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Teaser Stem cell-based organoid assays, enthusiastically used for genetic analysis of diverse CFTR mutations, are aiming to identify drug responders from patients with cystic fibrosis (CF) in vitro. The organoid response profiles will facilitate effective therapies for individual patients with CF.

Pharmacological analysis of CFTR variants of cystic fibrosis using stem cell-derived organoids

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Cystic fibrosis (CF) is a life-shortening genetic disease caused by mutations of *CFTR*, the gene encoding cystic fibrosis transmembrane conductance regulator. Despite considerable progress in CF therapies, targeting specific CFTR genotypes based on small molecules has been hindered because of the substantial genetic heterogeneity of CFTR mutations in patients with CF, which is difficult to assess by animal models *in vivo*. There are broadly four classes (e.g., II, III, and IV) of CF genotypes that differentially respond to current CF drugs (e.g., VX-770 and VX-809). In this review, we shed light on the pharmacogenomics of diverse CFTR mutations and the emerging role of stem cell-based organoids in predicting the CF drug response. We discuss mechanisms that underlie differential CF drug responses both in organoid-based assays and in CF clinical trials, thereby facilitating the precision design of safer and more effective therapies for individual patients with CF.

Introduction

CF, a lethal genetic disorder, affects ~85,000 individuals worldwide (www.cfww.org). Before 2012, managements of patients with CF mainly focused on improving symptoms, controlling infections, and ameliorating lung function [1,2]. Fortunately, a Phase III clinical trial demonstrated that the potentiator drug VX-770 (known as ivacaftor) was successful in patients with the CFTR-G551D mutation and proved the feasibility of using mutation-specific drugs for molecular intervention in CFTR mutations [3–6]. Results from CF clinical trials indicated that treatment with VX-770 resulted in improved CF symptoms, lung function, and quality of life. Furthermore,

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Kevin G. Chen Kevin Chen obtained his medical degree from Sichuan Medical College of Sichuan University. He finished his medical residence (in 1985) and Oral and Head-Neck Surgery training (in 1988) at the Second Affiliated Hospital and the Faculty of Stomatology, Xian Jiao-Tong University, respectively. From 1992 to 1993, he was a clinical fellow (with Willard E. Fee Jr.) in the Division of Otolaryngology and Head Neck Surgery at Stanford University School of Medicine. He began research on cancer genetics and drug resistance with Branimir Sikic at Stanford in 1993 and received his PhD. in the Program of Cancer Biology at Stanford in 2002. He continued studying drug resistance mechanisms in human cancer (with Michael Gottesman) as a postdoctoral research fellow at the National Cancer Institute. In 2005, he joined the NIH Stem Cell Unit, directed by Ronald McKay. Dr Chen was appointed as adjunct associate professor of Georgetown University in 2010, as a staff scientist at the NINDS in 2011, and an NINDS faculty member at the NIH in 2018. He has authored or co-authored one patent, 46 abstracts, and 54 articles in peer-reviewed journals and books. He has received several awards for his cancer and stem cell research. Currently, Dr Chen uses pluripotent stem cells to model various neuronal and vascular diseases *in vitro* for drug discovery and to optimize neuroectodermal and mesoendodermal differentiation in cell culture platforms for regenerative medicine.

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the clinical use of VX-770 combined with the corrector drug VX-809 (lumacaftor), termed Orkambi [7], began to expand therapeutic avenues to treat patients with CF by targeting the underlying pathological processes.

Noticeably, the underlying pathological processes of CF are driven by genomic mutations of *CFTR*, the gene encoding cystic fibrosis transmembrane conductance regulator [8,9]. VX-770 is a 'potentiator' that increases the probability of chloride channel opening in some CFTR variants, whereas VX-809, a pharmacological chaperone, aids CFTR protein folding and trafficking to the cell surface in certain types of CFTR variant. Both VX-770 and VX-809 have limited applications for treating patients with CF with the prevalent homozygous p.Phe508del (F508del) allele. Thus, the combinational drug Orkambi has been used to treat patients with CF with homozygous F508del [10]. Despite a substantial cost per year per patient for Orkambi, clinical trials showed only a modest improvement in these patients [10]. Among the various CF patient groups, there is great variability in drug responses to VX-770, VX-809, and Orkambi, from nonresponse to complete response [7,11]. The underlying causes of mixed drug responses and often unsatisfactory clinical outcomes might be attributed to a lack of understanding of the genetic background of CF patient groups, which have considerable genetic heterogeneity (e.g., coexistence of various rare mutations and complex alleles) (Table 1). Likewise, the prevalence of genetic heterogeneity in individual patients is difficult to assess preclinically in animal models. Moreover, drug–CFTR interactions have been poorly elucidated at the molecular level, which has hampered the rational design of the next generation of CF drugs for preclinical drug development.

Thus, there is an urgent need to develop clinically relevant functional assays *in vitro* and to identify responders from individual patients who have different *CFTR* mutations. The use of transgenic mice is not a feasible approach for theratyping diverse human *CFTR* mutations because of the differential thermostability and drug responses between the human and mouse *CFTR* orthologs [12]. In 1991, chloride secretory and current assays were developed using intestinal biopsies from children with CF [13], which provided the rationale for the subsequent development of rectal organoid studies of patients with CF [14,15]. Moreover, the advancement of human pluripotent and adult stem cell platforms and organoid-based disease modeling offers a better opportunity to develop reliable protocols for the expansion and banking of diverse types of organoid [16–19]. Thus, the current organoid platforms will allow us to study the functional pharmacogenomics of various CFTR mutations via organoid assays.

In this review, we discuss the genetic heterogeneity of CFTR mutations and clarify the applications of CF drugs that target various CFTR mutations. We discuss the roles of organoids in CF modeling and preclinical drug development, with a focus on major limitations and prospective resolutions related to this topic; and illuminate the utilities of CFTR organoids in the analysis of CFTR function and mutations. Additionally, we discuss drug–CFTR interaction data based on CFTR protein structures. Lastly, we highlight alternative therapeutic approaches for CF based on organoid analysis.

Elucidating the genetic heterogeneity of *CFTR* mutations

Currently (as of March 2019), there are ~374 annotated CFTR variants as determined from the study of 89,052 patients, 83% of which (312) have been defined as CF-causing variants (http://cftr2.

TABLE 1

Genetic analysis of CFTR mutations in organoids derived from patients with CF[a]

Row	CFTR mutations[b]		Mutation class[c]	Subject, number[e]	Organoid drug response (%)[f]		
	Allele 1 (F1)	Allele 2 (F2)			VX-809	VX-770	Orkambi
1	166delTA (NA)	3120G>A (~0.06%)	I/I	1	0	0	0
2	1811 + 1G > C (~0.01%)	1811 + 1G>C (~0.01%)	I/I	1	0	0.9	0.9
3	F508del (~70%)	711 + 1G>T (~0.2%)	II/I	1	3.8	5.3	24
4	F508del (~70%)	N1303K (~2%)	II/II	2	0.2	3.1	38
5	F508del (~70%)	R347P (~0.4%)	II/II + IV	2	−4.8	−3.8	42
6	F508del (~70%)	Class I	II/I	6	0.4	8.6	57
7	R334W (~0.3%)	R764X (0.02%)	IV/I	1	0.4	42	58
8	F508del (~70%)	F508del (~70%)	II/II	8	0	13	100
9	S1251 N (~0.09)	A455E (~0.4%)	III/II[d] + V	1	3.1	92	110
10	F508del (~70%)	TG(13)T(5) (~0.03%)	II/V	3	0	126	126
11	F508del (~70%)	G1249R (NA)	II/III[d] or IV[d]	2	4	127	141
12	F508del (~70%)	R117H-7T (~0.09%)	II/IV	3	18	146	160
13	F508del (~70%)	A455E (~0.4%)	II/II[d] + V	3	15	71	175
14	F508del (~70%)	S1251N (0.09%)	II/III	3	2	153	183

[a] Genetic analysis of CFTR mutations by organoid-based assays, determined at a subsaturating concentration of forskolin (i.e., 0.128 μM) [11]; Abbreviations: Class I, Class I mutations that include G542X, R1162X (two different subjects), W1282X, DELE2.3, and E60X; F, allele frequency in one copy of *CFTR*.

[b] Mutational frequencies in single copy (one allele) of *CFTR* based on www.cftr2.org (accessed on August 13, 2015).

[c] Mutation classes based on CFTR mutation databases (www.genet.sickkids.on.ca; www.cftrscience.com).

[d] Additional mutation classes based on functional CFTR measurements in rectal organoids [11]. Mutation classes are: no protein synthesis (I); impaired trafficking (II); defective channel gating (III); altered conductance (IV); and reduced amounts of functional CFTR (V).

[e] Subject numbers: numbers of patients with CF.

[f] Organoid drug response: drug response in the FIS assays of rectal organoids derived from patients with CF. Patients with the common CF mutation F508del (at one allele) combined with various variant classes, rare mutations, and complex alleles at another allele. Orkambi responses in rectal organoids derived from patients with CF with homozygous F508del in the FIS assay were used as 100% control for comparison.

org/). CF can be briefly classified into mild (e.g., R117H-T7), severe (e.g., F508del/F508del), and extremely severe (e.g., E60X/4015ATTTdel and F508del/G542X) conditions. In the following subsections, we discuss CFTR mutations in terms of their current classifications, genomic distributions, and prevalent alleles that occur with complex variants and rare mutations.

