From CFTR biology toward combinatorial pharmacotherapy: expanded classification of cystic fibrosis mutations

Gudio Veit[a], Radu G. Avramescu[a], Annette N. Chiang[b], Scott A. Houck[c], Zhiwei Cai[d], Kathryn W. Peters[e], Jeong S. Hong[f], Harvey B. Pollard[g], William B. Guggino[h], William E. Balch[i], William R. Skach[j], Garry R. Cutting[k], Raymond A. Frizzell[e], David N. Sheppard[d], Douglas M. Cyr[c], Eric J. Sorscher[l], Jeffrey L. Brodsky[b], and Gergely L. Lukacs[a,m,n]

[a]Department of Physiology, [m]Department of Biochemistry, and [n]GRASP, McGill University, Montréal, QC H3G 1Y6, Canada; [b]Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260; [c]Marsico Lung Institute, School of Medicine, University of North Carolina, Chapel Hill, NC 27514; [d]School of Physiology & Pharmacology, University of Bristol, Bristol BS8 1TD, United Kingdom; [e]Department of Cell Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261; [f]Department of Cellular, Developmental, and Integrative Biology, University of Alabama, Birmingham, AL 35294; [g]Department of Anatomy, Physiology and Genetics and Center for Medical Proteomics, Uniformed Services University of the Health Sciences, Bethesda, MD 20814; [h]Department of Physiology and [k]McKusick-Nathans Institute of Genetic Medicine, School of Medicine, Johns Hopkins University, Baltimore, MD 21205; [i]Department of Chemical Physiology, Skaggs Institute of Chemical Physiology, Scripps Research Institute, La Jolla, CA 92037; [j]Department of Biochemistry and Molecular Biology, Oregon Health and Science University, Portland, OR 97239; [l]Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322

ABSTRACT **More than 2000 mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) have been described that confer a range of molecular cell biological and functional phenotypes. Most of these mutations lead to compromised anion conductance at the apical plasma membrane of secretory epithelia and cause cystic fibrosis (CF) with variable disease severity. Based on the molecular phenotypic complexity of CFTR mutants and their susceptibility to pharmacotherapy, it has been recognized that mutations may impose combinatorial defects in CFTR channel biology. This notion led to the conclusion that the combination of pharmacotherapies addressing single defects (e.g., transcription, translation, folding, and/or gating) may show improved clinical benefit over available low-efficacy monotherapies. Indeed, recent phase 3 clinical trials combining ivacaftor (a gating potentiator) and lumacaftor (a folding corrector) have proven efficacious in CF patients harboring the most common mutation (deletion of residue F508, ΔF508, or Phe508del). This drug combination was recently approved by the U.S. Food and Drug Administration for patients homozygous for ΔF508. Emerging studies of the structural, cell biological, and functional defects caused by rare mutations provide a new framework that reveals a mixture of deficiencies in different CFTR alleles. Establishment of a set of combinatorial categories of the previously defined basic defects in CF alleles will aid the design of even more efficacious therapeutic interventions for CF patients.**

Monitoring Editor
David G. Drubin
University of California, Berkeley

Received: Oct 6, 2015
Revised: Nov 12, 2015
Accepted: Nov 23, 2015

DOI:10.1091/mbc.E14-04-0935

Address correspondence to: Gergely L. Lukacs (gergely.lukacs@mcgill.ca).

Abbreviations used: ABC, ATP-binding cassette; CF, cystic fibrosis; CFFT, Cystic Fibrosis Foundation Therapeutics, Inc.; CFTR, cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum; MSD, membrane-spanning domain; NBD, nucleotide-binding domain; PM, plasma membrane; PTC, premature termination codon.

INTRODUCTION

Cystic fibrosis (CF), caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), is characterized by a multiorgan pathology affecting the upper and lower airway, gastrointestinal and reproductive tracts, and endocrine system (Riordan *et al.*, 1989; Collins, 1992; Rowe *et al.*, 2005; Cutting, 2015). CF is one of the most common lethal autosomal-recessive diseases, with a prevalence of one in 3500 in the United States and one in 2500 in the European Union (Farrell, 2008; Pettit and Fellner, 2014). Lack of functional CFTR expression at the apical membrane of secretory epithelia results in defective Cl^- and bicarbonate secretion, coupled to enhanced Na^+ absorption and mucus secretion, which in airway epithelia leads to dehydration and acidification of the airway surface liquid (Tarran *et al.*, 2001; Chen *et al.*, 2010; Derichs *et al.*, 2011; Pezzulo *et al.*, 2012). As a consequence, impaired mucociliary clearance provokes recurrent infection and uncontrolled inflammation culminating in lung damage, which is the primary cause of morbidity and mortality in CF (Ratjen and Doring, 2003; Boucher, 2007; Stoltz *et al.*, 2015). CFTR is member of the ATP-binding cassette (ABC) subfamily C (ABCC7) (Kerr, 2002). It consists of two homologous halves, each containing a hexa-helical membrane-spanning domain (MSD1 and MSD2) and a nucleotide-binding domain (NBD1 and NBD2) that are connected by an unstructured regulatory domain (Riordan, 1993; Riordan *et al.*, 1989).

BIOLOGY OF CFTR MUTATION: TRADITIONAL CLASSIFICATION

CF is caused by ~2000 mutations in the *CFTR* gene with a wide range of disease severity (www.genet.sickkids.on.ca/home.html; www.cftr2.org; Sosnay *et al.*, 2013), which is further influenced by modifier genes (Collaco and Cutting, 2008; Cutting, 2010) and by the environmental and socioeconomic status of patients (Schechter *et al.*, 2001; Barr *et al.*, 2011; Taylor-Robinson *et al.*, 2014; Kopp *et al.*, 2015). The first classification of CF mutations into four classes according to their primary biological defect was proposed by Welsh and Smith in a landmark paper (Welsh and Smith, 1993). Currently, six major classes are distinguished (Rowe *et al.*, 2005; Zielenski and Tsui, 1995) (Figure 1).

Class I encompasses frameshift, splicing, or nonsense mutations that introduce premature termination codons (PTC), resulting in severely reduced or absent CFTR expression.

Class II mutations lead to misfolding, premature degradation by the endoplasmic reticulum (ER) quality-control system, and impaired protein biogenesis, severely reducing the number of CFTR molecules that reach the cell surface.

Class III mutations impair the regulation of the CFTR channel, resulting in abnormal gating characterized by a reduced open probability.

Class IV mutations alter the channel conductance by impeding the ion conduction pore, leading to a reduced unitary conductance (Sheppard *et al.*, 1993; Hammerle *et al.*, 2001).

Class V mutations do not change the conformation of the protein but alter its abundance by introducing promoter or splicing abnormalities (Highsmith *et al.*, 1994, 1997; Zielenski and Tsui, 1995).

Class VI mutations destabilize the channel in post-ER compartments and/or at the plasma membrane (PM), by reducing its conformational stability (Haardt *et al.*, 1999) and/or generating additional internalization signals (Silvis *et al.*, 2003). This results in accelerated PM turnover and reduced apical PM expression (Haardt *et al.*, 1999; Silvis *et al.*, 2003).

For many of the identified mutations, the disease liability is unknown, but efforts are under way to assess their functional consequence and clinical severity (www.cftr2.org; Sosnay *et al.*, 2013).

