Rescue of defective CFTR (dF508) is enhanced by targeting the ubiquitin proteasomal system

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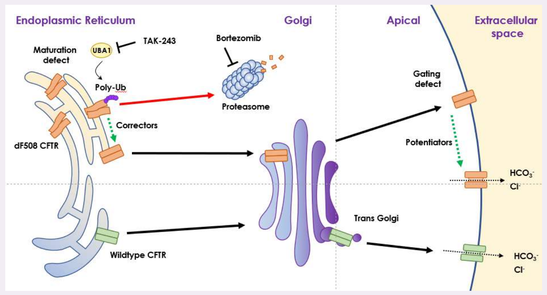
Introduction

The dF508 mutation represents the most common cause underlying cystic fibrosis. The resultant misfolding of the nascent cystic fibrosis transmembrane regulator (CFTR) protein and its subsequent proteasomal degradation lead to a deficiency in functional CFTR channels and Cl^- efflux at the apical cell membrane in ducts throughout the body (Veit *et al*. 2016). Small molecule drugs have been identified that rectify this protein misfolding ('correctors') and facilitate channel opening ('potentiators'), thereby restoring CFTR-mediated Cl^- efflux at the apical cell membrane and providing clinical improvement (Hanrahan *et al*. 2017). Nevertheless, enhanced efficacy remains a key research goal, and evidence indicates that this could be achieved by combining correctors/potentiators with modulators of the ubiquitin proteasomal system (UPS) that regulates CFTR protein degradation (Borgo *et al*. 2022). There is growing interest in the development of novel treatments that utilise this dual-target approach; we therefore set out to demonstrate that we could:

1. Measure currents elicited by wild-type (WT) CFTR in transiently transfected CHO-K1 cells using automated patch clamp (APC) electrophysiology.
2. Use the APC platform to confirm efficacy of CFTR correctors (VX-809, VX-445/VX-661) and a potentiator (VX-770) in CHO-K1 cells transiently transfected with dF508 CFTR.
3. Develop a 384-well halide-sensitive YFP assay for assessment of corrector efficacy in HEK293 cells transiently transfected with dF508 CFTR.
4. Use the YFP assay to determine if modulators of the UPS (bortezomib, TAK-243) enhance the efficacy of the CFTR corrector VX-809.



Materials and Methods

AUTOMATED PATCH CLAMP ASSAY

CHO-K1 cells were cultured according to standard cell culture protocols and transiently transfected using optimised protocols (Lipofectamine 3000) with either WT or dF508 CFTR to record whole-cell currents on the QPatch48 APC system (Sophion). Intracellular solution contained (in mM) 60 Cs-gluconate, 80 CsCl, 10 TES, 1 EGTA and 3.2 Mg_2ATP; pH 7.3. Extracellular solution contained (in mM) 145 NaCl, 4 CsCl, 1 $MgCl_2$, 1 $CaCl_2$ and 10 TES and 10 glucose; pH 7.4.

CFTR currents were elicited from a holding potential of -50 mV using a ramp protocol from -80 to +80 mV (Figure 1A, 15 second intersweep interval). Compounds were then applied during successive liquid phases to activate (forskolin, genistein), potentiate (VX-770), and inhibit CFTR ($CFTR_{inh}172$).

YFP QUENCH ASSAY

HEK293 cells stably expressing a halide sensitive YFP (Smith *et al*. 2017) were cultured according to vendor instructions and transiently transfected with either WT or dF508 CFTR as above. Cells were then seeded into 384 well plates at 30k cells/well and treated ± correctors/UPS modulators overnight.

The YFP quench assay was performed using the FLIPR Penta platform (Molecular Devices) at 37°C. Following CFTR activation (forskolin, 10 µM; VX-770, 3 µM), NaI (100 mM) was applied to induce YFP signal quench via CFTR-mediated I^- influx (Figure 4A). YFP fluorescence (F) was normalised to that upon NaI addition (at 423 seconds; F/F_{423}) and the linear rate of YFP quench measured (30 seconds; $Slope_{30}$). To control for non-CFTR mediated I^- influx, YFP quench rate in cells expressing YFP alone was subtracted.