Mutational or variant classes

CFTR variants are traditionally classified into six classes: Class I (protein synthesis defect); Class II (trafficking defect); Class III (gating defect); Class IV (conductance defect); Class V (reduced quantity); and Class VI (reduced stability) [20,21]. In general, these variants or mutations affect the electrostatics related to pore construction, protein folding (which stabilizes CFTR), cellular trafficking, ATP-binding or ATPase activities, and the interactions between nucleotide-binding domains (NBDs) and transmembrane domains (TMDs), which regulate ionic conduction and gating.

Mutation or variant distributions

Remarkably, a considerable number of CF-causing variants (~13) have been found asymmetrically at the interfaces between the NBD1 and the TMD2 domains (Fig. 1). These variants include S492F, I507del, F508del, G551D, R560K, R560S, R560T, A561E, G1061R, L1065P, R1066C, and R1066H. However, at the corresponding interface, where NBD2 interacts with an intracellular helix 2 (^{269}IQSVKAY^{275}, designated as IH2), there are only a few documented variants (i.e., S1255P and N1303K) [22]. The reason for this uneven distribution of variants or mutations is not well understood. Nonetheless, these variants lead to deficiencies in both protein folding and channel gating through the NBD–TMD interface, which couples phosphorylation-mediated ATP hydrolysis with channel gating (Fig. 1) [22–25].

The prevalent mutation CFTR-F508del

The most common mutation of CFTR is the deletion of phenylalanine at the residue 508 of CFTR (i.e., F508del), which occurs at a frequency of 70%. Approximately 50% of patients with CF are homozygous for F508del [26–29]. F508del impairs protein folding, stability, and recycling to the cell plasma membrane (after endocytosis), thus leading to CFTR intracellular retention, mislocalization, degradation, and eventually a decreased presence at the apical plasma membrane. Current drugs for patients with CF mainly target homozygous CFTR-F508del. However, patients with CF might have the common CF mutation F508del (at one allele) with complex heterozygous alleles, rare mutations, various variant classes (at the other allele) (Table 1) [30], which can result in different responses to current CF drugs.

Complex alleles in patients with CF

Complex alleles are defined by having at least two functional alterations located on the same parental gene [30]. For example, both R117H-T7 and TG13-T5 are the best-understood complex alleles of the *CFTR* gene [30]. Both T7 and TG13-T5 are intron 8 variants with T(n) and TG(n) tracts. In the case of R117H-T7, R117H (a relatively frequent mutation in patients with CF worldwide) is in a *cis* configuration with the polypyrimidine stretch T7. The R117H-T7 genotype results in a mild form of CF or no disease conditions at all [30,31]. Interestingly, a neutral variant (p.S912L) of CFTR, which had normal CFTR protein processing and function, was reported to be involved in a complex allele (p.S912L;p.G1244V) that contributed to a severe CF phenotype compared with p.G1244V alone [32].

Nevertheless, the functional consequences of these complex alleles coexisting with the prevalent F508del have not been well assessed *in vitro* or *in vivo*. Thus far, there are more than 20 exonic *cis* variants identified in F508del complex alleles, albeit at a low frequency [33–35]. Some of these F508del complex alleles in

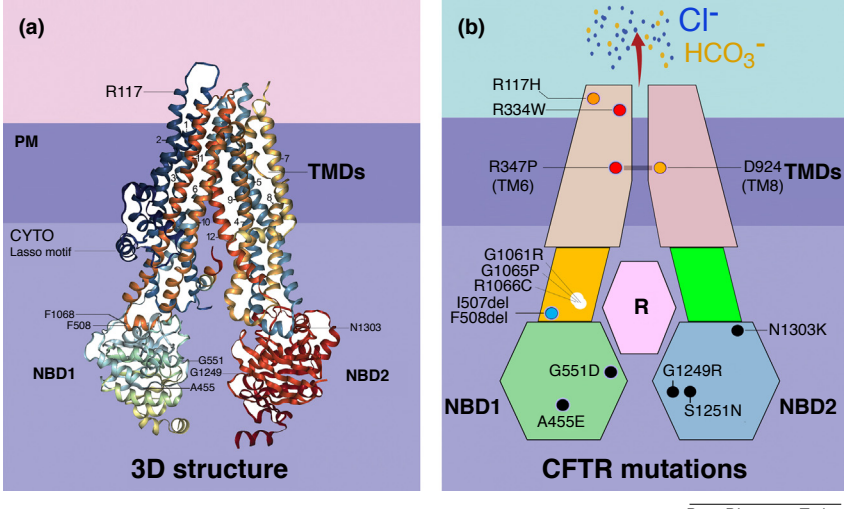

FIGURE 1

Cystic fibrosis transmembrane conductance regulator (CFTR) structure and mutational distributions. (a) Plasma membrane presentation of a 3D human CFTR protein structure [Protein Data Bank (PDB): 5UAK] labeled with indicated variants. The numbers indicate the α-helices that form the transmembrane domains (TMDs). (b) Simplified CFTR domain structures with two TMDs, two nucleotide-binding domains (NDB1 and NBD2), the R domain (R), and indicated representative mutations. Abbreviations: CYTO, cytoplasm; PM, plasma membrane.

patients with CF have been associated with a poor response to corrector and/or potentiator treatments [33]. However, the impact of many newly identified F508del complex alleles (e.g., p.F508del;p.L467F, p.F508del;p.R553Q, and p.F508del;p.I1027T) on CF therapeutic response remains to be determined. Taken together, the current data suggest that *CFTR* complex alleles contribute to the significant variability of the drug response in patients with CF.

Rare CFTR mutations

CFTR rare mutations occur at the coding region as well as noncoding genomic sites (that affect RNA splicing). For example, the class IV mutant R347P arises near a transmembrane region of CFTR (Fig. 1b). This mutation, which also causes a major defect in CFTR folding, now qualifies as a Class II mutation [36]. G1249R, located within the Walker A consensus of NBD2 (Fig. 1b), is both a Class III and IV mutant [11]. However, both F508del/G1249R and F508del/R347P could be considered as rare genotypes because of their low frequency. Moreover, their functional consequences are not well elucidated when these mutations orient in the *CFTR* gene *in trans* [11,37]. Other genomic mutations, such as 1811 + 1G > C/1811 + 1G > C, a homozygous mutation with a splicing defect in the *CFTR* gene, lead to a defect in CFTR protein synthesis from immature transcripts [38]. The presence of considerable heterogeneity in CFTR mutations represents a major challenge for CF therapeutics, suggesting the need for a more personalized approach to correlate the responsiveness of diverse CFTR mutations to pre-existing modulators and/or to develop new drugs for specific mutation classes.

Drug–response profiles in patients with CF with different CFTR mutations

VX-770

VX-770 was the first drug approved (on January 31, 2012) for the management of patients with CF aged 2 years and older (Fig. 2a) [39]. It functions as a potentiator of impaired CFTR channel gating (i.e., channel opening or closing) in patients with certain Class III mutations. Thus, patients with CF bearing the second most common mutation, G551D (~5%), show significant improvements in lung function (e.g., 10% increase in forced expiratory volume in 1 s, designated as FEV_1, from baseline) [40]. The underlying molecular mechanism for G551D, a Class III mutation occurring at the ABC signature motif (Fig. 1), is the deficiency in CFTR channel activity, although there is CFTR surface expression. The data suggested that VX-770 binds and potentiates certain Class III mutants (such as CFTR-G551D), promoting decoupling between ATP hydrolysis and gating cycles [41,42].

Thus far, there are at least 33 CFTR mutations (e.g., R117H, G178R, S1251N, and G1349D) that are responsive to VX-770 potentiation, which were approved for VX-770 treatment by the US Food and Drug Administration (FDA) in 2017 (www.drugs.com). As expected, VX-770 monotherapy failed to show tangible benefits in ~50% of patients with CF with the homozygous F508del (Class II/III/VI) mutation [26–29], in which only a limited amount of CFTR protein is present on the cell plasma membrane because of a severe defect in CFTR processing and trafficking [43].

VX-809

In contrast to VX-770, VX-809 (Fig. 2b) acts as a corrector of protein misfolding (a pharmacological chaperone), thus enhancing CFTR folding, processing, trafficking, and plasma membrane presentation. Recently, VX-809 was shown to directly bind to the human CFTR NBD1, thereby allosterically connecting the binding site with the NBD1-cytoplasmic loop 4 interface [44]. Thus, VX-809 might correct misfolding of mutant CFTRs (e.g., CFTR-F508del) through a mechanism that acts on TMD1 [45]. However, VX-809 alone did not result in a significant improvement in FEV_1, weight or body mass index, or sweat chloride reduction for patients with CF with homozygous CFTR- F508del in a Phase II clinical trial [46].

Orkambi

To synergize the effects of CF drugs on CFTR mutations, the combination of both VX-770 and VX-809 (i.e., Orkambi) was approved by the FDA on July 2, 2015 to treat patients with CF with the prevalent alleles F508del/F508del. However, clinical trials showed only a modest improvement, as evidenced by a 2.6–7.7% increase in FEV_1 and a 34–43% reduction in exacerbation rate [7,47].

Next-generation CFTR correctors

New CFTR correctors (similar to VX-809 in the mode of action), including the next-generation correctors VX-659, VX-661 (known as tezacaftor), and VX-445, were developed to rescue the function of CFTR-F508del with other mutations. Two triple combination regimens (VX-659–VX-661–VX-770 and VX-445–VX-661–VX-770) have shown potential in patients with F508del plus a minimal-function mutation (F508del–MF), which accounts for ~90% of all patients with CF with homozygous F508del genotypes [48,49]. For example, the VX-659–VX-661–VX-770 combination increased CFTR-F508del processing and trafficking significantly, enhanced chloride transport *in vitro*, resulted in a substantial increase in the percentage of predicted FEV_1 in clinical trials, a decrease in sweat chloride concentrations, and an increase in the scores on the CF questionnaire. Convincingly, in homozygous F508del patients who had already received the VX-661–VX-770 double regimen, the addition of either VX-659 or VX-445 significantly increased the percentage of predicted FEV_1 [48,49].