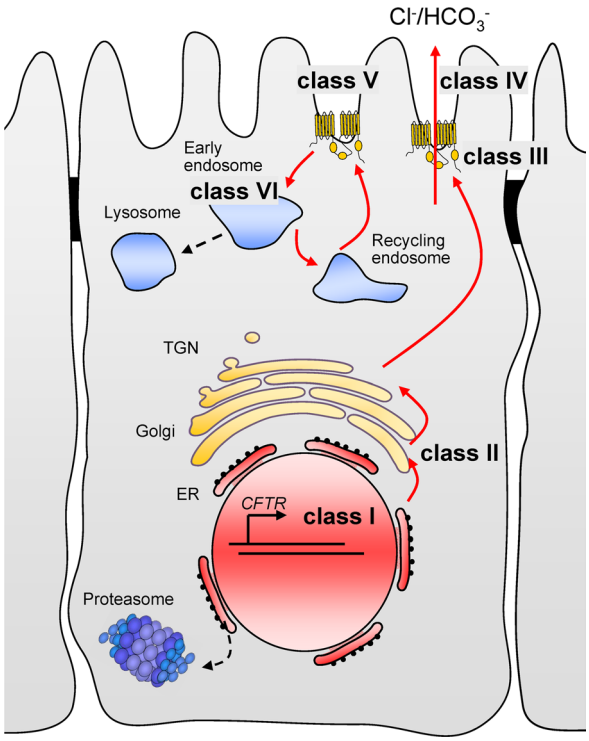

FIGURE 1: Traditional classification of CF mutations based on their cellular phenotype. Class I: protein synthesis defect; class II: maturation defect; class III: gating defect; class IV: conductance defect; class V: reduced quantity; and class VI: reduced stability. ER, endoplasmic reticulum; TGN, *trans*-Golgi network.

MUTATION CLASS–SPECIFIC PHARMACOTHERAPY

Defining the cellular and molecular pathology of CFTR mutations proved to be invaluable for development of small-molecule compounds targeting the underlying defect(s) in CF. The fact that some CFTR variants carrying class III or IV mutations can be expressed at the apical membrane of secretory epithelia at a density similar to that of the wild-type protein, although they are functionally impaired (e.g., G551D), led to the development of gating potentiators that increase the open probability and thereby the PM chloride conductance (Yang *et al.*, 2003). VX-770 (ivacaftor) is the first potentiator drug to be U.S. Food and Drug Administration approved for CF treatment; it directly targets the gating defect of the class III mutation G551D-CFTR (Van Goor *et al.*, 2009). This compound was developed by Vertex Pharmaceuticals in conjunction with Cystic Fibrosis Foundation Therapeutics, Inc. (CFFT), and shows remarkable clinical benefit in patients carrying the mutation in either one or two alleles (Van Goor *et al.*, 2009; Accurso *et al.*, 2010; Ramsey *et al.*, 2011). The approval of VX-770 was extended to eight additional class III mutations (G178R, S549N, S549R, G551S, G1244E, S1251N, S1255P, and G1349D) (Yu *et al.*, 2012; Vertex, 2014a) and recently to the class IV mutation R117H (Vertex, 2014b).

The prototypical class II mutation, ΔF508-CFTR (Phe508del), elicits a complex folding defect that compromises both NBD1 stability and the channel's cooperative domain assembly (Du and Lukacs, 2009; Du *et al.*, 2005; Mendoza *et al.*, 2012; Rabeh *et al.*, 2012). For many years, large-scale efforts have been under way to isolate correctors that act as pharmacological chaperones by directly binding to and promoting the biogenesis of class II CFTR mutations. The most promising corrector compound at present, VX-809 (lumacaftor), partially reverts the ΔF508-CFTR functional expression

defect by stabilizing the NBD1-MSD1/2 interface (Farinha *et al.*, 2013; Loo *et al.*, 2013; Okiyoneda *et al.*, 2013; Ren *et al.*, 2013), leading to a marked correction from 3 to 15% of wild-type channel activity in vitro (Van Goor *et al.*, 2011). A clinical trial, however, failed to observe significant clinical benefit in homozygous ΔF508-CFTR patients (Clancy *et al.*, 2012). Acute addition of VX-770 to VX-809-corrected ΔF508-CFTR doubled the PM activity in vitro (Van Goor *et al.*, 2011), and the combination therapy showed modest but significant clinical improvement (Boyle *et al.*, 2014; Wainwright *et al.*, 2015). Based on these results, the combination treatment has been approved for CF patients 12 years and older with two copies of the ΔF508 mutation (Vertex, 2015). Other class II mutations that can be corrected by VX-809 in vitro include E56K, P67L, E92K, R170G, L206W, V232D, F508G, and A561E (Caldwell *et al.*, 2011; Okiyoneda *et al.*, 2013; Ren *et al.*, 2013; Veit *et al.*, 2014; Awatade *et al.*, 2015).

Ribosomal read-through allows synthesis of full-length CFTR carrying class I mutations. To this end, ataluren (PTC124) was developed as a drug that promotes near-cognate aminoacyl-tRNA incorporation at PTCs (Lentini *et al.*, 2014; Welch *et al.*, 2007). Ataluren partially restores G542X-CFTR (class I) expression in a mouse model and modestly corrects CFTR function in nasal epithelia in patients with class I mutations (Du *et al.*, 2008; Sermet-Gaudelus *et al.*, 2010; Wilschanski *et al.*, 2011). In a recent phase 3 clinical trial, however, ataluren treatment failed to produce significant clinical benefit, perhaps due to an adverse drug–drug interaction with tobramycin, which is a commonly administered, inhaled antibiotic used to treat lung infections in CF patients (Kerem *et al.*, 2014).

LIMITATIONS OF CF MUTATION CLASSIFICATION

The efficacy of available monotherapies for some mutant alleles, which have been designated as class I, class II, or class III/IV mutations, is currently limited. This could be partly explained by the pleiotropic molecular defects caused by single mutations. Thus comprehensive mapping of the multiple molecular defects caused by a single or combination of mutant alleles could offer considerable advantage for improving therapeutic interventions and for future development of drug combinations. In the following list, we present a subset of mutations that display combinatorial molecular defects.

- **ΔF508:** The most prevalent class II mutation impairs CFTR conformational maturation and leads to its targeting for premature ER-associated degradation (Cheng *et al.*, 1990; Cyr, 2005; Kim and Skach, 2012; Lukacs *et al.*, 1994). However, ΔF508-CFTR molecules that either constitutively or following rescue procedures escape the ER quality control and accumulate at the PM of airway epithelia exhibit a channel-gating defect, which is a hallmark of class III mutations (Dalemans *et al.*, 1991), as well as accelerated turnover in post ER compartments and at the PM, a class VI mutation characteristic (Lukacs *et al.*, 1993). Unless the folding and conformational dynamics of the rescued ΔF508-CFTR are fully restored to that of the wild-type protein by pharmacological treatment, this mutation remains partially defective and requires correction of its gating and/or peripheral stability defect. Rescue of the gating defect can be achieved with potentiators (e.g., VX-770) (Van Goor *et al.*, 2009). Peripheral stabilization of the ΔF508-CFTR could be attained by 1) the peptide inhibitor iCAL36 (Cushing *et al.*, 2010), 2) preventing post-Golgi ubiquitination (Fu *et al.*, 2015; Okiyoneda *et al.*, 2010), 3) restoring autophagosome formation (Luciani *et al.*, 2012), or 4) modulating cellular protein homeostasis (Hutt *et al.*, 2010). Thus the most common mutant has multiple defects that extend beyond the features of a class II mutation.
- **W1282X:** This PTC represents a class I mutation, though recent studies suggest a more complex phenotype. First, the level of the W1282X transcript is reduced by nonsense-mediated RNA decay (Hamosh *et al.*, 1992; Linde *et al.*, 2007). Second, the PTC deletes part of the NBD2, which likely compromises NBD1-NBD2 dimerization and W1282X-CFTR folding and activity. Moreover, if the primary defect is corrected either with spontaneous or drug-induced read-through, some of the fully translated channel will contain nonconservative amino acid substitutions. These missense mutations may cause structural defects (class II characteristic), as suggested by the phenotype of CF patients with a missense mutation at the W1282 residue (Faucz *et al.*, 2007; Ivaschenko *et al.*, 1993; Visca *et al.*, 2008), as well as a gating defect (class III characteristic), which can be inferred based on W1282X-CFTR channel activation after exposure to VX-770 (Xue *et al.*, 2014).
- **P67L:** P67L is a mild class II mutation that results in attenuated CFTR biogenesis, as indicated by the reduced ratio between post-ER complex–glycosylated (band C) and ER-resident core-glycosylated protein (band B) (Ren *et al.*, 2013; Sosnay *et al.*, 2013; Van Goor *et al.*, 2014). Treatment with the corrector VX-809 increases the abundance of the complex-glycosylated form and PM density to nearly the level of WT-CFTR (Ren *et al.*, 2013; Veit *et al.*, 2014). However, the mutant channel is also sensitive in vitro to potentiator treatment (a class III characteristic), both in the presence and absence of corrector (Van Goor *et al.*, 2014; Veit *et al.*, 2014). Accordingly, treatment with VX-770 ameliorated the CF lung disease in a heterozygous P67L/ΔF508 patient (Yousef *et al.*, 2015).
- **R117H:** This mutation in conjunction with the 5T variant in the polythymidine tract in intron 8 was originally categorized as a class IV mutation, but it also exhibits a gating defect (class III trait) that, at least in part, can be rectified by VX-770 treatment (Sheppard *et al.*, 1993; Van Goor *et al.*, 2014). The R117H mutation also results in reduced complex-glycosylated CFTR expression, which is a class II characteristic (Fanen *et al.*, 1997; Sheppard *et al.*, 1993). This potentially explains the limited success of VX-770 treatment in patients carrying this mutation (Char *et al.*, 2014; Moss *et al.*, 2015).

