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Rescuing Mutant CFTR: A Multi-task Approach to a Better Outcome in Treating Cystic Fibrosis

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Abstract: Correcting multiple defects of mutant CFTR with small molecule compounds has been the goal of an increasing number of recent Cystic Fibrosis (CF) drug discovery programmes. However, the mechanism of action (MoA) by which these molecules restore mutant CFTR is still poorly understood, in particular of CFTR correctors, i.e., compounds rescuing to the cells surface the most prevalent mutant in CF patients - F508del-CFTR. However, there is increasing evidence that to fully restore the multiple defects associated with F508del-CFTR, different small molecules with distinct corrective properties may be required.

Towards this goal, a better insight into MoA of correctors is needed and several constraints should be addressed. The methodological approaches to achieve this include: 1) testing the combined effect of compounds with that of other (non-pharmacological) rescuing strategies (e.g., revertants or low temperature); 2) assessing effects in multiple cellular models (non-epithelial vs epithelial, non-human vs human, immortalized vs primary cultures, polarized vs non polarized, cells vs tissues); 3) assessing compound effects on isolated CFTR domains (e.g., compound binding by surface plasmon resonance, assessing effects on domain folding and aggregation); and finally 4) assessing compounds specificity in rescuing different CFTR mutants and other mutant proteins.

These topics are reviewed and discussed here so as to provide a state-of-the art review on how to combine multiple ways of rescuing mutant CFTR to the ultimate benefit of CF patients.

Keywords: Cystic fibrosis, F508del-CFTR, rescue, correctors, potentiators, endoplasmic reticulum retention, traffic mutant, misfolding protein.

INTRODUCTION

Cystic Fibrosis (CF), the most common*,* lethal monogenic disorder in Caucasians, is dominated by the respiratory disease, the main cause of morbidity and mortality, with airway obstruction by thick mucus and chronic infections, mostly by *Pseudomonas aeruginosa*, eventually leading to impairment of respiratory function [1]. Other CF symptoms include pancreatic dysfunction, elevated sweat electrolytes and male infertility, but there is wide clinical variability in organ involvement [2]. Current therapies are mostly aimed at treating such multi-organ CF symptoms, such as aggressive antibiotic strategies for the clinical management of major bacterial lung infections, especially by *Pseudomonas aeruginosa*, a hallmark of this disease. Nonetheless, these "symptomatic" therapies have significantly pushed forward mean age of survival of CF patients from early childhood in 1950's to late 30s at present [3]. Pointedly, these approaches, together with extensive lung transplant programmes, have led to the fact that in several countries close to (or over) 50% of CF patients are now adults. However, despite of these major therapeutic advances, the burden of CF care is still very high and life expectancy and quality of life of most CF patients are still limited.

The much ambitioned alternative is to address the basic CF defect(s) systemically with small molecules which can correct, if not all, at least most symptoms notably those involving the airways. Due to intensive high-throughput efforts by companies and a few academic labs, this promising approach has recently evolved as a reality [4]. However, the mechanism of action (MoA) by which these molecules restore mutant CFTR is still poorly understood, in particular, MoA for correctors, i.e., compounds rescuing to the cells surface, the most prevalent mutant in CF patients - F508del-CFTR.

CF is caused by dysfunction of a single gene comprising 27 exons which encode for the CF transmembrane conductance regulator (CFTR), a 1,480 amino-acid protein functioning as a chloride (Cl^-) channel at the apical membrane of epithelial cell [5, 6]. CFTR is a multidomain protein, member of the ABC transporter superfamily, which possesses two transmembrane domains (TMD1/2), two nucleotide binding domains (NBD1/2) that bind and hydrolyse ATP regulating the gating of the channel, and a regulatory domain (RD) with multiple phosphorylation sites [7, 8].

To date about 1,900 alterations have been described in the CFTR gene, most presumed to be pathogenic [9]. Although ultimately all CF disease-causing mutations result in defective cAMP-regulated Cl^- secretion by epithelial cells, this is however due to distinct molecular and cellular causes. CFTR mutations have thus been previously classified according to their functional defect with the expectation that mutations in the same class may benefit from the same CFTR-repairing therapeutic strategy [10, 11]. These functional classes have somewhat evolved as we gain mechanistic insight into the basic defects underlying CF-causing mutations [10-13]. Therefore, currently 6 classes (and two sub-classes) of mutations are considered to more adequately account for the possible CFTR functional defects, namely (see also Fig. **1**): **1)** defective synthesis caused by either nonsense mutations (class Ia) or splicing disruption (class Ib); **2)** defective CFTR protein traffic (class II, including F508del); **3)** a gating defect leading to absence of Cl^- channel opening (class III); **4)** a conductance defect leading to decreased channel opening (class IV); **5)** very low levels of normal protein (class V); and **6)** decreased cell surface stability (class VI). Elucidation of the molecular and cellular effects caused by such wide spectrum of CFTR mutations is very relevant to decide on the most adequate CFTR-modulator therapeutic strategy aimed at correcting the underlying basic defect [10, 11].

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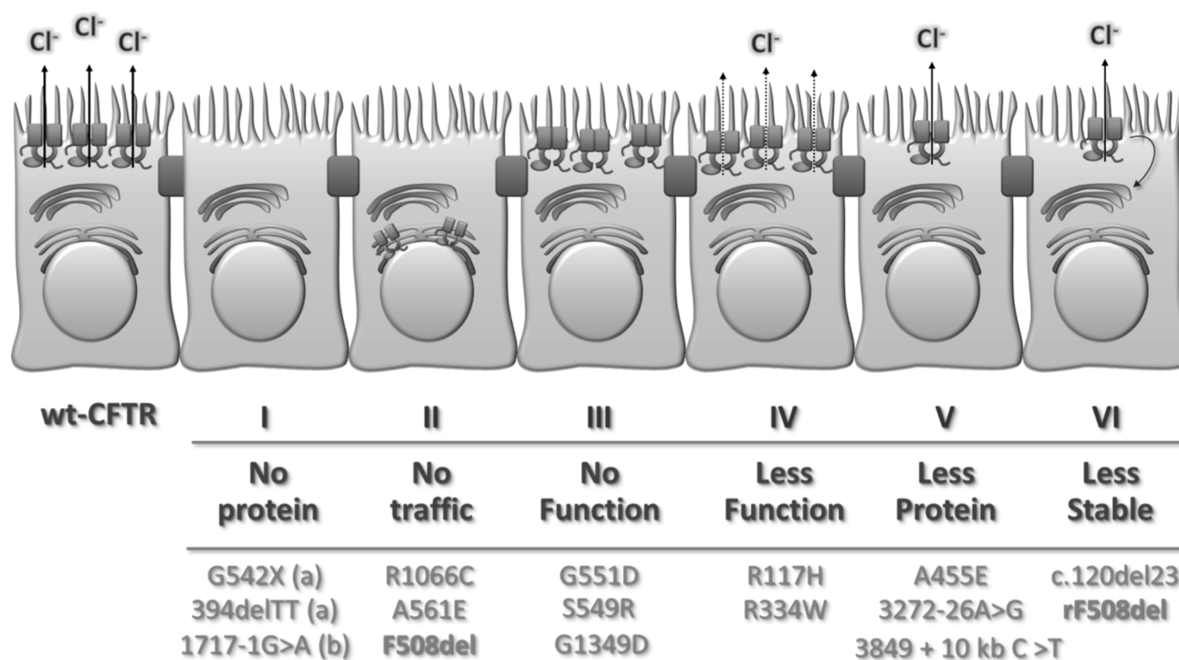