Thus, the above clinical data provide the basis for the combined use of multiple correctors to modulate the functionality of various rare missense mutations, highlighting a general strategy for wide-ranging applications. However, the mechanisms that underlie the above mixed drug responses in the homozygous F508del patient groups are not well understood. With regard to the evaluation of drug efficacy in patients with CF with considerable genetic heterogeneity, organoid-based disease modeling offers an opportunity to identify responders for personalized medicine because it faithfully retains the genetic and epigenetic signatures of original normal and pathological tissues (reviewed in Refs. [16–19]).

Roles of organoids in CF-modeling and pharmacogenomic analyses

Brief description of organoids

The current concept of an organoid is based on stem cell-derived 3D tissues that have organ-specific cell types and structures in *in*

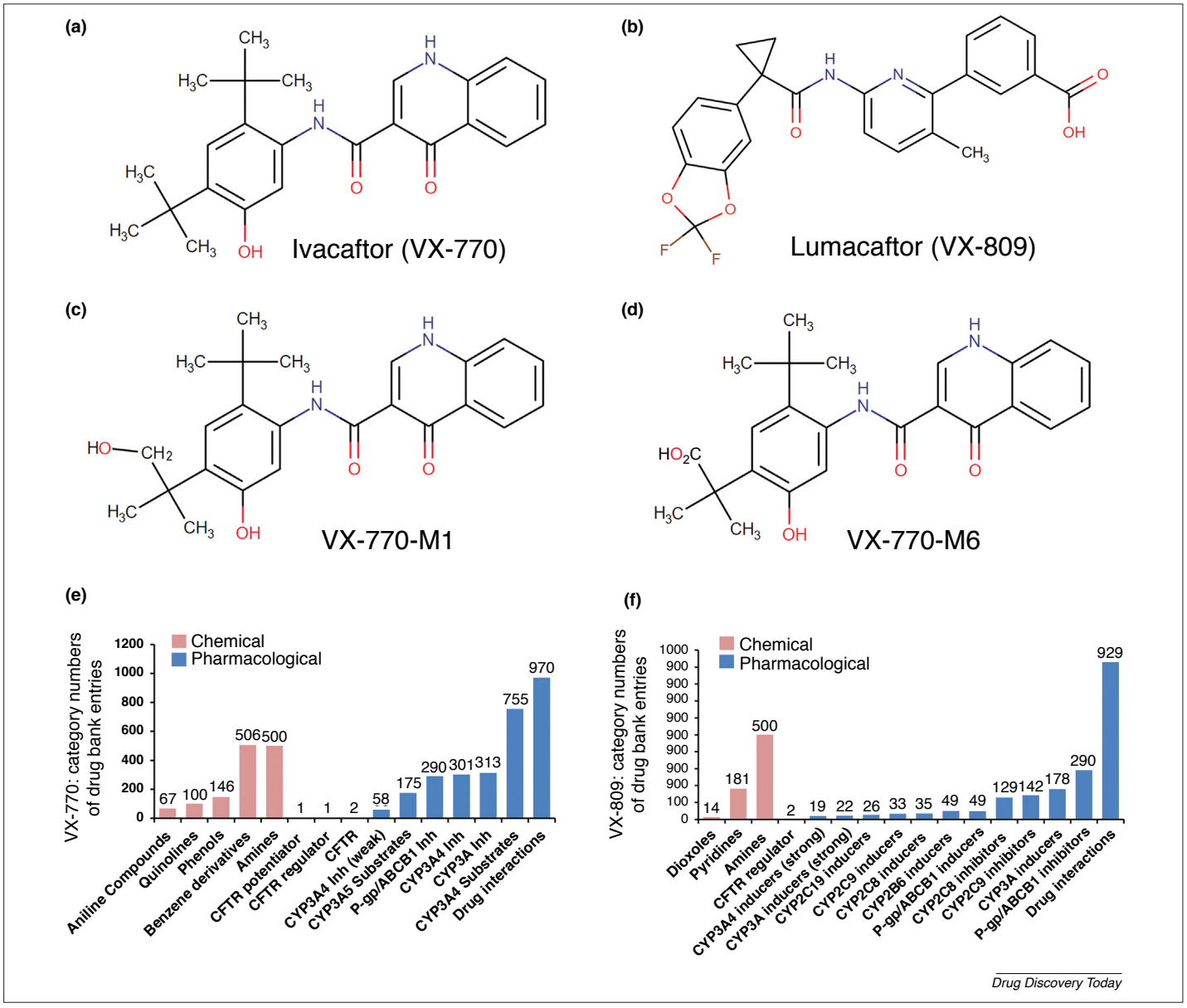

FIGURE 2

Two effective small molecules currently used to treat patients with cystic fibrosis. (a,b) Structure of VX-770 (known as ivacaftor and Kalydeco) and VX-809 (lumacaftor). (c,d) Two major metabolites, M1 and M6, of VX-770. (e,f) Chemical and pharmacological category analysis of both VX-770 and VX-809 (based on the DrugBank database, at www.drugbank.ca, on May 2019). Category numbers (labeled at the top of each column) indicate the number of chemical drugs (deposited in the drug bank) in that category. For example, VX-770 belongs to the cystic fibrosis transmembrane conductance regulator (CFTR) potentiator category, which has only one drug (i.e., VX-770) in the drug bank; and to the inhibitor of P-glycoprotein (P-gp)/ABCB1 [as shown in (e)], which currently has 290 similar drugs in the drug bank. The numbers of drug interactions with VX-809 or VX-770 are indicated in the last columns. Abbreviations: CYP, Cytochrome P450; Inh, inhibitor.

vitro cell culture. Stem cells in organoids have the capacity to self-renew, self-organize, and differentiate toward adult tissues under optimal growth conditions [16–19] (Fig. 3). Organoids can be generated from multiple stem cell resources, which include pluripotent stem cells, adult stem cells, and embryonic or adult tissues [16–19].

For example, intestinal stem cells, belonging to adult stem cells, are capable of self-renewal, self-organizing, and differentiating into intestinal cells (Fig. 3) [17]. *In vitro,* culture of these stem cells allows them to propagate and form closed organoids, which recapitulate the *in vivo* intestinal architecture with an internal lumen lined by adult stem cells and differentiated cells [15] (Fig. 3f). Stem cell-based organoids have been used to model human development and diverse types of disease [17]. Organoid systems have also been increasingly used to model CF and to develop functional assays for assessing CFTR function *in vitro* (Fig. 3g).

Organoid assays for CFTR activity

Many CF mutations have various mechanisms that lead to a dysfunctional CFTR. Currently, the classic patch-clamp is still the gold standard in defining the functionality of these CFTR mutations. The use of Fisher rat thyroid (FRT) or CF bronchial epithelial (CFBE) cells transfected with diverse CFTR variants

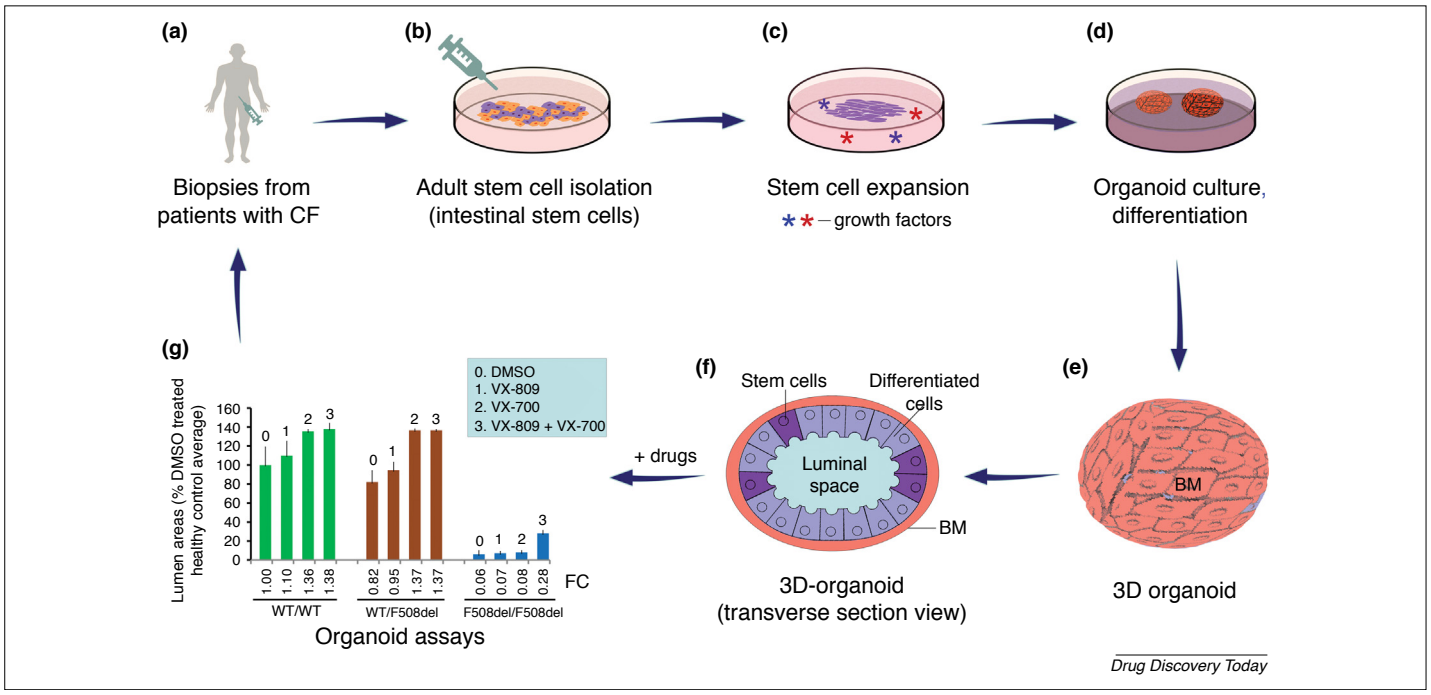

FIGURE 3

Scheme of organoid assays. (a) Biopsy from patients with cystic fibrosis (CF), (b) isolation of adult stem cells, (c) stem cell expansion, (d) organoid culture and differentiation, (e,f) representative organoid structure of an intestinal organoid (including a transverse section view), and (g) CF transmembrane conductance regulator (CFTR) functional assays of intestinal organoids derived from patients' specimens with various *CFTR* genotypes (i.e., WT/WT, WT/F508del, and F508del/F508del) by measuring lumen areas after indicated drug treatments. Abbreviations: BM, basement membrane of organoids; DMSO, dimethyl sulfoxide treatments as control; FC, fold changes related to wild-type control (column 1); VX-770, ivacaftor; VX-809, lumacaftor; WT, wild-type allele.

