In vitro assessment of triple combination therapy for the most common disease-associated mutation in cystic fibrosis

Han-I Yeh[1] and Tzyh-Chang Hwang[1,2,3]

[1]Dept of Pharmacology, National Yang Ming Chiao Tung University, Taipei, Taiwan. [2]Dept of Medical Pharmacology and Physiology, University of Missouri-Columbia, Columbia, MO, USA. [3]Dalton Cardiovascular Research Center, University of Missouri-Columbia, Columbia, MO, USA.

Corresponding author: Tzyh-Chang Hwang (HwangT@health.missouri.edu)

Shareable abstract (@ERSpublications)
F508del-CFTR, the most common disease-associated mutation in cystic fibrosis, may lose its responsiveness to the CFTR potentiator ivacaftor upon prolonged exposure to the newly developed CF drug Trikafta https://bit.ly/3A22MKQ

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Cystic fibrosis (CF), one of the most common lethal genetic diseases in those of European descent, is caused by loss-of-function mutations in the *CFTR* (cystic fibrosis transmembrane conductance regulator) gene, which encodes an epithelial ion channel, CFTR, that mediates chloride and bicarbonate transport across the cell membrane [1]. Patients with CF suffer from multiorgan dysfunctions, but mortality and morbidity are mainly caused by progressive respiratory impairment. 30 years after the breakthrough discovery of the *CFTR* gene, we are now witnessing the success of precision medicine in CF clinics prescribing small-molecule compounds targeting the CFTR protein [2]. These CFTR modulators have brought transformative effects on the health and well-being of CF patients and their families.

The first clinically available CFTR modulator was a potentiator called ivacaftor (VX-770 or Kalydeco), which improves chloride transport by facilitating channel opening [3–5]. While ivacaftor is highly effective in patients with gating-defective mutations (*e.g.* G551D), the majority of CF population carry the F508del mutation that causes multiple abnormalities, such as defective protein processing, gating and membrane stability, and thus require more complex medications. Small molecules that promote the surface expression of CFTR are known as CFTR correctors, and two of them, lumacaftor (VX-809) and tezacaftor (VX-661), soon reached the market in the form of combination therapy with ivacaftor, as Orkambi (lumacaftor and ivacaftor) and Symdeko (tezacaftor and ivacaftor), respectively [6–8].

The 2019 approval of a triple combination therapy (Trikfta in the USA, Kaftrio in Europe: a combination of two CFTR correctors, elexacaftor (VX-445) and tezacaftor (VX-661), plus the CFTR potentiator ivacaftor) was an important milestone in the history of our struggles against this debilitating illness. Trikafta brings significant improvement in lung function (measured in percentage of predicted forced expiratory volume in 1 s), with an impressive gain of 13.8% from the baseline compared to placebo in compound heterozygous (*i.e.* F508del-minimal function) patients, and 10% from the baseline compared to Symdeko in homozygous F508del patients [9, 10]. Treatment with Trikafta also results in a reduced number of pulmonary exacerbations and improved patient-reported quality of life. The approval of Trikafta for patients 12 years and older carrying at least one F508del allele now benefits up to 90% of the CF population. In June 2021, the US Food and Drug Administration approved the use of Trikfata for children from age 6 to 11 years who have at least one copy of the F508del mutation, with the goal of slowing down or preventing the irreversible progression of CF lung damage at an early age.

The speedy approval of Trikafta is based on its real clinical benefits, but how this combination therapy affects the function of F508del CFTR in airway epithelial cells has not been examined in detail. Previous

studies have shown that VX-770 reduces the correction efficacy of VX-809 and VX-661 [11, 12]. With the newly developed corrector VX-445, whose mechanism of action likely differs from the type I correctors (*e.g.* VX-809 and VX-661 [13]), possible interactions between VX-770 and the two correctors in Trikafta need to be evaluated. The study by Becq *et al*. [14] thus aimed to investigate the maturation and function of F508del-CFTR in airway epithelial cells pretreated with different combinations of the correctors, including VX-809, VX-661 and VX-445, and the potentiator VX-770.