Fig. (1). Classes of CF mutation that facilitate correction of the basic defect by CFTR modulators through a "mutation-specific" approach. Class I mutations, which abrogate protein production, often include mutations that generate premature stop codons, class Ia (e.g. G542X) that lead to mRNA degradation by nonsense-mediated decay or those affecting canonical splice sites class Ib (e.g. 1717-1G>A). Class II mutations (including the most prevalent, F508del) cause retention of a misfolded protein at the ER, and subsequent degradation in the proteasome. Class III mutants affect channel regulation, impairing channel opening (e.g. G551D). Class IV mutants exhibit reduced conduction: that is, decreased flow of ions (e.g. R334W). Class V mutants cause significant reduction in mRNA and/or protein levels – albeit with normal function – often through causing alternative splicing (e.g. 3272-26A>G). Class VI mutants cause significant plasma membrane instability and include F508del when rescued by most correctors (rF508del).

However, one single mutation, F508del (a 3bp deletion causing absence of phenylalanine 508) accounts for ~70% of CF chromosomes worldwide and is present in ~90% of patients [7, 14]. It is therefore, not surprising that most CF therapeutic efforts focus on the correction of this mutant.

THE MULTIPLE DEFECTS OF F508DEL-CFTR

The F508del mutation causes CFTR to fail reaching its proper location at the plasma membrane (PM) being mostly retained at the endoplasmic reticulum (ER) from where it is rapidly sent for degradation by the ubiquitin-proteasome pathway [15, 16]. Despite the multiple explanations regarding the basic defect caused by the Phe508 deletion, it is commonly accepted that it consists primarily in the failure of the mutant protein in acquiring a native conformation. Subsequently, such non-native conformation of F508del-CFTR is recognized by the ER quality control (ERQC), which thus actively precludes F508del-CFTR from exiting the ER. The ERQC machinery involves several molecular chaperones and co-chaperones from both the ER-membrane and the cytosol, namely: Hsp70/Hsc70 [17, 18], calnexin [19, 20], Hsp90 [21], Hdj-1/Hdj-2 [22], HspBP1 [23], CHIP[24], BAG-1 and -2 [25] and cysteine string proteins (Csp's) [26] shown to regulate the exit of CFTR from the ER and Hsc70/Hsp70-CHIP-mediated CFTR degradation – all of them playing an active role in the ERQC. According to our first model of the ERQC, F508del-CFTR is retained in the ER [20] at two distinct checkpoints, involving the Hsp70/Hsc70 and calnexin chaperone machineries, respectively. These checkpoints sequentially assess the folding status of CFTR, wild-type (wt) or mutant and target for degradation non-native protein conformations. Later, we postulated the existence of a third ERQC checkpoint controlling the active export of proteins at the ER exit sites (ERES) [27]. At this 3rd checkpoint F508del-CFTR export is prevented due to abnormal exposure of four ER retention motifs - the AFTs, arginine-framed tripeptides (RXR), whose substitution leads to F508del-CFTR rescue [28]. In contrast, active CFTR ER-export relies on exposure of a di-acidic ER exit motif (NBD1-located "DAD" motif), which is a cargo signal for Sec24-mediated COPII packing [29]. Alanine-substitution of the second "DAD" Asp-residue in CFTR abolished both its Sec24-CFTR association and ER exit [29]. Additional evidence supports F508del-CFTR as a conformational mutant with a partially functional ER exit code and DAA-CFTR as a trafficking mutant without a major conformational defect [30]. This multi-step mechanism accounting for both F508del-CFTR defect(s) and ERQC has been recently supported by other authors [31, 32].

Besides these cellular mechanisms preventing F508del-CFTR from reaching the cell surface, its ER retention is also, and primarily, a consequence of its intrinsic (protein-autonomous) abnormal folding. To date, we have identified in this mutant at least three major folding defects. Firstly, isolated CFTR F508del-NBD1 alone already possesses an intrinsic folding defect, as shown by: *i)* impaired F508del-NBD1 folding kinetics vs wt-NBD1 [33]; *ii)* ability of some NBD1 amino acid variants that solubilize isolated F508del-NBD1 *in vitro* to also rescue *in vivo* full-length F508del-CFTR to the PM [34]; *iii)* demonstration by surface-plasmon resonance that F508del-NBD1 is a potent substrate of Hsp70 with 5-fold higher affinity than the wt domain [35].

The second F508del-associated defect impairs CFTR interdomain-folding, namely: *i)* the NBD1-NBD2 dimerization interface (critical for channel activation and accounting for F508del-CFTR gating defect) [36], making it also a class III mutant (see above), and which can be rescued by the G550E revertant [27]; and *ii)* the interaction of NBD1 with 4th intracellular loop (ICL4) of TMD2 [37], shown to be reverted by either V510D [38] or R1070W which fill the pocket left empty by F508del [31]. Thirdly, when rescued F508del-CFTR reaches the cell surface, it still presents a reduced

half-life [39-41] due to both increased endocytosis [42] and reduced recycling [43], thus making it also a class VI mutant (see above).

Altogether, these defects account for the reduced function of ER-rescued F508del-CFTR as a Cl^- channel in comparison to its wt-counterpart [36]. Thus, great efforts have been put into the identification of strategies to rescue mutant protein. Three main strategies have been successfully described to rescue F508del-CFTR localization and function: *i)* low temperature (physical rescue), as F508del-CFTR has been shown to be temperature sensitive, *ii)* second-site mutations (genetic rescue) since F508del-CFTR is rescued by the presence of other mutations *in cis* that either correspond to the removal of retention signals to allow protein to escape ER quality control (ERQC) mechanism and mislocalization or to the correction of critical contact points in the three-dimensional structure of CFTR; and finally *iii)* small molecules (chemical rescue) that interact either directly with CFTR or with components of the cellular machinery have been shown to induce correct protein localization and also to augment channel activity.

LOW TEMPERATURE RESCUE

Soon after the identification of the CFTR gene, F508del-CFTR was shown to be a temperature sensitive mutation. This was first described as chloride channel activity was detected when F508del-CFTR was expressed in *Xenopus* oocytes, Vero cells and Sf9 insect cells [44]. As those cells lines are typically maintained at lower temperatures than mammalian cells, and because processing of nascent proteins can be sensitive to temperature, Denning *et al.* reported that the processing of F508del-CFTR reverts towards that of wt-CFTR as the incubation temperature is reduced [44].

