Editorial

The holy grail of cystic fibrosis research: pharmacological repair of the F508del-CFTR mutation

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Therapeutic strategies aimed at correcting the defect of the cystic fibrosis transmembrane conductance regulator (CFTR) ("CFTR-repair") constitute a new avenue towards the treatment of patients affected by Cystic Fibrosis (CF), the most common lethal monogenic disease in Caucasians. CF is caused by mutations in the *CFTR* gene, which codes for a 1480 amino-acid protein normally functioning as a chloride channel at apical membrane from epithelial cells (1). In the *CFTR* gene, more than 1900 mutations, most of which are disease-relevant, have been identified and then categorized in six different classes according to their functional impact (2). The phenotypic consequences of such genotypes comprise insufficiency of the exocrine pancreas, increased electrolytes in sweat, male infertility and—most prevalent—a debilitating and eventually lethal inflammation/infection-triggered respiratory dysfunction. Mutation-specific approaches are focusing on the identification of small molecules capable of correcting the deficient subcellular trafficking of the CFTR protein ("correctors": agents that ensure the expression of the mutated protein at the apical plasma membrane) or defective gating ("potentiators": agents that reinstate the channel function of mutated CFTR proteins that are orthotopically expressed).

The design of therapeutic strategies of CFTR repair might appear an easy task because restoring even less than 30% of CFTR function *in vivo* has been estimated to confer an at least partial clinical benefit to CF patients; in addition, this degree of CFTR repair is sufficient to prevent most if not all phenotypic manifestations in CF animal models (3-5). An orally available compound identified by high-throughput screening, the CFTR potentiator VX-770 (Ivacaftor, trade name Kalydeco, Vertex Pharmaceuticals), has been shown to efficiently rescue CFTR function (and hence to decrease chloride levels in sweat, a surrogate marker of CFTR channel function) and to improve lung function in CF patients harboring the plasma membrane (PM)-resident *G551D CFTR* mutant. This particular *CFTR* mutation, however, only affects 4-5% of CF patients worldwide (6), meaning that VX770 may only be used to cure a rather small fraction of CF patients. One single mutation, *F508del-CFTR*, accounts for about 70% of *CFTR* loss-of-function mutations and is present in approximately 85% of CF patients worldwide (1). The mutant protein transcribed from the *F508del-CFTR* gene is misfolded and is prematurely degraded before it reaches the PM. Despite of a mild gating defect that is linked to a subtly altered protein conformation, F508del-CFTR is rather functional if its degradation can be avoided and the protein is stabilized at the PM (7). However, although F508del-CFTR can be rescued at the PM by CFTR correctors *in vitro* (2), this mutant CFTR protein is unstable at the cell surface and rapidly redirected from endosomal recycling towards lysosomal delivery and degradation (8). In keeping with these *in vitro* results, the F508del corrector VX-809, which is endowed with a good rescuing efficacy *in vitro* and in primary cultures of lung cells from CF patients (9),

showed only modest efficacy in a Phase II clinical trial in CF patients homozygous for the *F508del-CFTR* mutant (10). In addition, only marginal effects on lung function have been observed in a Phase 3 randomized clinical trials aimed at testing the efficacy of a combination of the corrector VX-809 and the potentiator VX-770 (Traffic and Transport Phase 3 studies; http://investors.vrtx.com/releasedetail.cfm?ReleaseID=856185). Moreover, the effects of the combined VX-809/VX-770 treatment on sweat chloride levels were not disclosed, suggesting that the results were not significant. Because of the modest efficacy of such a combination regimen on F508del-CFTR homozygous patients, a new combination of another CFTR corrector, VX-661, and the potentiator VX-770 has been evaluated in a Phase 2 study, revealing a statistically significant improvement in lung function (http://investors.vrtx.com/releasedetail.cfm?ReleaseID =757597). However, preclinical evidence supporting the putative effectiveness of chronic administration of the potentiator VX-770 together with a CFTR corrector (either VX-809 or VX-661) is lacking.

