A new era in the treatment of cystic fibrosis: correction of the underlying CFTR defect

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Cystic fibrosis is caused by dysfunction or deficiency of the cystic fibrosis transmembrane conductance regulator (CFTR) protein, an epithelial chloride channel that has a key role in maintaining homoeostasis of the airway surface liquid layer in the lungs. More than 1900 *CFTR* mutations that might result in a disease phenotype have been identified; these can be grouped into classes on the basis of their effect on CFTR protein production, trafficking, function, and stability. In the past 2 years, landmark clinical trials have shown that correction of CFTR function leads to substantial clinical benefit for individuals with cystic fibrosis. These findings are ushering in a new era of cystic fibrosis treatments designed to correct the underlying CFTR defect caused by different mutation classes. With analysis of continuing trials and available patient registries, here we assess mutation types and the number and geographical distribution of patients who are likely to benefit from CFTR-correcting treatment.

Introduction

Cystic fibrosis transmembrane conductance regulator (CFTR) protein is found in the apical plasma membrane of airway, intestinal, and exocrine epithelial cells. One of CFTR's primary roles in the lungs is to maintain homoeostasis of the airway surface liquid layer through its function as a chloride channel and its regulation of the epithelial sodium channel ENaC.[1] This liquid layer lines the airways and allows cilia to protect the lungs through continuous mucociliary clearance. If an individual has genetic mutations that cause CFTR dysfunction, the CFTR chloride channel does not work correctly and ENaC is not appropriately regulated, resulting in increased fluid and sodium resorption from the airways and formation of a contracted viscous surface liquid layer.[1,2] This abnormality, defects in the innate lung defence,[3] and an intrinsic pro-inflammatory status,[4] form the basis for cystic fibrosis lung pathology,[5] which results in a progressive cycle of lung damage from recurrent mucous plugging, infection, and inflammation.

For the past 50 years, nearly all new pulmonary treatments for cystic fibrosis have targeted one of two characteristic components of the disease: viscous mucus or chronic airway infection. The hope was that if these components were addressed, the progressive cycle of airway obstruction, inflammation, and lung damage could be interrupted. Discussions since the discovery of the cystic fibrosis gene in 1989[6] of potential strategies to address the underlying chloride channel defect characteristic of cystic fibrosis have been frequent but have remained theoretical.[7,8] However, the results of a 2011 landmark phase 3 study of individuals with the Gly551Asp mutation showed that correction of the underlying channel defect of CFTR is possible and has clinical benefit in individuals with cystic fibrosis.[9] These results signal a new era in treatment of the disease, in which patient outcomes will be improved by correction of the underlying chloride channel defect. This advancement would represent a triumph of so-called bench-to-bedside medicine and lay a path for treatment of other genetic illnesses. However, to understand how these new treatments will work and what subsets of patients with cystic fibrosis are most likely to benefit, *CFTR* mutations and their effect on CFTR function must be understood.

CFTR mutation classes

More than 1900 *CFTR* mutations that might cause dysfunction of the CFTR protein and result in a cystic fibrosis phenotype have been identified. The disease has a classic recessive inheritance pattern; ie, an individual must have a pathological *CFTR* mutation on each chromosome to develop the disease phenotype. *CFTR* mutations can be grouped into six classes on the basis of their effect on CFTR protein production, trafficking, function, or stability (figure 1).[10] Class I mutations result in no functional CFTR protein being made and are mostly non-sense mutations that cause premature stop codons, resulting in production of truncated unstable RNA (eg, Gly542X, Trp1282X, and Arg553X).[11] Other class I mutations include canonical splice mutations and chromosomal deletions, which also result in no functional protein being made (621+1G→T and *CFTR*del2,3).[11]

Class II mutations are the commonest *CFTR* mutation type, and—often because of protein misfolding—prevent the correct trafficking of CFTR from the cell surface after production; as a result, minimum functional CFTR reaches the apical membrane. The most common class II mutation is Phe508del, a three-base pair deletion that causes a single aminoacid deletion from CFTR, subsequent protein misfolding, and failure of the Phe508del–CFTR to be trafficked to the cell surface. Almost 90% of individuals with cystic fibrosis worldwide have this mutation on at least one *CFTR* gene, and roughly 50% are homozygous for two Phe508del mutations. Other common class II mutations include Asn1303Lys, Ile507del, Arg560Thr, and Gly85Glu.[11]

