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2 **Modulators of CFTR. Updates on clinical development and future directions**

3

4 Emmanuelle Bardin¹, Alexandra Pastor^{2,3}, Michaela Semeraro⁴, Anita Golec¹, Kate Hayes⁵, Benoit
5 Chevalier¹, Farouk Berhal^{2,3}, Guillaume Prestat^{2,3}, Alexandre Hinzpeter¹, Christine Gravier-Pelletier^{2,3},
6 Iwona Pranke^{1*} and I Sermet-Gaudelus^{1,3,5,6,7*}

7 * Co last Authors

8 ¹. Institut Necker Enfants Malades. INSERM U1151, Paris, France.

9 ². LCBPT, UMR CNRS 8601, Paris, France.

10 ³. Université Paris Sorbonne, Paris France

11 ⁴. Centre d'Investigation Clinique, Unité de Recherche Clinique-CIC P1419, Hôpital Necker
12 Enfants Malades, Université de Paris, Paris France

13 ⁵. Clinical Trial Network, European Cystic Fibrosis Society, Belfast, Ireland

14 ⁶. Centre de Référence Maladies Rares, Mucoviscidose et maladies de CFTR, Hôpital Necker
15 Enfants Malades, Paris France

16 ⁷. European Respiratory Network Lung, Paris France

17

18 **Corresponding author:** Isabelle Sermet-Gaudelus, INSERM U 1151, 160 rue de Vaugirard,
19 Paris 75015, France. Isabelle.sermet@aphp.fr; Phone number: (+ 33)1 44 49 48 87

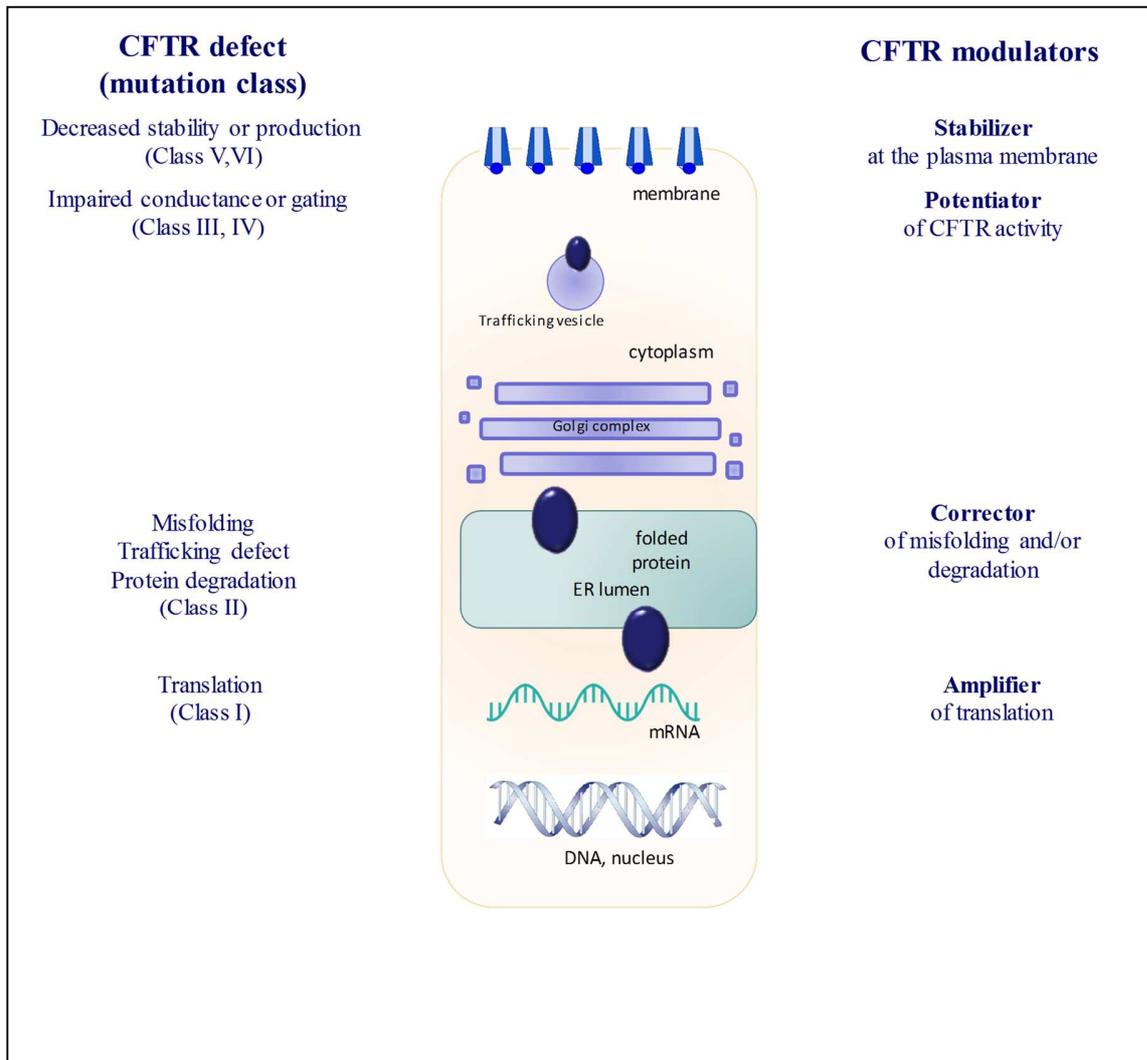
20 **Abstract**

21 Cystic fibrosis (CF) is the most frequent life-limiting autosomal recessive disorder in the Caucasian
22 population. It is due to mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*)
23 gene. Current symptomatic CF therapies which treat the downstream consequences of *CFTR* mutations
24 have increased survival. Better knowledge of the *CFTR* protein has enabled pharmacologic therapy
25 aiming to restore mutated *CFTR* expression and function. These *CFTR* “modulators” have revolutionized
26 the CF therapeutic landscape, with the potential to transform prognosis for a considerable number of
27 patients. This review provides a brief summary of their mechanism of action and presents a thorough
28 review of the results obtained from clinical trials of *CFTR* modulators.

29

30 **Graphical abstract**

31



32 **Key words:** Cystic Fibrosis, CFTR modulators, corrector, potentiator, ivacaftor, lumacaftor, tezacafor,
33 elexacaftor

34 **Introduction**

35 Cystic fibrosis (CF) is a life-limiting autosomal recessive disorder due to mutations in the Cystic Fibrosis
36 Transmembrane Conductance Regulator (*CFTR*) gene. It affects approximately 75,000 people in North
37 America, Europe and Australia. Recently, better knowledge of the CFTR protein structure and functional
38 consequences of mutations have enabled pharmacologic therapy aiming to restore CFTR expression and
39 function. This revolutionizes the approach in treating CF patients and should improve the prognosis for a
40 considerable number of patients. This review presents the results obtained from clinical trials of the
41 main CFTR modulators.

42

43 **CFTR channel, CF-causing mutations and CFTR modulators**

44 The CFTR protein – or ABCC7 – is the only member of the old family of human ATP-binding cassette
45 (ABC) transporters that functions as an anion channel. It is expressed in the apical membrane of the
46 epithelial cells of multiple exocrine organs where it contributes primarily to the active transport of
47 chloride (Cl⁻) and bicarbonate (HCO₃⁻) ions across epithelial cell membranes. It also impacts other ion
48 transport channels, notably the epithelial sodium channel (ENaC), which drives sodium (Na⁺) absorption.
49 CFTR is composed of two transmembrane domains (TMD), two intracellular subunits called nucleotide
50 binding domains (NBD) and a Regulator R domain [1]. TMD1 and TMD2 together make up the channel
51 pore through the membrane. Conformational changes of the TMDs and the opening of the channel are
52 induced by the binding of adenosine triphosphate (ATP) to NBD1 and NBD2 [2]. Conversely, dissociation
53 of ATP from the NBD domains leads to the closing of the channel and to the return to its basal state [3].
54 The regulatory (R) domain is unique amongst the 48 members of the ABC transporter family and
55 determines the channel activity [4]. The binding of ATP to NBD is enhanced by the phosphorylation of
56 the R domain, which is induced by cAMP-dependent protein kinases A and C. Maturation of a functional
57 CFTR channel requires multiple steps to achieve the complex folding and arrangement of its 5 domains
58 in the endoplasmic reticulum (ER), and further glycosylation in the Golgi apparatus. The CFTR protein is
59 thus prone to processing errors and a significant proportion (60 to 80%) is eliminated by the ER-
60 associated ubiquitin-dependent degradation system even in the wild-type (WT) cells (60-80%) [5]–[7].
61 Once matured CFTR proteins have reached the plasma membrane (PM), they undergo continuous
62 recycling through clathrin-dependent endocytosis at a rate of 10% per minute [8], [9].

63 Defective CFTR channel functioning is the primary cause of CF and its clinical consequences. Impairment
64 of the channel causes an ion imbalance as Cl⁻ secretion is disrupted whilst Na⁺ absorption increases,
65 causing a secondary osmotic uptake of water and therefore the dehydration of epithelial surface fluids
66 in multiple organs, including lungs, pancreas, vas deferens, liver, and intestine. In sweat glands, the
67 secretory coil duct excretes water, Na⁺ and Cl⁻, which is reabsorbed along the sweat duct. Defective CFTR
68 channel prevents reabsorption of Cl⁻, resulting in sweat with abnormally high Cl⁻ concentration. This

69 phenomenon led to the implementation of the diagnostic “sweat test” based on sweat Cl⁻
70 measurement. Whilst the digestive, reproductive and other systems are also impacted, most of the
71 morbidity and mortality associated with CF is due to its impact upon the respiratory system. Lack of
72 HCO₃⁻ excretion causes a pH decrease at the mucosal surface because reduced pH inhibits the activity of
73 antimicrobial peptides in the airway surface liquid [10]. These phenomena result in impaired host
74 defence in the airways. The dehydration of airway surfaces results in increased fluid viscosity, which
75 impairs mucociliary clearance, one of the lung’s innate defence mechanisms. Mucus accumulates in the
76 respiratory tract, allowing infection to initiate and persist. An excessive and inefficient inflammatory
77 response to microbiological infection contributes to this impaired airway defence and progressive lung
78 degradation [11], [12]. Early in life, a vicious circle of chronic bacterial infections and lung inflammation
79 is initiated, eventually leading to irreversible lung damage, which is responsible for more than 95% of
80 deaths amongst the CF population [13], [14]. In 2018, median age at death for people with CF was still in
81 the 30s in the US [15].