AN EXPANDED CLASSIFICATION OF MUTANT CFTR BIOLOGY

We propose a modification of the current classification scheme, which would entail permutations of the traditional class I–VI CF mutations. This expanded classification of the major mechanistic categories (Welsh and Smith, 1993; Zielenski, 2000; Rowe *et al.*, 2005) accommodates the unusually complex, combinatorial molecular/cellular phenotypes of CF alleles. It consists of 31 possible classes of mutations, including the original classes I, II, III/IV, V, and VI, as well as their 26 combinations, as depicted in the Venn diagram shown in Figure 2. For the sake of simplicity, class III and IV mutations, representing functional (gating and conductance, respectively) defects, are combined. For example, according to the expanded classification, G551D will be designated as a class III mutation as before (Welsh and Smith, 1993), while ΔF508 will be classified as class II–III–VI, W1282X as class I–II–III–VI, P67L as class II–III, and R117H as class II–III/IV, reflecting the composite defects in mutant CFTR biology (Figure 2 and Table 1).

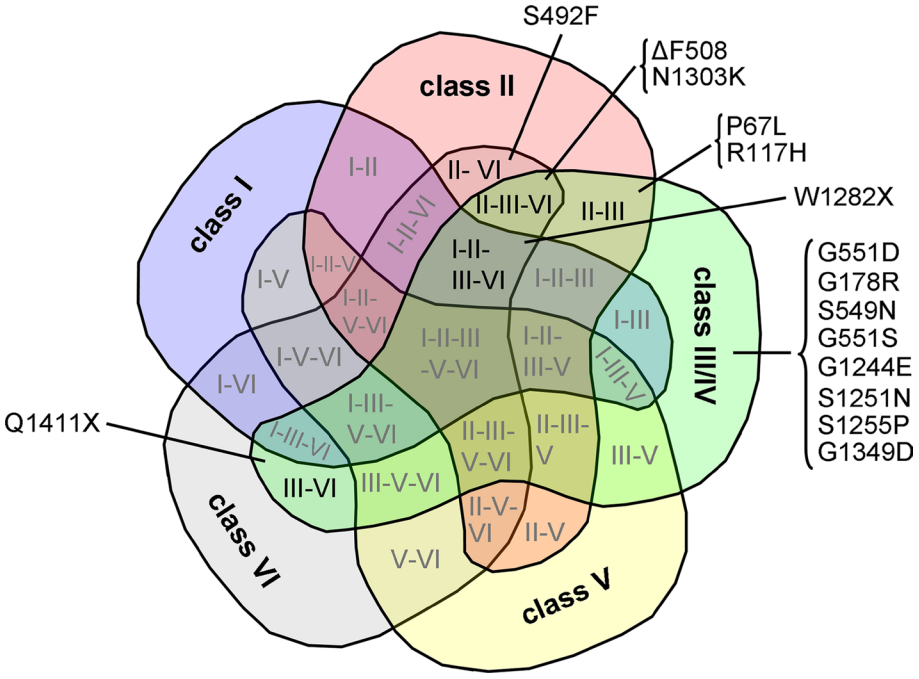

FIGURE 2: Refined classification of CF mutations accounting for complex phenotypes of major CFTR cellular defects. The Venn diagram indicates all combinations of mutation classes with selected examples. Possible combinations without identified mutation are indicated in gray.

A recent study by Vertex Pharmaceuticals successfully demonstrated that 24 of 54 tested missensse mutations display both a processing (class II) and gating (class III) defect in the Fischer rat thyroid epithelial expression system (Van Goor *et al.*, 2014). Characterization of several rare CF mutations is ongoing in laboratories of the CFTR2 Consortium, the CFTR Folding Consortium, CFFT, Vertex Pharmaceuticals, and many others (Caldwell *et al.*, 2011; Yu *et al.*, 2012; Sosnay *et al.*, 2013; Harness-Brumley *et al.*, 2014; Hong *et al.*, 2014; Van Goor *et al.*, 2014; Wang *et al.*, 2014; Awatade *et al.*, 2015). This work will likely provide further examples of combinatorial mechanistic defects exhibited by CF mutants.

THERAPEUTIC SUSCEPTIBILITY OF CF MUTATIONS WITH COMPLEX BIOLOGICAL DEFECTS

In-depth analysis of the biology of CF mutants distinguishes them according to their complex molecular pathology and suggests drug combinations for treatment of different patient populations. This process, called

Refined classification	Mutation	I	II	III/IV	V	VI	Model	Reference
I–II–III–VI	W1282X	$X^{1,2,3}$	$X^{2,5}$	$X^{2,4,5}$		X^{5}	[1]HNE [2]HBE [3]CFBE [4]CFBE [5]CFBE	[1]Hamosh *et al.*, 1992 [2]Cyr lab, unpublished[a] [3]Frizzell lab, unpublished[b] [4]Xue *et al.*, 2014 [5]Lukacs lab, unpublished[c]
II–III	M1V		X^{6}	X^{6}			[6]FRT	[6] Van Goor *et al.*, 2014
II–III	E56K		$X^{5,6}$	X^{6}			[5]CFBE[d] [6]FRT	[5]Lukacs lab, unpublished [6]Van Goor *et al.*, 2014
II–III	P67L		$X^{3,6,7,8,9,10}$	$X^{6,7,10}$			[3]CFBE [6]FRT [7]CFBE [8]Hek293 [9]HeLa [10]FRT	[3]Frizzell lab, unpublished [6]Van Goor *et al.*, 2014 [7]Veit *et al.*, 2014 [8]Ren *et al.*, 2013 [9]Sosnay *et al.*, 2013 [10]Sorscher lab, unpublished[e]
II–III	R74W		X^{6}	X^{6}			[6]FRT	[6]Van Goor *et al.*, 2014
II-III	E92K		$X^{3,5,6,8,16}$	X^{5}			[3]CFBE [5]CFBE [6]FRT [8]HEK293 [16]HEK293	[3]Frizzell lab, unpublished [5]Lukacs lab, unpublished [6]Van Goor *et al.*, 2014 [8]Ren *et al.*, 2013 [16]Brodsky lab, unpublished
II–III	P99L		X^{11}	X^{11}			[11]HeLa	[11]Sheppard *et al.*, 1996
II–III	D110H		X^{6}	X^{6}			[6]FRT	[6]Van Goor *et al.*, 2014
II–III	R117C		X^{6}	X^{6}			[6]FRT	[6]Van Goor *et al.*, 2014
II–III	R117H		$X^{2,3,12,13}$	$X^{2,6,12}$			[2]HBE [3]CFBE [6]FRT [12]FRT, HeLa [13]HeLa	[2]Cyr lab, unpublished [3]Frizzell lab, unpublished [6]Van Goor *et al.*, 2014 [12]Sheppard *et al.*, 1993 [13]Fanen *et al.*, 1997

TABLE 1: Examples for CF mutations with complex or classical cellular phenotypes.

