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Poster #52

Novel corrector-potentiator combinations for treating Cystic Fibrosis

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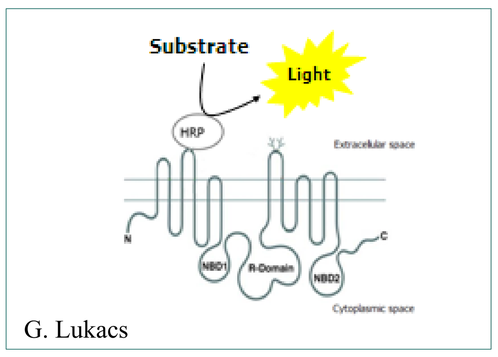
Corrector identification and characterization

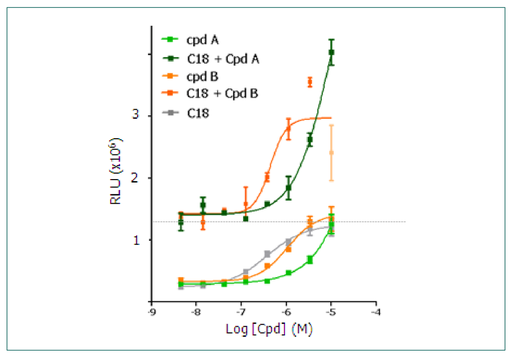
To address the most prominent cause of cystic fibrosis, two biomolecular activities are required to restore function to CFTR harboring the F508del mutation. 1) Correctors, which increase CFTR surface expression and 2) potentiators, which allow the effective opening of the CFTR channel. Maximum restoration of CFTR function through a combination of correctors and potentiators yields improved hydration of the lung surface and subsequent restoration of mucociliary clearance. To this end, we have identified novel corrector molecules that enhance the restoration of CFTR in combination with a known corrector (C18) and a novel potentiator (GLPG1837/ABBV-974[4]).

Two cellular systems were employed to identify novel corrector molecules. Galapagos and AbbVie compound libraries were examined across both CFBe41o- cells harboring HRP-tagged F508del CFTR[1] and U2OS cells harboring ProLink-tagged F508del CFTR[2] to identify molecules that could restore the surface expression of these tagged CFTR fusion proteins. Two hits representing distinct chemical series, represented here, were interrogated in additional localization and functional assays. The hits exhibited significant activity alone and in combination with C18.