The study of Cholon *et al*. published in *Science Translational Medicine* (11) aimed at providing the proof-of-concept for the therapeutic use of potentiators in CF patients carrying the *F508del-CFTR* mutation. However, the results of this study clearly indicate that the potentiator VX-770 may do more harm than good. Indeed, chronic (48 h) exposure of primary bronchial epithelial cells to VX-770 *in vitro* subverted the rescuing efficacy of the corrector VX-809 on F508del-CFTR in a concentration-dependent fashion, without affecting intracellular concentrations of VX-809. Chronic VX-770 administration also frustrated other tentatives of rescuing expression of the F508del-CFTR protein at the PM, namely, treatment with yet another corrector, VX-661, as well as prolonged exposure of the cells to low-temperature, as reported by Veit *et al*. in the same issue of *Science Translational Medicine* (12). Altogether, these results indicate that VX-770 is detrimental for the restoration of F508del-CFTR function, irrespective of the strategy through which rescuing the mutant protein at the cell surface is attempted.

Based on these premises, the question arises as to whether potentiators are warranted for the treatment of patients harboring F508del-CFTR. This particular *CFTR* mutant retains a sufficient channel function if rescued at the cell surface, but tends to be unstable at the PM as the peripheral quality control system rapidly accelerates its disposal (8). Thus, in principle, potentiators could be useful only if they increased PM stability of F508del-CFTR after rescue. Cholon *et al*. demonstrated that, although the short-term (acute) administration of VX-770 increased *in vitro* F508del-CFTR response to forskolin after rescue, as expected, chronic (48 h) exposure to VX-770 decreased F508del-CFTR stability at the PM, resulting in the accelerated removal of rescued *CFTR* mutant from the PM coupled to its degradation by proteases. Accordingly, chronic exposure to VX-770 paradoxically reduced the ability of pulses with the potentiator to enhance forskolin response, for the simple reason that the mutant protein was no longer resident at the PM.

The destabilizing effect of VX-770 on PM-resident CFTR was not limited to the intrinsically labile *F508del* mutant, but also extended to the stable and PM-resident *G551D* mutant. In spite of its largely reduced channel function, this *G551D* mutant CFTR protein is rather stable when it is present in the PM, presumably because the G residue in position 551 affects the conformation of the protein in a way that it becomes abnormally stable or "hyperstable" (11). However, chronic administration of VX-770 reduces PM stability of G551D CFTR *in vitro*. This finding contrasts with clinical observations clearly indicating that chronic administration of VX-770 *in vivo* is beneficial for patients carrying the *G551D CFTR* mutation, while is not effective in patients homozygous for the *F508del CFTR* mutation. These findings raise the intriguing question as to whether the beneficial effects of chronic administration of VX-770 on G551D are due to its action as a potentiator or, instead, as a CFTR destabilizer.

Cholon *et al*. (11) discussed the intriguing relationship between a "normal/ideal" stability and flexibility of CFTR at the cell surface and proper channel function. VX-770 was found to reduce the stability of wild-type CFTR which obviously represents the paradigm of a protein with "ideal" (physiological) stability. Chronic exposure (48 h) to VX-770 reduced the abundance of CFTR mature protein (Band C) at the PM and, consequently, decreased channel function in a concentration-dependent manner. In addition, Veit *et al*. (12) reported that a shorter (24 h) pretreatment with VX-770 did not affect protein kinase A-activated ion current nor the PM density of wild-type CFTR or G551D CFTR in human bronchial epithelial cells, although it decreased PM stability of F508del-CFTR, indicating a time-dependent destabilizing effect of VX-770 on different CFTR mutants.

The CFTR channel regulates a wide spectrum of physiological function. Obviously, wild-type CFTR does not require potentiators to properly act *in vivo*, correlating

with the fact that channel opening is finely tuned and can respond to a variety of environmental challenges, be they chemical or physical. Recently, CFTR has been identified as a mechanosensitive anion channel in different tissues, meaning that it can be activated by increases in membrane tension or stretch through a phosphorylation and ATP-independent mechanism (13,14). Thus, conformational restructuring of the protein caused by G551D mutation, which can perturb domain interactions, might impair the physiological adaptation of CFTR to permanently remodeling membrane lipids thereby interfering with balanced channel opening. The inherent low flexibility of G551D mutant (which is hyperstable) (15,16) might reduce its adaptation to either chemical (cAMP) or physical (tension) challenges that regulate channel opening. Thus, proper CFTR channel function might result from a delicate balance between stability and flexibility, and a physiological degree of CFTR flexibility might be required to ensure appropriate channel function.