enables robust CFTR current measurements, which are linearly related to CFTR function [42,50] (Table 2). Thus, it is also essential to combine the patch-clamp technique with newly developed assays to study CFTR processing, intracellular transport, and plasma membrane stability.

It is well established that adenylyl cyclase (ADCY) activation leads to activation of CFTR via the cAMP-protein kinase A pathway [25]. Thus, the ADCY activator forskolin-induced swelling (FIS) assay represents an emerging but powerful tool to measure CFTR activity based on organoids cultured *in vitro* [15]. A simple organoid has a spherical structure, in which the central lumen cavity is wrapped by an epithelial monolayer (Fig. 3e,f). For healthy organoids after forskolin induction, a rapid swelling phenotype, the amount of fluid secretion from the cells into the lumen can be indirectly estimated based on the measurements of the lumen areas of the organoids. In the case of CF organoids, a decreased or diminished swelling can be observed [15]. Thus, the FIS assay enables analyses of the effects of various CF mutations, which include prevalent allele(s) occurring with complex variants and rare mutations from individual patients with CF, on CFTR functions and CF drug response.

FIS assays of the predominant mutation F508del of CFTR

The FIS assay has revealed differential responses of organoids to a handful of CFTR drugs (such as C8, Corr-4a, and VRT-325) in addition to VX-809 and VX-770 [15]. In healthy controls, a 36% increase in lumen areas was observed relative to the wild-type CFTR baseline in the presence of either VX-770 alone or the VX-770 and VX-809 combination (Fig. 3g, columns 1–4), suggesting that VX-770 modulates (activates) wild-type CFTR gating activity.

In the case of the heterozygous *CFTR*-F508del genotypes (i.e., one CF and one wild-type allele), CFTR activity appears to be compensated by the wild-type *CFTR* allele. Hence, a 37% increase in lumen areas in the heterozygous condition results from modulatory effects on the wild-type CFTR, rather than the effects on the F508del allele (Fig. 3g, columns 6–8). However, we cannot rule out the possibility of other modifiers that influence ion and fluid transport processes, which enable CFTR proteins produced from one normal allele to increase their maximal activity.

With respect to the homozygous F508del genotype, the lumen area was reduced to 6% of the wild-type CFTR baseline (100%) (Fig. 3g, columns 1 and 9). The defective CFTR activity was slightly modulated by either VX-770 or VX-809 at a subsaturating forskolin concentration (0.128 μM) (Fig. 3g: columns 9–12). However, a swelling phenotype of patient organoids increased four–fivefold in the presence of a combination treatment with VX-809 and VX-770 (i.e., Orkambi) compared with an untreated F508del/F508del control (Fig. 3g: columns 9–12). Taken together, these organoid analyses delineate the modulatory effects of the approved CF drugs, in which VX-770 or VX-809 alone is not sufficient but their combination is effective for patients with homozygous CFTR-F508del.

It is unclear whether VX-809 could restore CFTR-F508del expression on the plasma membrane in this assay or whether CFTR-F508del present on the plasma membrane is in a dysfunctional state. Based on the capacity of VX-809 to enhance the plasma membrane presentation of misfolded CFTR proteins, we postulate that VX-809 at least partially corrects misfolded CFTRs from being recognized by quality control mechanisms, which degrade misfolded proteins. Moreover, CFTR-F508del might not be functional

TABLE 2

Roles of GEC lines and stem cell organoids from patients in CF research[a]

Category	GEC lines	Organoids from patients
Assay components	Cell sources: large amounts of CFTR variants expressed in FRT and CFBE cell lines	Cell sources: individually collected from biopsies
	Derivation: transfected or recombinant CFTR variant cell lines	Derivation: organoids derived from adult stem cells (e.g., intestinal stem cells)
	Physiological relevance: low physiological relevance (immortalized cell lines)	Physiological relevance: high, organoids recapitulate physiological states of cells
	Availability: commonly available	Availability: organoids conditionally available
Assay conditions and types	Grow as simple 2D monolayers	Usually as 3D complex aggregates
	Easy to apply CF drugs on apical and basolateral sides of cells	Difficult to inject drugs into lumen of organoids
	Adaptable for HTP drug screens	Difficult for HTP drug screens
Applications (functional measurements)	Used for measuring multiple physiological parameters	For limited parameters: e.g., FIS assays
	Compatible with patch clump-based methods for measuring CFTR activity (e.g., currents) in variants treated with CF drugs	Currently not compatible with patch clump-based methods
Applications (theratyping) and influencing factors	Used in CFTR2 project for correlating genotype function with CF clinical traits	Suitable for analyzing less common, rare, and private CFTR mutations
	Sensitive method to evaluate CF drug efficacy *in vitro,* need to evaluate likelihood of overestimating effects of drugs on CFTR activity	Generally, a sensitive method like using GEC lines; have forskolin concentration-dependent effects (insensitivity) to some CF drugs (e.g., VX-809); need to assess possibility of underestimating effects of drugs on CFTR activity
	Residual CFTR function and drug response: highly correlated, lower data variability because of controllable and consistent CFTR variant expression within normal human physiological limits	Residual CFTR function and drug response: highly correlated, with variations because of different organoid culture methods or measuring procedures of FIS assay
	Not applicable for certain CF variants or modulators that need presence of native human cellular factors for functional CFTR	FIS assay: has exceptional ability to test differences in residual CFTR activity or drug response between individuals
	Lack capacity to analyze effects of endogenous regulatory elements and large genetic alterations on CFTR function and drug response	Organoids: have advantage in assessing effects of CFTR native promoters, haplotypes, complex splicing mutations, and gene rearrangements
	Might have nonspecific effects because of off-target integration of variant cDNAs	Organoids contain differentiated cells, thus having mixed drug responses in FIS assay
Clinical relevance	GEC variants: represent 87% of individuals in CFTR2 database	Organoids: represent individual CF models
	Utility of GEC lines confirmed in some clinical trials, e.g., strong correlation between *in vitro* FRT results and clinical trials	Approved clinical relevance and correlation in personalized drug screens
	FDA accepted part of CFTR testing in FRT cells	FDA information not available
	Limited values for personalized study	Suitable for predicting clinical outcomes in rare genotypes and personalized drug screens

Abbreviations: CFBE, cystic fibrosis bronchial epithelial cells; CFTR2 project, clinical and functional translation of CFTR project; FRT, Fischer rat thyroid; HTP, high throughput.
[a] Based mainly on [11,15–17,40,50,51,63,64].

when it is moved to the cell membrane by the chaperone effect of VX-809. Nonetheless, the current organoid data provide a rationale for the combined use of VX-770 and VX-809 for patients with homozygous CFTR-F508del, which in turn validate the FIS assay as a useful functional assay for evaluation of drug response for diverse CFTR mutations. We will delineate below with organoid assays of CFTR mutations based on their prevalence and availability.

FIS assays of F508del with mutations at the NBDs

There are many advantages to using organoids for drug screening, particularly for patients with CF with rare mutations at NBD2. Concerning F508del/S1251N, S1251 is the last residue of the Walker A consensus (^{1244}GRTGSGK**S**$^{\mathbf{1251}}$) of NBD2 (Fig. 1b). Rectal organoids derived from patients with this genotype showed abrogated CFTR activity, similar to the homozygous CFTR-F508del level. Compared with homozygous F508del organoids treated with Orkambi (as 100% control), F508del/S1251N organoid activity was elevated by 53% and 83% by VX-770 alone and Orkambi, respectively (Table 1) [11]. These data suggest a key role of VX-770 in restoring CFTR activity by modulating the channel activity of the S1251N mutant at NBD2.

With respect to an extremely rare mutant genotype (i.e., F508del/G1249R), the rectal organoids derived from patients with CF ($N = 2$) with this genotype showed a strong response to VX-770 and Orkambi, exhibiting 27% and 41% increase in the FIS assay, respectively, compared with the homozygous CFTR-F508del organoids (as 100% control) (Table 1). However, there was only a 4% increase by VX-809 treatment in this FIS assay (Table 1) [11]. Based on this organoid assay, Beekman and colleagues treated two patients with the F508del/G1249R genotype with VX-770, which rapidly relieved the patients' symptoms, improved lung function, and elevated body weight [11]. Moreover, recent studies from the same group showed an individual correlation of *in vitro* response in organoids with either FEV_1 or sweat chloride concentrations or intestinal current measurements [51,52].

