Molecular targeting of CFTR as a therapeutic approach to cystic fibrosis

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One of the major challenges facing the pharmaceutical field is the identification of novel, ‘druggable’ targets common to distinct diseases that, despite their clinical diversity, share the same basic molecular defect(s) – thus, being termed ‘horizontal diseases’. Membrane proteins constitute one of the largest families in the human genome and, given their major roles in cells and organisms, they are relevant to common human disorders such as cardiovascular disease and cancer, but also to rare genetic conditions such as cystic fibrosis (CF). Here, we review therapeutic approaches to correcting the basic defect in CF, which is caused mainly by the intracellular retention of a misfolded protein, and focus on various recent drug-discovery strategies for this important and paradigmatic disease. These strategies have possible applications in many membrane protein disorders, including other channelopathies. The mechanisms of action of potent and specific compounds, representing promising drug leads for CF pharmacotherapy, are explained and discussed.

Introduction
One of the major challenges facing the pharmaceutical field is the identification and validation of novel, high-quality, ‘druggable’ targets common to distinct diseases that, despite being clinically diverse, share the same basic molecular defect(s). These are termed ‘horizontal diseases’ because of their common defective cellular phenotype(s). Examples include misfolding and trafficking disorders.

Membrane proteins constitute some of the largest families encoded in the human genome, including ATP-binding cassette (ABC) transporters, ion channels and G-protein-coupled receptors (GPCRs). Given their major roles in cells and organisms, from transport and communication to immunity and nervous system functions, membrane proteins are highly relevant to frequent human disorders such as cardiovascular and neurological disorders, cancer, chronic pain, obesity and diabetes, but also to rare genetic conditions [1]. GPCRs and ion channels are currently the most attractive targets for drug discovery [2,3].

Here, we provide a concise, up-to-date review of therapeutic approaches to cystic fibrosis (CF), with potential application in a wide range of diseases involving membrane proteins, particularly ABC transporters (see http://www.ncbi.nlm.nih.gov/books/bf.cgi?rid=mono_001.chapter.137). These proteins are central to cancer (resistance to drugs), metabolic disorders (hyperinsulinemia, dyslipidemias and lysosomal disorders), osteoporosis, hearing loss and many other disorders [4].

CF is the most common lethal monogenic disorder in Caucasians, estimated to affect one per 2500–4000 newborns [5]. Clinically, CF is dominated by chronic lung disease, which is the main cause of morbidity and mortality. Airway obstruction by thick mucus and chronic infection by Pseudomonas aeruginosa eventually lead to loss of pulmonary function [6]. Other CF symptoms include pancreatic dysfunction, elevated sweat electrolytes and male infertility.

CF is caused by dysfunction of a single gene encoding the CF transmembrane conductance regulator (CFTR), which is an ABC transporter (ABCC7) that functions as a Cl⁻ channel in the apical membrane of epithelial cells lining the target organs (lung, intestine and sweat gland). CFTR, probably the best-studied ion channel, is regulated by cAMP-dependent protein kinase (PKA) and ATP. Its 1480 amino acids constitute two transmembrane domains (TMDs), two nucleotide-binding domains (NBDs) and one regulatory domain (RD), which undergo multiple phosphorylations [7]. Despite impressive advances in elucidating the molecular basis of CF, most current therapies are still restricted to alleviating symptoms [8]. Thus, life expectancy and quality of life, although largely improved recently, are still limited for CF patients [6].

Approaches aimed at correcting the basic CF defect still hold promise for curing the disease. But what is the basic defect in CF? To date, ~1500 CFTR mutations have been described, most of which cause CF (see the CFTR Mutation Database: http://www.genet.sickkids.on.ca/cftr/app). Between these gene defects and the ultimate clinical phenotype of respiratory insufficiency, several events comprise the so-called ‘CF pathogenesis cascade’ (Figure 1a).

To correct such a variety of gene and protein defects effectively, CFTR mutants are grouped into functional classes (Figure 2b) that can be corrected through the same restoring strategy – an approach termed ‘mutation-specific therapy’ [9] (Box 1).

