Editorial

Interaction non grata between CFTR's correctors and potentiators

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Cystic fibrosis (CF), the most common life-shortening hereditary diseases in people of Caucasian ethnicity, is caused by mutations that render the CF transmembrane conductance regulator (CFTR) chloride channel dysfunctional (1,2). So far, more than 1,900 mutations have been discovered to cause CF (http://www.cftr2.org) (3,4), but approximately 90% of the CF patients are inherited with at least one copy of the ΔF508 mutation, the deletion of a single amino acid residue phenylalanine at position 508. Multiple defects of the CFTR channels have been associated with the ΔF508 mutation. It is now well established that the majority of misfolded ΔF508-CFTR proteins are degraded by the endoplasmic reticulum (ER) quality control machinery (5,6). This defect in protein maturation, which results in an insufficient amount of functional channels reaching the apical membrane of epithelial cells (7), is further exacerbated by the fact that once the ΔF508-CFTR channels do make it to the plasma membrane, their half-life is greatly shortened (7,8). Adding on top of these already intractable pathologies is a disrupted gating, i.e., a much reduced probability of the mutant channel being opened (9-11), for ΔF508 channels that stay long enough in the cell membrane to be recorded by electrophysiological means. The requirement of having drugs to correct all the underlying defects including misfolding, mislocalization, protein instability and malfunction of channel gating in ΔF508-CFTR leaves this most common disease-associated mutant thornier to be contended in our search for targeted CF therapy.

In the past decades, tremendous efforts and investments have been apportioned to developing pharmacological reagents for the treatment of CF. Mechanistically, the available investigational small-molecule CFTR modulators fall into two major classes: correctors (e.g., VX-809 and VX-661) and potentiators (e.g., flavones, xanthines, benzimidazoles and VX-770) (12-14). The goal of correctors is to rectify misprocessing of CFTR mutants with trafficking/stability defects; whereas potentiators are intended to restore the activity of channels residing in the cell membrane. Indeed, highly potent compounds in both classes were developed in the past few years! Amid the correctors, VX-809 (Lumacaftor) has been shown to significantly improve protein maturation of ΔF508-CFTR with an EC_{50} of ~80 nM (15). On the other hand, VX-770 (Ivacaftor), as a potentiator, has been reported to dramatically increase the activity of G551D-CFTR, the third most common pathogenic mutation (16). Lately, the FDA approved the use of Ivacaftor for the treatment of patients carrying the G551D mutation following successes in clinical trials (17,18). Recent mechanistic studies provide exquisite insights into how VX-770 works at a fundamental level (19-21): by shifting the gating equilibrium of CFTR's transmembrane domains (TMDs) to favor the open channel conformation, VX-770 not only increases the open probability of channels with disabled gating machinery (i.e., CFTR's two nucleotide binding domains, NBDs), but also enhances gating efficiency of channels with fully or partially functional NBDs due to an energetic coupling between TMDs and NBDs [see (20) for details]. Thus, VX-770 is deemed a universal CFTR potentiator (21), a proposition supported by experiments showing effects of VX-770 on numerous disease-associated mutations including ΔF508 (22).

In theory then, a significant symptomatic improvement is attainable for CF patients carrying the ΔF508 mutation once treated with a combination of corrector (i.e., VX-809 or VX-661) that increases the surface expression of CFTR and potentiator (i.e., VX-770) that enhances the opening

probability of the ΔF508 channel. Unexpectedly, despite promising *in vitro* studies with individual reagents (15,16), phase 2 and phase 3 clinical trials with a combination of VX-809 and VX-770 to patients carrying the ΔF508 mutation—although showed a significant improvement of clinical parameters—did not yield a comparable results that match those of the FDA-approved single agent therapy (VX-770) for patients carrying the G551D mutation (http://www.clinicaltrials.gov, NCT01225211 and NCT01531673). This latest clinical report thus raises the possibility of an undesirable drug interaction between VX-770 and VX-809. Indeed, two recent papers (23,24) present *in vitro* experimental data supporting the notion that a prolonged exposure of ΔF508-CFTR channels to VX-770 negatively impacts the effect of VX-809 on CFTR trafficking and stability, and hence may well explain the aforementioned conundrum in clinical trials.