1. Measurement of WT CFTR activity using automated patch clamp electrophysiology, QPatch 48

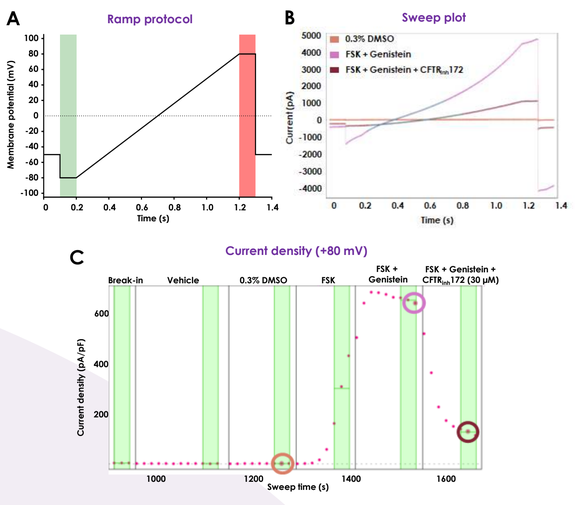


Figure 1. CFTR currents elicited in CHO-K1 cells transiently transfected with WT CFTR: A: Ramp protocol used to elicit CFTR currents. **B**: Sweep plot showing representative current traces following sequential treatment with vehicle (0.3% DMSO, orange trace), CFTR activation with forskolin (FSK, 10 µM) and genistein (30 µM, pink trace), and CFTR inhibition with the selective CFTR inhibitor $CFTR_{inh}172$ (30 µM, claret trace). **C**: Time course plot showing the current density at +80mV for each sweep during the liquid phases (annotated at top of graph).

2. Electrophysiological assessment of the potentiator VX-770 (ivacaftor) against WT CFTR

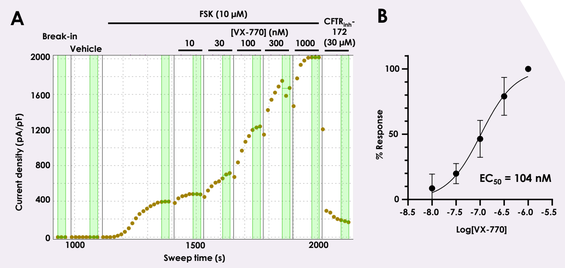


Figure 2. VX-770 elicits concentration-dependent increase in WT CFTR potentiation: Using the ramp protocol established in Figure 1, WT CFTR transiently transfected in CHO-K1 cells was activated with forskolin (FSK, 10 µM) followed by sequential application of the potentiator VX-770 from 0.01–1 µM in half-log unit increments. $CFTR_{inh}172$ was applied during the final liquid phase. **A:** Time course plot showing current density at +80mV for each sweep during the liquid phases (annotated at top of graph). **B:** Concentration-response curve of VX-770 elicited currents. Data represent mean ± S.D., N=7 independent recordings.

3. Electrophysiological assessment of the CFTR correctors VX-809, VX-445 and VX-661 using the QPatch 48

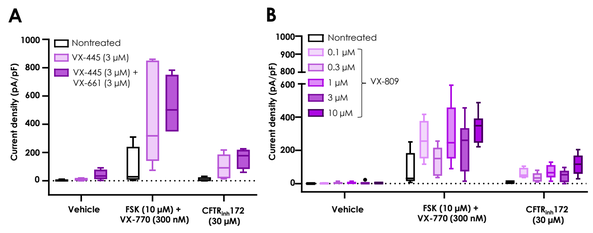


Figure 3. Known dF508 correctors (VX-809, VX-445, VX-661) elicit an increase in dF508 CFTR current density: A: Current density elicited in CHO-K1 cells transiently transfected with dF508 CFTR treated ± VX-445 (3 µM) or VX-445/VX-661 in combination (both 3 µM). **B.** Current density elicited in CHO-K1 cells transiently transfected with dF508 CFTR treated ± VX-809 (0.1–10 µM). Sequential liquid phases are indicated on the X axis (CFTR activation: FSK, 10 µM + VX-770, 300 nM; CFTR inhibition, $CFTR_{inh}172$, 30 µM). Boxes represent median ± interquartile range; whiskers represent non-outlier range; N=4–8 individual cells for each condition.