Continues

Refined classification	Mutation	I	II	III/IV	V	VI	Model	Reference
II–III	R170G		X[7,14]	X[7]			[7]CFBE [14]BHK	[7]Veit *et al.*, 2014 [14]Okiyoneda *et al.*, 2013
II–III	E193K		X[5]	X[5,6]			[5]CFBE [6]FRT[f]	[5]Lukacs lab, unpublished [6]Van Goor *et al.*, 2014
II–III	P205S		X[11]	X[11]			[11]HeLa	[11]Sheppard *et al.*, 1996
II–III	L206W		X[5,6,8]	X[5,6]			[5]CFBE [6]FRT [8]HEK293	[5]Lukacs lab, unpublished [6]Van Goor *et al.*, 2014 [8]Ren *et al.*, 2013
II–III	V232D		X[15]	X[15]			[15]HEK293	[15]Caldwell *et al.*, 2011
II–III	R334W		X[2,3,5]	X[2,5,6,12]			[2]COS-7 [3]CFBE [5]CFBE [6]FRT[f] [12]HeLa	[2]Cyr lab, unpublished [3]Frizzell lab, unpublished [5] Lukacs lab, unpublished [6]Van Goor *et al.*, 2014 [12]Sheppard *et al.*, 1993
II–III	I336K		X[6]	X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
II–III	T338I		X[5,6]	X[5,6]			[5]CFBE [6]FRT	[5]Lukacs lab, unpublished [6]Van Goor *et al.*, 2014
II–III	S341P		X[5,6]	X[5,6]			[5]CFBE [6]FRT	[5]Lukacs lab, unpublished [6]Van Goor *et al.*, 2014
II–III	A455E		X[3,6,16, 17]	X[6]			[3]CFBE [6]FRT [16]HEK293 [17]FRT, HeLa[d]	[3]Frizzell lab, unpublished [6]Van Goor *et al.*, 2014 [16]Brodsky lab, unpublished[g] [17]Sheppard *et al.*, 1995
II–III	S549R		X[3,5,18]	X[5,18]			[3]CFBE [5]CFBE [15]FRT	[3]Frizzell lab, unpublished [5]Lukacs lab, unpublished [18]Yu *et al.*, 2012
II–III	D579G		X[5,6]	X[5,6]			[5]CFBE [6]FRT	[5]Lukacs lab, unpublished [6]Van Goor *et al.*, 2014
II–III	R668C		X[6]	X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
II–III	L927P		X[6]	X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
II–III	S945L		X[6]	X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
II–III	S977F		X[6]	X[6]			[6]FRT	[6] Van Goor *et al.*, 2014
II–III	L997F		X[6]	X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
II–III	H1054D		X[6]	X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
II–III	R1066H		X[6]	X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
II–III	A1067T		X[6]	X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
II–III	R1070Q		X[6]	X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
II–III	R1070W		X[6,14]	X[6]			[6]FRT [14]BHK	[6]Van Goor *et al.*, 2014 [14]Okiyoneda *et al.*, 2013
II–III	F1074L		X[6]	X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
II–III	D1270N		X[6]	X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
II–VI	S492F		X[3,5,6]			X[5]	[3]CFBE [5]CFBE [6]FRT	[3]Frizzell lab, unpublished [5]Lukacs lab, unpublished [6]Van Goor *et al.*, 2014
II–III–VI	R347P		X[3,5,6]	X[5,6,12]		X[5]	[3]CFBE [5]CFBE [6]FRT [12]HeLa	[3]Frizzell lab, unpublished [5]Lukacs lab, unpublished [6]Van Goor *et al.*, 2014 [12]Sheppard *et al.*, 1993
II–III–VI	ΔF508		X[19]	X[20]		X[21]	[19]COS [20]Vero [21]CHO	[19]Cheng *et al.*, 1990 [20]Dalemans *et al.*, 1991 [21]Lukacs *et al.*, 1993

TABLE 1: Examples for CF mutations with complex or classical cellular phenotypes.

Continues

Refined classification	Mutation	I	II	III/IV	V	VI	Model	Reference
II–III–VI	A561E		X[6,22,23]	X[23]		X[23]	[6]FRT[d] [22]HBE[d] [23]BHK	[6]Van Goor *et al.*, 2014 [22]Awatade *et al.*, 2015 [23]Wang *et al.*, 2014
II–III–VI	L1077P		X[3,6,16,24]	X[24]		X[24]	[3]CFBE [6]FRT [16]HEK293 [24]CHO	[3]Frizzell lab, unpublished [6]Van Goor *et al.*, 2014 [16]Brodsky lab, unpublished [24]Sheppard lab, unpublished[h]
II–III–VI	N1303K		X[2,3,5,6,16,22,24]	X[24]		X[5]	[2]HBE [3]CFBE [5]CFBE [6]FRT [16]HEK293 [22]HBE [24]CHO[i]	[2]Cyr lab, unpublished [3]Frizzell lab, unpublished [5]Lukacs lab, unpublished [6]Van Goor *et al.*, 2014 [16]Brodsky lab, unpublished [22]Awatade *et al.*, 2015 [24]Sheppard lab, unpublished
III–VI	Q1411X			X[25]		X[26]	[25]BHK [26]Cos, BHK	[25]Gentzsch *et al.*, 2002 [26]Haardt *et al.*, 1999
II	A46D		X[6]				[6]FRT	[6]Van Goor *et al.*, 2014
II	G85E		X[3,6,16,24]				[3]CFBE [6]FRT [16]HEK293 [24]CHO	[3]Frizzell lab, unpublished [6]Van Goor *et al.*, 2014 [16]Brodsky lab, unpublished [24]Sheppard lab, unpublished
III	R352Q			X[5,6]			[5]CFBE [6]FRT[f]	[5]Lukacs lab, unpublished [6]Van Goor *et al.*, 2014
II	L467P		X[6]				[6]FRT	[6]Van Goor *et al.*, 2014
II	V520F		X[3,6,16]				[3]CFBE [6]FRT [16]HEK293	[3]Frizzell lab, unpublished [6]Van Goor *et al.*, 2014 [16]Brodsky lab, unpublished
II	A559T		X[6]				[6]FRT	[6]Van Goor *et al.*, 2014
II	R560S		X[6]				[6]FRT	[6]Van Goor *et al.*, 2014
II	R560T		X[3,6,16]				[3]CFBE [6]FRT [16]HEK293	[3]Frizzell lab, unpublished [6]Van Goor *et al.*, 2014 [16]Brodsky lab, unpublished
II	R560K		X[3]				[3]CFBE	[3]Frizzell lab, unpublished
II	Y569D		X[6]				[6]FRT	[6]Van Goor *et al.*, 2014
II	D614G		X[3]				[3]CFBE	[3]Frizzell lab, unpublished
II	L1065P		X[3,6]				[3]CFBE [6]FRT	[3]Frizzell lab, unpublished [6]Van Goor *et al.*, 2014
II	R1066C		X[3,6]				[3]CFBE [6]FRT	[3]Frizzell lab, unpublished [6]Van Goor *et al.*, 2014
II	R1066M		X[6]				[6]FRT	[6]Van Goor *et al.*, 2014
II	H1085R		X[6]				[6]FRT	[6]Van Goor *et al.*, 2014
II	M1101K		X[6]				[6]FRT	[6]Van Goor *et al.*, 2014
III	D110E			X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
III	G178R			X[18]			[18]FRT	[18]Yu *et al.*, 2012
III	R347H			X[6,7]			[6]FRT [7]CFBE	[6]Van Goor *et al.*, 2014 [7]Veit *et al.*, 2014
III	S549N			X[18]			[18]FRT	[18]Yu *et al.*, 2012
III	G551D			X[27, 28]			[27]CHO [28]L	[27]Bompadre *et al.*, 2007 [28]Yang *et al.*, 1993
III	G551S			X[18]			[18]FRT	[18]Yu *et al.*, 2012