The emphasis of these strategies is on a single mutation, F508del (included in class II; Figure 1b and Box 1), because it occurs in ~90% of CF patients. Accordingly, many efforts have focused on elucidating the molecular mechanism of F508del-CFTR dysfunction. CFTR without residue F508 is a particularly difficult folding substrate, the abnormal conformation of this mutant being recognized and retained
by the endoplasmic reticulum quality control (ERQC), which rapidly targets it for proteasomal degradation \[10,11\]. Thus, very little or no F508del-CFTR, depending on the cell type, reaches the cellular membrane. Nevertheless, rescuing this mutant to the cell surface would be therapeutically relevant because it retains some Cl\(^{-}\)/C\(_{0}\) channel function. The ERQC acting on F508del-CFTR has also been a good model with which to describe the key players of the ERQC – mostly molecular chaperones such as Hsp70/Hdj-2, calnexin, CHIP, Hsp90 and Hdj-1/Hsp40 (for review, see Ref. [10]) – and the mechanisms of retention and degradation, which are likely to be shared by several other mutant proteins [12,13].

Here, we focus on two types of recent and promising compound, emphasizing their mechanism of action: (i) compounds that rescue the trafficking defect of class II mutations (‘correctors’); and (ii) compounds that overcome or enhance the defective Cl\(^{-}\) channel activity of class III, IV and V CFTR mutants (‘potentiators’).

Because CFTR, in addition to being a channel, regulates epithelial ion transport by interfering with several other ion channels and transporters, we also describe a ‘bypassing’ approach to tackling drug discovery for CF. This strategy aims to correct the ionic imbalance in CF, which contributes to CF lung pathogenesis [14], through stimulation of bypassing ionic pathways that might compensate for the absence of functional CFTR.

The bypassing approach
Together with impaired cAMP-dependent Cl\(^{-}\) secretion, enhanced Na\(^{+}\) absorption occurs in CF airways [15] (Figure 1). This leads to hyperabsorption of fluid and electrolytes by the surface epithelium, and isotonic contraction of the airway surface liquid (ASL) – the thin aqeous layer covering the periciliated layer above airway epithelial cells [15]. Moreover, CFTR is involved in bicarbonate secretion, control of osmotic water permeability, electroneutral NaCl transport and many more aspects of epithelial cell physiology; thus, it is a true conductance regulator [16]. Given these multiple effects, correction of mutant CFTR, besides repairing a defective Cl\(^{-}\) channel, will also improve many other aspects of membrane transport. Moreover, CFTR-mediated Cl\(^{-}\) secretion might also be indirectly activated by increasing the secretory driving force for Cl\(^{-}\): for
example, by activation of basolateral $K^{+}$ channels. By causing hyperpolarization of the cell membrane potential, this enhances electrogenic luminal Cl$^{-}$ secretion [16].

Alternatively, activation of other, non-CFTR Cl$^{-}$ channels can circumvent a defective CFTR, at least in the airways. The most promising current strategy is to stimulate ATP-activated purinergic receptors with synthetic nucleotides that are more stable than ATP, such as INS365 (Figure 2), which was already tested in the clinical setting [16]. Such a strategy has the advantage of activating Ca$^{2+}$-activated Cl$^{-}$ secretion (CaCC) channels and inhibiting epithelial Na$^{+}$ channels (Figure 2), thus attenuating excessive Na$^{+}$ absorption and ASL dehydration [16]. Other potential tools for a bypassing pharmacotherapy comprise phosphodiesterase (PDE) inhibitors, sympathomimetic compounds, phosphatase inhibitors and compounds that act on other ionic pathways [8] (Figure 2).