In mutation classes III and IV, CFTR reaches the cell surface, but mutations prevent the channel from working correctly. Class III mutations cause a substantial reduction in CFTR chloride channel opening time and are often called gating mutations because the channel is
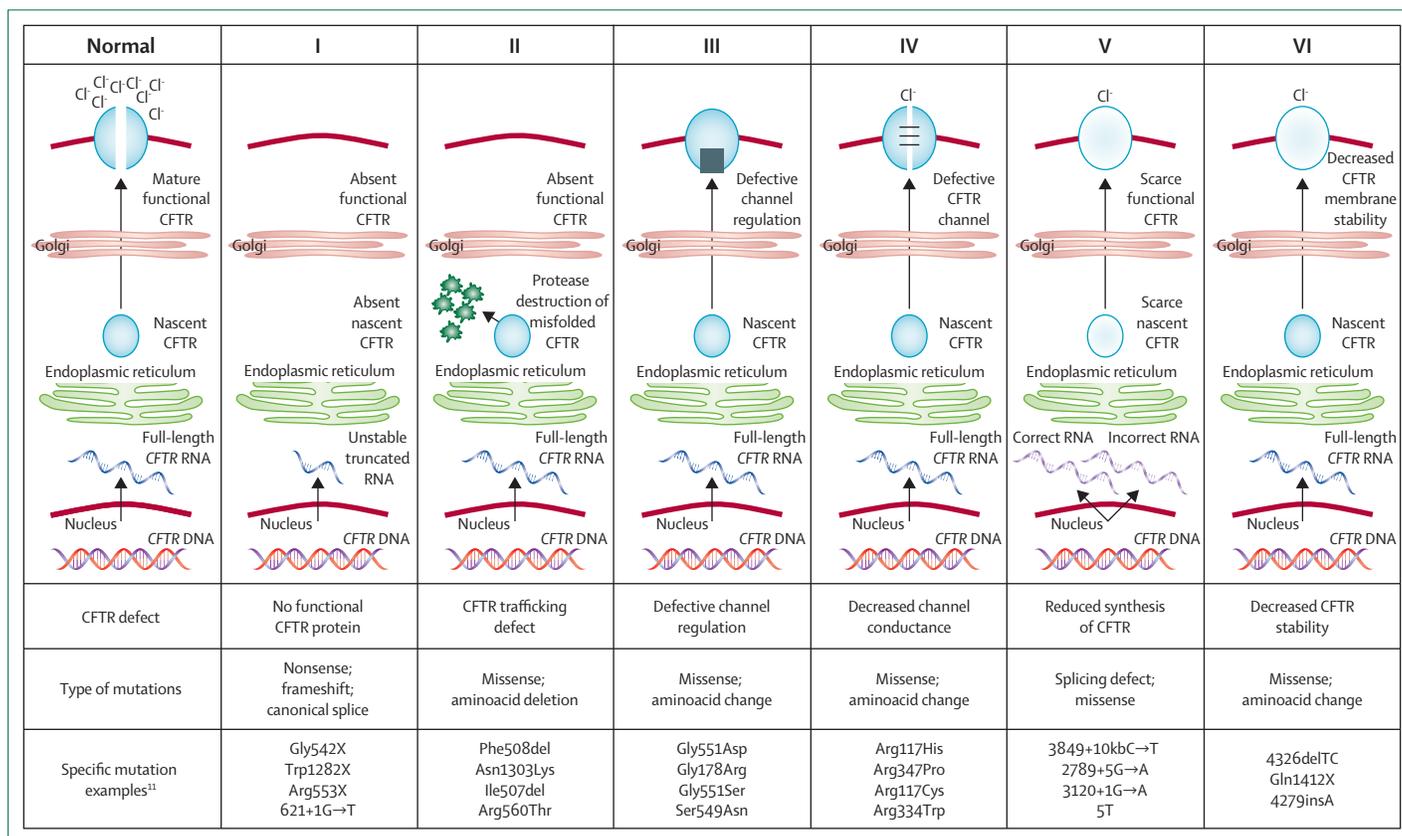
	Normal	I	II	III	IV	V	VI
CFTR defect		No functional CFTR protein	CFTR trafficking defect	Defective channel regulation	Decreased channel conductance	Reduced synthesis of CFTR	Decreased CFTR stability
Type of mutations		Nonsense; frameshift; canonical splice	Missense; aminoacid deletion	Missense; aminoacid change	Missense; aminoacid change	Splicing defect; missense	Missense; aminoacid change
Specific mutation examples[11]		Gly542X Trp1282X Arg553X 621+1G→T	Phe508del Asn1303Lys Ile507del Arg560Thr	Gly551Asp Gly178Arg Gly551Ser Ser549Asn	Arg117His Arg347Pro Arg117Cys Arg334Trp	3849+10kbC→T 2789+5G→A 3120+1G→A 5T	4326delTC Gln1412X 4279insA