TABLE 1: Examples for CF mutations with complex or classical cellular phenotypes.

Continues

Refined classification	Mutation	I	II	III/IV	V	VI	Model	Reference
III	F1052V			X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
III	K1060T			X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
III	D1152H			X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
III	S1235R			X[6]			[6]FRT	[6]Van Goor *et al.*, 2014
III	G1244E			X[18]			[18]FRT	[18]Yu *et al.*, 2012
III	S1251N			X[18]			[18]FRT	[18]Yu *et al.*, 2012
III	S1255P			X[18]			[18]FRT	[18]Yu *et al.*, 2012
III	G1349D			X[18,27]			[18]FRT [27]CHO	[18]Yu *et al.*, 2012 [27]Bompadre *et al.*, 2007

Superscript numbers refer to references in far-right column.
[a]S.A.H. and D.M.C., unpublished observations
[b]K.W.P. and R.A.F., unpublished observations.
[c]R.G.A., H.Xu, and G.L.L., unpublished observations.
[d]Does not exhibit a gating or conductance defect in this cell model.
[e]J.S.H. and E.J.S., unpublished observations.
[f]Does not exhibit a biogenesis defect in this cell model.
[g]A.N.C. and J.L.B., unpublished observations.
[h]Z.C. and D.N.S., unpublished observations.
[i]Does not exhibit a peripheral stability defect in this cell model.

TABLE 1: Examples for CF mutations with complex or classical cellular phenotypes. Continued

"theratyping" (Cutting, 2015), will pave the way to personalized medicine in CF. However, reliable prediction of the responsiveness of a mutant phenotype to pharmacotherapy could be challenging and is dependent on the cellular model system (Pedemonte *et al.*, 2010).

Emerging evidence also suggests that the efficacy of approved and preclinical drugs may vary with different mutations within the same class. For example, while nearly complete processing correction of P67L- and R170G-CFTR (class II) was achieved with VX-809 treatment (Okiyoneda *et al.*, 2013; Ren *et al.*, 2013; Veit *et al.*, 2014), VX-809 only partially reversed the folding defect of some other class II mutants; for example, N1303K and ΔF508 (Okiyoneda *et al.*, 2013; Awatade *et al.*, 2015). This differential susceptibility to correction is attributed to the nature of the primary folding/structural defect. According to one hypothesis, robust folding correction of ΔF508-CFTR requires corrector combinations to avert its NBD1-MSD1/2 interface and NBD1 stability defects (Mendoza *et al.*, 2012; Rabeh *et al.*, 2012; He *et al.*, 2013; Okiyoneda *et al.*, 2013). The N1303K mutation in NBD2 was not rescued by VX-809, and only modest processing was observed by targeting both the NBD1/MSDs and NBD2 interfaces with C4 and C18 (a VX-809 analogue) (Okiyoneda *et al.*, 2013; Rapino *et al.*, 2015).

Some of the class III mutations also respond differently to the gating potentiator VX-770. Although R347H- and T338I-CFTR cause severe functional defects with no or modest loss of protein expression, only R347H-CFTR is potentiated by VX-770 to near wild type–like conductance (Van Goor *et al.*, 2014). Likewise, the P5 potentiator activates ΔF508-CFTR, but it has no effect on G551D-CFTR chloride permeation (Yang *et al.*, 2003). Thus identification of mutation-specific novel potentiators or their combinations may further optimize channel rescue for specific class III/IV mutations. Additive enhancement of G551D-CFTR activity by the combination of the potentiators genistein and curcumin supports the feasibility of combining potentiators (Yu *et al.*, 2011). Likewise, we envision that mutation-specific read-through drugs will ultimately need to be combined with other correctors and potentiators, based on the pleiotropic defects associated with this class of mutations (as illustrated for W1282X above).

CONCLUDING REMARKS

The ultimate goal of theratyping is to achieve optimal correction of a specific mutant defect by selecting the most efficacious CFTR modulator(s), including corrector(s), potentiator(s), and/or read-through drugs, or a combination of these drugs. Based on accumulating observations, however, mechanistic subdivisions of some of the major classes of mutations (classes I, II, and III) may be necessary to further improve the success of drug-selection strategies. This will facilitate the theratyping of CF alleles and their combinations and expedite the identification and approval process for combination therapies. Theratyping has already proven successful in identifying class III mutations that are responsive to VX-770 (Yu *et al.*, 2012), leading to the approval of this drug for eight rare mutations besides G551D (Vertex, 2014a). In fact, the results of large-scale theratyping could be overlaid as a third dimension on the Venn diagram presented in Figure 2.

Thus, during the 22 years following the initial classification of CF mutations (Welsh and Smith, 1993), our understanding of the molecular complexity of CF alleles has evolved remarkably, establishing the need for an advanced mutation classification scheme in conjunction with personalized CF therapy.

ACKNOWLEDGMENTS

We thank the members of the CFTR Folding Consortium, the CFTR Theratype Group, C. M. Penland, and K. Tuggle (Cystic Fibrosis Foundation, Bethesda, MD) for their valuable support. The work described here was supported by the following institutions and grants: National Institutes of Health (NIH) NO1-HL28187 and IAA-A-HL-14-007.001 to H.B.P.; Cystic Fibrosis Foundation (CFF), NIH DK51870, TRDRP23RT-0012, and HL095524 to W.E.B.; NIH R01 DK, CFF CUTT13A1, and CUTTXX0 to G.R.C.; CFFT SHEPPA14XX0 and Cystic Fibrosis Trust to D.N.S.; NIH R01-DK068196, P30-DK072506,

and CFFT FRIZZE05X0 to R.A.F.; NIH RO1 GM56981 and CFFT CYR13XX0 to D.M.C.; the CFF Research Development Program, CFFT SORSCH05XXO, and SORSCH14XXO to E.J.S.; CFFT BRODSK13XX0 and NIH GM75061 to J.L.B.; CF Canada, CFFT Lukacs13XXO, NIH DK075302, and Canadian Institutes of Health Research to G.L.L. R.G.A. was supported by CF Canada Studentship; G.L.L. is a recipient of a Canada Research Chair.

REFERENCES

Accurso FJ, Rowe SM, Clancy JP, Boyle MP, Dunitz JM, Durie PR, Sagel SD, Hornick DB, Konstan MW, Donaldson SH, *et al.* (2010). Effect of VX-770 in persons with cystic fibrosis and the G551D-CFTR mutation. N Engl J Med 363, 1991–2003.

Awatade NT, Uliyakina I, Farinha CM, Clarke LA, Mendes K, Sole A, Pastor J, Ramos MM, Amaral MD (2015). Measurements of functional responses in human primary lung cells as a basis for personalized therapy for cystic fibrosis. EBioMedicine 2, 147–153.