Later, these data were confirmed by several groups [39, 45] and ever since many researchers have used this approach to recover some mutant protein in the cell surface for a great diversity of studies. Other examples of cell surface expression rescuing by low temperature include class I major histocompatibility complexes achieved by reducing the incubation temperature to 19-33ºC [46], or mutant forms of human tyrosinase (causing albinism) by incubation at 31ºC [47]. Another example comes from hyperinsulinemic hypoglycemia-causing mutations in the SUR1 subunit of pancreatic ATP-sensitive potassium channels, affecting channel activity and processing efficiency and reported to be totally rescued by incubation at 18ºC but only partially at 30ºC [48]. It is believed that many temperature sensitive mutants in most genetic disorders present a similar behaviour [49]. *In vitro*, lower temperature generally results in increased protein stabilization associated with higher folding efficiency [50]. However, *in vivo* the cellular mechanism of low temperature rescuing has not been fully elucidated.

Although this rescue of F508del-CFTR cannot be of direct therapeutic relevance, it was very important as it constituted the first demonstration that overcoming of F508del-CFTR trafficking block at the ER is possible and that it could perhaps be attempted by small molecules. Furthermore, to design pharmacological strategies aimed at rescuing mutant misfolding proteins to the cell surface, it is important to understand how low temperature restores the trafficking defect [51].

Rescue by low temperature was later used to elucidate some of the trafficking pathways followed by rescued CFTR on its way to the membrane. Gentzsch and cols [52] showed that surface CFTR enters several different routes, including an initial step to early endosomes shown to be dependent on a member of the Ras superfamily of monomeric G proteins (Rab's), namely on Rab5. Then these authors showed that CFTR undergoes either Rab11-dependent recycling back to the surface or Rab7-regulated movement to late endosomes or alternatively Rab9-mediated transit to the trans-Golgi network. In fact, modulation of different steps in the trafficking of CFTR suggested that F508del-CFTR has an altered structure detected in the distal as well as proximal secretory pathway.

Evidence from other studies at low temperature indicated however, that F508del-CFTR has a temperature-sensitive stability defect even in post-ER compartments, including the cell surface [40]. In fact, in BHK cells, although endocytosis of membrane rescued of F508del-CFTR was described to be equivalent to that of wt-CFTR, the mutant protein was shown to recycle to the plasma membrane much less efficiently than its wt counterpart [43]. This was later complemented by studies in the human bronchial CFBE cell line where endocytosis of temperature rescued F508del-CFTR was shown to be 6-fold more rapid that than of wt-CFTR [53].

Interestingly, rescue of F508del-CFTR seems to be a pleiotropic event, resulting from an overall change in the cell metabolism and trafficking machinery. A few reports have in fact shown that both mature and immature F508del-CFTR forms are present at the cell surface after low temperature incubation [54]. This trafficking of immature F508del-CFTR occurs even upon addition of the brefeldin A, an agent that induces Golgi disaggregation, suggesting the involvement of a Golgi-independent pathway, and that low temperature induces the appearance of a mixed population of mature and immature CFTR molecules at the plasma membrane through distinct pathways [54].

Although the mechanism by which low temperature rescues F508del-CFTR is still unclear, some authors argue that the export of the mutant protein from the ER requires a local biological "folding environment" that is sensitive to heat/stress-inducible factors and only to be found in some cell types [55]. Thus, the cell may require a "proteostasis" (protein homeostasis) environment (in part distinct from that of the wt pathway) so as to restore F508del-CFTR insertion into COPII vesicles [55]. This is actually supported by recent studies reporting that low temperature, in contrast to other rescuing agents, causes a profound change in the transcriptome of a CF human bronchial epithelial (HBE) cell line [56]. Others report also that the unfolded protein response (UPR) induction and repression of some cell-metabolic pathways (*e.g.*, those induced by a cold-shock) are the major responses that (in BHK cells) may generate a favourable cellular environment to promote F508del-CFTR rescue [57].

Interestingly, the rescue of F508del-CFTR was recently shown to be related to the association of CFTR to other proteins at the cell membrane, possibly in micro-membrane domains. In CF, the distinctive enhanced sodium (Na^+) absorption through the epithelial Na^+ channel (ENaC) is generally attributed to the failure of mutated CFTR to restrict ENaC-mediated Na^+ transport. Although the mechanism for this regulation is still controversial, a close association of wt-CFTR and wt-ENaC has been reported [58]. This association is however much weaker for membrane-located F508del-CFTR but can be rescued by both partial correction of its trafficking defect by low temperature [59].

REVERTANTS –SECOND SITE MUTATIONS RESCUING THE ORIGINAL TRAFFICKING DEFECT

Genetic manipulation has also been described to rescue F508del-CFTR trafficking defect. This consists in introducing second-site mutation(s) in *cis* with the F508del mutation to achieve rescuing of the CFTR mutant to the cell surface. The first studies reporting that the abnormal conformation produced by F508del could be partially corrected by second-site alterations in the protein, were carried out in yeast by Teem and cols [60, 61]. They employed a screen using a chimeric yeast STE6 (mating factor)/CFTR construct bearing the F508del mutation which led to a defect in mating. These authors showed that R553M, R553Q and R555K partially restore the yeast defective mating. Moreover, they showed that, when introduced into human F508del-CFTR, these "revertant mutations" partially restored the processing and the loss of apical Cl^- current associated with the mutant protein. For the R555K, single-channel measurements showed that correction of CFTR func-

tion was achieved by prolonging the duration of bursts of activity [61].

Further *in vitro* studies with isolated F508del-NBD1 bearing R553M later identified this revertant mutation as a "superfolder" which counteracts the misfolding effect of F508del on the temperature-dependent folding yield *in vitro*, although not significantly altering the free energy of stability [62]. This was the first demonstration that there is a correlation between NBD1 (lack of) solubility and full-length CFTR (mis)folding and (mis)processing.

Further investigation of the sequence of NBD1 by those authors allowed them to identify two more revertant mutations, I539T and G550E, proximal to and within the conserved "ABC signature motif" (LSGGQ motif) of NBD1, respectively. G550E mutation was shown to increase the sensitivity of F508el-CFTR to activation by cAMP agonists and to block the enhancement of its activity by IBMX [63]. The same mutation was later shown to dramatically correct the gating defect characteristic of the mutant [27].

Most of the original second-site mutations identified were in fact located within NBD1 region that includes the "ABC signature motif" or in close proximity to this site. However, evidence for a more universal mechanism of rescue, related to the trafficking machinery, came from studies on the trafficking of subunits of a mammalian ATP-sensitive potassium K^+ channel [64]. The channel is formed by four regulatory subunits and four K^+ ion channel subunits, and proper function requires co-expression of all eight subunits. Sequence analysis combined with mutagenesis studies identified the motif RXR (arginine – any amino acid – arginine) that when present, blocks the trafficking of channel subunits to the cell surface, causing their retention in the ER [64]. The similarity of this motif to the well-known KKXX motif (*e.g.*, KDEL) responsible for the retention of ER-resident proteins at the organelle, suggests that a common mechanism may prevent the trafficking of proteins containing either motif.

However, unlike KKXX motifs, the use of RXR motifs as retrieval signals has only been documented for proteins with a final location other than the ER. The RXR motif only acts as retrieval signal when present in misassembled or misfolded proteins, suggesting that the motifs are buried in assembled proteins. The masking of RXR-motifs in the mature state of proteins (by acquisition of folded conformations or covered by interacting proteins) appears to be the mechanism that makes these motifs acting as retrieval signals in the ERQC and not as constitutive retention signals [64].