Thus, these studies demonstrate the clinical importance of the organoid-based assays for the evaluation of drug efficacy at the personalized medicine level. However, the size of organoid samples used for this FIS assay was limited because of the availability of these rare mutations in patients with CF. CRISPR-based gene editing could enable genetic engineering of such rare mutations more routinely for preclinical assessments of drug efficacy. However, CRISPR-edited stem cells and organoids might gain new mutations, because of CRISPR-mediated off-target sites [53] compared with native patient cells and organoids.

FIS assays of F508del with complex alleles

As summarized in Table 1 and discussed in an earlier section, rectal organoids derived from patients with CF with the common CF mutation F508del (in one allele) combined with complex heterozygous alleles showed differential responses to VX-809, VX-770, and Orkambi. F508del/TG13-T5 organoids responded well to VX-770 alone, with a 26% increase in the FIS assay compared with the homozygous CFTR-F508del control, but not to VX-809 (Table 1, row 10). Moreover, the addition of VX-809 to VX-770 (i.e., Orkambi) had no additional modulatory effects on CFTR activity, suggesting the existence of unknown genetic or epigenetic factors that underlie enhanced CFTR activity. However, F508del/R117H-T7 organoids responded to VX-809 alone, exhibiting an 18% increase in CFTR activity in this assay, which is the highest response rate observed in this cohort study (Table 1, row 12). Thus, the effect of Orkambi on F508del/R117H-T7 organoids results from the additive effects of both VX-809 and VX-770.

FIS assays of non-F508del mutations

Regarding the rare A455E/S1251N genotype, A455 is located two amino acids upstream of the Walker A consensus (^{458}GSTGAGKT^{465}) of NBD1, whereas S1251 is within the Walker A consensus of NBD2 (Fig. 1). Therefore, the mutated genotype could be considered as a homozygous Walker A mutant, which might contribute to a dysfunctional CFTR because of abrogated ATP hydrolysis. In contrast to the potentiator VX-770, which reverted CFTR-A455E/S1251N activity to the level (100%) of homozygous CFTR-F508del organoids treated with Orkambi, the protein-misfolding corrector VX-809 did not have any significant effect on CFTR-A455E/S1251N activity (Table 1, row 9) [11]. These data suggest that VX-770, not VX-809, exerts its modulatory effects by targeting the Walker A motifs of both NBD1 and NBD2.

Organoid-based assays of noncoding genomic mutations

In this case, a homozygous mutation with a splicing defect in the *CFTR* gene (e.g., 1811 + 1G > C/1811 + 1G > C) [38] showed no response to VX-809, VX-770, or Orkambi (Table 1, row 2). This result was because of a defect in CFTR protein synthesis from immature CFTR transcripts. Thus, current CF drugs will not work in this context. Nevertheless, the above genotype can be used as a negative control when performing the FIS assay. Thus, development of drugs that target post-transcriptional or translational levels might be an alternative approach.

In summary, FIS assays with different CFTR mutations revealed differential organoid responses to current CF drugs, which are useful for predicting the drug response in patients with CF with considerable genetic heterogeneity. These organoid data also enable us to unveil many new features of various CF drugs, including complicated drug interaction patterns (e.g., addition, synergy, or interference) based on a specific genotype. By comparing homozygous CFTR-F508del organoids with the organoids generated from compound heterozygotes (e.g., CFTR-F508del plus a gating mutation), we would be able to determine the contribution of a gate mutation to the residual and potentiator-induced FIS activity (Table 1). Organoid models might also offer an opportunity to study the functionality of diverse CFTR mutations *in vitro,* thereby facilitating the design of the next generation of CF drugs with high efficacy and precision.

CFTR organoid studies with molecular and structural insights

Newly defined structural properties of CFTR

Among the 48 human ABC transporters, CFTR is probably the only ABC transporter that has ATP-gated ion channel activity. Recently, cryo-electron microscopy (cryo-EM) revealed that both zebrafish and human CFTRs have a structure similar to other ABC transporters [22–24,54,55]. The cryo-EM density maps also revealed two unique atomic structures of CFTRs: a biochemically characterized regulatory (R) domain and a previously unrecognized N-terminal Lasso motif (Fig. 1a) [22–24,55]. The R domain appears to have a crucial role in the regulation of CFTR channel activity, with inhibition of the channel when it is dephosphorylated or activation of the channel once it is phosphorylated [25].

Concerning the functionality of the R domain, it was recently shown that the release of a phosphorylated R domain between the two NDBs likely activates the ATPase site(s) via ATP binding to the phosphorylated conformation, thus facilitating the open-channel conformation of the ion conduction pathway [24]. Moreover, it is unknown how the Lasso motif interacts with the R domain. This newly discovered motif was found to be partially integrated into TMD1 adjacent to the R domain, suggesting a role of the Lasso motif in regulating channel gating via interactions with the R domain [22–24]. Potential molecule interactions with this interface might provide new clues for the molecular correction of CFTR mutations.

Molecular structures of CFTRs and their implications in CFTR organoid studies

Lucas and colleagues illuminated the molecular targets of available correctors and classified these correctors into three major classes, in which Class I, II, and III (chemical chaperone) correctors stabilize the NBD1–TMD1 or NBD1–TMD2 interfaces, NBD2, and F508del-NBD1, respectively [14]. For example, VX-809, C18, and C3, which are designated as Class I correctors, specially target the NBD1–TMD2 interface over the NBD1 energetic defect [14]. The above classification, which is different from that of mutation classes discussed in this review, is particularly useful in guiding mechanism-based corrector combinations, which aim to restore CFTR misfolding and function. Thus, CF therapies by correcting CFTR domain assembly, particularly by stabilizing NBD1–TMD1/2 energetic defects, represent a useful strategy for correcting the F508del mutation that prevents CFTR trafficking to the plasma membrane as well as non-F508del mutations that affect NBD–TMD assembly.

Noticeably, current intestinal organoid data support the above NBD–TMD energetic stabilization strategy. The common features, which underlie current CF drugs that act on various CF mutations, are associated with the additive or synergistic effects of these drugs on the NBD–TMD or F508del–NBD1 interfaces (Table 1). For example, VX-809, identified as a promising corrector of CFTR-F508del misprocessing, showed no effects on homozygous CFTR-F508del organoids (Table 1, row 8), consistent with its limited clinical benefits as monotherapy. However, the complete insensitivity of CFTR-F508del organoids to VX-809 alone is in conflict with its well-established effect on primary F508del CF-HBE cells grown at an air–liquid interface [56]. Thus, it is important to determine whether each individual method could optimally assess the effects of CF drugs on wild-type and mutant CFTR activity under the interference with native and non-native cellular backgrounds (Table 2).

Nonetheless, VX-809 significantly modulated CFTR activity in the organoids with F508del plus the NBD1 mutation A455E (F508del/A455E) (Table 1, row 13). This modulatory effect was lower in CFTR genotypes that contained NBD2 mutations (e.g., F508del/S1251N, F508del/G1249R, and S1251N/A455E) (Table 1, rows 9, 11, and 14). These data suggest that VX-809 alone preferentially targets NBD1 mutations. By contrast, the effects of VX-770 on the above NBD2 mutant-containing alleles (F508del/S1251N, F508del/G1249R, and S1251N/A455E) were significantly elevated, and exhibited an 89%, 123%, and 151% increase in lumen areas, respectively, compared with VX-809 (Table 1, rows 9, 11, and 14), indicating that VX-770 exerts its effects on NBD1, NBD2, and the F508del–NBD2 interface. Furthermore, the pharmacological effects of Orkambi on homozygous F508del and F508del/A455E organoids appear to result from a synergistic action of VX-809 and VX-770 (Table 1, rows 8 and 13). These data suggest that Orkambi medicates its modulatory effects on CFTR mutations at the two NBDs and the F508del–NBD1 interface via allosteric synergism.

Thus, the NBD–TMD interfaces provide an opportunity for a rational drug design, aiming to target critical folding steps. Re-establishing CFTR-F508del folding and function requires the correction of NBD1 energetics and domain interface interactions [57]. Using structure-based mutagenesis and biochemical crosslinking experiments, VX-809, the cotranslation-based misfolding corrector, was shown to promote interactions between the cytoplasmic loop 1 and the NBD1 of CFTR [58]. Direct binding of CFTR correctors to CFTR was shown in a biochemical linage experiment using modified correctors [59]. VX-809 also displays secondary binding activity after its partial rescue of CFTR-F508del to the cell surface, which enhances its function by stabilizing CFTR-F508del in an active state.

Recently, structure-guided combination therapy using compounds targeting distinct structural defects of CFTR was shown to synergistically rescue mutant expression and function up to ~50–100% of the wild-type CFTR level [60]. Such high levels of CFTR activity should be suitable for achieving pronounced clinical benefits in patients with CF. Taken together, defined structural properties of CFTR and molecular analysis of CFTR function by intestinal organoids might provide insights into the molecular intervention of CFTR functions and preclinical drug development. Nevertheless, many previously unrecognized limitations may influence the outcomes of drug testing and screens in organoid studies.

Organoid-based CFTR assays: limitations and future resolutions

Currently available approaches to test approved CFTR modulators comprise the use of primary cell culture [39], nasal spheroids [61,62], and genetically engineered cell lines expressing large amounts of CF mutations [40,42,50,63,64] (Table 2 and Table S1 in the supplemental information online). Thus far, these strategies remain vital for pharmaceutical and academic applications, which have provided data to validate the use of VX-770 to 38 CF mutations [42]. The cell line data have also revealed that the VX-809 and VX-770 combination restored most functional properties of the 59 CFTR mutants [50]. Currently, CFTR functional measurements and theratyping (using FRT and CFBE cell lines transfected with CFTR variants) are implemented routinely in the CFTR2 project, which aims to analyze as many as 159 less common, 361 rare, and 1120 private CFTR mutations [50,64]. Therefore, the combined use of FRT and CFBE cell lines (transfected with CFTR variants) with stem cell-based organoids would accelerate the precision use of current or new CF drugs for the management of patients with CF with diverse CFTR mutations.