The destabilizing action of VX-770 at the PM of epithelial cells could be beneficial for the hyperstable *G551D* mutant but detrimental for the normally stable wild-type CFTR and the inherently hypostable *F508del* mutant. In this perspective, normalizing the PM stability of different CFTR mutations (for instance by rendering hyperstable G551D more labile or by improving the stability of labile F508del) may reestablish a normal, close-to-physiological state.

The paper of Cholon *et al*. is of great relevance for the design of clinical trials and for drug discovery programs in CF repair. First, Cholon *et al*. explain the modest efficacy of the combination of VX-809 (Lumacaftor) and VX-770 (Ivacaftor) that was observed in advanced clinical trials enrolling CF patients homozygous for *F508del-CFTR*. Second, Cholon *et al*. unravel the mechanisms through which VX-770 may also mediate undesirable effects. Chronic treatment with VX-770 reduces the UTP-stimulated activity of Ca^{2+}-activated Cl^- channels (CACC), which provide an adaptive mechanism to the lack of CFTR channel function. For these reasons, VX-770 may provide an additional disadvantage for F508del homozygous CF patients.

Moreover, this study reinforces the notion that CFTR repairing therapies must first aim at improving CFTR function and, consequently, at ameliorating other disease manifestations, such as infection/inflammation and lung function. Chronic administration of VX-770 inhibits the epithelial sodium channel (ENaC)-mediated absorption of Na^+ by human bronchial epithelial cells that may improve electrolyte homeostasis, hence resulting in a putative beneficial effect on lung inflammation and respiratory function in a CFTR-independent fashion. In this perspective, the improvement of primary outcomes in recent clinical trials (www.vrtx.com) in the absence of reported rescue of CFTR function, might be due to putative off-target effects of treatment.

CF research is currently characterized by the febrile search of new generation correctors that should be able to efficiently stabilize F508del-CFTR at the PM after rescue. Moreover investigators are attempting to identify a novel class of potentiators that should be capable of increasing CFTR channel function without decreasing the stability of the CFTR protein. Meanwhile, more general therapeutic strategies advocating the improvement of proteostasis have emerged (17). These strategies have been designed to improve the cellular environment perturbed by the lack of a functional CFTR instead of directly targeting the mutant CFTR protein. As a matter of fact, recent evidence indicates that CFTR is a key player of proteostasis of epithelial cells and that the loss of CFTR function in the epithelial environment disables autophagy and derails proteostasis (18). Restoring disabled autophagy in CF by genetic manipulation or by means of small molecules (such as cysteamine, that acts as a modulator of proteostasis) is effective in rescuing and stabilizing F508del-CFTR at the PM beyond washout in several preclinical disease models, including F508del-CFTR homozygous primary cells and CF mice (19,20). Notably, combined treatment with cysteamine and the over-the-counter green tea flavonoid, epigallocatechin gallate, simultaneously targets two molecules that reduce F508del-CFTR stability at the PM: (I) the autophagic substrate p62/SQSTM1, an ubiquitin-binding protein that can sequester CFTR from the PM; (II) the master protein kinase CK2 that favors the proteolytic destruction of CFTR (20). This combination treatment has been recently translated to the clinics in a pilot trial involving 10 F508del-CFTR homozygous patients. In this phase II trial, cysteamine plus epigallocatechin gallate restored autophagy and improved CFTR function from nasal epithelial cells *in vivo*, correlating with a decrease of chloride concentrations in sweat, as well as with a reduction of the abundance of inflammatory cytokines in patients' sputum (20). These findings indicate that it is feasible to correct the F508del-CFTR defect without the need of potentiators, simply by rescuing the mutant CFTR protein and then normalizing its PM stability.