4. Development of a halide-sensitive YFP assay to measure CFTR activity and identify dF508 correctors

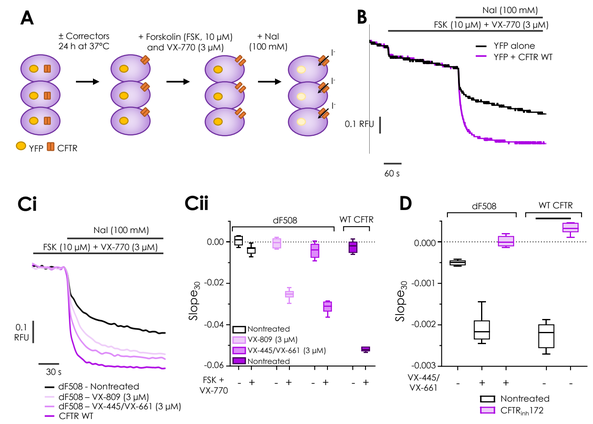


Figure 4. Development and validation of a halide-sensitive YFP assay to assess CFTR corrector efficacy: A: Schematic representation of FLIPR assay paradigm. **B:** Representative traces of assay performed in HEK293 cells stably expressing a halide-sensitive YFP. **C:** Truncated representative traces (**Ci**) and quantified $Slope_{30}$ (**Cii**) of YFP-expressing HEK293 cells transfected with dF508 or WT CFTR treated ± known correctors. **D:** Quantified $Slope_{30}$ for YFP-expressing HEK293 cells transfected with CFTR WT or dF508 (**D**) following pre-treatment ± $CFTR_{inh}172$ (30 µM) and stimulation with forskolin and VX-770. Boxes represent median ± interquartile range; whiskers represent non-outlier range. N=8–12 internal replicates for all conditions.

5. Assessment of the CFTR correctors VX-809, VX-445 and VX-661 using YFP quench assay

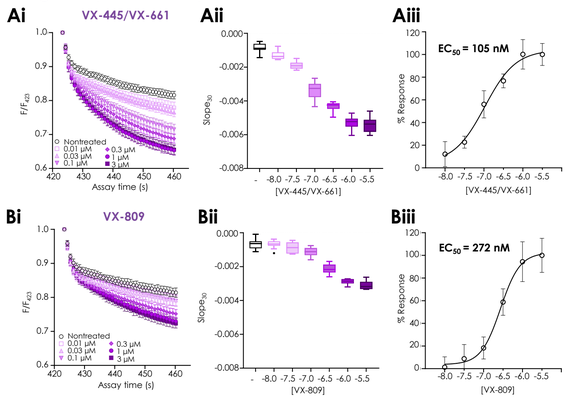


Figure 5. VX-809 and VX-445/VX-661 elicit concentration-dependent increases in dF508 CFTR activity in YFP quench assay: Truncated representative traces (**Ai, Bi,**) and $Slope_{30}$ (**Aii, Bii**) in YFP-expressing cells transfected with dF508 CFTR and treated with VX-445/VX-661 (0.01–3 µM) and VX-809 (0.01–3 µM), respectively. Concentration-response curves were determined for VX-445/VX-661 (**Aiii**) and VX-809 (**Biii**). CFTR activation with FSK 10 µM + VX-770 3 µM. Data represent mean ± S.D. (**Ai, Bi, Aiii, Biii**) or median ± interquartile and non-outlier ranges (**Aii, Bii**). N=11 internal replicates for all conditions.

6. The UPS modulators bortezomib and TAK-243 enhance corrector (VX-809) efficacy

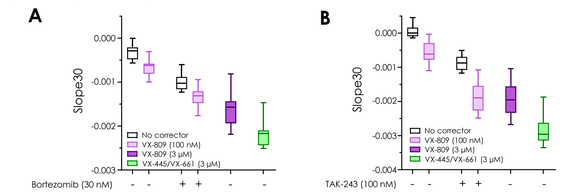


Figure 5. The UPS modulators bortezomib and TAK-243 enhance correction conferred by VX-809: $Slope_{30}$ was determined in YFP-expressing cells transfected with dF508 CFTR and treated with VX-809 (100 nM) in the presence and absence of either bortezomib (30 nM, **A**) or TAK-243 (100 nM, **B**). CFTR activation by FSK 10 µM + VX-770 3 µM. Boxes represent median ± interquartile range; whiskers represent non-outlier range. N=10–24 internal replicates for all conditions.

Summary

- VX-445, VX-661 and VX-809 rescue dF508 CFTR in transiently transfected CHO-K1 and HEK-293 cells in a concentration-dependent manner.
- Treatment with UPS modulators (bortezomib and TAK-243) increases the YFP quench rate in non-treated and VX-809 (100 nM) pre-treated cells.
- Modulation of the UPS may prove to be beneficial for the regulation of dF508 CFTR to the plasma membrane.

References

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Hanrahan JW, *et al*. Curr Opin Pharmacol. 2017;34:105-11.

Smith E, *et al*. Assay Drug Dev Technol. 2017;15(8):395-406.

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