Figure 1: Classes of *CFTR* mutations

present at the cell surface, but is rarely open. Gly551Asp is the most common class III mutation.[11] Class IV mutations also result in CFTR protein at the surface, but the channel function is reduced even when open. Examples of class IV mutations are Arg117His and Arg347Pro.[11] In class V mutations, overall CFTR function is inadequate because of reduced amount of normal CFTR at the surface. Class V mutations are most commonly intron mutations that reduce efficiency of CFTR production by affecting splicing (eg, 3849+10kbC→T and 2789+5G→A).[11] Class VI mutations are rare, but result in reduced amounts of functional CFTR at the cell surface because of decreased stability of mature CFTR at the cell membrane (4326delTC).

Treatments to correct the basic CFTR defect

To understand emerging treatments designed to correct the basic CFTR defect, the mechanisms of CFTR dysfunction in the different mutation classes need to be understood. The underlying CFTR difficulties in some *CFTR* mutations will be harder to address than others—particularly class I and II mutations in which little or no CFTR is present at the cell surface. Encouragingly, all six classes presently have emerging treatments or clinical trials in progress or both, with potential to address the underlying CFTR defect.

Class I mutations are the most challenging CFTR defect to address because they cause absence of stable CFTR protein. To correct CFTR function in patients with these mutations will need either replacement of the defective *CFTR* gene or changes in how the protein is made; both of these strategies are being pursued. The first method—replacement of the defective *CFTR* gene—is only possible with gene-addition therapy; however, previous attempts at stable transduction of pulmonary epithelia with adenoviral and adeno-associated viral vectors have had little success.[12] The UK Gene Therapy Consortium is doing a phase 2 trial[13] of cystic fibrosis with a non-viral lipid vector for DNA delivery; this protocol is monthly dosing by inhalation for 1 year (registered at ClinicalTrials.gov, NCT01621867). The enrolment goal is 130 participants, with results expected in 2014. The consortium is simultaneously developing an alternate lentiviral vector as a second DNA delivery option. The advantage of the gene-addition therapy approach is its potential use in all mutation classes—unless the intrinsic proinflammatory state of cystic fibrosis cells is closely linked to the accumulation of abnormal CFTR protein seen in some classes, in which case, gene insertion and expression of normal CFTR in addition to mutant CFTR protein might not fully resolve the cystic fibrosis pathology.[8]