Chang *et al.* showed that export-incompetent CFTR displays multiple AFT sequences. Moreover, those authors demonstrated that inactivation of 4 of these motifs by replacement of arginine residues at positions R29, R516, R555, and R766 with lysine residues simultaneously (4RK) caused mutant F508del CFTR protein to escape ERQC and function at the cell surface. R29K alone can also induce some effect, although at lower levels [30]. These findings suggest that interference with recognition of these signals may be helpful for the management of CF [28]. Factors detecting AFTs act as sensors for the presence of aberrant structures may also act as cargo-adaptors to the COPII coat-protein complex, the protein coating vesicles that are destined for delivery to the ER-**G**olgi **i**ntermediary **c**ompartment (ERGIC). Alternatively, or in addition, COPII proteins may also interact with positive export signals on nascent CFTR. Similarly, association with COPI vesicles that are responsible for retrograde retrieval may be mediated by putative negative signals [28].

Rescue of F508del-CFTR by 4RK was shown to involve an increase in CFTR channel activity by prolonging channel openings, but with limited impact on overall channel open probability [27]. 4RK was thus suggested to allow F508del-CFTR to escape ER retention retrieval mediated by AFTs that constitute a checkpoint for ERQC, as described above, but not so much to improve the folding of F508del-CFTR [27]. Further studies, mutating these four AFTs in all possible combinations, revealed that simultaneous inactivation of two of them (R29K and R555K) is necessary and sufficient to overcome F508del-CFTR ER retention [65].

The role of the ERQC in the rescuing by the AFTs was further evidenced by a study in which peptides that mimic these motifs were used [66]. In fact, these peptides seem to overcome mistrafficking mediated by aberrant exposure of these motifs as they elicit robust rescue of F508del-CFTR in cell lines and in respiratory epithelial tissues by transduction of RXR motif-mimetics, showing that abnormal accessibility of this motif is a key determinant of mistrafficking of the major CF-causing mutant [66].

Another set of second-site mutations was identified as enabling F508del-CFTR to be rescued from the plasma membrane by a totally different approach. To better understand at a molecular level how the F508del-CFTR mutation perturbs the structure of NBD1, Lewis and cols elucidated the crystal structure of F508del-NBD1 [67, 68]. However, to promote domain solubilisation and, hence, crystallization, a series of mutations was incorporated into the human NBD1 sequence. Some of these mutations (F409L, F429S, F433L, H667R) represented sequence changes between CFTR of human origin and those from other species, whereas others (G550E, R553Q, and R555K) had been previously identified as F508del-CFTR revertant mutations as described above.

Examination of the original wt- and F508del-NBD1 structures revealed little or no structural changes besides deformation of the local surface topography around the F508 residue [67, 68]. However, it remains plausible to envisage that a structure of F508del-NBD1 with more conformational differences, namely dynamic differences, than just those at the surface described would likely further disrupt such inter-domain interactions, in particular those involving NBD1. Nevertheless, because the original F508del-NBD1 construct used for the crystal structure contained several F508del revertant mutations, further F508del-NBD1 structures without these known revertants were then produced [67, 68]. However, these new F508del-NBD1 crystal structures still required either two (F494N/Q637R; Protein Data Bank [PDB] ID code: 2BBT) or three (F429S/F494N/ Q637R; PDB ID code: 2BBS) additional mutations for domain solubility and, hence, crystal formation. Given the ability of solubilizing mutations to increase the yield of NBD1 and facilitate its crystallization, we hypothesized that these mutations might also promote the folding of both isolated NBD1 and full-length CFTR protein. To test this idea, the effects of the mutations F494N/Q637R and F429S/F494N/Q637R on wt- and F508del-CFTR were assessed on: (1) the *in vivo* folding yield of NBD1, (2) the processing and trafficking of the full-length CFTR protein, and (3) the Cl^- channel function of CFTR. Data thus obtained showed that these so-called "solubilizing mutations" partially rescue the trafficking and attenuate gating defects of F508del-CFTR [34]. These data further confirmed that mutations improving NBD1 solubilisation *in vitro* also impact on the folding and processing of full-length CFTR *in vivo*.

A noteworthy study by Serohijos *et al.* using molecular modelling proposed a 3D structure for CFTR in which Phe-508 mediates a tertiary interaction between the surface of NBD1 and an intracellular loop (ICL4) in the C-terminal of membrane-spanning domain 2 (MSD2). This model, which was experimentally supported, evidenced for the first time a crucial cytoplasmic membrane interface, which is dynamically involved in regulation of channel gating, while explaining the known sensitivity of CFTR assembly to many disease-associated mutations in ICL4 as well as NBD1. Moreover, it provided a sharply focused target for small molecules to treat CF, namely F508del-CFTR [37].

Correction of this critical NBD1-ICL4 site was later found to be effective in rescuing F508del-CFTR, probably at the level of domain assembly [31]. The described interaction involves a cluster of aromatic residues (F508, F1068, F1074, Y1073) that is disrupted

upon deletion of F508. Thus, if the aromatic cluster is rescued by the substitution of arginine 1070 by a tryptophan residue (R1070W), the mutant protein is partially rescued.

Furthermore, Loo *et al.* found that replacement of Val510 by acidic residues (*e.g.*, V510D) promoted maturation of F508del-CFTR [38]. Those authors proposed that the V510D mutation in NBD1 promotes maturation and stabilizes mutant CFTR at the cell surface through formation of a salt-bridge with Arg1070 in MSD2 [38].

CFTR CORRECTORS – RESCUING CLASS II MUTANTS

Rescue of F508del-CFTR has been a major target for a series of drug discovery programs aimed at identifying new compounds that are able to correct the trafficking as well as the gating defect of this mutant protein. Over the years, chemical and pharmacological approaches have been used to rescue F508del-CFTR dysfunction(s). The approaches used can be used into two main strategies: 1) "protein non-autonomous" approaches that help mutant-CFTR to fold by generally favouring the cellular milieu or "cellular proteostasis", i.e., through modulation of interacting proteins such as chaperones, [69]; and 2) "protein autonomous" (specific) approaches, i.e., using small molecules that rescue folding of CFTR mutants (F508del in particular), putatively allowing them to function correctly at the plasma membrane.

Due to their non-specificity, the former approach includes chemical chaperones, or "proteostasis modulators", *i.e.*, molecules that correct the overall cellular proteostasis environment in which the mutant protein folds and functions. Molecules attempting rescue of mutant CFTR by the second approach, in turn, are termed pharmacological chaperones (or pharmaco-chaperones). Ideally, a pharmacological chaperone would act both as a CFTR corrector (*i.e.*, promoting mislocalized protein to the plasma membrane) and as a CFTR potentiator (*i.e.*, increasing CFTR Cl^- channel function upon stimulation).