82

83 **Classification of CF-causing mutations**

84 The CFTR protein is composed of 1 480 amino acids that are encoded by the *CFTR* gene located on the
85 long arm of chromosome 7. Mutations in both *CFTR* alleles lead to CF, which is the most common
86 genetic life limiting disease affecting the Caucasian population. There are currently 2103 variants of the
87 *CFTR* gene reported in the CFTR Mutation Database (CFTR1 Database [16]) and 442 mutations have
88 documented clinical consequences (CFTR2 Database [17]). CF-causing mutations are classified into 6
89 categories, according to their impact on the production, trafficking, functioning or stability of the CFTR
90 channel [18]. Mutations belonging to classes I, II and III usually result in little to no CFTR activity, leading
91 to severe clinical outcomes, whilst mutations from classes IV, V and VI allow significant residual CFTR
92 function leading to milder phenotypes.

93 **Class I mutations**, also known as protein production mutations, are often due to a premature
94 termination codon (PTC) caused by nonsense mutations, which engender unstable messenger RNA
95 (mRNA) that is rapidly eliminated by the nonsense mRNA decay (NMD) surveillance system [19]–[21].
96 The small proportion of mRNA that may escape NMD is usually translated into shortened and non-
97 functional proteins resulting in the absence of CFTR channels. Other genetic errors included in class I are
98 large insertions/deletions of genetic material and alterations of splicing sites.

99 **Class II mutations**, also known as protein processing mutations, lead to abnormal processing and
100 trafficking of the CFTR protein, which result in the absence of CFTR channels at the PM. The most
101 common mutation, legacy name F508del, belongs to this class. Overall prevalence of F508del is around
102 80% of the CF population worldwide, yet there are important geographical disparities, from 30% in
103 Turkey to above 95% in Denmark [22]. This in-frame deletion of phenylalanine at position 508

104 (p.Phe508del) causes abnormal folding of the F508del-CFTR-NBD1 protein generating its thermo-
105 instability. This hinders adequate assembly of NBD1 with the two TMD domains and makes CFTR prone
106 to degradation by the proteasome [23]. The very few proteins that reach the apical PM present defects
107 in channel gating and are highly unstable [24].

108 **Class III mutations**, also called gating mutations, allow the generation of CFTR proteins that can be
109 expressed at the PM, but they display a drastic decrease in opening probability due to failure of the ATP-
110 activation of the channel. The most common class III mutation is G551D (c.1652G>A). The substitution of
111 glycine by aspartate interferes with the junction of NBD1 and NBD2, thereby impeding the binding of
112 ATP, which leads to a 100-fold decrease in the opening probability of the channel compared to the WT
113 channel [25].

114 **Class IV mutations**, also known as conduction mutations, primarily impact the TMD domains, leading to
115 a decrease in ion conductance. CFTR proteins are present at the PM but display a decreased function.
116 R117H (c.350G>A) is the most frequent class IV mutation and affects 0.7% of patients [25].

117 **Class V mutations** reduce the density of operational CFTR channels at the PM because of promoter
118 mutations limiting transcription, splicing abnormalities or missense mutations, all yielding aberrant
119 mRNA products [25]. In contrast to Class I splicing mutation, a small quantity of viable mRNA is still
120 generated allowing residual CFTR function. The most frequent mutation is 3849+10kb C→T (c.3718-
121 2477C>T), globally carried by 0.6% of patients.

122 **Class VI mutations** are characterised by the production of fairly functional CFTR proteins with a low
123 overall concentration at the cell surface. Alterations in the protein conformation and additional
124 endocytic signals confer the proteins with a high instability at the cell surface [26], [27]. The enhanced
125 endocytic rate leads to a globally reduced amount of CFTR channels operating at the PM.

126 There are multiple limitations in this classification including the fact that mutations may lead to several
127 defects and thus belong to various classes. In this way, the F508del mutation, which entails deficiency in
128 the trafficking, functioning, and stability of the CFTR protein may be classified as class II, III and VI [28].
129 Furthermore, individuals with CF may carry different mutations on each allele, leading to multiple
130 potential combinations of defects.

131

132 **Classes of CFTR modulators**

133 This classification has been crucial in understanding the pathophysiology of the CFTR channel, steering
134 research for precision therapies targeting the original cause of CF. Indeed, precision therapies pursue
135 various objectives inspired by this classification: enhancing the production of proteins, restoring
136 acceptable folding, gating, ion conductance or stability, and overcoming the insertion of PTCs. Classes I
137 and II induce little or no production of CFTR proteins, which has been qualified as minimal function (MF)
138 activity and represents a considerable rescue challenge. Classes IV, V and VI enable the generation of

139 some functional channels associated with residual function (RF) activity and milder clinical outcomes.
140 This has led to the concept of proteic therapy modulating the expression and/or the activity of the CFTR
141 channel. Those drugs are designed to rectify specific defaults stemming from mutations on the *CFTR*
142 gene. Depending on the targeted defect, they have distinct modes of action [29].

143 **Correctors** support the trafficking to the cell surface of mutated CFTR proteins resulting from class II
144 mutations and increase the amount of CFTR channels at the apical PM. Two strategies may be employed
145 to prevent the degradation of defective proteins: pharmaceutical chaperones directly binding to the
146 misfolded protein and probably correcting the folding and the resulting thermostability of the protein
147 or proteostasis regulators that modulate protein homeostasis and the cellular quality control system.

148 **Potentiators** intend to enhance ion transport of CFTR proteins that are present at the PM but are not
149 functional. This can be achieved through interaction with CFTR in order to prolong the open state of the
150 channel.

151 **Amplifiers** stimulate protein expression by improving mRNA stability and assisting CFTR transcription or
152 translation.

153 **Stabilisers** restore the stability of class VI-produced proteins and decrease endocytosis at the PM,
154 leading a higher quantity of CFTR channels at the surface. This can be achieved by anchoring the
155 defective CFTR protein at the PM or establishing stabilising interactions with other components of the
156 membrane.

157 Rescue of the most common mutation, F508del, requires acting upon several defaults: aid folding of the
158 protein to restore the channel stability, escape the ER quality control system, stabilise the channel at the
159 PM, and enhance its functional activity.

160 This review intends to thoroughly present the results obtained with CFTR modulators in clinical trials.
161 Results focus, for comparability and ease of reading on 2 endpoints: the assessment of the CFTR channel
162 activity based on the measurement of sweat Cl⁻ concentration, and lung function based on percent
163 predicted forced expiratory volume in 1 second (ppFEV₁).

164 Listed clinical trials are interventional and exclude roll-over and observational studies. Least square
165 mean differences are reported (i) between the treated group and the baseline, and (ii) between the
166 treated group and the controls, according to data availability and relevance. **Tables 1 to 3** summarise
167 the clinical outcomes obtained with the first modulators and dual combinations currently approved in
168 Europe; **Tables 4 and 5** present the results obtained with the next-generation modulators at different
169 stages of development, and **Table 6** the list of reported trials.

170

171 **Approved CFTR modulators**

172 **1. CFTR potentiator, VX-770**

173 The first potentiator, VX-770, Ivacaftor, is based on a 4-(1*H*)-quinolinone connected to a di-*tert*-butyl-
174 substituted phenol by an amide linkage (**Figure 1**). VX-770 was selected following high-throughput (HTS)
175 pharmaceutical screening on CFTR Cl⁻ transport activity. The compound demonstrated an increase in the
176 activity of both WT and defective CFTR cells with specific mutations affecting the activation of the
177 channel, such as G551D [30]. *In vitro* assays revealed that VX-770 prolongs the duration of the CFTR
178 channel opening even in the absence of ATP [31], [32], suggesting a potentiation mechanism
179 independent of ATP and NBD domains. Recent studies demonstrated that the binding of VX-770 to CFTR
180 occurs at the interface of the two TMDs [33], [34]. Yet, the mechanism of action behind VX-770's action
181 remains to be fully elucidated. Interestingly, VX-770 also rescues other ABC proteins such as ABCB4,
182 suggesting that it is not CFTR specific [35].

183 The first proof of concept clinical trial was performed in 2007, in adults carrying the G551D mutation
184 and showed a dose dependant improvement in sweat Cl⁻, as well as other parameters of CFTR function
185 such as nasal potential difference, and amazingly, respiratory function (**Table 1**). This clinical trial
186 demonstrated for the first time the proof of concept that restoring CFTR function was associated with
187 clinical benefits in patients. VX-770 was further shown to increase the ppFEV₁ in subjects carrying at
188 least one G551D [36] but not in F508del homozygous patients [37] (**Table 1**). Subsequent clinical trials
189 consistently demonstrated the efficacy of VX-770 in decreasing sweat Cl⁻ concentration, improving lung
190 function and nutritional status as well as decreasing the number of exacerbations in patients carrying at
191 least one gating mutation [38]–[40]. Occurrence of adverse events was globally comparable in the
192 ivacaftor and placebo groups and most frequently included cough, headache, fever, nausea, rash and
193 pulmonary exacerbations in the treated group [38]–[40]. Young children also showed a good response
194 to VX-770 with a decrease in sweat Cl⁻ concentrations, and possible changes in pancreatic function, as
195 assessed by an increase in pancreatic elastase [41]. Nevertheless, abnormal liver function was found in a
196 significant number of children enrolled, highlighting necessity of careful monitoring [41]. Studies in
197 patients with RF mutations did not demonstrate drastic improvements, but a decrease in sweat Cl⁻ in
198 people with one or two R117H mutations was still highly favourable, as well as an increase in ppFEV₁ in
199 adults [42], [43]. Interestingly, a recent promising study followed 26 patients carrying an RF mutation
200 with a severe CF phenotype who had compassionate access to VX-770. The potentiator was found to
201 safely improve respiratory function with a sustained increase of about 10 points in ppFEV₁ and a
202 significant decrease in antibiotic therapies after one year of treatment [44].