**Figure 2.** Pharmacological compounds used in therapeutic strategies aimed at circumventing the ion channel defect in CF airways. (a) Enhanced Na$^{+}$ conductance in surface airway epithelial cells leads to excessive absorption of electrolytes. The responsible Na$^{+}$ channel, ENaC, can be blocked by specific inhibitors such as amiloride, benzamil and phenamil, and probably by activation of PKC. The activation of purinergic receptors by ATP or UTP inhibits ENaC. (b) Stimulation of an alternative, CaCC channel in CF airway epithelial cells by stimulation of luminal P2Y$_{2}$ purinergic receptors with ATP or UTP via a cascade of events that involves activation of PLC and breakdown of phosphatidylinositol (4,5)-bisphosphate (PIP$_{2}$) to $\gamma$-myo-inositol (1,4,5)-trisphosphate (IP$_{3}$) to $\gamma$-myo-inositol (1,4,5)-trisphosphate (IP$_{3}$). (c) Increase of the electrical driving force of luminal Cl$^{-}$ secretion by stimulation of the basolateral Ca$^{2+}$-activated K$^{+}$ channel SK4 by the benzimidazol compound 1-EBIO, or activation of CaCC-regulated K$^{+}$ channels (KvLQT1) by agonists of the cAMP pathway, such as $\beta$-adrenoceptor compounds, or PDE blockers such as amrinone and milrinone.

**Box 1. Mutation-specific therapies for treating CF**

‘Protein-repair’ or ‘mutation-specific’ therapy has become an important area of drug discovery for CF. This approach relies on grouping CFTR mutations into functional classes, namely [62]: (i) class I mutations, which abrogate protein production and are often nonsense mutations (i.e. they generate premature stop codons that lead to mRNA degradation by nonsense-mediated decay); (ii) class II mutations (including the most prevalent, F508del), which lead to a processing defect caused by retention by the ERQC and subsequent degradation; (iii) class III mutants, which impair the process of CFTR channel opening (gating); and (iv) class IV mutants with reduced anion conductance [63].

Several strategies adopting this approach are currently under experimental testing or have already progressed to the clinical setting [9].

- **Class I**
  Aminoglycoside antibiotics have been reported to suppress premature termination codons by enabling the incorporation of an amino acid, thus permitting translation to continue until the normal termination of the transcript. An example is the clinical trial carried out in patients with CF and CFTR stop mutations through gentamicin-induced correction [8].

- **Class II**
  Chemical, molecular or pharmacological chaperones, generally called ‘correctors’ [36], were reported to stabilize protein structure and promote folding, enabling cell-surface expression of processing mutants.

- **Class III**
  CFTR activators such as alkylxanthines (CPX) and the flavonoid genistein overcome these class III defects, acting as channel ‘potentiators’. F508del-CFTR is also a class III mutant because membrane-localized channels also exhibit impaired gating [34,38,50].

- **Class IV**
  Compensation for reduced conductance can be achieved by increasing the overall cell-surface content of these mutants (promoting their traffic) or/and through increased stimulation of the existing channels with potentiators.

- **Class V**
  Splicing factors that promote normal exon inclusion or factors that promote abnormal exon skipping increase levels of properly spliced transcripts. Potentiators are also useful for these mutants because they enhance the activity of normal channels already at the cell surface.

**CFTR traffic compounds (correctors)**

The discovery of ‘CFTR correctors’ (i.e. agents that rescue cell-surface expression of F508del-CFTR) has been anticipated to be more challenging than that of potentiators because of the complexity of trafficking processes and the multiplicity of intervenients involved [17]. According to the proposed mechanism of action, correctors fall into one of three different categories (Table S1 in the supplementary material online): (i) chemical chaperones (i.e. compounds that mimic the effects of molecular chaperones); (ii) compounds that putatively target molecular chaperones by affecting their levels or their interaction with the defective protein; and (iii) pharmacochaperones (which are similar to chemical chaperones, except target-specific).

**Chemical chaperones**

Osmotically active molecules such as glycerol, dimethylsulfoxide (DMSO), taurine, betaine and myo-inositol, which generally suppress protein-folding defects [18], are also effective at rescuing F508del-CFTR cell-surface expression in cellular systems [8,9]. However, these compounds are nonspecific and require high concentrations (millimolar) and prolonged incubation to be effective (Table S1 in the supplementary material online). Nevertheless, these findings encouraged the screening of small-molecule...
libraries for more-potent and more-specific compounds. Using high-throughput screening (HTS), Vertex (http://www.vpharm.com/) identified the quinazoline VX-325 – which is among the most effective F508del-CFTR correctors [4], being 100-fold more potent than previously described correctors such as benzo[c]quinolizinium compounds (MPBs) or sildenafil [4,19,20]. Although VX-325 seems to act on folding, its observed effect on F508del-CFTR is not specific [20].