CHEMICAL CHAPERONES AS CORRECTORS

Mimicking the activity of molecular chaperones towards promoting protein folding has been attempted by the use of small organic molecules termed "chemical chaperones" [70]. Like molecular chaperones, they do not provide any direct information to the folding process, but seem to positively influence the rate and/or fidelity of the folding, probably by stabilizing the properly folded form or an unstable intermediate of the polypeptide [70]. Most of the molecules with chemical chaperoning activity play this role naturally inside living cells to cope with adverse changes in the environment, namely those leading to protein denaturation/ aggregation. Some of these cellular compounds are also called "compatible osmolytes" [70] since they accumulate within the cell at rather high concentrations without disturbing protein function, in fact with a protective role towards native protein conformations [70]. Glycerol is included in this group.

It was demonstrated by Welch and cols [71] and by Kopito and cols [72] that when F508del-CFTR expressing cells are incubated with glycerol, they express the mature form of CFTR (*i.e.*, post-Golgi) and also display CFTR-mediated cAMP-activated transport. Glycerol was also described to facilitate the *in vitro* folding of NBD1 by preventing the appearance of off-pathway intermediates [73]. For glycerol, like for other polyhydric alcohols (polyols) in general, a mechanism has been proposed by which the polyol is excluded from the immediate vicinity of a polypeptide and as a result, at high concentrations, it will increase the relative hydration around the polypeptide. In response to this effect, the protein will tend to decrease its relative surface area by an increase in self-association or tighter packing. This hydrophobic effect enhances the stability of the protein reducing its potential to unfold in response to thermal or chemical treatments [71].

Another chemical chaperone also successfully shown to rescue F508del-CFTR to the cell surface is trimethylamine N-oxide (TMAO). Together with other methylamines, TMAO is referred to as a "counteracting osmolyte", as it is produced to offset the protein denaturing effects of urea [71]. Brown *et al.* have shown that TMAO exhibits properties similar to glycerol regarding F508del-CFTR rescuing. This approach has also been tested in one animal model [74] by Fischer *et al.* who indeed examined whether TMAO corrects the F508del-CFTR trafficking defect in the F508del-CF mouse. Because in rodents the severe effects of F508del mutation are most prevalent in the intestine, measurements of rectal potential differences (RPD) were performed in control mice and in F508del-CFTR mice. Administration of TMAO over 24h by subcutaneous injection revealed a significant increase in forskolin-activated RPD hyperpolarization in the F508del-CFTR vs control mice, consistent with a partial rescuing of the folding defect associated with the mutant protein [74].

More recently, proteostasis modulating strategies have been developed mostly by Balch and cols that define CF as a consequence of defective recognition of CFTR by the proteostasis network [75]. In line with this concept, the same group has described correction of F508del-CFTR by down-regulation of the Hsp90 co-chaperone Aha1 [76, 77] and by the histone deacetylase 7 (HDAC) inhibitor SAHA [78].

SCREENING FOR PHARMACOLOGICAL CHAPERONES AS CORRECTORS

In recent years, however, the greatest efforts to identify corrector compounds have been put into high-throughput screening (HTS) of small-molecule libraries. One of the first reports came from Verkman and Galietta [79] who used a fluorescent functional assay (a YFP variant which is halide-sensitive) to detect rescue of F508del-CFTR expressed on transfected Fisher Rat Thyroid (FRT) epithelial cells and thus identify small-molecule correctors. After screening 150,000 chemically diverse compounds and more than 1,500 analogs of active compounds, several classes of correctors (aminoarylthiazoles, quinazolinylaminopyrimidinones, and bisaminomethylbithiazoles) with micromolar potency were confirmed, all them exhibiting greater effects than low temperature. Correction was seen within 3-6 h of treatments and persisted for more than 12 h after washout. Biochemical studies suggested that the compounds may act through enhancing F508del-CFTR folding at the ER and stability at the cell surface. One of the compounds identified through this screen was Compound 4a (Corr-4a), one of the most used correctors in the recent years.

Soon after, Van Goor and cols [80] reported the identification of two classes of novel, potent small molecules identified by a similar CFTR-mediated anion flux assay in NIH/3T3 cells applied to the screening of compound libraries. The first class of correctors were quinazolinones (VRT-422 original hit and medicinal chemistry optimized hit VRT-325) which restored function of F508del-CFTR in both recombinant cells and cultures of HBE cells isolated from CF patients The compounds in this class partially correct the trafficking defect by facilitating exit from the ER and restore F508del-CFTR-mediated Cl^- transport to more than 10% of that observed in non-CF HBE cell cultures [80], a level expected to result at least in partial clinical benefit in CF patients [81]. The second class of compounds included the pyrazole VRT-532 which potentiates cAMP-mediated gating of F508del-CFTR and achieves single-channel activity similar to wt-CFTR [80]. The CFTR-activating effects of the two mechanisms are additive and support the rationale of a drug discovery strategy based on rescue of the multiple basic defect responsible for CF [80]. Results from this study in fact led to novel compounds being classified according to these two classes (correctors and potentiators) and provided the background for the nowadays spread definition of corrector – a compound that acts by promoting the appearance of mutated CFTR at the cell membrane,

by correcting its folding and/or trafficking [80]. The corrector molecule then identified - VRT-325- was subsequently widely used by others in research. VRT-325 was thus shown by Clarke and cols to correct other misprocessed CFTR mutants such as R258G, S945L, and H949Y and to be partially specific, while another quinazoline derivative, the posttraumatic stress disorder (PTSD) drug prazosin (Minipress™), did not rescue those misprocessed CFTR mutants. However, this compound could also rescue misprocessed mutants of P-glycoprotein [82]. The same group used also a cysteine cross-link approach to show that VRT-325 corrected the ICL4 mutants Q1071P and H1085R by promoting a cross-link pattern similar to that observed with mature wt-CFTR, suggesting that the compound acts by repairing the folding defects in the TMDs [83]. More recently, studies with VRT-325 suggested that the biosynthetic rescue mediated by this compound may be conferred (at least in part) by direct modification of the structure of the mutant protein, leading to a decrease in its ATP-dependent conformational dynamics [84].

In search of specific compounds, the corrector activity of previously described compounds potentiator VRT-532 [80] and Compound 2b (Corr-2b) [79] was further exploited and both were confirmed to rescue CFTR processing mutants but not a P-glycoprotein processing mutant [85]. The results obtained suggest that the mechanism by which these two compounds promote mutant CFTR folding is through direct interaction with mutant proteins. This was a pioneer work in identifying a dual action (corrector/ potentiator) lead compound (VRT-532) for the treatment of CF [86]. Later, VRT-532 was shown to cause a minor but significant decrease in the trypsin susceptibility of the full-length F508del-CFTR protein and a fragment encompassing the second half of the protein, suggesting it interacts direct with Phe508 to modify its ATPase activity and/or stabilize a structure that promotes the channel open state, this possibly also accounting for its efficacy as a corrector [87].

Some of these initial compounds were found to be toxic to cell cultures and unfortunately for the majority of them the efficiency of corrector-induced maturation of F508del-CFTR observed for the different molecules appeared to be too low for therapeutic value.