However, it is essential to understand the *pros* and *cons* of using organoids for CFTR functional measurements and theratyping in comparison with FRT and CFBE cell lines. We provide a summary of the potential applications and limitations of intestinal organoids and transfected CFTR variant cell lines in Table 1, which serves as a reference for CF researchers when conducting drug screens, novel compound development, and electrophysiological assays. Several major limitations of organoid-based assays (e.g., lacking host environment-related signal factors) have been discussed in previous reviews [16,18]. In the following subsections, we focus on several crucial limitations, which have not been previously well elucidated. These issues are associated with the bioavailability of CF drugs related to drug metabolism, pharmacological limitations associated with drug transport, systemic circulation and drug distribution *in vivo*, microenvironmental cues in an organoid assay *in vitro*, and tissue type-dependent requirements of CFTR activity.

Bioavailability of CF drugs associated with metabolism

Organoids do not have an *in vivo* environment. Hence, the uptake of drugs observed in epithelial cells of organoids might not reflect the oral administration of CF drugs. Following oral administration, VX-809, mostly excreted unchanged in the feces, is not extensively metabolized (www.drugbank.ca/drugs/DB09280). In the case of VX-770, only a small fraction (~12%) is absorbed in the intestine, whereas most (~88%) is eliminated in the feces (www.drugbank.ca/drugs/DB08820). VX-770 is extensively metabolized by the cytochrome P450 CYP3A and has two metabolites, termed M1 and M6 (Fig. 2c,d), which only have 1/6 and 1/15 of the potency of VX-770, respectively. Hence, VX-770-M6 is considered to be a pharmacologically inactive form (www.drugbank.ca/drugs/DB08820).

Thus, to mimic the *in vivo* effects of VX-770 on organoid cells, VX-770 could be replaced with the active metabolite VX-770-M1 at corresponding concentrations as measured *in vivo*. We need to place VX-770-M1 basolaterally in the culture medium, rather than injecting it into the luminal cavity of organoids (e.g., rectal organoids). However, given that tested CF drugs are administered

on the surface of the human respiratory tract, the route of CF drug candidates in unmetabolized forms might need to be injected into the luminal cavity of the organoid. Conceivably, optimal drug concentrations and routes of drug application in organoid-based assays would have a significant impact on experimental outcomes.

Predominant pharmacological limitations: roles of ABCB1

The linkage of the CF drugs to the multidrug transporter ABCB1 is evidenced from pharmacokinetic analysis of the serum concentration of VX-770 from data sets in a drug bank (www.drugbank.com). These documented drugs are likely the substrates or regulators of ABCB1 (Fig. 2e,f).

Thus, the above data from the drug bank indirectly suggest that VX-770 is a substrate and/or inhibitor of ABCB1 (Fig. 2e). Indeed, a recent investigation of the effects of VX-770 on human ABCB1 suggested that VX-770 is a potential inhibitor of both ABCB1 and cytochrome P450. The nucleotide-free ABCB1 appeared to strongly bind to VX-770 labeled with a cysteine-reactive fluorescent reporter dye [65]. Also, VX-770 stimulates ATPase activity of the purified ABCB1 and competes with the transport of Hoechst 33,342 [65], a fluorescent substrate of both ABCB1 and ABCG2. These studies suggest that increased serum concentrations of many clinical drugs (drug–drug interactions) are mediated by the inhibitory effects of VX-770 on ABCB1. Likewise, these data also imply that VX-770 could be efficiently effluxed as a substrate by ABCB1 in patients with CF, which would likely explain its limited intestinal absorption.

Moreover, it has been shown that the therapeutic effects of VX-770 on patients with CF could be reversed after withdrawal of CF drugs [11], suggesting that long-term medication is needed for patients with CF. Likely, the longer-term use of an ABCB1-like substrate, such as VX-770, would induce ABC transporter-medicated drug resistance. Hence, safe ABC transporter inhibitors (such as curcumin) should be considered in combination with tested CF drugs in an organoid assay. Curcumin, a widely used herbal supplement and food flavoring, can bind and inhibit multiple ABC transporters (including ABCB1, ABCC1, and ABCG2) and modulate various channel proteins (including voltage-gated potassium and calcium channels, volume-regulated anion channels, and aquaporin-4) (reviewed in Ref. [66]).

An early study indicated that oral administration of curcumin can correct CF defects in homozygous CFTR-F508del mice [67]. It was further shown that curcumin activates CFTR mutants (e.g., G55D1 and W1282X), which are defective for normal ATP-gating channels, by a novel mechanism that is independent of ATP-binding and dimerization of the NBDs. This mechanism possibly involves activation of the R domain, as described earlier [68]. Thus, curcumin could be considered in combination with VX-770 or Orkambi to modulate CF drug efficacy and to circumvent potential drug resistance in patients with CF.

CF drug bioavailability and the necessity of drug synergy

As discussed earlier, CF drug bioavailability is significantly reduced by poor absorption, enzymatic drug metabolism, and drug transporters. Hence, suboptimal CF drug concentrations might prevail *in vivo*. Thus, synergistic modulation of CFTR activity is imperative and could be made possible by two chemically distinct potentiators [e.g., VX-770 and 5-nitro-2-(3-phenylpropylamino) benzoate] [69] and some less toxic chemicals (e.g., curcumin and genistein) [70]. Synergy-based small-molecule screens could be used to identify drugs that maximize the efficacy of current correctors [70], although the synergy is not yet understood at the molecular level. How to maximally modulate mutant CFTR activity to the wild-type CFTR level remains a robust research area, in which we should take crucial microenvironmental factors and tissue type-dependent requirements into considerations when designing new organoid assays.

Microenvironmental cues and organoid assays

Besides its predominant chloride channel activity, CFTR also functions as a bicarbonate (HCO_3^-) channel that is essential for HCO_3^- secretion, airway fluid secretion, extracellular alkalization, and host defense [71–75]. However, this function has been long neglected in current CF studies. There is at least one outstanding question to be addressed: would intracellular and extracellular bicarbonate affect the functionality of CFTR?

The selectivity of HCO_3^- efflux by CFTR depends on both intracellular and extracellular chloride concentrations. At higher intracellular chloride concentrations $[Cl^-]_i$ (>10 mM), CFTR functions as a chloride channel that has restricted permeability to HCO_3^- [71–73]. However, at lower $[Cl^-]_i$ (<10 mM), the CFTR chloride channel gains the permeability to efflux HCO_3^- into the lumen [76,77]. It appears that the permselectivity of CFTR chloride to the bicarbonate channel is positively regulated by activation of the WNK-SPAK kinase cascade under low $[Cl^-]_i$ [77].

Moreover, the above permselectivity switch is also controlled by external chloride. Following a time delay after the withdrawal of extracellular chloride, the CFTR channel switched into a bicarbonate-permeable mode [71], consistent with the report that the addition of external chloride to cells incubated in a chloride-free medium failed to efflux HCO_3^- because of the impermeability [71]. Thus, HCO_3^- in the luminal cavity and basal lateral space of an organoid culture might influence assay results. Future research should take microenvironmental bicarbonate into consideration when conducting FIS assays and drug screens.

Tissue type-dependent CFTR activity in organoid assays

Although pulmonary insufficiency is the major cause of mortality in patients with CF, the epithelial cells of the lung are not the first or the most commonly affected cell types in patients with CF. In fact, epididymal, vas deferens epithelial, and pancreatic cells are affected earlier in life in most patients. It is conceivable that different tissues might have differential requirements for CFTR activity to maintain tissue homeostasis. For example, vas deferens and pancreas are particularly sensitive to reduced CFTR activity. R117H homozygous patients who have infertility (because of the absence of the vas deferens) usually do not have other CF clinical signs.

Thus, organoids derived from epithelial cells of the male reproductive system might have higher requirements for CFTR activity than those of epithelial organoids derived from both the lung and intestine. Accordingly, organoids derived from diverse tissues (e.g., vas deferens, pancreas, liver, and lung) should be established and used for CFTR mutational analysis. The levels of CFTR activity in the presence and absence of CF drugs, which are achieved in organoid assays, are crucial for predicting therapeutic effects of

small molecules in patients with CF. Hence, combined use of CFTR correctors and potentiators to achieve optimal CFTR activity in a defined tissue is important to treat individual patients with CF with differential tissue involvements.

Alternative therapeutic approaches

Given the complexity of genetic heterogeneity and the limitations of small chemical-based therapeutics, alterative therapeutic approaches should also be encouraged to meet the needs of the CF community. The novel strategies should target nonsense mutations of CFTR, utilize CFTR-mediated gene therapy, and exploit new CFTR modifiers.

Promote premature termination codon read-through

Nonsense mutations occur within the coding sequence of *CFTR* mRNA transcripts, thereby producing premature stop codons (PTCs) that lead to the truncation and deficiency of CFTR proteins. The compound PTC124 (Ataluren) was reported to promote the PTC read-through of the CFTR stop codon in the IB3.1 cell line (derived from a patient with CF) and to recover the expression of the CFTR protein [78]. The development of new drugs that promote PTC read-through offers opportunities to treat patients with CF with multiple rare genetic mutations (e.g., F508del/E60X, F508del/G542X, F508del/R1162X, and E60X/4015delATTT). However, current PTC read-through drugs, such as PTC124 and geneticin, have limited suppressor function and, hence, might be insufficient to restore CFTR function as tested in CF intestinal organoids. In addition to ongoing efforts in developing PTC drugs, re-emerging gene therapy technologies might provide new avenues for patients with refractory CF [79].