Additional compounds have been described as rescuing the traffic defect of F508del-CFTR but, because they are CFTR specific and/or were first described as potentiators, they are mentioned in the subsequent sections.

**Targeting molecular chaperones**

Provided that there is clear evidence that disruption of a given chaperone or ERQC-intervenient with a substrate of interest is of therapeutic relevance, a decrease in the levels of the chaperone or ERQC-intervenient can be specifically achieved using antisense RNA, which is an emerging therapeutic strategy [11]. Alternatively, interactions between chaperones and client proteins could be pharmacologically manipulated, and have proven to be successful in cancer therapy [21]. Consistent with this, deoxyspergualin (DSG) [22] and 4-phenylbutyrate (4-PBA) [23,24] were described as disrupting Hsc70/Hsp70-substrate interactions and, thus, facilitating membrane trafficking of F508del-CFTR [11]; this strategy, however, was not effective in another study [25]. Moreover, butyrate was shown to cause a significant decrease in anion secretion in Calu-3 cells [24]. Thus, instead of stimulating, butyrate might inhibit the anion secretory capacity of human CFTR-expressing epithelial cells, although it might enhance CFTR transcription.

Similarly, disruption of the interaction of CFTR with the endoplasmic reticulum (ER) chaperone calnexin was described as the major mechanism of action underlying the cell-surface rescue of F508del-CFTR by both curcumin [26] and the ER α-glucosidase I and II inhibitor N-butyl-deoxyo-jirimycin (miglustat) [27,28]. The latter is used in Gaucher disease therapy as an inhibitor of ceramide-specific glycosyltransferases [29]. However, these results are controversial because F508del-CFTR correction by curcumin was confirmed by only some investigators [30–32]. Also, it has not been proven that the effect of miglustat is caused by disruption of the CFTR–calnexin interaction, and indeed specific calnexin depletion by RNA interference (RNAi) causes a major decrease in the processing efficiency of wild-type CFTR [12]. Thus, these proposed chaperone-mediated mechanisms of action would probably cause further folding impairment of F508del-CFTR [10–12,25,57].

**Pharmacochaperones**

The identification of compounds that specifically rescue a misfolded protein (pharmacochaperones) followed pioneering work using mutant V_{2} vasopressin receptors that cause nephrogenic diabetes insipidus [2]. Rescue by pharmacochaperones is generally achieved through the specific binding of ligands (agonists or blockers) to unfolded mutants to favor energetically their folding. With regard to CF, this concept indicates that activators or potentiators that directly bind to CFTR might also act as correctors [11]. However, in our investigations, the potentiator genistein did not rescue the processing defect of F508del-CFTR, even after prolonged incubation [33].

Compounds that probably bind to CFTR and that have been shown to correct the abnormal localization of F508del-CFTR in nasal epithelial cells from F508del-homozygous patients include MPBs [34] and sildenafil (Viagra®) [35]. Although sildenafil is an inhibitor of phosphodiesterase (PDE5), the high doses required for correction makes this an improbable mechanism of action for F508del-CFTR rescue. Additional classes of correctors or potentiators recently identified by HTS include aminobenzothiazoles, aminopyrlythiazoles, quinazolinylaminopyrpyrimidiones and bisaminomethylbithiazoles [36] (Table S1 in the supplementary material online). Only bisaminomethylbithiazoles effectively rescued F508del-CFTR to the cell surface in polarized human bronchial epithelial cells, achieving a maximal correction of ~8% of the normal CFTR function and a sustained response (~80% after 24 h). The mechanism of action of this class is not yet fully described, but it seems to facilitate the ER exit of F508del-CFTR by: (i) enhancing folding; (ii) decreasing degradation; and (iii) increasing plasma membrane stability [36]. Because there are multiple ERQC steps at which CFTR folding can be assessed [12,13], it remains to be elucidated at which stage these compounds exert their effect.

The pyrazole potentiator VX-532 [4-methyl-2-(5-phenyl-1H-pyrazol-3-yl)-phenol] is among the most specific F508del-CFTR correctors [4,19,20]. Because VX-532 also enhances channel activity (see later), it might bind directly to CFTR.