Barr HL, Britton J, Smyth AR, Fogarty AW (2011). Association between socioeconomic status, sex, and age at death from cystic fibrosis in England and Wales (1959 to 2008): cross sectional study. Br Med J 343, d4662.

Bompadre SG, Sohma Y, Li M, Hwang TC (2007). G551D and G1349D, two CF-associated mutations in the signature sequences of CFTR, exhibit distinct gating defects. J Gen Physiol 129, 285–298.

Boucher RC (2007). Airway surface dehydration in cystic fibrosis: pathogenesis and therapy. Annu Rev Med 58, 157–170.

Boyle MP, Bell SC, Konstan MW, McColley SA, Rowe SM, Rietschel E, Huang X, Waltz D, Patel NR, Rodman D (2014). A CFTR corrector (lumacaftor) and a CFTR potentiator (ivacaftor) for treatment of patients with cystic fibrosis who have a phe508del *CFTR* mutation: a phase 2 randomised controlled trial. Lancet Respir Med 2, 527–538.

Caldwell RA, Grove DE, Houck SA, Cyr DM (2011). Increased folding and channel activity of a rare cystic fibrosis mutant with CFTR modulators. Am J Physiol Lung Cell Mol Physiol 301, L346–L352.

Char JE, Wolfe MH, Cho HJ, Park IH, Jeong JH, Frisbee E, Dunn C, Davies Z, Milla C, Moss RB, *et al.* (2014). A little CFTR goes a long way: CFTR-dependent sweat secretion from G551D and R117H-5T cystic fibrosis subjects taking ivacaftor. PLoS One 9, e88564.

Chen JH, Stoltz DA, Karp PH, Ernst SE, Pezzulo AA, Moninger TO, Rector MV, Reznikov LR, Launspach JL, Chaloner K, *et al.* (2010). Loss of anion transport without increased sodium absorption characterizes newborn porcine cystic fibrosis airway epithelia. Cell 143, 911–923.

Cheng SH, Gregory RJ, Marshall J, Paul S, Souza DW, White GA, O'Riordan CR, Smith AE (1990). Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell 63, 827–834.

Clancy JP, Rowe SM, Accurso FJ, Aitken ML, Amin RS, Ashlock MA, Ballmann M, Boyle MP, Bronsveld I, Campbell PW, *et al.* (2012). Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the *F508del-CFTR* mutation. Thorax 67, 12–18.

Collaco JM, Cutting GR (2008). Update on gene modifiers in cystic fibrosis. Curr Opin Pulm Med 14, 559–566.

Collins FS (1992). Cystic fibrosis: molecular biology and therapeutic implications. Science 256, 774–779.

Cushing PR, Vouilleme L, Pellegrini M, Boisguerin P, Madden DR (2010). A stabilizing influence: CAL PDZ inhibition extends the half-life of ΔF508-CFTR. Angew Chem Int Ed Engl 49, 9907–9911.

Cutting GR (2010). Modifier genes in Mendelian disorders: the example of cystic fibrosis. Ann NY Acad Sci 1214, 57–69.

Cutting GR (2015). Cystic fibrosis genetics: from molecular understanding to clinical application. Nat Rev Genet 16, 45–56.

Cyr DM (2005). Arrest of CFTR ΔF508 folding. Nat Struct Mol Biol 12, 2–3.

Dalemans W, Barbry P, Champigny G, Jallat S, Dott K, Dreyer D, Crystal RG, Pavirani A, Lecocq JP, Lazdunski M (1991). Altered chloride ion channel kinetics associated with the ΔF508 cystic fibrosis mutation. Nature 354, 526–528.

Derichs N, Jin BJ, Song Y, Finkbeiner WE, Verkman AS (2011). Hyperviscous airway periciliary and mucous liquid layers in cystic fibrosis measured by confocal fluorescence photobleaching. FASEB J 25, 2325–2332.

Du K, Lukacs GL (2009). Cooperative assembly and misfolding of CFTR domains in vivo. Mol Biol Cell 20, 1903–1915.

Du K, Sharma M, Lukacs GL (2005). The ΔF508 cystic fibrosis mutation impairs domain-domain interactions and arrests post-translational folding of CFTR. Nat Struct Mol Biol 12, 17–25.

Du M, Liu X, Welch EM, Hirawat S, Peltz SW, Bedwell DM (2008). PTC124 is an orally bioavailable compound that promotes suppression of the human *CFTR*-G542X nonsense allele in a CF mouse model. Proc Natl Acad Sci USA 105, 2064–2069.

Fanen P, Labarthe R, Garnier F, Benharouga M, Goossens M, Edelman A (1997). Cystic fibrosis phenotype associated with pancreatic insufficiency does not always reflect the cAMP-dependent chloride conductive pathway defect. Analysis of C225R-CFTR and R1066C-CFTR. J Biol Chem 272, 30563–30566.

Farinha CM, King-Underwood J, Sousa M, Correia AR, Henriques BJ, Roxo-Rosa M, Da Paula AC, Williams J, Hirst S, Gomes CM, Amaral MD (2013). Revertants, low temperature, and correctors reveal the mechanism of F508del-CFTR rescue by VX-809 and suggest multiple agents for full correction. Chem Biol 20, 943–955.

Farrell PM (2008). The prevalence of cystic fibrosis in the European Union. J Cyst Fibros 7, 450–453.

Faucz FR, Gimenez J, Ramos MD, Pereira-Ferrari L, Estivill X, Raskin S, Casals T, Culpi L (2007). Cystic fibrosis in a southern Brazilian population: characteristics of 90% of the alleles. Clin Genet 72, 218–223.

Fu L, Rab A, Tang L, Bebok Z, Rowe SM, Bartoszewski R, Collawn JF (2015). ΔF508 CFTR surface stability is regulated by DAB2 and CHIP-mediated ubiquitination in post-endocytic compartments. PLoS One 10, e0123131.

Gentzsch M, Aleksandrov A, Aleksandrov L, Riordan JR (2002). Functional analysis of the C-terminal boundary of the second nucleotide binding domain of the cystic fibrosis transmembrane conductance regulator and structural implications. Biochem J 366, 541–548.

Haardt M, Benharouga M, Lechardeur D, Kartner N, Lukacs GL (1999). C-terminal truncations destabilize the cystic fibrosis transmembrane conductance regulator without impairing its biogenesis. A novel class of mutation. J Biol Chem 274, 21873–21877.

Hammerle MM, Aleksandrov AA, Riordan JR (2001). Disease-associated mutations in the extracytoplasmic loops of cystic fibrosis transmembrane conductance regulator do not impede biosynthetic processing but impair chloride channel stability. J Biol Chem 276, 14848–14854.

Hamosh A, Rosenstein BJ, Cutting GR (1992). CFTR nonsense mutations G542X and W1282X associated with severe reduction of CFTR mRNA in nasal epithelial cells. Hum Mol Genet 1, 542–544.

Harness-Brumley C, Millen L, Huerta C, Schmidt A, Wigley W, Sosnay PR, Cutting GR, Thomas PJ (2014). Groups of Cftr2 disease-causing mutations that respond to specific modulators. Pediatr Pulmonol 49, 220–220.

He L, Kota P, Aleksandrov AA, Cui L, Jensen T, Dokholyan NV, Riordan JR (2013). Correctors of ΔF508 CFTR restore global conformational maturation without thermally stabilizing the mutant protein. FASEB J 27, 536–545.

Highsmith WE, Burch LH, Zhou Z, Olsen JC, Boat TE, Spock A, Gorvoy JD, Quittel L, Friedman KJ, Silverman LM, *et al.* (1994). A novel mutation in the cystic fibrosis gene in patients with pulmonary disease but normal sweat chloride concentrations. N Engl J Med 331, 974–980.