CFTR-mediated gene therapy in patients with CF

As indicated, VX-770 has a half-life of 12 h following a single-dose oral administration (www.drugbank.ca/drugs/DB08820). Thus, a therapeutic drug regime that requires patients to take such drugs long term is inconvenient and can cause adverse effects. Recent clinical advances in gene therapy, based on safely applying various viral vectors [e.g., recombinant adeno-associated viral vector (rAAV)] in clinical trials, provide a new prospect to treat patients with CF in a mutation-independent manner [80]. Indeed, a recent study used rAAV-mediated gene transfer of a truncated CFTR (i.e. CFTR without the R-domain, designated as CFTR-ΔR) to successfully rescue the CF phenotype in CF patient-derived organoids and the nasal mucosa of CF mice [81]. This study highlights the clinical potential of rAAV-CFTR-ΔR in the management of patients with all types of CF, with the hope of achieving a cure.

CFTR modifiers as therapeutic targets

An increasing body of evidence suggests that altered non-CFTR genetic factors also contribute to the severity of clinical phenotypes of patients with CF. Several known modifiers of CF include the epithelial sodium channel (ENaC), transforming growth factor-β1 (TGF-β1), mannose-binding lectin (MBL), and β2-adrenergic receptor (reviewed in Ref. [82–84]). Markedly, the modes of action of these CFTR modifiers differ in terms of their underlying mechanisms. ENaC specifically transports epithelial Na^+. However, TGF-β1 is an intriguing cytokine that has both pro- and anti-inflammatory properties in addition to its effects on cell proliferation and differentiation. MBL, a serum protein, participates in innate immunity. Lastly, β2-adrenergic receptor, similar to the intestinal organoid stimulant forskolin, activates ADCY activity that exerts effect on airway reactivity.

Nonetheless, these modifiers could provide insight into the pathophysiological basis of CFTR organoid assays as well as the rationale for multi-target-based molecular interventions. For example, in patients with CF, the dehydrated secretions of the airway epithelial surface of the lung are caused, at least partially, by the ENaC, which facilitates Na^+ hyperabsorption in the presence of reduced Cl^- secretion mediated by CFTR. Thus, various approaches to inhibit ENaC expression and function have been proposed to restore the surface liquid volume of the airway, allowing normal mucociliary clearance of the respiratory tract in patients with CF [85]. Although the interactions between ENaC and CFTR are not well understood, the inhibition of ENaC-mediated Na^+ hyperabsorption would likely improve the therapeutic efficacy of CFTR drugs [86,87]. Therefore, organoid-based assays combined with these modifiers should be considered for individual patients with CF.

Concluding remarks

CFTR mutations can cause the synthesis deficiency, misfolding, and altered trafficking of CFTR proteins, resulting in the absence of CFTR on the cell surface, all of which contribute to a significant reduction in chloride channel activities. The emergence of stem cell-based organoid assays has begun to enable evaluations of the precision intervention of lethal genetic diseases, such as CF, by providing personalized drug screens [16,88]. Importantly, the effects of CF corrector and potentiator drugs, which restore CFTR cell surface expression and modulate CFTR-mediated chloride channel activity, respectively, can be synergized when used in combination.

Unraveling the complicated interactions between small molecules and various mutant CFTR proteins using organoid-based technologies enables us to understand the mechanisms of action of CFTR in high-resolution contents. Such approaches would also yield important insights into the development of precise potentiators, modulators, and chaperones. Combined therapeutic approaches with multiple correctors, potentiators, and chaperones offers a new direction to maximize CF drug efficacies and clinical benefits, potentially leading to a cure of patients with CF in the near future.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.drudis.2019.05.029.

References

1 O'Sullivan, B.P. and Freedman, S.D. (2009) Cystic fibrosis. *Lancet* 373, 1891–1904
2 Kuk, K. and Taylor-Cousar, J.L. (2015) Lumacaftor and ivacaftor in the management of patients with cystic fibrosis: current evidence and future prospects. *Ther. Adv. Respir. Dis.* 9, 313–326
3 Ramsey, B.W. *et al.* (2011) A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N. Engl. J. Med.* 365, 1663–1672
4 Accurso, F.J. *et al.* (2010) Effect of VX-770 in persons with cystic fibrosis and the G551D-CFTR mutation. *N. Engl. J. Med.* 363, 1991–2003
5 Davies, J.C. *et al.* (2013) Efficacy and safety of ivacaftor in patients aged 6 to 11 years with cystic fibrosis with a G551D mutation. *Am. J. Respir. Crit. Care Med.* 187, 1219–1225
6 Rowe, S.M. *et al.* (2014) Clinical mechanism of the cystic fibrosis transmembrane conductance regulator potentiator ivacaftor in G551D-mediated cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 190, 175–184
7 Wainwright, C.E. *et al.* (2015) Lumacaftor-ivacaftor in patients with cystic fibrosis homozygous for Phe508del CFTR. *N. Engl. J. Med.* 373, 220–231
8 Riordan, J.R. *et al.* (1989) Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 245, 1066–1073
9 Rommens, J.M. *et al.* (1989) Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 245, 1059–1065
10 Mayer, M. (2016) Lumacaftor-ivacaftor (Orkambi) for cystic fibrosis: behind the 'breakthrough'. *Evid. Based Med.* 21, 83–86
11 Dekkers, J.F. *et al.* (2016) Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis. *Sci. Transl. Med.* 8 344ra384
12 Bose, S.J. *et al.* (2019) Differential thermostability and response to cystic fibrosis transmembrane conductance regulator (CFTR) potentiators of human and mouse F508del-CFTR. *Am. J. Physiol. Lung Cell Mol. Physiol* . http://dx.doi.org/10.1152/ajplung.00034.2019 Published online April 10, 2019
13 Veeze, H.J. *et al.* (1991) Ion transport abnormalities in rectal suction biopsies from children with cystic fibrosis. *Gastroenterology* 101, 398–403
14 Okiyoneda, T. *et al.* (2013) Mechanism-based corrector combination restores DeltaF508-CFTR folding and function. *Nat. Chem. Biol.* 9, 444–454
15 Dekkers, J.F. *et al.* (2013) A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat. Med.* 19, 939–945
16 Chen, K.G. *et al.* (2018) Pluripotent stem cell platforms for drug discovery. *Trends Mol. Med.* 24, 805–820
17 Clevers, H. (2016) Modeling development and disease with organoids. *Cell* 165, 1586–1597
18 Fatehullah, A. *et al.* (2016) Organoids as an *in vitro* model of human development and disease. *Nat. Cell Biol.* 18, 246–254
19 Lancaster, M.A. and Knoblich, J.A. (2014) Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* 345 1247125
20 Veit, G. *et al.* (2016) From CFTR biology toward combinatorial pharmacotherapy: expanded classification of cystic fibrosis mutations. *Mol. Biol. Cell* 27, 424–433
21 Sosnay, P.R. *et al.* (2013) Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. *Nat. Genet.* 45, 1160–1167
22 Zhang, Z. and Chen, J. (2016) Atomic structure of the cystic fibrosis transmembrane conductance regulator. *Cell* 167, 1586–1597
23 Liu, F. *et al.* (2017) Molecular structure of the human CFTR ion channel. *Cell* 169 85–95 e88
24 Zhang, Z. *et al.* (2017) Conformational changes of CFTR upon phosphorylation and ATP binding. *Cell* 170, 483–491
25 Cheng, S.H. *et al.* (1991) Phosphorylation of the R domain by cAMP-dependent protein kinase regulates the CFTR chloride channel. *Cell* 66, 1027–1036
26 Cheng, S.H. *et al.* (1990) Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell* 63, 827–834
27 Dalemans, W. *et al.* (1991) Altered chloride ion channel kinetics associated with the delta F508 cystic fibrosis mutation. *Nature* 354, 526–528
28 Lukacs, G.L. *et al.* (1993) The delta F508 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
29 Sharma, M. *et al.* (2001) Conformational and temperature-sensitive stability defects of the delta F508 cystic fibrosis transmembrane conductance regulator in post-endoplasmic reticulum compartments. *J. Biol. Chem.* 276, 8942–8950
30 Castellani, C. *et al.* (2008) Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. *J. Cyst. Fibros.* 7, 179–196
31 Cuppens, H. *et al.* (1998) Polyvariant mutant cystic fibrosis transmembrane conductance regulator genes. The polymorphic (Tg)m locus explains the partial penetrance of the T5 polymorphism as a disease mutation. *J. Clin. Invest.* 101, 487–496
32 Clain, J. *et al.* (2005) A neutral variant involved in a complex CFTR allele contributes to a severe cystic fibrosis phenotype. *Hum. Genet.* 116, 454–460
33 Baatallah, N. *et al.* (2018) Cis variants identified in F508del complex alleles modulate CFTR channel rescue by small molecules. *Hum. Mutat.* 39, 506–514
34 Lucarelli, M. *et al.* (2017) A new targeted CFTR mutation panel based on next-generation sequencing technology. *J. Mol. Diagn.* 19, 788–800
35 Ivanov, M. *et al.* (2018) Targeted sequencing reveals complex, phenotype–correlated genotypes in cystic fibrosis. *BMC Med. Genomics* 11 (Suppl. (1)), 13
36 van Willigen, M. *et al.* (2019) Folding-function relationship of the most common cystic fibrosis–causing CFTR conductance mutants. *Life Sci. Alliance* 2 e201800172
37 Dijkstra, D.J. *et al.* (1994) A novel mutation (G1249R) in exon 20 of the CFTR gene. *Hum. Mutat.* 4, 161–162
38 Dujardin, G. *et al.* (2011) Splicing defects in the CFTR gene: minigene analysis of two mutations, 1811+1G>C and 1898+3A>G. *J. Cyst. Fibros.* 10, 212–216
39 Van Goor, F. *et al.* (2009) Rescue of CF airway epithelial cell function *in vitro* by a CFTR potentiator, VX-770. *Proc. Natl. Acad. Sci. U. S. A.* 106, 18825–18830
40 Yu, H. *et al.* (2012) Ivacaftor potentiation of multiple CFTR channels with gating mutations. *J. Cyst. Fibros.* 11, 237–245
41 Jih, K.Y. and Hwang, T.C. (2013) Vx-770 potentiates CFTR function by promoting decoupling between the gating cycle and ATP hydrolysis cycle. *Proc. Natl. Acad. Sci. U. S. A* 110, 4404–4409
42 Van Goor, F. *et al.* (2014) Effect of ivacaftor on CFTR forms with missense mutations associated with defects in protein processing or function. *J. Cyst. Fibros.* 13, 29–36
43 Flume, P.A. *et al.* (2012) Ivacaftor in subjects with cystic fibrosis who are homozygous for the F508del-CFTR mutation. *Chest* 142, 718–724
44 Hudson, R.P. *et al.* (2017) Direct binding of the corrector VX-809 to human CFTR NBD1: evidence of an allosteric coupling between the binding site and the NBD1: CL4 interface. *Mol. Pharmacol.* 92, 124–135
45 Ren, H.Y. *et al.* (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
46 Clancy, J.P. *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
47 Boyle, M.P. *et al.* (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
48 Davies, J.C. *et al.* (2018) VX-659-tezacaftor-ivacaftor in patients with cystic fibrosis and one or two Phe508del alleles. *N. Engl. J. Med.* 379, 1599–1611
49 Keating, D. *et al.* (2018) VX-445-tezacaftor-ivacaftor in patients with cystic fibrosis and one or two Phe508del alleles. *N. Engl. J. Med.* 379, 1612–1620
50 Han, S.T. *et al.* (2018) Residual function of cystic fibrosis mutants predicts response to small molecule CFTR modulators. *JCI Insight* 3, 121159
51 Berkers, G. *et al.* (2019) Rectal organoids enable personalized treatment of cystic fibrosis. *Cell Rep.* 26 1701–1708 e1703
52 de Winter-de Groot, K.M. *et al.* (2018) Stratifying infants with cystic fibrosis for disease severity using intestinal organoid swelling as a biomarker of CFTR function. *Eur. Respir. J.* 52, 1702529
53 Zhang, X.H. *et al.* (2015) Off-target effects in CRISPR/Cas9–mediated genome engineering. *Mol. Ther. Nucleic Acids* 4, e264
54 Esser, L. *et al.* (2017) Structures of the multidrug transporter P-glycoprotein reveal asymmetric ATP binding and the mechanism of polyspecificity. *J. Biol. Chem.* 292, 446–461
55 Kim, Y. and Chen, J. (2018) Molecular structure of human P-glycoprotein in the ATP-bound, outward-facing conformation. *Science* 359, 915–919