203 VX-770 (trade name Kalydeco®, Vertex pharmaceuticals) was granted marketing authorisation in the
204 United States (early 2012), Europe and Canada (late 2012), and then in Australia, and New Zealand
205 (2013). The American Food and Drug Administration (FDA) and the European Medicines Agency (EMA)
206 have approved the clinical use of VX-770 to treat patients from four-months old carrying one of the nine
207 gating mutations: G178R, S549N, S549R, G551D, G551S, G1244E, S1251N, S1255P, G1349D (G970R was

208 not approved) or the conduction mutation R117H, which have all been tested through clinical trials. In
209 addition to these, the FDA further authorised 23 RF mutations based on clinically proved benefits and
210 further *in vitro* assays: A455E, E193K, R117C, A1067T, F1052V, R347H, D110E, D110H, F1074L, R352Q,
211 G1069R, R1070Q, D579G, K1060T, R1070W, D1152H, L206W, S945L, D1270N, P67L, S977F, E56K, R74W;
212 and 5 splice mutations 711+3A→G, 3272-26A→G, E831X, 2789+5G→A, 3849+10kBc→T. These have not
213 yet been approved by the EMA. Indeed, the FDA and the EMA slightly differ in their application
214 requirements. There are precedents of the FDA granting authorisation based on *in vitro* data whilst the
215 EMA has so far demanded clinical trial data. This may hamper timely access to new treatments for
216 European patients carrying rare mutations. Notably, the HIT-CF consortium is currently discussing with
217 the EMA to qualify rectal organoids as relevant personalised *ex-vivo* models to predict the efficacy of
218 innovative drugs [45].

219

220 **2. CFTR correctors**

221 2.1. VX-809

222 The first corrector demonstrating safe and efficient rescue of misfolded F508del-CFTR protein *in vitro*
223 was VX-809, lumacaftor [35]. As for VX-770, VX-809 was selected by HTS screening. This compound is
224 based on a di-fluorobenzodioxolyl-cyclopropane linked to a substituted arylpyridine through an amide
225 linkage (**Figure 1**). Its mode of action is yet to be elucidated but several studies indicate that it may
226 stabilise the folding of the molecule either by direct binding to NBD1 [46] or through promoting
227 interactions between TMD1 and NBD1 [47]. Interestingly, VX-809 was shown to rectify trafficking of
228 another deficient ABC transporter (ABC4), which shares large homology with CFTR NBDs, indicating that,
229 similarly to VX-770, VX-809 is not CFTR-specific [48].

230 VX-809 alone failed to demonstrate clinical effect on patients heterozygous for F508del [49] (**Table 2**).
231 Indeed, rescued F508del-CFTR may reach the PM but shows reduced cell surface stability and low open
232 probability. Therefore, VX-809 was combined with the potentiator VX-770 to further aid channel
233 opening. Phase 2 [50] and longer phase 3 [51] studies showed slight but significant positive effects,
234 notably on lung function for F508del homozygous patients, together with a significant increase in body
235 mass index (BMI) and a reduced number of exacerbations as well as hospitalizations. Subsequent studies
236 on children aged 6 to 11-years, consistently confirmed an improved functioning of the channel with a
237 reduction in sweat Cl⁻ of more than 20mmol/L and a decrease in lung clearance index (LCI) around 1 unit
238 [52], [53]. Importantly, follow-up studies now confirm the benefits (and the safety) of long-term therapy
239 with a rate of decline in lung function 42% slower than in matched registry controls [54]. A phase 3b
240 open-label prospective study evaluating the benefit of this combination for patients with advanced lung
241 disease (ppFEV₁<40%) observed more frequent respiratory adverse occurrences which is a source of
242 concern. Study conclusions recommended treatment initiation at a lower dose [55] (NCT02390219,

243 results not reported in **Table 2**). Common adverse reactions associated with treatment were indeed
244 respiratory based, such as dyspnoea, chest tightness or haemoptysis. Abnormal liver function tests and
245 increased blood pressure were also reported but did not lead to severe adverse events [42], [51]–[54].
246 It should be noted however, that improvements as a result of VX-809 treatment were not
247 commensurate with VX-770-responsive patients, (e.g ~4% versus ~10% improvement for ppFEV₁). This
248 has been initially attributed to inhibition of VX-809 by VX-770 hindering the overall rescue of F508del-
249 CFTR [56]. In addition, VX-770 metabolites and VX-809 induce cytochrome CYP3A4 activity, decreasing
250 plasma concentration of VX-770 (see below for more details) [57].
251 The combination VX-809/VX-770 (trade name Orkambi®, Vertex Pharmaceuticals) was approved both by
252 the FDA and the EMA in 2015 for patients from two years old. Later, real-life data further revealed
253 frequent respiratory adverse effects and drug intolerance which led to discontinuation of the treatment
254 in some cases [58], [59]. Importantly, clinical response may also vary significantly amongst patients
255 bearing the same genotype, the mechanism of which is yet not clearly understood but, supporting the
256 need for personalised therapeutic approaches and assessment [50], [51].

257

258 2.2. VX-661

259 VX-661, or tezacaftor, is a corrector designed on the basis of the chemical structure of VX-809 yet with
260 improved pharmacokinetics and less side-effects. The skeleton remains the same, but the pyridine ring
261 has been replaced by a functionalised indole moiety (**Figure 1**).

262 VX-661 monotherapy led to some improvement in lung function in F508del homozygous patients [60]
263 (**Table 3**), slightly higher than VX-809 [49] but displayed similar effect in association with potentiator VX-
264 770 in F508del homozygotes [50], [60]. Patients compound heterozygous F508del with G551D or RF
265 mutations appeared more responsive than F508Del homozygous to the VX-661/VX-770 combination
266 with a confirmed decrease in sweat Cl⁻ and consistent increase in ppFEV₁, in contrast to VX-809/VX-770
267 which did not display a significant response (**Table 3.**) [60], [61]. Reported adverse effects included
268 respiratory events of mild severity such as pulmonary exacerbation, cough, and increased sputum, but
269 also included nausea, diarrhea, headache, and fatigue. These were less frequent in the treated group
270 compared to placebo [60]–[62]. Moreover, patients encountered less respiratory adverse effects and
271 drug-drug interactions with the VX-661/VX-770 co-treatment than previously reported with VX-809/VX-
272 770 [42], [51]. Further studies confirmed VX-661 safety and potential benefit on younger patients [63].

273 The VX-661/VX-770 co-therapy (trade name Symdeko® or Symkevi®, Vertex Pharmaceuticals) received
274 marketing authorisation in 2018. To date, the combination is approved in Europe for patients above 12
275 years old homozygous for F508del and heterozygous with one of the following 10 RF mutations : P67L,
276 R117C, L206W, R352Q, A455E, D579G, S945L, S977F, R1070W, D1152H; or one of the 4 splice
277 mutations: 2789+5G>A, 3272-26A>G, 3849+10kbC>T, 711+3A>G. Authorisation was granted in the

278 United States for patients from six years old and for the 12 additional mutations: E56K, R74W, A1067T,
279 E193K, D110H, R347H, D110E, F1052V, F1074L, K1060T, D170N, E831X.

280

281 2.3. VX-445 and the advent of Vertex triple combination

282 VX-770 and combinations with first generation correctors (VX-809 or VX-661) now constitute the
283 standard of care with sustainable clinical benefits and serve as the benchmark to evaluate novel
284 treatments. However, clinical improvement remains moderate, notably in lung function. F508del-CFTR
285 function is incompletely restored and many mutations, especially related to minimal CFTR function, are
286 still lacking suitable treatments. Advances in the last two years have shown the importance of targeting
287 different CFTR sites to maximise the corrector effect. Therefore, pharmaceutical companies now aim for
288 combinatory therapies [64]. Vertex Pharmaceuticals selected 4 compounds showing correcting potential
289 combined with VX-661 and VX-770 for further evaluation: VX-152, VX-440, VX-445 and VX-659. VX-445
290 (elexacaftor) and VX-659 (bamocafort) were further shortlisted because of their better pharmacological
291 properties and long-term safety. Both molecules present a different structure than VX-661/809. They
292 display a common pyrazolo pyrrolidinopyridine moiety connected respectively to a pyrazolylsulfonyl or a
293 phenylsulfonyl group through an amide appendage (**Figure 1**). Since they were anticipated to act on
294 different binding sites than the first-generation correctors, they were tested in combination to
295 supplement the action of the potentiator VX-770 and an old-generation corrector. VX-661 was chosen
296 for its enhanced pharmacokinetics and lower activation of cytochrome CYP3A4 compared to VX-809.

297 Both triple combinations underwent phase 2 clinical trials in patients homozygous for F508del and
298 heterozygous with a MF mutation [65], [66]. They demonstrated spectacular improvements with a
299 minimum decrease of -40mmol/L in sweat Cl⁻ and at least 10% improvement in ppFEV₁ (**Table 4**).
300 Importantly, and unexpectedly, the benefits gained in patients heterozygous for F508del and an MF
301 mutation, for whom no therapeutic options were available at the time, were comparable to those of
302 F508del homozygous patients. The majority of patients experienced mild to moderate adverse effects
303 that did not lead to treatment discontinuation. They were similar for both triple combinations and
304 comparable to those of individual CFTR modulators as well as representative of CF disease, i.e. cough,
305 increased sputum production, infective pulmonary exacerbation, haemoptysis, and fever. In addition,
306 reactions that were significantly more frequent in the treated group and call for caution included rash,
307 slight increase in blood pressure, elevated levels of aminotransferases, and elevated levels of creatine
308 kinase in serum that were often associated with exercise [65]–[67]. At this point, it is not known
309 whether it was one specific compound or their association that was responsible for these adverse
310 events. Phase 3 clinical trials further tested the combination of VX-661, VX-445 and VX-770 and
311 confirmed the benefits and the safety profile on a larger cohort of patients above 12 years old, F508del-
312 homozygous and -heterozygous [67], [68] (**Table 4**). Nevertheless, assessment of liver function is

313 recommended prior to initiating treatment, as well as a close follow-up during the first year of
314 treatment with the triple combination.