Accordingly, the idea of combined effect of more than one compound emerged as one of the topics to be further developed. In fact, if the multiple correctors identified bound to different protein sites, they may have an additive effect on maturation. Along these lines, Wang and cols tested whether expression of F508del-CFTR in the presence of combinations of three different classes of corrector molecules would increase its maturation efficiency. To this end, compounds VRT-325, Corr-2b and Corr-4a were tested. When combined with either 2b or 4a, VRT-325 doubled the steady-state maturation efficiency of F508del-CFTR (to levels up to 40-50% of wt-CFTR) [88]. This was the first clear evidence that, at least for F508del-CFTR, corrector molecules acting at different steps along the folding pathway may provide the rationale basis for a combination therapy approach to human protein folding disorders.

As the studies towards the clarification of the MoA of the identified compounds proceeded, other large-scale screenings were still being performed and reported in the literature. Robert and cols used a novel HTS assay to screen 42,000 compounds [89] and identified several sildenafil analogs as F508del-CFTR correctors, subsequently to initial sildenafil (Viagra™) effect described by Dormer and cols [90]. Furthermore, Robert and cols tested some structural analogs of sildenafil, having the sulfonylpiperazine KM11060 been found to be surprisingly potent, both in biochemical and functional assays [89]. However, this compound was also found to be unspecific as it also rescued G268V, a processing mutant of P-glycoprotein [91]. Moreover, recent studies have reported that the high doses of these sildenafil analogs which are required for CFTR recovery might not be suitable as therapeutic drugs for CF lung disease [92].

Large-scale screening approaches were also complemented by other studies describing advances in chemical synthesis, based on previously identified molecules. Accordingly, based on the earlier identification of bithiazoles Corr-2b and Corr-4a [79], Yoo and cols described progresses in the production of a 148-member methyl-bithiazole-based library, highlighting the critical role of the bithiazole substructure to identify novel compounds with low micromolar potencies [93, 94].

The chemical synthesis/modification approach allowed also the design of a hybrid molecule incorporating a linker (fragment 13) between fragments of previously described potentiators (PG01) and correctors (Corr-4a). This linker can thus be cleaved by intestinal enzymes under physiological conditions, thus providing proof-of-concept that small-molecule potentiator-corrector hybrids can potentially be used as a single drug therapy for CF in patients carrying the F508del mutation [95].

Reports on compounds with combined corrector/potentiator action also came from *in silico* structure-based screening approaches utilizing homology models of CFTR that, besides evidencing some novel F508del-CFTR correctors, allowed the identification of dual-acting compounds [96]. More recently, Pedemonte *et al.* also reported the characterization of aminoarylthiazoles (AATs) as compounds having dual activity on the rescue of F508del-CFTR but also on CFTR bearing G1349D or G551D mutations, which cause only a gating defect [97].

Further studies aimed at identifying the MoA of the compounds, using different cell lines (see below) and variable assays, started to build-up on the hypothesis that maximal correction of F508del-CFTR requires a chemical corrector that acts by three distinct effects: 1) promotes folding and exit from the ER (class II defect); 2) improves channel gating (class III defect); and 3) enhances surface stability (class VI defect) [98].

The most efficient and thus "gold standard" for F508del-CFTR correction since it was described in 2011, is the investigational drug VX-809 (Vertex Pharmaceuticals Inc., San Diego, CA, USA). VX-809, a CFTR corrector that was advanced into clinical development for the treatment of CF, improved F508del-CFTR processing in the ER in cultured HBE cells isolated from patients with CF homozygous for F508del [99]. Furthermore, this compound enhanced Cl^- secretion to approximately 14% of non-CF HBE cells, a level associated with mild CF in patients with less disruptive CFTR mutations. F508del-CFTR corrected by VX-809 exhibited biochemical and functional characteristics similar to normal CFTR, including biochemical susceptibility to proteolysis, residence time in the plasma membrane, and single-channel open probability [99]. VX-809 was more efficacious and selective for CFTR than all the previously reported CFTR correctors [99].

VX-809 is already in Phase III clinical trial, however, despite its impressive rescue levels in primary cultures of lung cells from CF patients, the success of this compound in F508del-homozygous patients so far resorted has been modest and its MoA still poorly understood.

STRATEGIES TO ELUCIDATE THE MECHANISM OF ACTION (MOA) OF CORRECTORS

Recent reports in the literature concerning the different strategies for CFTR correction have pointed out that probably only through combined strategies will therapeutic efficacy levels be achieved. Several studies reported on the additivity of CFTR correction by different genetic revertants [100, 101], thus suggesting that correction of F508del-CFTR will need to contemplate different contact sites in the protein structure along with cellular folding checkpoints that may be crucial in CFTR folding, trafficking, processing and ultimately membrane stability.

Rabeh and cols showed that correction of individual defects alone is insufficient to restore F508del-CFTR biogenesis. Instead,

both F508del-NBD1 energetics and the NBD1-MSD2 (membrane-spanning domain 2) interface stabilization are required to achieve wt-like folding, processing, and transport function. This is suggestive of a synergistic role of NBD1 energetics and topology in CFTR-coupled domain assembly [102].

Recently, we have explored the MoA of VX-809 by analysing its synergetic/additive effect with those of two other small-molecule correctors (VRT-325, Corr-4a) and of other rescuing strategies such as low temperature and genetic revertants (Farinha & Amaral, unpublished). Our results provide insight into VX-809 MoA and elucidate on the scope for further F508del-CFTR rescuing by other compounds. Indeed, such recent data show that VX-809 adds up to the two other correctors in rescuing F508del-CFTR and also with low temperature. In contrast, VX-809 (and the 2 other correctors) evidences variably effects with genetic revertants, supporting its putative binding at the interface of NBD1 with TMD2 ICL4. Moreover, protein modelling supported this interpretation of experimental results. These data lead us to conclude that there is scope for correction beyond VX-809 at distinct F508del-CFTR conformational sites/ cellular folding checkpoints. Combined therapeutic strategies rescuing F508del-CFTR may thus be required to reach the threshold of functional CFTR necessary to avoid CF.

CHOICE OF THE MOST ADEQUATE MODEL TO ASSESS EFFICACY

One of the major problems in the identification of novel molecules aimed at correcting F508del-CFTR is the choice of the adequate cellular model(s) to assess the effects of the compound. Indeed, the efficacy of a compound has been often reported to differ among cell lines and larger differences arise when the molecule is applied to patient-derived materials or ultimately in clinical trials.

We have (Table **1**) analysed the effect of a panel of previously described correctors (kindly provided by CFFT) in two different cell lines stably expressing F508del-CFTR – the human bronchial epithelial CFBE cell line and the hamster fibroblast BHK cell line. Results shown in (Fig. **2**) illustrate that some compounds (C2, C3, C4) rescue the appearance of mature CFTR in both cell lines, whereas others only promote the rescue of mutant CFTR in one of the cell lines (C1, C6, C7 in CFBE cells, C13, C14, C15, C17, P1 in BHK cells). These data lead us to conclude, as other authors, on the cell line specificity regarding F508del-CFTR rescue by most of the correctors tested.

Rowe and cols examined the activity of F508del-CFTR stably expressed in polarized CFBE41o human airway cells and Fischer rat thyroid (FRT) cells following treatment with low temperature and a panel of small molecule correctors (including Corr-4a, VRT-325 and VRT-640). Results identified important differences in F508del-CFTR activation in polarizing epithelial models of CF [103].