**Stimulators of CFTR channel activity (potentiators)**

Compounds that stimulate pre-activated CFTR channel activity (potentiators) can be grouped into three major classes, covering chemicals and potential drugs that have been identified through: (i) conventional approaches (i.e. through single observations) or hypothesis-driven (H-D) approaches; (ii) HTS; and (iii) exploring naturally occurring compounds.

**Conventional approaches**

Using a standard approach, potentiators of CFTR channel activity are discovered through single observations or by testing known activators of other ion channels. Such an H-D approach led to the identification of several stimulators of Cl− currents, mediated by wild-type or mutant CFTR, with a relatively low input of work and money; however, this type of approach is likely to miss unknown but potent CFTR potentiators. Nevertheless, it has a higher chance of identifying compounds already in clinical use and, therefore, closer to clinical application.

Compounds thus identified include [8,37] (Table S2 in the supplementary material online): (i) alkylxanthines [3-isobutyl-1-methylxanthine (IBMX), 8-cyclopentyl-1,3-dipropylxanthine (CPX or DPCPX) and 1,3-diallyl-8-cyclohexylxanthine (DAX)]; (ii) phenanthrolines and
benzoquinolines (1,10-phenanthroline, 7,8- and 5, 6-benzoquinolines, and 4-chloro-benzo[F]isoquinoline (CBIQ)); (iii) flavonoids; (iv) MPB derivatives; (v) phloxin B; (vi) benzimidazolones [NS004, NS1619, 1-ethyl-2-benzimidazolone (EBIO) and 5,6-dichloro-1-ethyl-1,3-dihydro-2H-benzimidazol-2-one (DCEBIO)]; (vii) psoralens; and (viii) N-acetyl-L-cysteine.

Xanthines do not activate CFTR by stimulating adenosine receptors or by inhibiting PDE activity and increasing intracellular cAMP levels, but apparently by binding (directly but differentially) to NBD1 of wild-type CFTR and F508del-CFTR after pre-activation by PKA. Despite conflicting results [38,39], CPX was clinically tested but no benefit was demonstrated [38].

1,10-Phenanthroline affects neither the generation of cAMP nor the concentration of intracellular calcium ([Ca\(^{2+}\)]\(_{i}\)) in colonic epithelial cells; however, as is the case with benzoquinolines, it activates basolateral Ca\(^{2+}\) and cAMP-regulated K\(^{+}\) channels. Therefore, phenanthrolines are useful lead compounds for adjunct therapy in CF, with dual actions on CFTR and basolateral K\(^{+}\) channels [40]. CBIQ, which acts on CFTR Cl\(^{-}\) and Ca\(^{2+}\)-activated KCNn4 K\(^{+}\) channels, activates short-circuit currents and increases the open probability of CFTR (EC\(_{50}\) ~ 4 \(\mu\)M) [41]. MPBs probably act through direct CFTR binding, and some (e.g. MPB-91) are K\(_{ATP}\) channel inhibitors [42]. One study could not detect any activating effects of MPB-07 on G551D-CFTR [43]. The fluorescein derivative phloxine B stimulates wild-type CFTR and F508del-CFTR by increasing the open probability of the pre-phosphorylated channel; that is, by enhancing CFTR activity. The binding of ATP to NBDs regulates opening (NBD1) and closing (NBD2) of the channel. Phloxine B slows the rate of channel closure by binding to NBD2, thereby slowing ATP dissociation [44].

The benzimidazolone NS004 changes the gating of CFTR in a phosphorylation-dependent manner [39]. NS004 and NS1619 shorten the closed time of the channel after PKA-dependent pre-stimulation [39]. In addition, benzimidazolones activate basolateral K\(^{+}\) channels, thus further supporting Cl\(^{-}\) secretion [39]. Finally, N-acetyl-L-cysteine, which has long been used as a mucolytic agent, was also found to activate CFTR Cl\(^{-}\) conductance [8]. Overall, using conventional approaches, a diverse group of chemicals has been identified as potentiators of CFTR, but the respective mechanisms of action have been described for only a few of the compounds.