	Treatment/potential treatment	Trial*	Phase	Status
Class I treatments				
Nonsense (stop codons)	Ataluren (PTC 124)	NCT00803205 (PTC 124)	3	Completed
Nonsense (stop codons)	Ataluren (PTC 124)	NCT01140451 (ePTC 124)	3	In progress
Class II treatments				
Phe508del	VX-809/Ivacaftor combination	NCT01225211 (VX 809-102)	2	In progress
Phe508del	VX-809/Ivacaftor combination	NCT01225211 (VX 809-103)	3	2013
Phe508del	VX-661/Ivacaftor combination	NCT01531673 (VX 661-101)	2	In progress
Phe508del	N6022 (N30 Pharma)	N6022-1CF1-04	1	2013
Class III treatments				
Gly551Asp	Ivacaftor monotherapy	NCT00909532 (VX 770-102)	3	Completed[9]
Other gating†	Ivacaftor monotherapy	NCT01614470 (VX 770-111)	3	In progress
Class IV treatments				
Arg117His	Ivacaftor monotherapy	NCT01614457 (VX 770-110)	3	In progress
Class V and VI treatments	Ivacaftor monotherapy	..	..	..
All classes	Gene addition therapy	NCT01621867	2	In progress

*NCT number represents ClinicalTrials.gov reference number.†Gly178Arg, Gly551Ser, Ser549Asn, Ser549Arg, Gly970Arg, Gly1244Glu, Ser1251Asn, Ser1255Pro, and Gly1349Asp. Gly551Asp ivacaftor monotherapy (registered at ClinicalTrials.gov, NCT00909532) is approved for clinical use.

Table: **Present treatments and potential treatments by** *CFTR* **mutations**

The second strategy for class I mutations is to address the premature stop codons present in most patients. PTC Therapeutics (South Plainfield, NJ, USA) has done this with a small molecular compound ataluren (previously PTC124), which allows read-through of premature stop codons, particularly the codon in Gly542X UGA.[14,15] Successful read-through by ribosomes would allow formation of full-length functional CFTR, insertion at the surface, and restoration of CFTR function. Initial studies with measurements of nasal chloride transport suggested ataluren was effective;[16,17] however, the 2012 phase 3 trial of ataluren in 238 patients with cystic fibrosis with stop mutations did not reach its primary endpoint of improvement in forced expiratory volume in 1 s (FEV_1) at 48 weeks, except in a subset of individuals who were not simultaneously taking nebulised aminoglycoside antibiotics.[18] Since aminoglycosides have the potential to competitively inhibit ataluren, investigators and the sponsoring company PTC Therapeutics are considering further testing.

Future therapeutic strategies for class II mutations will likely take advange of the new treatment developed for class III mutations, ivacaftor (previously called VX-770). This small molecule increases the opening time of CFTR present at the cell surface in class III mutations, and thereby increases chloride flux through the CFTR channel. This drug was originally identified as a potentiator of CFTR function in cell culture with respiratory epithelial cells that carried a single Gly551Asp mutation.[19] Recent phase 3 clinical trials showed substantial clinical benefits

of ivacaftor in 144 individuals with cystic fibrosis with at least one Gly551Asp mutation, and led to rapid approval of ivacaftor by the US Food and Drug Administration and the European Medicines Agency. Patients given ivacaftor orally, twice a day, had mean absolute improvement of 10·5% in predicted FEV_1% within 2 weeks of treatment initiation, which was maintained throughout the 48-week treatment period.[9] Treated participants also had a 55% reduction in the incidence of pulmonary exacerbations, a mean weight gain of 2·7 kg, pronounced improvement in measured quality of life (p<0·001), and a mean reduction of sweat chloride concentration of 48·7 mmol/L.[9] A subsequent trial[20] of 52 children with cystic fibrosis aged 6–11 years with at least one Gly551Asp mutation showed a nearly identical effect on lung function, weight, and sweat chloride, with the mean improvement in absolute FEV_1% predicted as 10·0% at week 48 (relative improvement 15·1%). The G551D Observational Study (GOAL; NCT01521338) is in progress and will assess in Gly551Asp patients the effect of ivacaftor on other outcomes of interest including sputum inflammatory mediators, mucociliary clearance, gastrointestinal pH, and sweat rate. Results are expected in late 2013.