Highsmith WE Jr., Burch LH, Zhou Z, Olsen JC, Strong TV, Smith T, Friedman KJ, Silverman LM, Boucher RC, Collins FS, Knowles MR (1997). Identification of a splice site mutation (2789 +5 G > A) associated with small amounts of normal CFTR mRNA and mild cystic fibrosis. Hum Mutat 9, 332–338.

Hong JS, Mahiou J, Liang F, Bihler HJ, Mense M, Lukacs GL, Wen H, Sorscher EJ (2014). Epithelial models encoding diverse Cftr2 mutations for studies of disease mechanism and drug discovery. Pediatr Pulmonol 49, 277–278.

Hutt DM, Herman D, Rodrigues AP, Noel S, Pilewski JM, Matteson J, Hoch B, Kellner W, Kelly JW, Schmidt A, *et al.* (2010). Reduced histone deacetylase 7 activity restores function to misfolded CFTR in cystic fibrosis. Nat Chem Biol 6, 25–33.

Ivaschenko TE, Baranov VS, Dean M (1993). Two new mutations detected by single-strand conformation polymorphism analysis in cystic fibrosis from Russia. Hum Genet 91, 63–65.

Kerem E, Konstan MW, De Boeck K, Accurso FJ, Sermet-Gaudelus I, Wilschanski M, Elborn JS, Melotti P, Bronsveld I, Fajac I, *et al.* (2014). Ataluren for the treatment of nonsense-mutation cystic fibrosis: a randomised, double-blind, placebo-controlled phase 3 trial. Lancet Respir Med 2, 539–547.

Kerr ID (2002). Structure and association of ATP-binding cassette transporter nucleotide-binding domains. Biochim Biophys Acta 1561, 47–64.

Kim SJ, Skach WR (2012). Mechanisms of CFTR Folding at the endoplasmic reticulum. Front Pharmacol 3, 201.

Kopp BT, Sarzynski L, Khalfoun S, Hayes D Jr., Thompson R, Nicholson L, Long F, Castile R, Groner J (2015). Detrimental effects of secondhand smoke exposure on infants with cystic fibrosis. Pediatr Pulmonol 50, 25–34.

Lentini L, Melfi R, Di Leonardo A, Spinello A, Barone G, Pace A, Palumbo Piccionello A, Pibiri I (2014). Toward a rationale for the PTC124 (Ataluren) promoted readthrough of premature stop codons: a computational approach and GFP-reporter cell-based assay. Mol Pharm 11, 653–664.

Linde L, Boelz S, Nissim-Rafinia M, Oren YS, Wilschanski M, Yaacov Y, Virgilis D, Neu-Yilik G, Kulozik AE, Kerem E, Kerem B (2007). Nonsense-mediated mRNA decay affects nonsense transcript levels and governs response of cystic fibrosis patients to gentamicin. J Clin Invest 117, 683–692.

Loo TW, Bartlett MC, Clarke DM (2013). Corrector VX-809 stabilizes the first transmembrane domain of CFTR. Biochem Pharmacol 86, 612–619.

Luciani A, Villella VR, Esposito S, Gavina M, Russo I, Silano M, Guido S, Pettoello-Mantovani M, Carnuccio R, Scholte B, *et al.* (2012). Targeting autophagy as a novel strategy for facilitating the therapeutic action of potentiators on ΔF508 cystic fibrosis transmembrane conductance regulator. Autophagy 8, 1657–1672.

Lukacs GL, Chang XB, Bear C, Kartner N, Mohamed A, Riordan JR, Grinstein S (1993). The ΔF508 mutation decreases the stability of cystic fibrosis transmembrane conductance regulator in the plasma membrane. Determination of functional half-lives on transfected cells. J Biol Chem 268, 21592–21598.

Lukacs GL, Mohamed A, Kartner N, Chang XB, Riordan JR, Grinstein S (1994). Conformational maturation of CFTR but not its mutant counterpart (ΔF508) occurs in the endoplasmic reticulum and requires ATP. EMBO J 13, 6076–6086.

Mendoza JL, Schmidt A, Li Q, Nuvaga E, Barrett T, Bridges RJ, Feranchak AP, Brautigam CA, Thomas PJ (2012). Requirements for efficient correction of ΔF508 CFTR revealed by analyses of evolved sequences. Cell 148, 164–174.

Moss RB, Flume PA, Elborn JS, Cooke J, Rowe SM, McColley SA, Rubenstein RC, Higgins M (2015). Efficacy and safety of ivacaftor in patients with cystic fibrosis who have an Arg117His-CFTR mutation: a double-blind, randomised controlled trial. Lancet Respir Med 3, 524–533.

Okiyoneda T, Barriere H, Bagdany M, Rabeh WM, Du K, Hohfeld J, Young JC, Lukacs GL (2010). Peripheral protein quality control removes unfolded CFTR from the plasma membrane. Science 329, 805–810.

Okiyoneda T, Veit G, Dekkers JF, Bagdany M, Soya N, Xu H, Roldan A, Verkman AS, Kurth M, Simon A, *et al.* (2013). Mechanism-based corrector combination restores ΔF508-CFTR folding and function. Nat Chem Biol 9, 444–454.

Pedemonte N, Tomati V, Sondo E, Galietta LJ (2010). Influence of cell background on pharmacological rescue of mutant CFTR. Am J Physiol Cell Physiol 298, C866–C874.

Pettit RS, Fellner C (2014). CFTR modulators for the treatment of cystic fibrosis. P T 39, 500–511.

Pezzulo AA, Tang XX, Hoegger MJ, Alaiwa MH, Ramachandran S, Moninger TO, Karp PH, Wohlford-Lenane CL, Haagsman HP, van Eijk M, *et al.* (2012). Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. Nature 487, 109–113.

Rabeh WM, Bossard F, Xu H, Okiyoneda T, Bagdany M, Mulvihill CM, Du K, di Bernardo S, Liu Y, Konermann L, *et al.* (2012). Correction of both NBD1 energetics and domain interface is required to restore ΔF508 CFTR folding and function. Cell 148, 150–163.

Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Drevinek P, Griese M, McKone EF, Wainwright CE, Konstan MW, *et al.* (2011). A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N Engl J Med 365, 1663–1672.

Rapino D, Sabirzhanova I, Lopes-Pacheco M, Grover R, Guggino WB, Cebotaru L (2015). Rescue of NBD2 mutants N1303K and S1235R of CFTR by small-molecule correctors and transcomplementation. PLoS One 10, e0119796.

Ratjen F, Doring G (2003). Cystic fibrosis. Lancet 361, 681–689.

Ren HY, Grove DE, De La Rosa O, Houck SA, Sopha P, Van Goor F, Hoffman BJ, Cyr DM (2013). VX-809 corrects folding defects in cystic fibrosis transmembrane conductance regulator protein through action on membrane-spanning domain 1. Mol Biol Cell 24, 3016–3024.

Riordan JR (1993). The cystic fibrosis transmemebrane conductance regulator. Annu Rev Physiol 55, 609–630.

Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, *et al.* (1989). Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 245, 1066–1073.

Rowe SM, Miller S, Sorscher EJ (2005). Cystic fibrosis. N Engl J Med 352, 1992–2001.

Schechter MS, Shelton BJ, Margolis PA, Fitzsimmons SC (2001). The association of socioeconomic status with outcomes in cystic fibrosis patients in the United States. Am J Respir Crit Care Med 163, 1331–1337.

