Characterization of novel potentiators

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Poster #23

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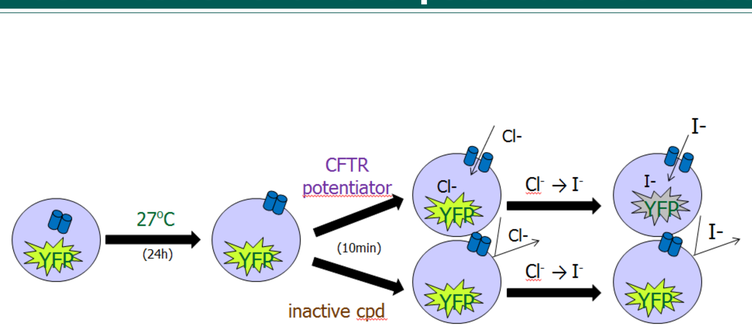
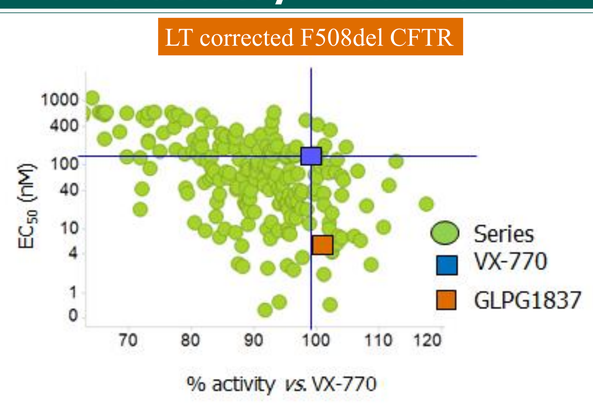
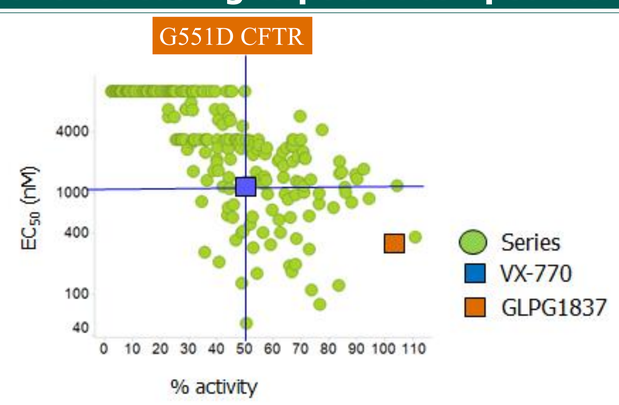
We previously described the identification of novel potentiators[1]. In short, using YFP-based high-throughput screening assay, we identified novel compounds that can potentiate cAMP-dependent activity of low temperature-rescued F508del CFTR in CFBe41o- cells. A series of reagents was developed further, with improvement in the potency of compounds down to <10nM for a number of new analogues. Potency and activity on G551D CFTR was measured for all compounds.

Identification of a novel potentiator series using YFP-Halide assay – Medicinal Chemistry efforts leading to potent compounds

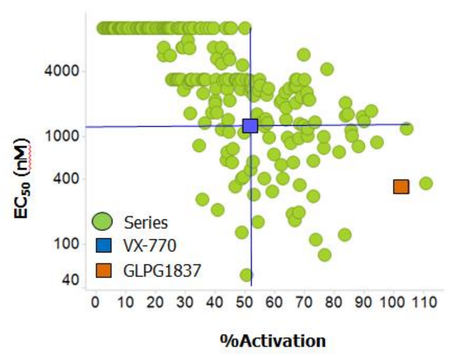




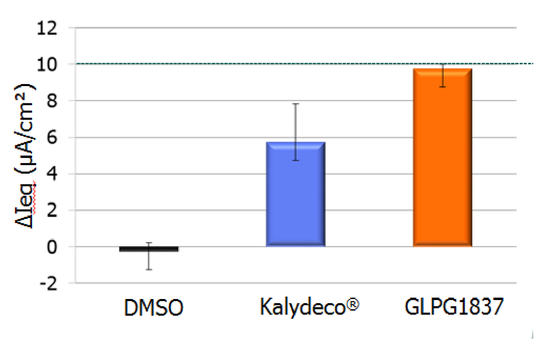


Many compounds showed a maximal activity on G551D exceeding that of VX-770 in a YFP-halide assay with up to 250% of the maximum obtained by VX-770. Furthermore, the compounds showed higher potency compared to VX-770 in the same assay. A broad set of compounds was evaluated in parallel on low temperature-rescued F508del CFTR and G551D CFTR and a good correlation between potencies in these two assays was observed.