315 These results clearly created immense hope within the CF community. Following these outstanding
316 results, the FDA soon granted a broad marketing authorisation in the United States (Autumn 2019)
317 under the trade name Trikafta® (Vertex Pharmaceuticals) for all patients aged 12 years and older, with
318 at least one F508del mutation. The FDA is currently reviewing supplemental *in vitro* data to expand the
319 indication to some rare mutations [69]. Trials are being completed to assess efficacy and safety on
320 F508del-heterozygous with a gating or RF mutation (NCT04058353) and in younger children, aged 6 to
321 11 years of age (NCT03691779) (**Table 7**). In the summer 2020, Vertex press-released positive results of
322 the Phase 3 study of Trikafta® in patients aged 12 years and older, compound heterozygotes for F508del
323 and one gating mutation or one RF mutation. ppFEV₁ increased significantly from baseline (after a 4-
324 week run-in of treatment on ivacaftor or tezacaftor/ivacaftor) through 8 weeks of treatment by 3.7
325 percentage point (p<0.0001) as well as a significant mean within-group reduction of 22 mmol/L from
326 baseline in sweat Cl⁻ (p<0.0001) [70]. The EMA has approved the combination under the trade name
327 Kaftrio® (Vertex Pharmaceuticals) for patients homozygous for F508del or heterozygous with a broad
328 range of MF mutations only (August 2020). The recent available clinical data on RF mutations is currently
329 being evaluated through a post-authorisation procedure [71].

330

331 **CFTR modulators in the pipeline**

332 **1. Vertex pipeline**

333 Several promising compounds are currently being investigated in order to offer alternatives to VX-770 to
334 potentiate gating mutations. Vertex pharmaceuticals is working on VX-561 (also called deutivacaftor,
335 formerly CTP-656), an analogue of VX-770 in which one of the *tert*-butyl groups was replaced by a *per*-
336 deuterated one (**Figure 1**). This altered version of VX-770 has shown similar pharmacologic potency in
337 preliminary studies. Furthermore, the metabolic stability with regards to cytochrome activity was
338 increased *in vitro* and *in vivo*, suggesting it could be prescribed once daily only, contrary to VX-770,
339 which must be administered every 12h [72]. Following these encouraging data, VX-561 was included in
340 phase 2 clinical trials evaluating two possible triple combinations. Substitution of VX-770 by VX-561 in
341 the triple combinations VX-445/VX-661/VX-770 [65] or VX-659/VX-661/VX-770 [66] led to equally
342 positive clinical outcomes (**Table 4**). A phase 2 clinical trial started mid-2019 to assess the effects of VX-
343 561 alone on gating mutations in comparison to VX-770 in adults 18 years and older (NCT03911713).

344 The company is also developing another corrector, VX-121, which has reached clinical phase 2. It is being
345 assessed in combination with VX-661, VX-770, and/or VX-561 (NCT03768089 and NCT03912233). Its
346 structure is still not available at the Drug bank.

347

348 2. AbbVie pipeline

349 Galapagos NV, had initiated the development of several potentiators, whose development is being
350 continued by AbbVie Inc, notably ABBV-974, ABBV-2451, and ABBV-3067 (formerly GLPG-1837, GLPG-
351 2451, and GLPG-3067, respectively).

352 Their chemical structures differ significantly from that of VX-770 (**Figure 1**). ABBV-974 is built on a
353 pyrazole ring connected through an amide bond to a substituted thiophen-carboxamide. ABBV-2451 is
354 based on a phenylsulfonyl aminopyridine connected through an amide linkage to a 2-hydroxypropyl
355 group. Both potentiators enhanced the activity of the corrected F508del-CFTR channel by increasing
356 opening time and shortening basal state conformation [73]. Combined treatment of ABBV-974 or ABBV-
357 2451 with VX-770 did not lead to an increase in channel activity, suggesting a common binding site on
358 the protein [73]. Similar to VX-770, ABBV-974 was confirmed to bind to the same site between the two
359 TMDs and to display a mechanism independent of ATP hydrolysis [33], [34]. ABBV-974 and ABBV-2451
360 improved CFTR activity in class III and IV mutants *in vitro* to a level that was at least comparable to the
361 benchmark VX-770 [73], [74]. A phase 2a clinical trial assessed the clinical safety, pharmacokinetics and
362 efficacy of ABBV-974 in patients with at least one G551D allele, after withdrawal from standard of care
363 VX-770 treatment and a short washout period [75]. The one-week VX-770 washout was not a barrier to
364 patients' participation and overall, was well tolerated. VX-770 withdrawal resulted in a decline in lung
365 function, which was restored to pre-washout levels after four weeks of ABBV-974 treatment. Sweat Cl⁻
366 decreased in a dose-dependent manner (**Table 5**), confirming that ABBV-974 enhances the activity of
367 G551D-CFTR, though respiratory adverse effects were reported. Although most of adverse events were
368 mild and typical of CF disease, they seemed to be dose-dependent as they were more frequent during
369 the high-dose (500mg) treatment period. A significant number of participants (38.5%) had an abnormal
370 increase in aminotransferase levels. Four of the 26 treated patients experienced severe effects calling
371 for caution in future trials: fatigue, cough, increased blood creatine phosphokinase and pulmonary
372 exacerbation [75].

373 Another potentiator, ABBV-3067, is currently being tested alone and in combination with a corrector
374 (ABBV-2222) in a phase 2 clinical trial with adult patients homozygous for F508del (NCT03969888).
375 AbbVie is pursuing the development of the correctors ABBV-2222, ABBV-2737 and ABBV-3221 (formerly
376 GLPG-2222, GLPG-2737, GLPG-3221, respectively); some of them have reached an advanced stage of
377 validation. Chemical structure of ABBV-2222, also called galifactor, presents similarities with the first-
378 generation correctors (**Figure 1**). Compared to VX-809, the pyridine appendage has been replaced by a
379 functionalised chromane moiety in ABBV-2222. The latter demonstrated efficiency to correct on
380 F508del-CFTR in homozygous bronchial epithelial cells [76], [77]. In a two-fold phase 2a clinical trial
381 patients heterozygous for F508del and a gating mutation received ABBV-2222 alone, whilst F508del-
382 homozygous subjects had ABBV-2222 in combination with VX-770 [78]. ABBV-2222 at a high dose

383 markedly decreased sweat Cl⁻ concentrations in heterozygous patients and, to a lesser extent, in
384 homozygous patients on VX-770 (**Table 5**). Impact on lung function was weak but comparable to the
385 results obtained with a single first-generation corrector such as VX-809 [49], [50] (**Table 2, 3 and 5**).
386 Common reported treatment-related adverse events were headache, cough, pulmonary exacerbation,
387 sputum quantity and diarrhea [78].

388 Other on-going studies are currently examining safety and efficacy of dual or triple combinations of the
389 second-generation corrector ABBV-2737 with the potentiators ABBV-2451 or ABBV-3067. *In vitro* studies
390 previously proved the ability of ABBV-2737 to increase the number of CFTR channels expressed at the
391 PM, notably in combination with the potentiator ABBV-3067 and the corrector ABBV-2222 [79].
392 Although ABBV-2737 seemed to facilitate the F508del protein escape from the quality control system of
393 the cell, it yielded to a more rigid conformation presenting opening defects similar the G551D-CFTR
394 channel that required the addition of ABBV-3067. ABBV-2737 significantly improved F508del-CFTR
395 activity, compared to the dual combination ABBV-3067/ABBV-2222, indicating that ABBV-2737 and
396 ABBV-2222 have distinct modes of action. It further demonstrated its added value in F508del
397 homozygous patients already treated with Orkambi® (VX-770/VX-809) [80]. The phase 2a clinical trial led
398 to a significant decrease in sweat Cl⁻ concentration and a small improvement in lung function compared
399 to the dual combination (**Table 5**). The most common adverse events were upper respiratory tract
400 infection and headache. There were no serious adverse effects related to treatment leading to
401 treatment discontinuation and no impact on liver function as previously reported with Orkambi® [51],
402 [80].

403 An early stage clinical study is currently testing the triple combination of the two correctors ABBV-2737
404 and ABBV-2222 with the potentiator ABBV-2451 (NCT03540524, FALCON study). ABBV-3221, a second-
405 generation corrector (**Figure 1**) is a promising molecule, which enhances CFTR function *in vitro* in
406 combination with ABBV-2222 corrector and ABBV-974 potentiator [81]. This compound displays an
407 original structure around a polyfunctionalised proline core as compared to the previous ones.

408

409 **3. Proteostasis pipeline**

410 Since the association of several correctors demonstrated outstanding clinical benefits, pharmaceutical
411 companies have been testing triple combinations. Proteostasis Therapeutics Inc. (PTI) is the first
412 company to elaborate a cocktail involving a potentiator, a corrector and an amplifier that has reached
413 clinical phase 2.

414 The amplifier PTI-428 (or nesolicaftor) was identified through the phenotypic HTS of thousands of
415 compounds. Nesolicaftor, displays a linear polyheteroaromatic structure distributed around a
416 cyclobutane (**Figure 1**). PTI-428 was tested *in vitro* in combination with the potentiator VX-770 and the
417 corrector VX-809 and proved to add onto their activity, indicating a different mode of action [82], [83]. It

418 was demonstrated to selectively enhance the production of defective *CFTR* mRNA *in vitro* across various
419 mutations, including F508del and some rare mutations, by targeting the defects hindering synthesis at
420 early stages [82]. Biochemical and proteomic studies revealed that the amplifier improves *CFTR* mRNA
421 stability and the fraction of *CFTR* mRNA associated with polysomes. Pull-downs identified that the
422 amplifier binds to the poly(rC)-binding protein 1 (PCBP1) [84]. It was further shown that this increase in
423 the production of misfolded F508del-*CFTR* protein did not trigger ER-associated cellular stress responses
424 [82]. Yet, the amplification of F508del-*CFTR* biosynthesis did not increase expression of the *CFTR*
425 channel at the PM indicating that the produced protein remains mostly misfolded and non-functional
426 [83]. Early stage clinical trials are in progress to evaluate the safety and efficacy of PTI-428 in CF patients
427 in addition to stable treatment with ivacaftor (NCT03258424), lumacaftor/ivacaftor (NCT02718495), or
428 tezacaftor/ivacaftor (NCT03591094).