In another study, Sondo *et al.* reported that, when compared to other cell types, CFBE41o- shows the lowest response to correctors such as Corr-4a and VRT-325, and that at least Corr-4a does not cause a profound change in cell transcriptome [56]. Results suggest that F508del-CFTR correctors acting by altering the cell transcriptome may be particularly active in heterologous expression systems

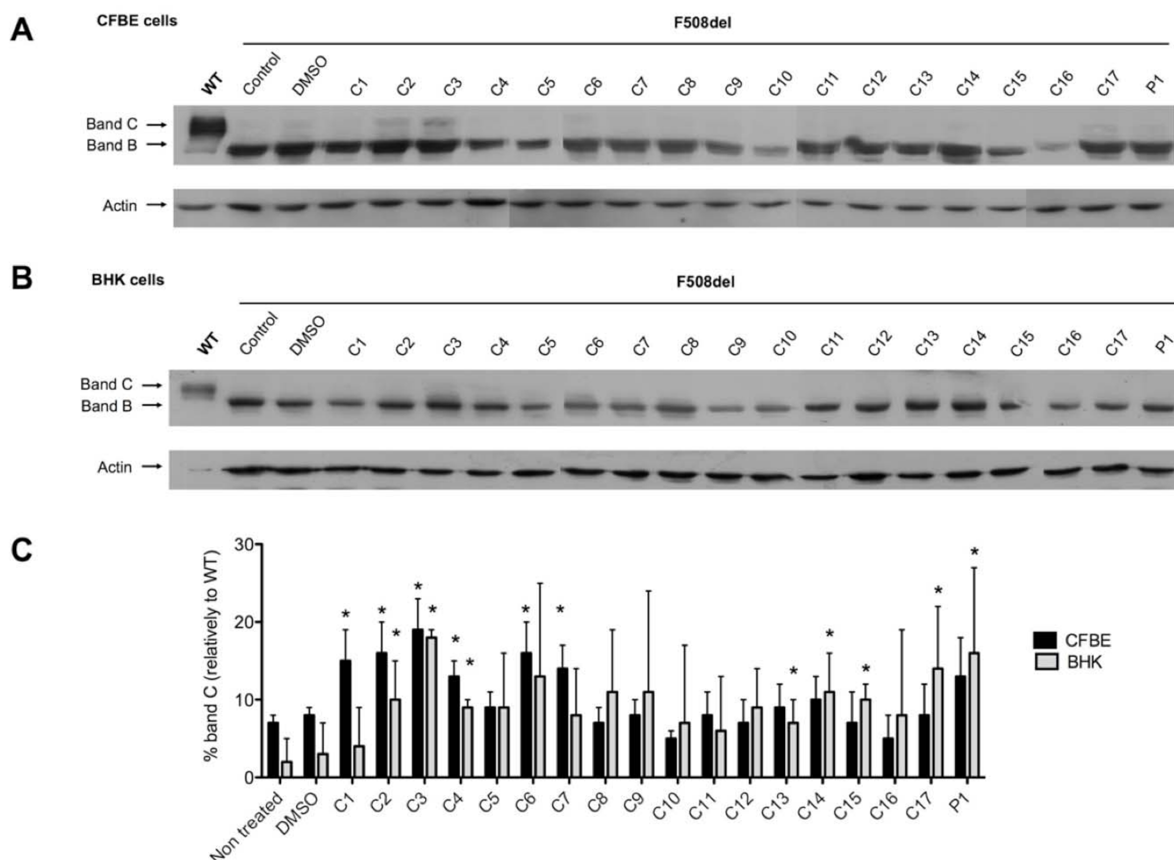

Fig. (2) Rescue of F508del-CFTR in CFBE and BHK cell lines by a panel of correctors. (**A**) CFBE or (**B**) BHK cell lines stably expressing CFTR bearing F508del were incubated for 24h with 10 μM of each compound (except for C3 6.7μM). CFTR protein was analysed by Western Blot with the anti-CFTR 596 mAb. Data are representative of n = 5-7 independent experiments. (**C**) Densitometry was used to calculate for each condition the percentage of band C to total CFTR expressed. Data were normalized to wt-CFTR. Asterisks indicate significant difference from the F508del-CFTR variant with DMSO ($p<0.05$).

Table 1. Panel of Correctors Tested (Provided by Cystic Fibrosis Foundation Therapeutics, Inc.)

ID	Chemical Name	Common Name	Reference
P1	4-Methyl-2-(5-phenyl-1H-pyrazol-3-yl)-phenol	VRT-532	[80]
C1	6-(1H-Benzoimidazol-2-ylsulfanylmethyl)-2-(6-methoxy-4-methyl-quinazolin-2-ylamino)-pyrimidin-4-ol	-	[79]
C2	2-{1-[4-(4-Chloro-benzensulfonyl)-piperazin-1-yl]-ethyl}-4-piperidin-1-yl-quinazoline	VRT-640	Vertex patent
C3	4-Cyclohexyloxy-2-{1-[4-(4-methoxy-benzensulfonyl)-piperazin-1-yl]-ethyl}-quinazoline	VRT-325	[83]
C4	N-[2-(5-Chloro-2-methoxy-phenylamino)-4'-methyl-[4,5']bithiazolyl-2'-yl]-benzamide	Corr 4a	[79]
C5	4,5,7-trimethyl-N-phenylquinolin-2-amine	Corr 5a	[79]
C6	N-(4-bromophenyl)-4-methylquinolin-2-amine	Corr 5c	[79]
C7	2-(4-isopropoxypicolinoyl)-N-(4-pentylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide	Compound 48	[120]
C8	N-(2-fluorophenyl)-2-(1H-indol-3-yl)-2-oxoacetamide	-	Vertex patent
C9	7-chloro-4-(4-(4-chlorophenylsulfonyl)piperazin-1-yl)quinoline	KM11060	[89]
C10	7-chloro-4-(4-(phenylsulfonyl)piperazin-1-yl)quinoline	KM11057	[89]
C11	(Z)-N'-(3,4-dihydroxybenzylidene)-3-hydroxy-2-naphthohydrazide	Dynasore	[121]
C12	N-(4-fluorophenyl)-4-p-tolylthiazol-2-amine	Corr 2i	[79]
C13	N-(2-(3-acetylphenylamino)-4'-methyl-4,5'-bithiazol-2'-yl)benzamide	Corr 4c	[79]
C14	N-(2'-(2-methoxyphenylamino)-4-methyl-5,5'-bithiazol-2-yl)benzamide	Corr 4d	[79]
C15	N-phenyl-4-(4-vinylphenyl)thiazol-2-amine	Corr 2b	[79]
C16	2-(6-methoxy-4-methylquinazolin-2-ylamino)-5,6-dimethylpyrimidin-4(1H)-one	Corr 3d	[79]
C17	N-(2-(5-chloro-2-methoxyphenylamino)-4'-methyl-4,5'-bithiazol-2'-yl)pivalamide	Compound 15Jf	[93]

but markedly less effective in native epithelial cells [56]. The same authors in Pedemonte *et al.* [104] assessed potency, efficacy and MoA for panel of correctors and potentiators on two different cell lines, FRT (rat) and human A549 cells, to conclude that effect of correctors (but not so much of potentiators) was strongly affected by cell background. The gold standard to validate compound efficacy are primary cultures of HBE cells derived from CF patients [105, 106]. Still, the efficacy of ~25% activity shown for VX-809 in F508del-HBE cells isolated from seven CF F508del-homozygous patients [99] would lead to predict higher efficacy than the modest results actually reported in clinical trials for this compound. Several reasons may account for this, among which tissue penetrance and bioavailability of compounds. An appealing alternative is thus to use native tissues, such as rectal biopsies used for CF diagnosis and prognosis [107, 108] to assess bioavailability/efficacy of compounds. Indeed, the feasibility of this approach, testing compounds *ex vivo* directly on human tissues has been recently demonstrated [109]. In the near future it may constitute, not only the most physiologically relevant way to validate efficacy of novel therapeutic compounds, but also the most expedite method to identify patients who will likely be responders to approved drugs through a personalized-medicine approach [11].