Results for ivacaftor in Gly551Asp patients were groundbreaking because they showed that correction of CFTR chloride transport improves clinical outcomes, but only 4% of individuals with cystic fibrosis carry this mutation. However, ivacaftor could be given to more patients if it was shown to be beneficial in other class III and perhaps class IV and V mutations. In-vitro studies of ivacaftor do suggest potential benefit in other mutations; eg, other gating mutations similar in effect to Gly551Asp, such as Ser1251Asn, Ser549Asn, and Gly178Arg.[21] Patients with these mutations might show clinical benefit, and a phase 3 clinical trial of ivacaftor in other class III mutations is in progress (registered at ClinicalTrials.gov, NCT01614470). However, only 1% of patients carry these other gating mutations; more patients carry class IV mutations and some of these mutations might also be responsive to ivacaftor.[22] One of these class IV mutations is R117H, which is present in roughly 2% of individuals with cystic fibrosis worldwide. A clinical trial of ivacaftor is in progress in this mutation group (registered at ClinicalTrials.gov, NCT01614457). The effect of this drug in class V and VI mutations is unknown; however, ivacaftor improves chloride conductance even in wild-type CFTR,[19] which suggests that patients with these mutations could benefit, although clinical trials are yet to begin. In the future, in addition to being grouped by their mechanism of CFTR dysfunction, *CFTR* mutations might also be grouped by their ability to respond to available CFTR-correcting drugs.

Even if most patients with class III and VI mutations had clinical benefit from ivacaftor therapy, this group would account for less than 15% of individuals with cystic fibrosis. Class II mutations, the most common,

are the most important *CFTR* mutations to target. Although ivacaftor increases opening time and chloride conductance for Phe508del-CFTR in vitro, the drug alone is not sufficient to provide clinical benefit in patients homozygous for Phe508del[23] because the primary issue in Phe508del—protein misfolding—prevents CFTR from reaching the cell surface. Lumacaftor (VX-809) and VX-661 are two new small molecular compounds that have shown in cell culture and in early phase 2 studies that they can increase the amount of Phe508del–CFTR that is trafficked to the cell surface.[24] However, even when trafficked to the cell surface, Phe508del–CFTR might not have a fully optimised function as a chloride channel. With lumacaftor or VX-661 to help the movement of Phe508del CFTR to the cell surface, and ivacaftor to

increase opening time and chloride conductance, the amelioration of the underlying Phe508del-CFTR defect might be possible. In-vitro studies of lumacaftor-ivacaftor in Phe508del respiratory epithelia have shown that lumacaftor alone increases CFTR-mediated chloride transport to roughly 15% of wild type, and that addition of ivacaftor increases transport to nearly 30% of wild type[25]—a concentration probably associated with change in clinical outcomes, but only if a similar effect is also seen in vivo. This drug combination is under investigation in a phase 2 study of individuals with the Phe508del mutation; full results have not yet been published, but initial results suggest a beneficial effect on lung function in Phe508del homozygotes.[26] Plans for a phase 3 study are underway. Additionally, a phase 2 trial of Phe508del homozygotes with alternate corrector
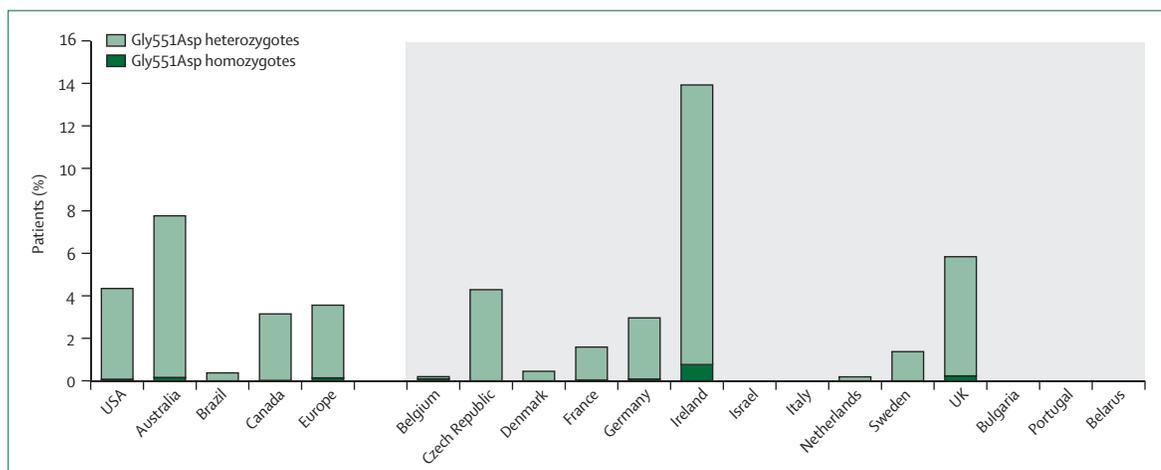