429 Proteostasis Therapeutics is also developing a third-generation corrector, PTI-801 or posenacaftor and a
430 potentiator, PTI-808, or dirocaftor. PTI-808 is an analogue of VX-770 and VX-561 in which *tert*-butyl
431 groups have been replaced by trimethylsilyl groups. PTI-801 displays a benzofuranyl quinoline central
432 motif (**Figure 1**). The triple combination, PTI-428, PTI-801 and PTI-808 proved to enhance F508del-*CFTR*
433 activity *in vitro*. A phase 1/2 clinical study (NCT03500263) showed an improvement of 8% in ppFEV₁ and
434 a decrease of 29 mmol/L in sweat Cl⁻ in adults homozygous for F508del after 4 weeks of treatment
435 compared to the placebo (communication by Proteostasis Therapeutics). The F508del heterozygotes
436 showed a range of response in clinical response, suggesting specificity towards a number of definite
437 mutations, but as a whole, the effect on lung function was not significant. The combination is currently
438 being tested *ex vivo* on intestinal organoids grown from rectal biopsies taken from more than 500 CF
439 patients with ultra-rare mutations as part of the HIT-CF Europe project [45]. A selection of these patients
440 whose organoids showed a positive response will be invited to participate in CHOICES, a clinical phase 3
441 study planned to start in the last quarter of 2020 (Proteostasis Therapeutics).

442

443 **Daily challenges of *CFTR* modulator therapies**

444 **Compliance**

445 The first and often underestimated challenge of *CFTR* modulator treatment is compliance [85]. Indeed,
446 patients who report symptomatic improvement may reduce their adherence to baseline therapies, such
447 as physiotherapy or nutritional support. Moreover, patients who gain too much weight or have side
448 effects such as acne may decide to discontinue the drug. To evaluate the extent of these phenomena,
449 more accurate patient-reported outcomes discriminating between benefits and side effects are needed
450 (Cooke et al, personal data).

451 **Drug interactions**

452 CF patients routinely take a significant number of medications, therefore drug interactions involving
453 CFTR modulators must be carefully considered in order to obtain high local (bronchial secretions)
454 concentrations.

455 Pharmacokinetics of CFTR modulators mainly involves the family of P450 cytochrome (CYP450) enzymes
456 (CYP3A4 and CYP3A5). Cytochrome enzymes may be induced (increased expression) by drugs or food
457 (usually, but not only, substrates of the cytochrome such as phenobarbital, phenytoin, rifampicin,
458 glucocorticoids terfenadine, elexriptan, St. John's Wort), resulting in enhanced metabolism, and thus
459 lower levels of its substrate compounds. They may be inhibited by a number of compounds, such as
460 azole antifungal therapies (voriconazole, ketoconazole, miconazole, itraconazole), clarithromycin,
461 erythromycin, diltiazem, midazolam, verapamil, goldenseal root, grapefruit. This results in increased
462 drug levels. Moreover, several genetic polymorphisms can involve the *CYP* gene leading to variations of
463 that enzyme's activity.

464 The enzyme CYP3A4 metabolises VX-770 to less active metabolite M1 and inactive metabolite M6 and
465 the enzyme CYP3A5 metabolises VX-661 to less active metabolites M1, M2 and M5. On the other hand,
466 they do not impact VX-809 levels [39], [40]. Therefore, concomitant treatment of Kalydeco[®], Symdeko[®]
467 or even VX-770-containing Orkambi[®] with CYP3A4 inhibitors increases the exposure to VX-770/VX-661,
468 whilst concomitant treatment with an inducer decreases the exposure.

469 CYP3A expression is strongly induced by VX-809 and, to a lesser extent, by VX-770 metabolites, but not
470 by VX-661. The combination VX-770/VX-809 is thus both a CYP3A4 substrate (VX-770) and a strong
471 inducer of other *CYP* (VX-809 and VX-770 metabolites). Therefore, the level or the activity of drugs
472 usually metabolised by CYP4 should be carefully monitored in patients treated by Orkambi[®], which may
473 lead to a decrease in efficacy, for example of oestroprogestatives, antifungals, or clarithromycin [86].

474 These examples demonstrate that drug interactions must be carefully monitored by clinicians for CF
475 patients in order to avoid lack of efficacy, toxicity and associated adverse effects.

476

477 **Future directions**

478 In less than 10 years, dramatic advances in CFTR science has opened new pathways to a therapeutic
479 landscape reshuffle. These modulators have deeply transformed CF therapeutic approaches and will
480 undoubtedly positively impact prognosis. The number of CF patients who are candidates for highly
481 effective CFTR modulation is now over 80%. The portfolio of efficient molecules is increasing
482 dramatically, and future personalised therapy is now a reality. Given the different combinations
483 available in a near future for the same mutation, new tools are now needed to identify the optimal
484 association of molecules. To best tailor treatment to each patient, models are being developed from
485 patient cells. Two- or three-dimension primary cell cultures should help to recapitulate the
486 transcriptomic and proteomic background of the patient and its impact on individual metabolism and

487 efficiency of the drugs. This includes 2D primary cultures from nasal brushings and/or 3D spheroids from
488 intestinal or respiratory cells. These techniques should help to tailor the most efficient therapy to a
489 given patient and initiate the era of novel personalised therapies. However, the question remains
490 whether these patient-derived models are representative of clinical phenotypes. Whilst organoids and
491 primary cells may provide information about the “correctability” of a given mutation as a preclinical
492 model [87], [88], it remains to be proved whether they can predict the clinical response at an individual
493 level. Recent publications [89]–[91] did not provide evidence for patients carrying the same mutation
494 treated by CFTR modulators of the relationship between the variation of the ppFEV1 and the Forskolin-
495 Induced response of rectal organoids incubated with the drug. A study is ongoing to establish a potential
496 relationship between the improvement in ppFEV1 at 6 months of Orkambi® in F508del homozygous
497 patients and the variation of CFTR function in their primary nasal cells upon lumacafor/ivacaftor
498 (PREDICT-CF study, NCT03894657).

499 Future challenges also lie in study design for increasingly asymptomatic patients and implementation of
500 new surrogate endpoints. Not only are naïve patients rarer and rarer, they may also be reluctant to
501 interrupt an efficient treatment to participate in trials if their condition is improved by modulators.
502 Therefore, studies should be designed as non-inferiority trials and be as short as possible. The sweat test
503 remains one of the most sensitive biomarkers, widely used during clinical trials for its dynamic range and
504 its capacity to adjust quickly. Sweat Cl⁻ concentration was previously shown to differ at the basal state
505 depending on the mutation class, with notably lower concentrations for class IV mutations [92]. More
506 recently, changes in sweat Cl⁻ concentration were related to variations in other interesting biomarkers,
507 such as nasal potential difference (NPD) and intestinal current measurements (ICM) in patients initiating
508 Orkambi® treatment [93], [94]. However, no correlation with clinical outcomes could be established.
509 Importantly, one study in patients treated by Orkambi® showed that the change in CFTR activity in 2D
510 nasal primary cultures upon lumacaftor/ivacaftor was correlated to that of intestinal biopsies sampled in
511 the same patient at 6 months of treatment. Moreover, in this pilot study, this variation was also
512 correlated to changes in ppFEV1 at 6 months of treatment. Conversely, it was not related to variations in
513 sweat Cl⁻ concentration or CFTR-related Cl⁻ transport in NPD [95]. Other outcome measures such as lung
514 imaging, lung clearance index as well as “Omics” biomarkers should be further investigated.

515 Last but not least, the CF community, including healthcare staff, researchers, as well as people with CF
516 and Patient Advocacy Associations, continue in their collaborative endeavours for equitable global
517 access to these expensive molecules for all people with CF. CF is at the cutting edge of basic and
518 translational research; these results should have important implications well beyond the science of CFTR
519 modulation as a paradigm for other rare disease pharmacological therapies.

520

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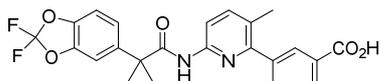
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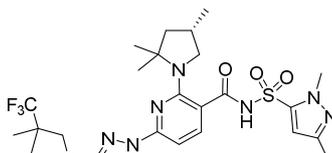
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801 **Figure 1.** Structure of correctors, potentiators and amplifier

