus, the FDA also approved Ivacaftor as a treatment for CF subjects aged greater than 6 years old with at least one copy of *R117H-CFTR* (60). Additionally, Ivacaftor is currently being tested in patients carrying non-R117H residual function mutations. The phase II clinical trial showed encouraging results with improvement of lung function and a decreased sweat chloride level; a phase III clinical trial will surely be planned shortly (Vertex release 852372).

As introduced above, the p.Phe508del (p.F508del) mutation is the most common class II mutation. Worldwide, 70% of the CF patients carry at least one p.F508del *CFTR* allele, and 50% are homozygotes for this mutation. It results in the misfolding of the protein leading to rapid degradation of the protein in the cell, preventing its expression at the cell surface. Molecules that could prevent this degradation are called "correctors", and would allow CFTR to be sufficiently expressed at the cell surface. Vertex developed the VX-809 (or Lumacaftor), a corrector which may achieve this goal. In fact, using bronchial epithelial cells harboring the p.F508del homozygous mutation, VX-809 increased the chloride transport (55-58, 61). A phase II clinical trial has shown a modest beneficial effect on the sweat chloride levels in patients homozygous for the p.F508del mutation (62). This suggested that merely restoring CFTR expression was not sufficient, and that the function of CFTR had to be promoted by a potentiator. Consequently, a phase II trial in adults homozygous or heterozygous for the p.F508del mutation was realized using the combination of Ivacaftor and Lumafactor. This association was well tolerated, and, in the homozygous p.F508del patients, improved lung function (gain of 8.6% percent-predicted FEV_1), and significantly decreased the sweat

chloride level (63). However, no FEV_1 improvement was observed for the p.F508del heterozygous patients. Two international phase III studies encompassing ~1100 patients homozygous for p.F508del ended this year, and promising results have been shown at the last North-American CF Conference (NACFC 2014, Atlanta) but are unpublished as of yet (Vertex press release) (64, 65). Interestingly, another promising compound similar to Lumacaftor, VX-661, has also been developed by Vertex, and clinical trials are underway. Interim results of a phase II, double-blind, placebo-controlled study of VX-661 (various daily doses) alone or in combination with Ivacaftor have been reported for p.F508del homozygous patients (66). The treatment was well tolerated and led to an improvement of the percent-predicted FEV_1 (maximum 9% of gain with a combination VX-661 and Ivacaftor), and a decreased sweat chloride level.

Although the efficacy of Ivacaftor has been proven, the question of the cost-effectiveness, *i.e.* affordability compared to long-term benefit of this personalized treatment, for life, must be taken into account and is debatable (67, 68). This drug is indeed highly expensive (around 311,000 US $/year) and the actual knowledge of its efficacy would support a long-term (all life-long) prescription. However, to corroborate that the benefits seen in the clinical trials are indeed sustained long-term, a prospective follow-up of the treated patients should be realized and reported. Then, if the long-term benefit is ascertained, as a society, we would be both ethically and morally obliged to assure the access to these personalized therapies for all CF patients that would benefit.

Pharmacogenomics

Even if specific CF protein therapies are emerging, inter-individual variation to drug treatment is unfortunately expected and has to be considered in relation to the 4P medicine. In this context, the future upsurge of highthrough-put genomic data concerning

pharmacogenomics in CF may contribute to the development of personalized treatment across specific subgroups of CF patients. Importantly, Kalydeco® is mainly metabolized by the cytochrome P450 family 3, subfamily A (CYP3A) and early in its development, Kalydeco® and metabolites have been shown to be able to inhibit CYP3A, and other cytochromes (CYP2C8, CY2C9 and CYP2D6). A recent drug-drug interaction (DDI) study examined the effect of Kalydeco® on sensitive substrates of CYP2C8 (rosiglitazone), CYP3A (midazolam), CYP2D6 (desipramine), and P-glycoprotein (P-gp) (digoxin). Also, a DDI study of Kalydeco® combined with oral contraceptives was realized that indicated that there was no need for dose adjustment. Kalydeco® is a weak inhibitor of CYP3A and P-gp, but has no effect on CYP2C8 and CYP2D6 (69). Thus, caution must be exercised with co-administration of CYP2C9, CYP3A, and/or Pgp substrates. Although CYP SNPs may affect the level of enzyme production, to date, no genomic studies have been performed to assess the effect of CYP variants on the pharmacokinetics of Kalydeco® in CF patients. Interestingly, CYP3A also interacts with widely used drugs for CF patients, such as macrolides such as azithromycin (70) or Tacrolimus (71). A recent study has shown that Tacrolimus clearance was 40% faster in CYP3A5 expressers compared to non-expressers. Pharmacogenetics testing is a growing current and future challenge that would be predictive for efficacy and for adverse drugs effects.

Conclusion

35 years following the discovery of *CFTR*, personalized medicine is not merely just a concept for the treatment of cystic fibrosis but a reality for the patients. Protein specific therapy is a clear step-ahead in terms of therapeutic options for CF patients, particularly in regards to the new correctors, potentiators and readthrough drugs. Finally, the highthrough-put genomic data, new genome editing tools, and stem cell regenerative medicine are exciting

and will hopefully further evolve into new affordable therapeutics and rise to meet the needs

of CF patients.

Acknowledgements

Figure legends

Figure 1. CFTR mutations classes (I to V), their functional consequences and their potential specific therapies. CFTR is composed of 2 nucleotide-binding domains (NBD1 and NBD2), two transmembrane domains (TMD1 and TMD2) and one regulatory (R) domain.

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