Figure 2: Percentage of patients heterozygous (light green) or homozygous (dark green) for the Gly551Asp mutation
Registries: USA 2010 n=26 293, 90·6% genotyped; Australia 2009 n=2348; 78% genotyped; Brazil 2009 n=1249, 41·6% genotyped; Canada 2009 n=4901; Europe: UK, Ireland, and Sweden 2009, other European countries and Israel 2007, n=23 766; 93·4% genotyped. No bar means that the mutation was not detected in the country.
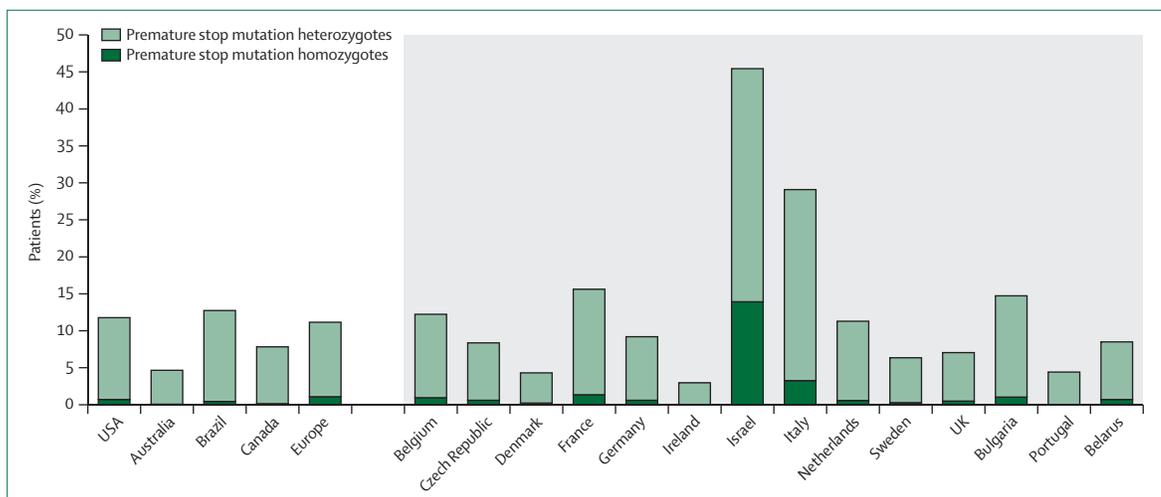

Figure 3: Percentage of patients heterozygous (light green) or homozygous (dark green) for a premature stop mutation
Registries: USA 2010 n=26 293, 90·6% genotyped; Australia 2009 n=2348, 78% genotyped; Brazil 2009 n=1249, 41·6% genotyped; Canada 2009 n=4901 genotyped; Europe: UK, Ireland and Sweden 2009, European countries and Israel 2007, n=23 766; 93·4%.

Search strategy and selection criteria

We searched PubMed for articles published between Jan 1, 1971, and Aug 31, 2012, with the terms "cystic fibrosis", "CFTR mutation class", and "cystic fibrosis drug therapy". We searched ClinicalTrials.gov with the term "cystic fibrosis". We included articles published in English, French, and German.

VX-661 in combination with ivacaftor is in progress. Other new Phe508del correctors are also being developed, and some will enter phase 1 testing in 2013.

Evidence suggests that, in the future, patients with class II mutations could benefit from a combination of many correctors to improve CFTR trafficking to the cell surface.[27] Presently identified correctors seem to act on different stages of the trafficking process and might have an additive effect on increasing CFTR at the cell surface when used together. Potential to address the underlying CFTR defect in all cystic fibrosis mutation classes exists (table). Some of these treatments, particularly for mutations in classes III and IV, will probably be available in the very near future. Results of Phe508del studies suggest that treatment for class II mutations could be available in the upcoming years, whereas class I mutation treatments might need longer to develop.