**HTS**

A major advance in the identification of potentially therapeutic small molecules is the use of HTS of large and diverse chemical libraries of direct activators or stimulators of CFTR [45]. Such high-throughput screens are possible through an automated fluorescence-based assay: for example, cells expressing the halide indicator yellow fluorescent protein. To screen for F508del-CFTR activators, cells require pre-incubation at 27°C (for 24 h) for cell-surface expression of the mutant. A large number of potent potentiators of mutant CFTR was thus identified, including: (i) benzoquinolizinium compounds, benzoflavones and isoxazoles (from flavone and MPB lead libraries, yielding the 223 flavonoid, quinolizinium and related heterocyclic compounds); (ii) phenylglycines and sulfonamides; (iii) benzothiophenes and benzofurans; (iv) 1,4-dihydropyridines (DHPs, from a library of approved drugs); (v) pyrazole derivatives (VX-532); and (vi) pyrrolopyrazines (Table S2 in the supplementary material online).

The most potent compounds are currently benzoquinolizinium compounds, benzoflavones and isoxazoles, 2-(4-pyridinium)benzo[h]4H-chromen-4-one bisulfate (UCcf029) and 3-(3-butyryl)-5-methoxy-1-phenylpyrazole-4-carbaldehyde (UCcf180), which are derived from the novel structural class of fused pyrazolo heterocycles, and UCcf339 (Table S2 in the supplementary material online). They have an activity that is ~10 times that of the reference drug genistein. Although the naturally derived flavone apigenin remains the most potent activator of G551D-CFTR [46], the novel classes of isoxazolones and isoxazolines might deliver higher-potency compounds [47].

Phenyglycines and sulfonamides (potencies >100 nM) improve F508del-CFTR, G551D-CFTR and G1349D-CFTR gating, but only after cAMP activation [48,49]. Because phenyglycines are rapidly metabolized in hepatic microsomes, they probably require aerosol administration. Tetrahydrobenzothiophene (EC\(_{50}\) < 100 nM), benzoferan, pyramidinetrione, dihydropyridine and anthraquinone compounds putatively bind to CFTR NBD1 directly [50].

Antihypertensive DHPs such as nifedipine, nicardipine, nimodipine, isradipine, nifedipine, felodipine and nigrudin, which already have European Agency for the Evaluation of Medicinal Products (EMEA: http://www.ema.europa.eu/) and US Food and Drug Administration (FDA: http://www.fda.gov/) approval, potentiate F508del-CFTR at low doses [51] (Table S2 in the supplementary material online). Potentiation is independent of voltage-dependent Ca\(^{2+}\) channels, which are the original pharmacological target of antihypertensive DHPs.

The pyrazole VX-532 is among the most potent and efficacious potentiators of F508del-CFTR and G551D-CFTR [19]. Pyrrolopyrazine derivatives (6-phenylpyrrolo[2,3-b]pyrazines) such as 7-N-butyryl-6-(4-hydroxyphenyl)[5H]pyrrolo[2,3-b]pyrazine (RP107) potentiate wild-type CFTR, G551D-CFTR and F508del-CFTR in human airway epithelial cells and colonic epithelium [37]. Thus, HTS has identified several diverse molecules that now require in-depth analysis to determine their mechanisms of action and efficacy in the native epithelium.

**Naturally derived compounds**

The search for naturally derived compounds is driven by the idea that natural plants, herbs, fruits and food components contain active molecules that accumulate in the human body, reaching concentrations at which they might interfere with CFTR activity. The rationale is to bypass time- and cost-intensive validation procedures before EMEA or FDA approval. This approach has identified the following compounds: (i) phytoflavonoids (genistein, apigenin, kaempferol and quercetin); (ii) capsaicin; (iii) *Phyllanthus acidus* extract; and (iv) ginsenosides, limonoids and vitamin C (Table S2 in the supplementary material online).