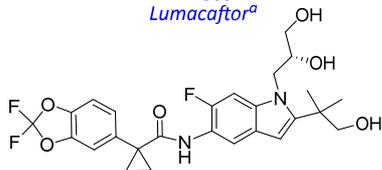
Correctors



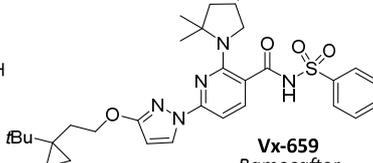
Vx-809
Lumacaftor^a



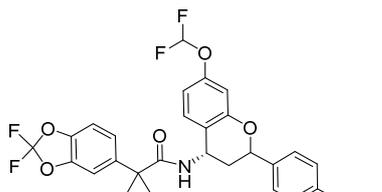
Vx-445
Elexacaftor^a



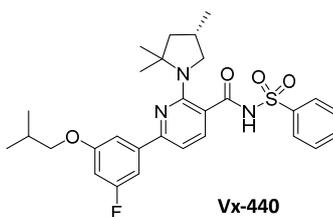
Vx-661
Tezacaftor^a



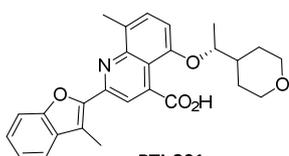
Vx-659
Bamacaftor



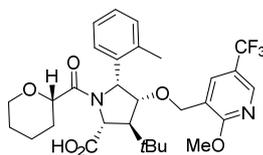
ABBV-2222
Galicaftor



Vx-440
Olacaftor



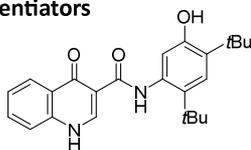
PTI-801



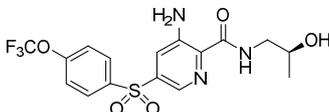
ABBV-3221

^a: approved drugs

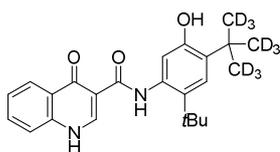
Potentiators



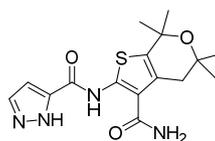
Vx-770
Ivacaftor^a



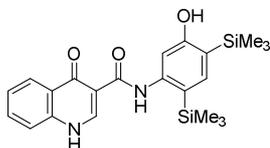
ABBV-2451



Vx-561
Deutivacaftor



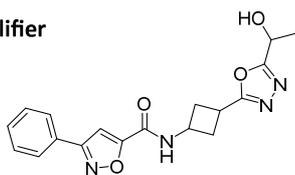
ABBV-974



PTI-808
Dirocaftor

^a: approved drug

Amplifier



PTI-428

Table 1. Interventional clinical studies on the potentiator VX-770 (IVA)

Ref.	Clinical Trial Period	Genotype	Age (yrs)	Control	N total (incl. controls)	N Intervention	VX-770 dose	Treatment duration (weeks)	Sweat Cl ⁻ change* (mmol/L)			ppFEV ₁ change* (%)		
									(i) from baseline	(ii) from control	p-value	(i) from baseline	(ii) from control	p-value
[36]	2007-2008	1 or 2x G551D	≥18	Placebo (cross-over)	(9)		25mg BID	2	-33.8 (-43.4 to -24.2)	NC	<0.0001	+4.7* (-2.7 to +12.2)	NC	NS
							75mg BID	2	-42.0 (-49.9 to -34.1)	NC	<0.0001	+9.5* (+4.1 to +14.8)	NC	NS
							150mg BID	2	-46.0 (-53.9 to -38.2)	NC	<0.0001	+10.8* (+5.6 to +15.9)	NC	NS
							250mg BID	2	-27.1 (-39.7 to -14.6)	NC	0.05	+12.0* (+3.8 to +20.1)	NC	NS
							150mg BID	4	-44.2 (-51.1 to -37.3)	NC	0.02	+10.6* (+6.5 to +14.8)	NC	NS
				(5)		250mg BID	4	-28.2 (-39.0 to -17.4)	NC	0.03	+9.4* (+3.4 to +15.4)	NC	NS	
[37]	2009-2013	2x F508del	≥12	Placebo (parallel)	140 (28)		150mg BID	16	-2.7 (0.6)	-2.9 (1.4)	0.04	+1.5 (0.5)	+1.7 (1.2)	NS
[38]	2009-2012	1 or 2x G551D	>12	Placebo (parallel)	161 (78)		150mg BID	48	-48.7 (1.2)	-48.1 (1.7)	<0.0001	+10.1 (0.7)	+10.5 (1.0)	<0.0001
[39]	2009-2011	1 or 2x G551D	6-11	Placebo (parallel)	52 (26)		150mg BID	48	-56.0 (2.5)	-54.3 (3.7)	<0.0001	+10.7 (1.9)	+10.0 (2.7)	0.0006
[43]	2012-2013	1 or 2x R117H	≥6	Placebo (parallel)	70 (34)		150mg BID	24	-26.3 (1.4)	-24.0 (-28.0 to -19.9)	<0.0001	+2.6 (1.2)	+2.1 (-1.1 to +5.4)	NS
[40]	2012-2013	1 or 2x non-G551D mutation [§]	≥6	Placebo (cross-over)	39 (39)		150mg BID	8	-55.8 (24.9)	-49.2 (-57.0 to -41.4)	<0.0001	8.13 (9.9)	+10.7 (+7.3 to +14.1)	<0.0001
[41]	2013-2014	1 or 2x gating mutation	2-5	Open label	34 (N/A)		<14kg: 50mg BID ≥14kg: 75mg BID	24	-46.9 (26.2)	N/A	N/A	N/A	N/A	N/A
[42]	2015-2017	F508del + RF	≥12	Placebo (cross-over)	248 (160)		150mg BID (8)	8	-4.9 (-6.7 to -3.0)	-4.5 (-6.7 to -2.3)	<0.0001	+4.4 (+3.5 to +5.3)	+4.7 (+3.7 to +5.8)	<0.0001
[96]	2016-2017	1 or 2x gating mutation	1-2	Open label	19 (N/A)		<14kg: 50mg BID ≥14kg: 75mg BID	24	-73.5 (17.5)	N/A	N/A	N/A	N/A	N/A

OD = Once daily

BID = Twice daily

N/A = Not applicable

NC = Not communicated

NS = Not significant

RF = Residual function mutation

yrs = Years

#Absolute changes given as the least squares mean difference between the treated group and (i) its baseline characteristics (within patient changes) or (ii) the control group (e.g. changes vs placebo).

When standard deviation was not communicated, 95% confidence intervals are given within brackets. *p*-values are given between treated and control groups.

*Relative change

§G178R. S549N. S549R. G551S. G970R. G1244E. S1251N. S1255P or G1349D

Table 2. Interventional clinical studies on VX-809 (LUM) combinations

Ref.	Clinical Trial Period	Genotype	Age (yrs)	Control	N total (incl. N controls)	Intervention	Treatment duration (weeks)	Sweat Cl ⁻ change ^a (mmol/L)			ppFEV ₁ change ^a (%)		
								(i) from baseline	(ii) from control	p-value	(i) from baseline	(ii) from control	p-value
[49]	2009	2x F508del	≥18	Placebo (parallel)	93 (19)	25mg LUM OD	4	+0.9 (-3.3 to +5.2)	+0.1 (-12.3 to 0.0)	NS	-1.6 (-4.7 to +1.4)	IC	NS
						50mg LUM OD	4	-3.8 (-8.0 to +0.4)	-4.6 (-12.3 to 0.0)	NS	-0.4 (-3.5 to +2.7)	IC	NS
						100mg LUM OD	4	-5.3 (-9.6 to -1.0)	-6.1 (-12.3 to 0.0)	<0.05	+0.2 (-3.0 to +3.3)	IC	NS
						200mg LUM OD	4	-7.4 (-11.6 to -3.2)	-8.2 (-14.3 to -2.1)	<0.01	-0.2(-3.1 to +2.8)	IC	NS
[50]	2010-2014	2x F508del	≥18	Placebo (parallel)	159 (42)	*200mg LUM OD followed by 200mg LUM OD +150mg IVA BID	2 +1	-6.7 (-11.1 to -2.4)	-5.0 (-11.6 to +1.5)	NS	+3.1 (+0.1 to +6.1)	2.8 (-1.3 to +7.0)	NS
						*200mg LUM OD followed by 2/ 200mg LUM OD +250mg IVA BID	2 +1	-12.6 (-17.2 to -7.9)	-10.9 (-17.6 to -4.2)	0.002	+0.5 (-2.8 to +3.8)	0.3 (-4.2 to +4.7)	NS
						*200mg LUM OD followed by 200mg LUM OD +250mg IVA BID	4 +4	-4.4 (-8.5 to -0.3)	-5.1 (-10.7 to +0.5)	NS	+1.8 (-1.3 to +4.9)	3.8 (-0.4 to +8.1)	NS
						*400mg LUM OD followed by 2/ 400mg LUM OD +250mg IVA BID	4 +4	-9.1 (-3.3 to -4.9)	-9.8 (-15.5 to -4.2)	<0.001	+0.6 (-2.5 to +3.8)	2.7 (-1.7 to +7.0)	NS
						*600mg LUM OD followed by 2/ 600mg LUM OD +250mg IVA BID	4 +4	-8.9 (-13.1 to -4.7)	-9.6 (-15.3 to -4.0)	0.001	+3.6 (+0.4 to +6.8)	5.6 (+1.2 to +10.0)	0.01
						* 400mg LUM OD followed by 2/ 400mg LUM BID +250mg IVA BID	4 +4	-10.3 (-16.7 to -4)	-11.1 (-18.5 to -3.7)	0.004	+2.2 (-2.3 to +6.7)	4.2 (-1.3 to +9.6)	NS
[42], [50]	2010-2014	1 or 2x F508del	≥18	Placebo (parallel)	153 (69)	* 600mg LUM OD followed by 2/ 600mg LUM OD +250mg IVA BID	4 +4	-5.2 (-9.8 to -0.7)	-6.0 (-11.9 to 0.0)	0.05	-1.7 (-5.1 to +1.8)	0.3 (-4.2 to +4.9)	NS
						400mg LUM BID +250 IVA BID	8	-11.8 (1.3)	-11.0 (-14.5 to -7.6)	<0.0001	-0.6 (0.8)	-0.6 (-1.7 to +2.9)	NS
[51]	2013-2014	2x F508del	>12	Placebo (parallel)	559 (187)	600mg LUM OD +250mg IVA BID	24	N/A	N/A	N/A	+3.6 (0.5)	4.0 (+2.6 to +5.4)	<0.0001
						400mg LUM BID +250 IVA BID	24	N/A	N/A	N/A	+2.2 (0.5)	2.6 (+1.2 to +4.0)	0.0003
[51]	2013-2014	2x F508del	≥12	Placebo (parallel)	563 (187)	600mg LUM OD +250mg IVA BID	24	N/A	N/A	N/A	+2.5 (0.5)	2.6 (+1.2 to +4.1)	0.0004
						400mg BID +250 IVA BID	24	N/A	N/A	N/A	+2.9 (0.5)	3.0 (+1.6 to +4.4)	<0.0001
[53]	2013-2015	2x F508del	5-11	Open label	58 (N/A)	200mg BID +250mg IVA BID	24	-24.8 (-29.1 to -20.5)	N/A	N/A	+2.5 (-0.2 to + 5.2)	N/A	N/A
[52]	2015-	2x	5-11	Placebo	206	200mg BID +250mg IVA BID	24	-20.0 [§] (-22.0 to -18.1)	-20.8 [§] (-23.4 to -18.2)	>0.0001	+1.1 (-0.4 to +2.6)	2.4 (+0.4 to +4.4)	0.02

2016	F508del	(parallel)	(101)								
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OD = Once daily

BID = Twice daily

IVA= Ivacaftor. VX-770

LUM = Lumacaftor. VX-809

N/A = Not applicable

NS = Not significant

yrs = Years

[#]Absolute changes given as the least squares mean difference between the treated group and (i) its baseline characteristics (within patient changes) or (ii) the control group (e.g. changes vs placebo).