Specifically, Veit *et al.* (24) showed that chronic exposure of cells expressing the ΔF508 mutant to VX-770 diminished the effects of VX-809 or VX-661 on ΔF508 protein maturation in both immortalized cell lines and primary human CF bronchial epithelial cells. This negative impact of VX-770 on ΔF508 maturation occurs at multiple levels: VX-770 not only dampens folding efficiency of ΔF508 proteins at the ER, but also stifles metabolic stability of ΔF508-CFTR in the post-ER compartments, resulting in an ultimate reduction of the number of mature CFTR channels in the plasma membrane. As this detrimental effect of VX-770 on CFTR trafficking is also seen in other trafficking defective mutants (such as R347H-, R107G- and P67L-CFTR), this report apparently serves as a cautionary note for future applications of this type of combination regimen in CF treatment. On the other hand, since this adverse effect of VX-770 can be overcome by so-called second-site mutations that supposedly stabilize the interface between CFTR's two NBDs, there remains optimism for an eventual correction of the obstinate missteps in ΔF508 protein trafficking.

While the basic observations with the ΔF508-CFTR in Veit *et al.* were reproduced in Cholon *et al.* (23), these two reports differ in one major respect—whether the trafficking of wild-type (WT) CFTR is impaired by VX-770. Cholon *et al.* reported that VX-770 reduced WT CFTR maturation, but neither WT nor G551D CFTR was affected by VX-770 in Veit *et al.* This issue needs to be solved before one can contemplate using VX-770 to treat secondary CFTR deficiencies due to environmental factors or other pathological conditions (25). A second, more minor difference is the concentration range of VX-770 applied. While Cholon *et al.* demonstrated a significant difference in corrector-treated cells with 1 versus 5 μM VX-770, no further reduction was seen in long-term treatment with more than 100 nM VX-770 in the presence of VX-809 in Veit *et al.* Although this technical aspect seems trivial, it may partly account for the discrepancy found in these two reports. We shall also beg to ask what the truly relevant concentration of VX-770 should be to faithfully translate *in vitro* results to *in vivo* conditions.

No doubt, the past few years have witnessed amazing advancements of personalized medicine in CF therapy. These two papers, however, timely remind us of the mounting challenges ahead on our path to overcome this debilitating disease. When we rightfully acknowledge the extensive work by Veit *et al.* and Cholon *et al.* that unravels the mechanism underpinning the interactions between VX-770 and VX-809, their results also aptly usher us back to bench research from some exciting successes in CF clinics. A swathe of looming questions begs for answers. For example, although in silico studies have suggested a binding site for the effect of VX-770 on ΔF508 trafficking (24), this binding site is apparently different from the one proposed for its potentiation effects on CFTR gating (20). It will be important to know if the same binding site(s) accounts for the negative effect on ΔF508 trafficking by many structurally diverse CFTR potentiators tested in Veit *et al.* Interestingly, one of the potentiators examined in Veit *et al.* did not reverse VX-809-induced improvement of ΔF508 trafficking. Is this due to intrinsic structural difference amid these molecules and thus different sites for their actions? Or the apparent difference is simply because different efficacy in promoting ΔF508 activity? Without careful, more quantitative studies of those compounds at a single-channel level, questions like this will be difficult to answer. Nonetheless, it is perhaps a consensus that the field will benefit from further development of new CFTR potentiators and correctors as well as rigorous studies of the mechanisms of these reagents. Equipped with a plethora of advanced technologies that can assess all different aspects of CFTR biochemistry and physiology, we shall be optimistic that useful compounds that complement rather than antagonize each other will be found in the offing.

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