Patient populations likely to benefit from CFTR-correcting treatment

Examination of presently available patient databases allows a more accurate assessment of the number and geographical distribution of patients likely to benefit from CFTR-correcting treatment. These databases show striking heterogeneity between countries in the frequency of specific *CFTR* mutations. In total, Gly551Asp is found in 3–4% individuals with cystic fibrosis; the mutation is rare in some countries, but in others such as Ireland, Australia, and the UK, prevalence is as high as 14%, 8%, and 6%, respectively (figure 2). A similar example of heterogeneity is seen with the class I premature termination codon mutations, which are found in about 10% of individuals with cystic fibrosis worldwide. In northern European countries, these mutations are less common, whereas in Italy, their prevalence is 29% and 45% in Israel (figure 3). Some countries—eg, Canada and France—will have particular interest in the present Arg117His trial of ivacaftor because of raised prevalence of this mutation in their newborn screening programmes.[28,29] This heterogeneous distribution of mutations highlights the importance of large patient registries and clinical trial networks to speed up the clinical phase of cystic fibrosis drug development.[30,31] Irrespective of whether a trial of ivacaftor is aimed at improvement of CFTR function in patients with gating mutations or a trial of ataluren is aimed at improvement of CFTR function in those with premature termination codons, knowing the distribution of CFTR genotypes allows identification of countries whose patients are most likely to benefit from new therapies and directs clinical trials to high prevalence countries for optimum trial efficiency.

Despite continuing efforts, many individuals with cystic fibrosis have not been genotyped or have not had sufficient genotyping to identify both of their *CFTR* mutations. The percentage of fully genotyped patients with cystic fibrosis varies in Europe from less than 70% in several eastern European countries to 99% in Denmark and 100% in Sweden.[32] To maximise effect of CFTR-modifying therapies in the future, resources need to be committed to increase the percentage of fully genotyped patients. A second challenge will be development of guidelines for appropriate use of new mutation-specific treatments such as ivacaftor. This drug is very expensive and as the number of individuals who can benefit from it increases, regulatory assessors will probably need clear guidelines for its use to restrict costs; eg, if ivacaftor is found to be beneficial in individuals with cystic fibrosis and lung disease and the Arg117His mutation. Although Arg117His associated with intron splicing abnormality 5T results in cystic fibrosis lung disease[33]—and can occasionally cause lung disease in association with 7T[34]—many individuals with Arg117His/7T will not have cystic fibrosis lung disease and are unlikely to need treatment.[29] Continuing education of both caregivers and the cystic fibrosis community is essential to ensure appropriate use of new drugs and longitudinal studies will be needed to show long-term safety in patients started on treatment. An additional challenge is that some CFTR mutations seem to have characteristics of more than one mutation class; eg, Phe508del-*CFTR*, which is associated with both abnormal trafficking (class II) and abnormal chloride channel gating (class IV).[35] Correction of mutations with characteristics of more than one mutation class might need a combination of treatments. Finally, mutation class is not yet known for many of the rare *CFTR* mutations, which makes it difficult to identify the most appropriate treatment strategy.

The CFTR2 project aims to identify the functional and clinical characteristics of all *CFTR* mutations, starting with the most common. Even in its early stage, this database provides a great resource for researchers, clinicians, and patients who want to understand mutation class and potential treatment approaches for specific *CFTR* mutations.

Conclusions

Therapeutic developments suggest that the next few years will be a more exciting time for cystic fibrosis treatments than any other in history. A growing number of patients with cystic fibrosis are likely to benefit from the CFTR potentiator ivacaftor, and a substantially larger number will benefit if corrector treatment is found to be efficacious in patients with Phe508del. This focus on correction of the underlying CFTR defect will reshape

the approach to treatment of cystic fibrosis and provide a road map that can be used for other genetic illnesses.

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