Sermet-Gaudelus I, Boeck KD, Casimir GJ, Vermeulen F, Leal T, Mogenet A, Roussel D, Fritsch J, Hanssens L, Hirawat S, *et al.* (2010). Ataluren (PTC124) induces cystic fibrosis transmembrane conductance regulator protein expression and activity in children with nonsense mutation cystic fibrosis. Am J Respir Crit Care Med 182, 1262–1272.

Sheppard DN, Ostedgaard LS, Winter MC, Welsh MJ (1995). Mechanism of dysfunction of two nucleotide binding domain mutations in cystic fibrosis transmembrane conductance regulator that are associated with pancreatic sufficiency. EMBO J 14, 876–883.

Sheppard DN, Rich DP, Ostedgaard LS, Gregory RJ, Smith AE, Welsh MJ (1993). Mutations in CFTR associated with mild-disease-form Cl^- channels with altered pore properties. Nature 362, 160–164.

Sheppard DN, Travis SM, Ishihara H, Welsh MJ (1996). Contribution of proline residues in the membrane-spanning domains of cystic fibrosis transmembrane conductance regulator to chloride channel function. J Biol Chem 271, 14995–15001.

Silvis MR, Picciano JA, Bertrand C, Weixel K, Bridges RJ, Bradbury NA (2003). A mutation in the cystic fibrosis transmembrane conductance regulator generates a novel internalization sequence and enhances endocytic rates. J Biol Chem 278, 11554–11560.

Sosnay PR, Siklosi KR, Van Goor F, Kaniecki K, Yu H, Sharma N, Ramalho AS, Amaral MD, Dorfman R, Zielenski J, *et al.* (2013). Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. Nat Genet 45, 1160–1167.

Stoltz DA, Meyerholz DK, Welsh MJ (2015). Origins of cystic fibrosis lung disease. N Engl J Med 372, 351–362.

Tarran R, Grubb BR, Parsons D, Picher M, Hirsh AJ, Davis CW, Boucher RC (2001). The CF salt controversy: in vivo observations and therapeutic approaches. Mol Cell 8, 149–158.

Taylor-Robinson DC, Thielen K, Pressler T, Olesen HV, Diderichsen F, Diggle PJ, Smyth R, Whitehead M (2014). Low socioeconomic status is associated with worse lung function in the Danish cystic fibrosis population. Eur Respir J 44, 1363–1366.

Van Goor F, Hadida S, Grootenhuis PD, Burton B, Cao D, Neuberger T, Turnbull A, Singh A, Joubran J, Hazlewood A, *et al.* (2009). Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. Proc Natl Acad Sci USA 106, 18825–18830.

Van Goor F, Hadida S, Grootenhuis PD, Burton B, Stack JH, Straley KS, Decker CJ, Miller M, McCartney J, Olson ER, *et al.* (2011). Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. Proc Natl Acad Sci USA 108, 18843–18848.

Van Goor F, Yu H, Burton B, Hoffman BJ (2014). Effect of ivacaftor on CFTR forms with missense mutations associated with defects in protein processing or function. J Cyst Fibros 13, 29–36.

Veit G, Avramescu RG, Perdomo D, Phuan PW, Bagdany M, Apaja PM, Borot F, Szollosi D, Wu YS, Finkbeiner WE, *et al.* (2014). Some gating potentiators, including VX-770, diminish ΔF508-CFTR functional expression. Sci Transl Med 6, 246ra97.

Vertex (2014a). U.S. Food and Drug Administration approves KALYDECO™ (ivacaftor) for use in eight additional mutations that cause cystic fibrosis. Press release, February 21, 2014. http://investors.vrtx.com/releasedetail.cfm?ReleaseID=827435 (accessed 1 October 2015).

Vertex (2014b). U.S. Food and Drug Administration approves KALYDECO® (ivacaftor) for use in people with cystic fibrosis ages 6 and older who have the R117H Mutation. Press release, December 29, 2014. http://investors.vrtx.com/releasedetail.cfm?ReleaseID=889027 (accessed 1 October 2015).

Vertex (2015). FDA approves ORKAMBI™ (lumacaftor/ivacaftor)—the first medicine to treat the underlying cause of cystic fibrosis for people ages 12 and older with two copies of the F508del mutation. Press release, July 2, 2015. http://investors.vrtx.com/releasedetail.cfm?ReleaseID=920512 (accessed 1 October 2015).

Visca A, Bishop CT, Hilton SC, Hudson VM (2008). Improvement in clinical markers in CF patients using a reduced glutathione regimen: an uncontrolled, observational study. J Cyst Fibros 7, 433–436.

Wainwright CE, Elborn JS, Ramsey BW, Marigowda G, Huang X, Cipolli M, Colombo C, Davies JC, De Boeck K, Flume PA, *et al.* (2015).

Lumacaftor-ivacaftor in patients with cystic fibrosis homozygous for Phe508del CFTR. N Engl J Med 373, 220–231.
Wang Y, Liu J, Loizidou A, Bugeja LA, Warner R, Hawley BR, Cai Z, Toye AM, Sheppard DN, Li H (2014). CFTR potentiators partially restore channel function to A561E-CFTR, a cystic fibrosis mutant with a similar mechanism of dysfunction as F508del-CFTR. Br J Pharmacol 171, 4490–4503.
Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, Trifillis P, Paushkin S, Patel M, Trotta CR, Hwang S, *et al*. (2007). PTC124 targets genetic disorders caused by nonsense mutations. Nature 447, 87–91.
Welsh MJ, Smith AE (1993). Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell 73, 1251–1254.
Wilschanski M, Miller LL, Shoseyov D, Blau H, Rivlin J, Aviram M, Cohen M, Armoni S, Yaakov Y, Pugatsch T, *et al*. (2011). Chronic ataluren (PTC124) treatment of nonsense mutation cystic fibrosis. Eur Respir J 38, 59–69.
Xue X, Mutyam V, Tang L, Biswas S, Du M, Jackson LA, Dai Y, Belakhov V, Shalev M, Chen F, *et al*. (2014). Synthetic aminoglycosides efficiently suppress cystic fibrosis transmembrane conductance regulator nonsense mutations and are enhanced by ivacaftor. Am J Respir Cell Mol Biol 50, 805–816.
Yang H, Shelat AA, Guy RK, Gopinath VS, Ma T, Du K, Lukacs GL, Taddei A, Folli C, Pedemonte N, *et al*. (2003). Nanomolar affinity small molecule correctors of defective ΔF508-CFTR chloride channel gating. J Biol Chem 278, 35079–35085.
Yang Y, Devor DC, Engelhardt JF, Ernst SA, Strong TV, Collins FS, Cohn JA, Frizzell RA, Wilson JM (1993). Molecular basis of defective anion transport in L cells expressing recombinant forms of CFTR. Hum Mol Genet 2, 1253–1261.
Yousef S, Solomon GM, Brody A, Rowe SM, Colin AA (2015). Improved clinical and radiographic outcomes after treatment with ivacaftor in a young adult with cystic fibrosis with the P67L CFTR mutation. Chest 147, e79–e82.
Yu H, Burton B, Huang CJ, Worley J, Cao D, Johnson JP Jr., Urrutia A, Joubran J, Seepersaud S, Sussky K, Hoffman BJ, Van Goor F (2012). Ivacaftor potentiation of multiple CFTR channels with gating mutations. J Cyst Fibros 11, 237–245.
Yu YC, Miki H, Nakamura Y, Hanyuda A, Matsuzaki Y, Abe Y, Yasui M, Tanaka K, Hwang TC, Bompadre SG, Sohma Y (2011). Curcumin and genistein additively potentiate G551D-CFTR. J Cyst Fibros 10, 243–252.
Zielenski J (2000). Genotype and phenotype in cystic fibrosis. Respiration 67, 117–133.
Zielenski J, Tsui LC (1995). Cystic fibrosis: genotypic and phenotypic variations. Annu Rev Genet 29, 777–807.