CFTR POTENTIATORS – ACTIVATING CFTR CHANNEL WITH CLASS III, IV OR V MUTATIONS

Along with the efforts in the finding of a corrector for F508del-CFTR, developments have also been made towards the identification of channel potentiators. In contrast to correctors, there is a relatively large number of potentiators, as it it easier to find small molecules that further activate CFTR channels already present at the cell surface than those rescuing the folding and/or trafficking of F508del-CFTR so as to increase its cell surface density. As described above (see Introduction), potentiators will be of therapeutic benefit to patients with mutant CFTR that exhibits defective gating (class III) or conductance (class IV).

The most representative CFTR mutant of class III is G551D, occurring in 3-4% of CF patients worldwide. Class IV mutations, i.e., those associated with reduced Cl^- conductance such as R117H or R334W individually account for worldwide frequencies < 1% [110]. Patients carrying Class IV mutations are also expected to benefit from correctors, as stimulation of the overall cellular CFTR conductance can be achieved by increasing the overall cell surface content of these mutants, thus compensating for the reduced conductance of each individual channel.

Class V mutants, mostly including splicing mutations that generate both aberrant and correctly spliced mRNAs in variable amounts among patients, e.g., 3272-26A>G [81], originate residual levels of normal CFTR. The major aim for therapy in these cases is to increase levels of correctly spliced transcripts. However, modulating the splicing process is very complex and tissue specific. Thus increasing the activity of wt-CFTR can also be envisaged.

Compounds like genistein, xanthines and others like milrinone and trimethylamine N-oxide (TMAO) can stimulate CFTR conductance [111]. IBMX (3-isobutyl-1- methylxanthine) an inhibitor of phosphodiesterase, seems to enhance F508del-CFTR function both by direct binding and by increasing cAMP cellular levels [112]. HTS has also identified several different potentiators such as VRT-532 (described above) and VX-770 [113]. The latter (generic drug

name Ivacaftor and trade name Kalydeco™), was recently approved by both the North-American Food and Drug Administration (FDA) and the European Medicines Agency (EMEA), as the first drug to address the underlying defect of CF. However, approval is just for 4-5% of CF patients, i.e., those bearing the G551D mutation. VX-770 is an orally bioavailable CFTR potentiator that was shown, in recombinant cells, to increase CFTR channel open probability in both the F508del processing mutation and the G551D gating mutation. VX-770 also increased Cl^- secretion in primary cultures CF HBE cells carrying the G551D gating mutation on one allele and the F508del processing mutation on the other allele by approximately 10-fold, to approximately 50% of that observed in primary HBE cells isolated from individuals without CF. Furthermore, VX-770 reduced excessive Na^+ and fluid absorption to prevent dehydration of the apical surface and increased cilia beating in these epithelial cultures [114]. Studies were then performed to evaluate the safety and adverse-event profile of VX-770. Results showed that VX-770 was associated with within-subject improvements in CFTR and lung function [115]. Further results highlighted that VX-770 was associated with improvements in lung function at 2 weeks that were sustained through 48 weeks. Substantial improvements were also observed in the risk of pulmonary exacerbations, patient-reported respiratory symptoms, weight, and concentration of sweat Cl^- [116].

Although approved only for the G551D patients, VX-770 was also shown to have a similar effect on all CFTR forms with gating defects supporting investigation of the potential clinical benefit of Ivacaftor in CF patients who have CFTR gating mutations beyond G551D [117]. Moreover, this drug could be useful for CF patients with Class IV (reduced conductance) or Class V mutations. This can be tested directly *ex vivo* on patients tissues, like rectal biopsies to directly assess efficacy of compound for individual CF patients/mutations (see above).

CFTR STABILIZERS – CAN WE ALSO IMPROVE CLASS VI MUTANTS?

Class VI includes mutations decreasing stability of CFTR present at the plasma membrane or affecting the regulation of other channels. Although there have been rare mutations investigated in a few patients like the c.120del23 deletion which abrogates part of the N-terminal of CFTR [118], most class VI mutants should be considered as severe. Interestingly, when rescued to the plasma membrane by novel small-molecule correctors, F508del-CFTR is also intrinsically unstable [39]. This can actually also account for limited success of the VX-809 corrector in clinical trials. We have recently shown that restoring F508del-CFTR intracellular localization by correctors can be dramatically improved through a novel pathway involving stimulation of signalling by the endogenous small GTPase Rac1 [119]. Indeed, we found that such enhanced anchoring retains F508del-CFTR at the cell surface, after rescuing by chemical correctors boosting the modest restoration of its function up to 30% of wt channel levels in human airway epithelial cells. This finding indicates that surface anchoring and retention is a major target pathway for CF pharmacotherapy, namely to achieve maximal restoration of F508del-CFTR in patients and in combination with correctors.

CONCLUSION

As a consequence of multiple HTS efforts for drug discovery, several novel compounds emerged as promising effective drugs against the basic defect in CF. Most significantly, the first CFTR modulator, developed based on the understanding of the gating defect has been approved by FDA and EMEA for the clinical setting. Based on the current "drug pipeline", these compounds are expected to rise in numbers very soon and there are great expectations in the scientific, clinical and patients communities alike for the first clinically-approved corrector of F508del-CFTR.

Meanwhile, a few innovative in-progress approaches seek more mechanistic insight into CF disease and a more efficient track to drugs, as combination therapy appears to be the most promising approach for F508del-CFTR. In parallel, the establishment of good predictive pre-clinical assays and adequate therapy trial endpoints for "CFTR-modulator" therapies is indispensable to bring only the best compounds to the clinical setting.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

AFT	=	Arginine-framed tripeptide (motif)
CF	=	Cystic Fibrosis
CFTR	=	CF transmembrane conductance regulator
ERGIC	=	ER-Golgi **i**ntermediary **c**ompartment
ER	=	Endoplasmic reticulum
ERQC	=	ER quality control
HBE	=	Human bronchial epithelial (cells)
HTS	=	High-throughput screening
ICL	=	Intracellular loop
MoA	=	Mechanism of action
MSD	=	Membrane-spanning domain
NBD	=	Nucleotide binding domain
RPD	=	Rectal potential differences
UPR	=	Unfolded protein response

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