When standard deviation was not communicated, 95% confidence intervals are given within brackets. *P*-values are given between treated and control groups.

^{*}Intervention involved two consecutive treatment steps, duration in weeks are given accordingly for each step.

[§]Change given at week 4. Change at week 24 = -21.6 (1.3).

Table 3. Interventional clinical studies on VX-661 (TEZ) combinations

Ref.	Clinical Trial Period	Genotype	Age (yrs)	Control	N total (incl. N controls)	Intervention (weeks)	Treatment duration (weeks)	Sweat Cl ⁻ change# (mmol/L)			ppFEV ₁ change# (%)		
								(i) from baseline	(ii) from control	p-value	(i) from baseline	(ii) from control	p-value
[60]	2012-2014	2x F508del	≥12	Placebo (parallel)	194 (38)	10mg TEZ OD	4	+3.9 (-0.5 to +8.3)	+4.8 (-0.3 to +9.8)	NS	+3.3 (-0.8 to +7.3)	+3.6 (-0.2 to +7.4)	NC
						30mg TEZ OD	4	-4.8 (-9.8 to +0.2)	-3.9 (-9.5 to +1.7)	NS	+0.2 (-3.7 to +4.1)	+1.8 (-2.0 to +5.5)	NC
						100mg TEZ OD	4	-20.4 (-24.8 to -16.1)	-19.6 (-24.6 to -14.6)	>0.0001	+1.6 (-2.3 to +5.5)	+1.7 (-2.0 to +5.5)	NC
						150mg TEZ OD	4	-10.5 (-14.5 to -6.4)	-9.6 (-14.4 to -4.8)	0.0001	+2.3 (-1.3 to +6.0)	+2.7 (+0.9 to +6.3)	NC
						10mg TEZ OD +150mg IVA BID	4	-5.1 (-8.0 to -2.1)	-4.2 (-8.1 to -0.3)	0.03	+2.0 (-0.7 to +4.6)	+1.4 (-1.4 to +4.3)	NC
						30mg TEZ OD +150mg IVA BID	4	-6.0 (-9.0 to +3.0)	-5.1 (-9.0 to -1.3)	0.01	+3.0 (+0.4 to +5.6)	+3.0 (+0.2 to +5.9)	NC
						100mg TEZ OD +150mg IVA BID	4	-6.0 (-9.1 to -3.0)	-5.2 (-9.2 to -1.2)	0.01	+4.4 (+1.7 to +7.2)	+3.9 (+0.9 to +6.8)	NC
						150mg TEZ OD +150mg IVA BID	4	-2.6 (-5.7 to +0.4)	-1.8 (-5.7 to +2.2)	NS	+4.1 (+1.4 to +6.9)	+3.8 (+0.8 to +6.7)	NC
						100mg TEZ OD +50mg IVA BID	4	-6.1 (-10.8 to -1.3)	-4.9 (-11.2 to +1.4)	NC	+0.8 (-2.0 to +3.6)	-0.5 (-4.1 to +3.0)	NC
		50mg TEZ BID +150mg IVA BID	4	-7.9 (-12.3 to -3.5)	-6.7 (-12.9 to -0.5)	0.04	+1.6 (-1.3 to +4.4)	+0.8 (-2.8 to +4.5)	NC				
		F508del + G551D	≥12	Placebo (parallel)	14 (4)	100mg TEZ OD +150mg IVA BID	4	-7.0 (-14.2 to +0.1)	-17.2 (-31.8 to -2.7)	0.02	+5.2 (+1.7 to +8.7)	+3.2 (-4.1 to +10.5)	NS
[62]	2015-2017	2x F508del	≥12	Placebo (parallel)	510 (259)	100mg TEZ OD +150mg IVA BID	24	-9.9 (-10.9 to -8.9)	-10.1 (-11.4 to -8.8)	NC	+3.4 (+2.7 to +4.0)	+4.0 (+3.1 to +4.8)	<0.0001
[61]	2015-2017	F508del + RF	≥12	Placebo & Kalydeco® (cross-over)	248 (placebo=61; Kalydeco®=156)	100mg TEZ OD +150mg IVA BID	8	-9.9 (-11.8 to -8.0)	a) -9.5 (-11.7 to -7.3) b) -5.1 (-7.0 to -3.1)	<0.0001 NC	+6.5 (+5.6 to +7.3)	a) +6.8 (+5.7 to +7.8) b) +2.1 (+1.2 to +2.9)	<0.0001 NC
[63]	2016-2018	1 or 2x F508del + RF or gating mutation [§]	5-11	Open label	70 (N/A)	<40kg: 50mg TEZ OD +75mg IVA BID >=40kg: 100mg TEZ OD +150mg IVA BID	24 24	-14.5 (-17.4 to -11.6)	N/A	N/A	+0.9 (-0.6 to +2.3)	N/A	N/A

OD = Once daily

BID = Twice daily

IVA= Ivacaftor. VX-770

TEZ = Tezacaftor. VX-661

N/A = Not applicable

NC = Not communicated

NS = Not significant

RF = Residual function mutation

yrs = Years

Kalydeco® treatment = 150mg IVA BID

[§]Gating mutations responsive to VX-770

[#]Absolute changes given as the least squares mean difference between the treated group and (i) its baseline characteristics (within patient changes) or (ii) the control group (e.g. changes vs placebo).

When standard deviation was not communicated, 95% confidence intervals are given within brackets. *P*-values are given between treated and control groups.

Table 4. Interventional clinical studies on Vertex triple combinations

Ref.	Clinical Trial Period	Genotype	Age (yrs)	Control	N total (incl. N controls)	Intervention (weeks)	Treatment duration (weeks)	Sweat Cl ⁻ change# (mmol/L)			ppFEV ₁ change# (%)		
								(i) from baseline	(ii) from control	p-value	(i) from baseline	(ii) from control	p-value
[66]	2017-2018	F508del + MF	≥18	Placebo (parallel)	63 (10)	80mg VX-659 OD +100mg TEZ OD +150mg IVA BID	4	-45.7 (4.3)	NC	NC	+10.2 (2.7)	NC	NC
						240mg VX-659 OD +100mg TEZ OD +150mg IVA BID	4	-43.8 (3.4)	NC	NC	+12.0 (2.0)	NC	NC
						400mg VX-659 OD +100mg TEZ OD +150mg IVA BID	4	-51.4 (3.2)	NC	NC	+13.3 (1.9)	NC	NC
						200mg VX-561 OD +100mg TEZ OD +400mg VX-659 OD	4	-38.1 (3.0)	NC	NC	+12.2 (1.9)	NC	NC
		2x F508del	≥18	Symdeko® (parallel)	29 (11)	*100mg TEZ OD +150mg IVA BID 400mg VX-659 OD +100mg TEZ OD +150mg IVA BID	4	-42.2 (2.2)	NC	NC	+9.7 (1.5)	NC	NC
[65]	2017-2018	F508del + MF	≥18	Placebo (parallel)	63 (10)	50mg ELE OD +100mg TEZ OD +150mg IVA BID	4	-38.2 (4.2)	NC	NC	+11.1 (2.1)	NC	NC
						100mg ELE OD +100mg TEZ OD +150mg IVA BID	4	-33.2 (2.8)	NC	NC	+7.9 (1.4)	NC	NC
						200mg ELE OD +100mg TEZ OD +150mg IVA BID	4	-39.1 (2.9)	NC	NC	+13.8 (1.4)	NC	NC
						150mg VX561 OD +200mg ELE OD +100mg TEZ OD	4	-33.6 (2.8)	NC	NC	+11.7 (1.6)	NC	NC
		2x F508del	≥18	Symdeko® (parallel)	29 (11)	*100mg TEZ OD +150mg IVA BID 200mg ELE OD +100mg TEZ OD +150mg IVA BID	4	-39.6 (2.8)	NC	NC	+11.0 (1.5)	NC	NC
[67]	2018-2019	F508del + MF	≥12	Placebo (parallel)	405 (204)	200mg ELE OD +100mg TEZ OD +150mg IVA BID	24	-42.2 (-44.0 to -40.4)	-41.8 (-44.4 to -39.3)	<0.001	+13.9 (+12.8 to +15.0)	+14.3 (+12.7 to +15.8)	<0.001
[68]	2018	2x F508del	≥12	Symdeko® (parallel)	107 (52)	1*/ 100mg TEZ OD +150mg IVA BID 2/ 200mg ELE OD +100mg TEZ OD +150mg IVA BID	4	-43.4 [§] (-46.9 to -40.0)	-45.1 (-50.1 to -40.1)	<0.0001	+10.4 [§] (+8.6 to +12.2)	+10.0 (+7.4 to +12.6)	<0.0001

OD = Once daily

BID = Twice daily

IVA = Ivacaftor, VX-770

TEZ = Tezacaftor, VX-661

ELE = Elexacaftor, VX-445

MF = Minimal function mutation

NC = Not communicated

Symdeko® treatment = 100mg TEZ OD +150mg IVA BID

yrs = Years

[§]Baseline taken after 4 weeks of Symdeco® treatment

[#]Absolute changes given as the least squares mean difference between the treated group and (i) its baseline characteristics (within patient changes) or (ii) the control group (e.g. changes vs placebo).