The authors provided evidence that the correction of F508del-CFTR by Trikafta is dampened due to the presence of VX-770. Both Western blot and immunofluorescence analysis show that the expression of F508del-CFTR treated with VX-445 and VX-661 (referred to as 2VX) is diminished by the addition of VX-770 (*i.e.* 3VX) in human CF bronchial epithelial cell line CFBE41o-. A similar observation was made with homozygous human bronchial epithelial (HBE) cells by Keating *et al*. [9]. The negative impact of prolonged exposure to VX-770 on folding efficiency and plasma membrane stability of F508del-CFTR has been previously established [11, 12], and it is perhaps not surprising that Trikafta faces the same problem. What is puzzling, however, is why VX-770 abrogates the effect of correctors on F508del-CFTR, but not on wild-type- or G551D-CFTR. Veit *et al*. [12] proposed that the unstable NBD1-NBD2 interface in F508del-CFTR [15] is responsible for VX-770-mediated destabilisation, but it seems hard to explain how VX-770 achieves the destabilising effect with its binding site located in the transmembrane domains [16, 17]. Moreover, the replacement of glycine with aspartate at position 551 (G551D) indeed destabilises the NBD interface [18], without rendering the channels to the adverse effect of VX-770 [12]. A recent study by Laselva *et al*. [19] did point to an alternative binding site for VX-770 at the region around the fourth intracellular loop (ICL4) that interacts with F508 and other nearby residues, mutations of which cause folding defects. Even if this new binding site for VX-770 could account for the effect of VX-770 on the folding efficiency of F508del-CFTR, it would still be puzzling why VX-770 diminished the effect of correctors on other folding-defective mutations at positions farther away from this very region (*e.g.*,E92K, S341P and D614G [20]). A more complete understanding of the mechanism for VX-770-induced destabilisation may await future solution of the atomic structures of F508del-CFTR and/or other folding-defective mutant CFTR.

Measuring the short-circuit currents of human airway epithelial cells, Becq *et al*. [14] also demonstrated that the 3VX-treated cells are unresponsive to acute addition of VX-770. Similar results were obtained by Veit *et al*. [13], who also showed that some other Triakfta-treated rare mutations with folding defects, including G85E, M1101K and N1303K, *etc*., do not respond to acute application of VX-770, either. In contrast, the 2VX-incubated F508del cells can be potentiated by acute addition of VX-770, and the maximum current is greater than that of 3VX-treated cells. Although it is unclear why VX-770 fails to potentiate 3VX-treated F508del-CFTR activities, the authors at least ruled out the possibility that these channels had already assumed a maximally possible function with no room for further improvement: they showed that the activity of the 3VX-treated F508del-CFTR can be enhanced by genistein or Cact-A1, two other CFTR potentiators. Indeed, pharmacological synergy for different CFTR potentiators has been shown repeatedly [21–25]. Therefore, further optimisation of CF treatment can be attained by developing novel potentiators that can replace or work in synergy with VX-770, without negative impact on channel folding/stability.

These *in vitro* data, when extrapolated to *in vivo* conditions, may bear immediate clinical significance as patients taking Trikafta are in a state similar to prolonged exposure to VX-770, VX-661 and VX-445 (*i.e.* 3VX-corrected). The authors thus argued that, despite the well-documented efficacy of VX-770 as a potentiator, chronically Trikafta-rescued F508del-CFTR may somehow lose its responsiveness to VX-770. This finding implies that the necessity of the Trikfta's evening tablet, which contains only ivacaftor, might need further evaluation.

Of note, this work by Becq *et al*. [14] compares the expression and function of F508del channels treated with 2VX or 3VX, where the two correctors VX-445 and VX-661 are always applied together. VX445 is a type III corrector, as opposed to the type I correctors VX809 and VX661 [13], and should work through a different mechanism of action. The susceptibility of VX-445-rescued F508del channels to VX-770's destabilising effect awaits further experiments.

Interestingly, a recent report by Laselva et al. [26] revealed that VX-445 is both a corrector and a potentiator. Thus, the gating function of Trikafta-corrected F508del-CFTR is not only enhanced by VX-770 but also by VX-445, making the comparison between 2VX- and 3VX-treated F508del-CFTR even more complicated. One needs to consider if potentiation by VX-445 is affected by the presence of VX-770, as well as if prolonged treatment with VX-445 might also render the channel unresponsive to

acute addition of VX-445, as in the case of VX-770. While the interactions between VX-770 and dual-acting VX-445 are beyond the scope of this study, the authors have successfully raised our awareness of VX-770's acute and chronic effects on Trikafta-rescued F508del-CFTR, which lays the foundation for future investigation into the intricate interactions between CFTR correctors and potentiators.

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