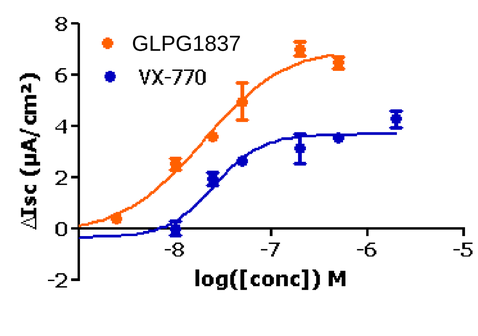
Higher efficacy confirmed on Bronchial Epithelial cells derived from G551D/F508del patients using TECC



YFP-Halide assay in HEK293 cells transfected with G551D CFTR. Potency of compounds versus % activation is represented



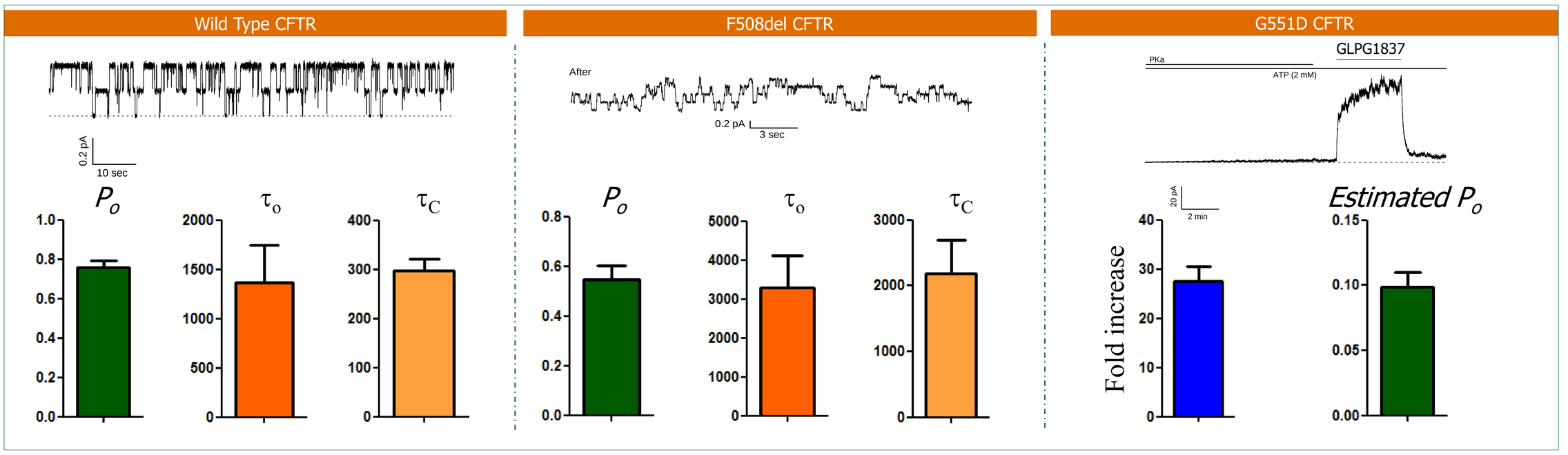
G551D/F508del HBE were grown for 21 days in ALI culture, treated with forskolin and 2 μM potentiator.



G551D/F508del HBE were grown for 21 days in ALI culture, treated with forskolin and a concentration range of potentiator.

GLPG1837 was further characterized in detail with the patch-clamp technique. In inside-out patches excised from CHO cells transiently expressing WT, G551D or F508del CFTR, applications of the compound resulted in reversible potentiation of the activity of the channels pre-activated with protein kinase A and ATP. For WT CFTR, the P_o in the presence of 3 μM GLPG1837 is 0.78 ± 0.03 (n = 8) with open time (τ_o) and closed time (τ_C) constants of 1479 ± 387 ms and 292 ± 26 ms respectively, a result compatible with those seen with VX-770[2]. However, a 27.5 ± 3.0 fold increase of macroscopic G551D CFTR currents was observed (~10 fold for VX-770 in Jih and Hwang, 2013). For F508del, the P_o was dramatically increased to 0.55 ± 0.05 (n = 9) with τ_o = 3290 ± 819 ms and τ_c = 2182 ± 516 ms.

Patch-clamp with GLPG1837



Conclusions

Galapagos has developed a novel potentiator series with superior channel opening activity *versus* VX-770. GLPG1837, the compound currently in Phase 2 clinical studies, was further characterized *in vitro* with the patch-clamp technique. The increased channel activity observed in primary cells derived from G551D/F508del patients was confirmed with an increased open probability of the heterologously-expressed G551D channel in excised inside-out patches.

References

1. Conrath K., *et al.* NACFC 2013 poster 41
2. Jih and Hwang. PNAS, 2013, 110(11): 4404-4409.

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