When standard deviation was not communicated, 95% confidence intervals are given within brackets. *P*-values are given between treated and control groups.

^{*}Intervention involved two consecutive treatment steps, duration in weeks are given accordingly for each step.

Table 5. Interventional clinical studies on compounds currently under investigation.

Investigated compound	Ref.	Clinical Trial Period	Genotype	Age (yrs)	Control	N total (incl. N controls)	Intervention (weeks)	Treatment duration (weeks)	Sweat Cl ⁻ change# (mmol/L)			ppFEV ₁ change# (%)		
									(i) from baseline	(ii) from control	p-value	(i) from baseline	(ii) from control	p-value
ABBV-974 (GLPG-1837) Potentiator	[75]	2016-2017	1 or 2x F551D	>18	Open label	26 (N/A)	*125mg	BID	-11.6 (-17.9 to -5.2)	N/A	N/A	+0.0 (-1.3 to +1.4)	N/A	N/A
							250mg	BID+1	-15.1 (-19.6 to -10.7)	N/A	N/A	+0.6 (-1.5 to +2.6)	N/A	N/A
							500mg	BID	+2	-28.8 (-39.1 to -18.4)	N/A	N/A	+2.8 (+0.2 to +5.3)	N/A
ABBV-2222 Corrector	[78]	2017	2x F508del	>18	Placebo (parallel)	59 (11)	50mg OD	4	-5.8 (3.1)	-3.3 (-11.6 to +5.0)	NS	-0.1 (1.5)	+1.1 (-3.1 to +5.4)	NS
							100mg OD	4	-6.6 (3.3)	-4.1 (-12.8 to +4.6)	NS	-0.3 (1.5)	+0.6 (-3.5 to +4.8)	NS
							200mg OD	4	-18.3 (2.5)	-15.8 (-23.2 to -8.3)	<0.0001	0.0 (1.3)	+1.0 (-2.9 to +4.9)	NS
							400mg OD	4	-8.8 (2.5)	-6.3 (-13.9 to +1.2)	NS	+1.3 (1.3)	+2.3 (-1.6 to +6.2)	NS
							F508del + gating mutation	2017	Kalydeco® (parallel)	37 (7)	150mg ABBV-2222 OD	4	-3.8 [§] (2.6)	-9.4 (-18.6 to -0.2)
300mg ABBV-2222 OD	4	-6.0 [§] (2.7)	-11.7 (-21.1 to -2.2)	0.02	+2.2 [§] (1.3)	+3.0 (-1.5 to +7.5)					NS			
ABBV-2737 Corrector	[80]	2017-2018	2x F508del	>18	Orkambi® (parallel)	22 (8)	75mg BID ABBV-2737	4	-22.1 [§] (4.5)	-19.6 (-36.0 to -3.2)	0.02	+1.7 [§] (1.1)	+3.4 (-0.5 to +7.3)	NS

OD = once daily

BID = twice daily

IVA = ivacaftor, VX-770

LUM = lumacaftor, VX-809

TEZ = tezacaftor, VX-661

ELE = elexacaftor, VX-445

MF = Minimal function mutation

N/A = Not applicable

NC = Not communicated

NS = Not significant

yrs = Years

Kalydeco® treatment = 150mg IVA BID

Orkambi® treatment = 400mg LUM BID +250mg IVA BID

[§]Baseline = Kalydeco® treatment

[§]Baseline = Orkambi® treatment

[#]Absolute changes given as the least squares mean difference between the treated group and (i) its baseline characteristics (within patient changes) or (ii) the control group (e.g. changes vs placebo).

When standard deviation was not communicated, 95% confidence intervals are given within brackets. *P*-values are given between treated and control groups.

^{*}Intervention involved three consecutive treatment steps, duration in weeks are given accordingly for each step.

Table 6. List and references of reported clinical studies

Ref.	Clinical trial number	Study name
Clinical studies on the potentiator VX-770 (IVA)		
[36]	NCT00457821	
[37]	NCT00953706	DISCOVER
[38]	NCT00909532	STRIVE
[39]	NCT00909727	ENVISION
[43]	NCT01614457	KONDUCT
[40]	NCT01614470	KONNECTION
[41]	NCT01705145	KIWI
[42]	NCT02392234	
[96]	NCT02725567	ARRIVAL
Clinical studies on VX-809 (LUM) combinations		
[49]	NCT00865904	
[50]	NCT01225211	
[42], [50]	NCT01225211	
[51]	NCT01807923	TRAFFIC
[51]	NCT01807949	TRANSPORT
[53]	NCT01897233	
[52]	NCT02514473	
Clinical studies on VX-661 (TEZ) combinations		
[60]	NCT01531673	
[62]	NCT02347657	
[61]	NCT02392234	
[63]	NCT02953314	
Clinical studies on Vertex triple combinations		
[66]	NCT03224351	
[65]	NCT03227471	
[67]	NCT03525444	
[68]	NCT03525548	
Clinical studies on compounds currently under investigation		
[75]	EudraCT 2015-003291-77	
[78]	NCT03119649	FLAMINGO
[78]	NCT03045523	ALBATROS
[80]	NCT03474042	PELICAN

Table 7. Interventional clinical trials in progress on approved compounds for broadening clinical indications

Clinical trial number	Study title	Status
Clinical studies on the potentiator VX-770 (IVA)		
NCT02725567	A Phase 3, 2 Part, Open-Label Study to Evaluate the Safety, Pharmacokinetics, and Pharmacodynamics of Ivacaftor in Subjects With Cystic Fibrosis Who Are Less Than 24 Months of Age and Have a CFTR Gating Mutation	Recruiting
Clinical studies on VX-770/VX-809 (IVA/LUM)		
NCT03601637	A Phase 3, 2-part, Open-label Study to Evaluate the Safety and Pharmacokinetics of Lumacaftor/Ivacaftor in Subjects 1 to Less Than 2 Years of Age With Cystic Fibrosis, Homozygous for F508del	Recruiting
Clinical studies on VX-770/VX-661 (IVA/TEZ)		
NCT03624101	Novel Therapeutic Approaches for Treatment of CF Patients With W1282X Premature Termination Codon Mutations	Recruiting
Clinical studies on VX-770/VX-661/VX-445 (IVA/TEZ/ELE)		
NCT04058353	A Phase 3, Randomized, Double-blind, Controlled Study Evaluating the Efficacy and Safety of VX-445 Combination Therapy in Subjects With Cystic Fibrosis Who Are Heterozygous for the F508del Mutation and a Gating or Residual Function Mutation (F/G and F/RF Genotypes)	Completed
NCT03691779	Evaluation of VX 445/TEZ/IVA in Cystic Fibrosis Subjects 6 Through 11 Years of Age	Completed
NCT04353817	A Phase 3b, Randomized, Placebo-controlled Study Evaluating the Efficacy and Safety of Elexacaftor/Tezacaftor/Ivacaftor in Cystic Fibrosis Subjects 6 Through 11 Years of Age Who Are Heterozygous for the F508del Mutation and a Minimal Function Mutation (F/MF)	Recruiting
NCT04509050 BEGIN study	A Prospective Study to Evaluate Biological and Clinical Effects of Significantly Corrected CFTR Function in Infants and Young Children	Recruiting
NCT04378153 SIMPLIFY study	A Master Protocol to Test the Impact of Discontinuing Chronic Therapies in People With Cystic Fibrosis on Highly Effective CFTR Modulator Therapy	Recruiting
Clinical studies on VX-770 and the combinations VX-770/VX-809 and VX-770/VX-661		
NCT03587961	Personalized Theratyping Trial for off-label mutations	Recruiting

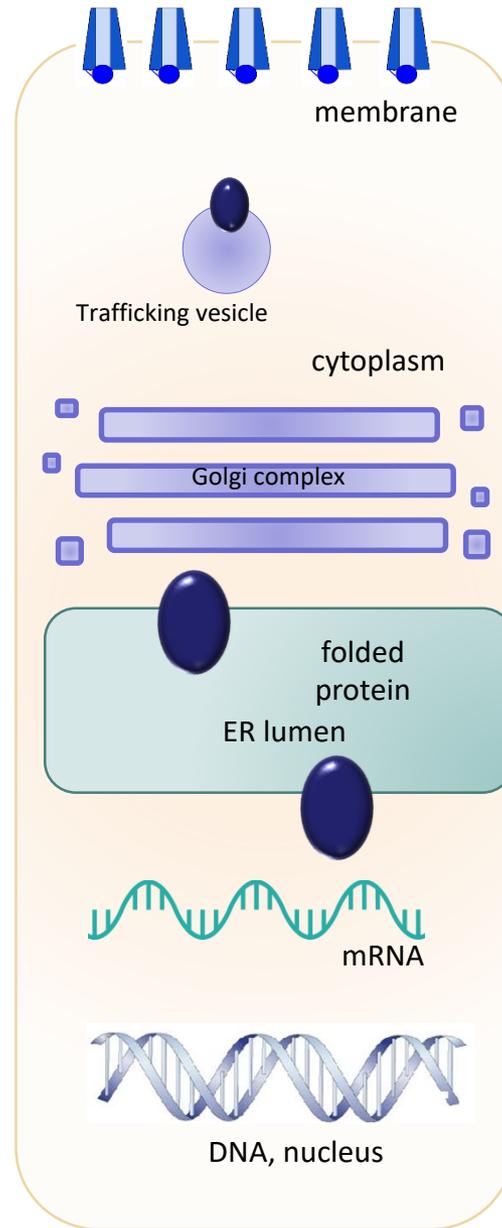
CFTR defect (mutation class)

Decreased stability or production
(Class V,VI)

Impaired conductance or gating
(Class III, IV)

Misfolding
Trafficking defect
Protein degradation
(Class II)

Translation
(Class I)



CFTR modulators

Stabilizer
at the plasma membrane

Potentiator
of CFTR activity

Corrector
of misfolding and/or
degradation

Amplifier
of translation