Regardless of the effects of VX-770, the study of Cholon

et al. provides a number of issues that we must reflect on. The results by Cholon *et al*. clearly prove how important a rigorous stepwise translational approach is to avoid initiating premature (and costly) clinical trials. Only a combination of optimal preclinical models, critical experimentation and alert biomarker discovery programs, may pave the way to successful pharmacological developments. In spite of the dominant role of the F508del-CFTR mutation, CF is a highly heterogeneous disease requiring a highly personalized approach to maximize therapeutic benefit.

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References

1. Ratjen F, Döring G. Cystic fibrosis. Lancet 2003;361:681-9.
2. Amaral MD, Farinha CM. Rescuing mutant CFTR: a multi-task approach to a better outcome in treating cystic fibrosis. Curr Pharm Des 2013;19:3497-508.
3. Ramalho AS, Beck S, Meyer M, et al. Five percent of normal cystic fibrosis transmembrane conductance regulator mRNA ameliorates the severity of pulmonary disease in cystic fibrosis. Am J Respir Cell Mol Biol 2002;27:619-27.
4. Kerem E. Pharmacologic therapy for stop mutations: how much CFTR activity is enough? Curr Opin Pulm Med 2004;10:547-52.
5. Stoltz DA, Rokhlina T, Ernst SE, et al. Intestinal CFTR expression alleviates meconium ileus in cystic fibrosis pigs. J Clin Invest 2013;123:2685-93.
6. Harrison MJ, Murphy DM, Plant BJ. Ivacaftor in a G551D homozygote with cystic fibrosis. N Engl J Med 2013;369:1280-2.
7. Lukacs GL, Verkman AS. CFTR: folding, misfolding and correcting the ΔF508 conformational defect. Trends Mol Med 2012;18:81-91.
8. Okiyoneda T, Barrière H, Bagdány M, et al. Peripheral protein quality control removes unfolded CFTR from the plasma membrane. Science 2010;329:805-10.
9. Van Goor F, Hadida S, Grootenhuis PD, et al. Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. Proc Natl Acad Sci U S A 2011;108:18843-8.
10. Clancy JP, Rowe SM, Accurso FJ, et al. Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation. Thorax 2012;67:12-8.
11. Cholon DM, Quinney NL, Fulcher ML, et al. Potentiator ivacaftor abrogates pharmacological correction of ΔF508 CFTR in cystic fibrosis. Sci Transl Med 2014;6:246ra96.
12. Veit G, Avramescu RG, Perdomo D, et al. Some gating potentiators, including VX-770, diminish ΔF508-CFTR functional expression. Sci Transl Med 2014;6:246ra97.
13. Zhang WK, Wang D, Duan Y, et al. Mechanosensitive gating of CFTR. Nat Cell Biol 2010;12:507-12.
14. Gray MA. CFTR is a mechanosensitive anion channel: a real stretch? Cellscience 2010;7:1-7.
15. He L, Aleksandrov AA, Serohijos AW, et al. Multiple membrane-cytoplasmic domain contacts in the cystic fibrosis transmembrane conductance regulator (CFTR) mediate regulation of channel gating. J Biol Chem 2008;283:26383-90.
16. Aleksandrov AA, Kota P, Cui L, et al. Allosteric modulation balances thermodynamic stability and restores function of ΔF508 CFTR. J Mol Biol 2012;419:41-60.
17. Hutt DM, Herman D, Rodrigues AP, et al. Reduced histone deacetylase 7 activity restores function to misfolded CFTR in cystic fibrosis. Nat Chem Biol 2010;6:25-33.
18. Luciani A, Villella VR, Esposito S, et al. Defective CFTR induces aggresome formation and lung inflammation in cystic fibrosis through ROS-mediated autophagy inhibition. Nat Cell Biol 2010;12:863-75.
19. Luciani A, Villella VR, Esposito S, et al. Targeting autophagy as a novel strategy for facilitating the therapeutic action of potentiators on ΔF508 cystic fibrosis transmembrane conductance regulator. Autophagy 2012;8:1657-72.
20. De Stefano D, Villella VR, Esposito S, et al. Restoration of CFTR function in patients with cystic fibrosis carrying the F508del-CFTR mutation. Autophagy 2014;10:2053-74.

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