56 Van Goor, F. *et al.* (2011) Correction of the F508del-CFTR protein processing defect *in vitro* by the investigational drug VX-809. *Proc. Natl. Acad. Sci. U. S. A.* 108, 18843–18848

57 Rabeh, W.M. *et al.* (2012) Correction of both NBD1 energetics and domain interface is required to restore DeltaF508 CFTR folding and function. *Cell* 148, 150–163

58 Loo, T.W. and Clarke, D.M. (2017) Corrector VX-809 promotes interactions between cytoplasmic loop one and the first nucleotide-binding domain of CFTR. *Biochem. Pharmacol.* 136, 24–31

59 Sinha, C. *et al.* (2015) Capturing the direct binding of CFTR correctors to CFTR by using click chemistry. *Chembiochem* 16, 2017–2022

60 Veit, G. *et al.* (2018) Structure-guided combination therapy to potently improve the function of mutant CFTRs. *Nat. Med.* 24, 1732–1742

61 Brewington, J.J. *et al.* (2018) Generation of human nasal epithelial cell spheroids for individualized cystic fibrosis transmembrane conductance regulator study. *J. Vis Exp* 134, e57492

62 Guimbellot, J.S. *et al.* (2017) Nasospheroids permit measurements of CFTR-dependent fluid transport. *JCI Insight* 2, 95734

63 Raraigh, K.S. *et al.* (2018) Functional assays are essential for interpretation of missense variants associated with variable expressivity. *Am. J. Hum. Genet.* 102, 1062–1077

64 McCague, A.F. *et al.* (2019) Correlating cystic fibrosis transmembrane conductance regulator function with clinical features to inform precision treatment of cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 199, 1116–1126

65 Lingam, S. *et al.* (2017) Investigation of the effects of the CFTR potentiator ivacaftor on human P–glycoprotein (ABCB1). *Sci. Rep.* 7, 17481

66 Zhang, X. *et al.* (2014) Effects of curcumin on ion channels and transporters. *Front. Physiol.* 5, 94

67 Egan, M.E. *et al.* (2004) Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. *Science* 304, 600–602

68 Wang, W. *et al.* (2007) Curcumin opens cystic fibrosis transmembrane conductance regulator channels by a novel mechanism that requires neither ATP binding nor dimerization of the nucleotide-binding domains. *J. Biol. Chem.* 282, 4533–4544

69 Lin, W.Y. *et al.* (2016) Synergistic potentiation of cystic fibrosis transmembrane conductance regulator gating by two chemically distinct potentiators, ivacaftor (VX-770) and 5-nitro-2-(3-phenylpropylamino) benzoate. *Mol. Pharmacol.* 90, 275–285

70 Phuan, P.W. *et al.* (2014) Synergy-based small-molecule screen using a human lung epithelial cell line yields DeltaF508-CFTR correctors that augment VX-809 maximal efficacy. *Mol. Pharmacol.* 86, 42–51

71 Shcheynikov, N. *et al.* (2004) Dynamic control of cystic fibrosis transmembrane conductance regulator Cl(-)/HCO3(-) selectivity by external Cl(-). *J. Biol. Chem.* 279, 21857–21865

72 Linsdell, P. *et al.* (1997) Permeability of wild-type and mutant cystic fibrosis transmembrane conductance regulator chloride channels to polyatomic anions. *J. Gen. Physiol.* 110, 355–364

73 Poulsen, J.H. *et al.* (1994) Bicarbonate conductance and pH regulatory capability of cystic fibrosis transmembrane conductance regulator. *Proc. Natl. Acad. Sci. U. S. A.* 91, 5340–5344

74 Shah, V.S. *et al.* (2016) Airway acidification initiates host defense abnormalities in cystic fibrosis mice. *Science* 351, 503–507

75 Shamsuddin, A.K.M. and Quinton, P.M. (2019) Concurrent absorption and secretion of airway surface liquids and bicarbonate secretion in human bronchioles. *Am. J. Physiol. Lung Cell Mol. Physiol.* 316, L953–L960

76 Jung, J. and Lee, M.G. (2014) Role of calcium signaling in epithelial bicarbonate secretion. *Cell Calcium* 55, 376–384

77 Park, H.W. *et al.* (2010) Dynamic regulation of CFTR bicarbonate permeability by Cl- i and its role in pancreatic bicarbonate secretion. *Gastroenterology* 139, 620–631

78 Pibiri, I. *et al.* (2015) Enhancement of premature stop codon readthrough in the CFTR gene by Ataluren (PTC124) derivatives. *Eur J. Med. Chem.* 101, 236–244

79 Lueck, J.D. *et al.* (2019) Engineered transfer RNAs for suppression of premature termination codons. *Nat. Commun.* 10, 822

80 Cooney, A.L. *et al.* (2018) Cystic fibrosis gene therapy: looking back, looking forward. *Genes* 9, E538

81 Vidovic, D. *et al.* (2016) rAAV-CFTRDeltaR rescues the cystic fibrosis phenotype in human intestinal organoids and cystic fibrosis mice. *Am. J. Respir. Crit. Care Med.* 193, 288–298

82 Merlo, C.A. and Boyle, M.P. (2003) Modifier genes in cystic fibrosis lung disease. *J. Lab. Clin Med.* 141, 237–241

83 Slieker, M.G. *et al.* (2005) Disease modifying genes in cystic fibrosis. *J. Cyst. Fibros.* 4 (Suppl. (2)), 7–13

84 Collaco, J.M. and Cutting, G.R. (2008) Update on gene modifiers in cystic fibrosis. *Curr. Opin. Pulm. Med.* 14, 559–566

85 Butler, R. *et al.* (2015) ENaC inhibitors for the treatment of cystic fibrosis. *Pharm. Pat. Anal.* 4, 17–27

86 Shei, R.J. *et al.* (2018) The epithelial sodium channel (ENaC) as a therapeutic target for cystic fibrosis. *Curr. Opin. Pharmacol.* 43, 152–165

87 Moore, P.J. and Tarran, R. (2018) The epithelial sodium channel (ENaC) as a therapeutic target for cystic fibrosis lung disease. *Expert Opin. Ther. Targets* 22, 687–701

88 Gorshkov, K. *et al.* (2018) Advancing precision medicine with personalized drug screening. *Drug Discov. Today* 24, 272–278