Although flavonoids are inhibitors of tyrosine kinases and phosphatases, their effects on CFTR are probably
independent of these activities, resulting from direct binding to an NBD of phosphorylated CFTR [8]. Genistein is also effective in human nasal epithelia – particularly on G551D-CFTR – restoring ENaC downregulation but, at higher concentrations, it inhibits ion transport [38,39]. However, in our studies with native epithelial tissues from CF patients, genistein was relatively ineffective [8]. Genistein is currently under Phase I pilot testing in co-administration with 4-PBA. Capsaicin, which is present in chilli peppers, was suggested to activate intestinal fluid secretion by stimulating sensory neurons and releasing neurotransmitters. Although it acts on transient receptor potential receptors and influences intracellular Ca\textsuperscript{2+}-signaling, capsaicin directly activates wild-type CFTR, G551D-CFTR and F508del-CFTR as effectively as does genistein and with a similar mechanism of action. It seems to be particularly useful for treating CF-associated gastrointestinal disorders [52].

The extract from the traditional Thai herbal medicinal plant P. acidus activates Cl\textsuperscript{–} secretion and inhibits Na\textsuperscript{+} absorption through several parallel mechanisms [53]. This extract was effective in murine native tissues expressing wild-type CFTR or F508del-CFTR and, thus, might provide a novel complementary nutraceutical treatment for CF lung disease. Ginsenosides and pseudoginsenosides stimulate Ca\textsuperscript{2+}-activated Cl\textsuperscript{–} channels through phospholipase C (PLC) activation and intracellular Ca\textsuperscript{2+} mobilization [53]. Moreover, some ginsenosides increase the level of nitric oxide, which activates K\textsuperscript{+} and Ca\textsuperscript{2+} channels and Cl\textsuperscript{–} secretion via wild-type CFTR and F508del-CFTR [53]. Citrus limonoids, identified in a yeast-based drug discovery bioassay for natural products, also increase Cl\textsuperscript{–} conductance in epithelial cells to an extent comparable to genistein [54]. A similar effect was recently shown for vitamin C [55].

Future perspectives

From a total of 132 clinical trials involving CF patients (see http://www.clinicaltrials.gov and http://www.cf.org/treatments/Pipeline/), only a minority is aimed at correcting the basic defect in CF, which might explain why the impact of these approaches on the clinical setting is still low.

However, growing attention is being given to innovative approaches. The genomic era has introduced a basic shift into experimentation by enabling researchers to look comprehensively at biological systems. A major transformation in drug-target discovery results from coupling genomic tools with automation [56]. Examples include a recent proteomic approach for assessing global CFTR protein interactions (CFTR interactome), by which a co-chaperone of Hsp90, Aha, was identified as a potential target for CF therapy [57]. Most promising are postgenomic screens aimed at identifying protein targets that modify the defective localization and/or function of mutant CFTR [57].

Gene therapy for CF, although recovering from the abyss that followed the original high expectations, has not yet met the standards of a promising new treatment. Recent advances report moderate success for non-viral formulations and indicate integrating lentiviruses as most promising in terms of efficacy [58]. Another emerging approach for curing CF is stem- or progenitor-cell therapy with ex vivo corrected cells. Murine embryonic stem cells were recently shown to differentiate into airway epithelial cells, originating a well-differentiated airway epithelium [59]. However, while effective gene and stem-cell therapies are still far from being a viable option, greater expectations lie on pharmacological strategies.

Thus, several novel compounds seem to be promising leads for developing effective drugs against the basic defect in CF. Nevertheless, significant improvements in terms of potency, specificity, efficacy and toxicity are required to minimize the risk of side effects. Additional high-throughput screens of small-molecule collections are probably needed for identifying novel chemical scaffolds, thus circumventing the current toxicity issues.

Elucidating the mechanism of action for each novel compound is essential for approval as a drug. To this end, studies that characterize the basic mechanisms underlying the disease are most important. These can, in turn, indicate innovative H-D therapeutic approaches. Pre-clinical characterization of novel compound efficacy is done mainly in rodents, whose airway physiology and anatomy are remarkably different from those of humans. The use of larger models such as pigs [60], and validation using human tissues ex vivo [61] can circumvent these problems.

Moreover, establishing adequate therapy endpoints is indispensable for assessing drug efficacy and clinical validation; however, this issue awaits further progress in the CF field. Additionally, pharmacogenomics can help to distinguish responders from non-responders through the identification of global effects. With such a concerted action, it might be possible to achieve the threshold of CFTR activity that is believed to be sufficient for curing CF (~10% of that found in non-CF individuals) [17].

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Supplementary data

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