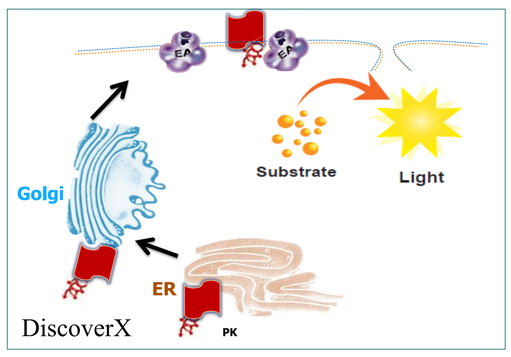
CFTR F508del – HRP in CFBe41o-



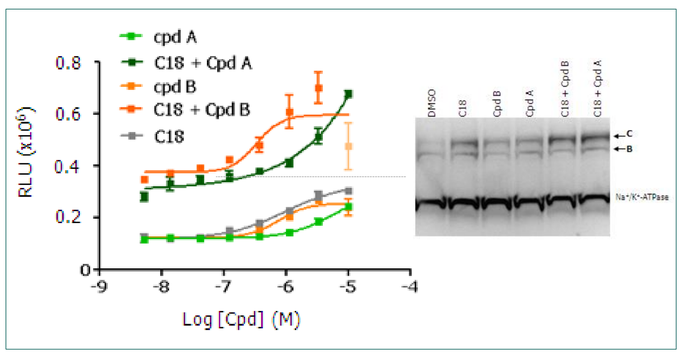
CFTR F508del – HRP in CFBe41o-



CFTR F508del – ProLink in U2OS



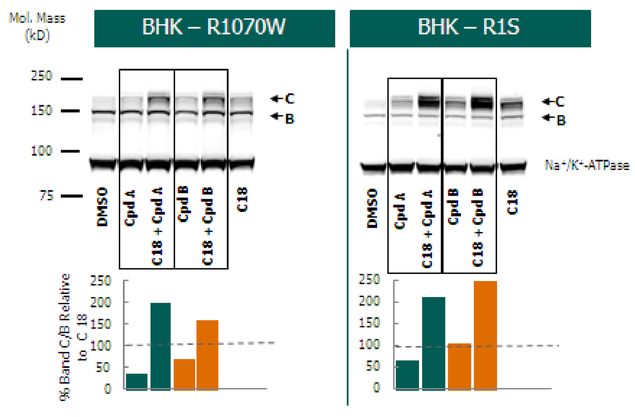
CFTR F508del – ProLink in U2OS



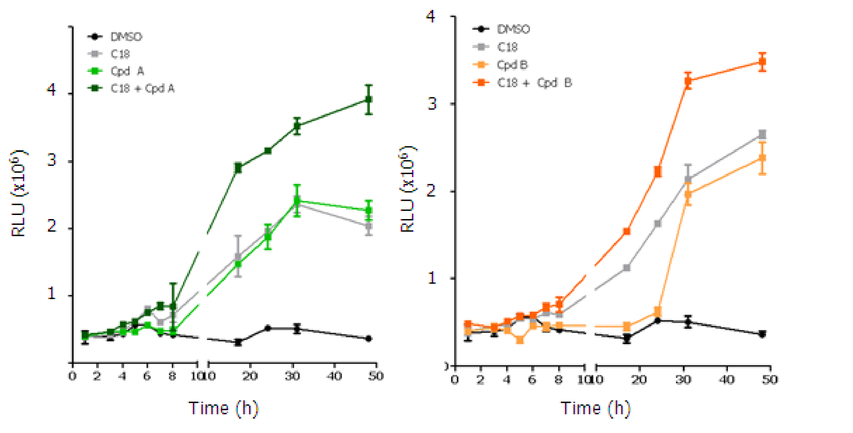
Novel correctors promote CFTR surface expression/stabilization alone or in combination with C18

Representative compounds from two series were characterized in their ability to promote CFTR glycosylation (Band C) and surface stabilization. Compounds A and B enhanced Band C formation in the presence of either R1070W or R1S suppressor mutations expressed in BHK cells[3]. Kinetic studies of Compounds A and B in the CFBe41o- cells harboring HRP-tagged F508del CFTR[1] demonstrated differences in the correction rates, suggesting that each compound functions by a distinct mechanism(s). Additional kinetic studies using the same compounds and cell system but using Brefeldin to block addition of newly synthesized F508del CFTR at the plasma membrane, showed differences in F508del CFTR levels.

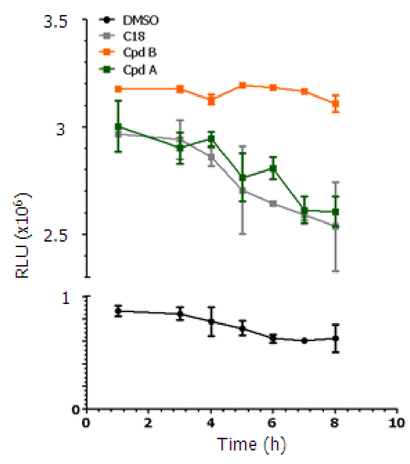
Band B/C in F508del suppressor mutants



Correction of HRP-F508del CFTR in time



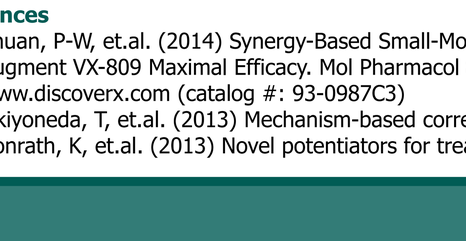
Residence time of HRP-F508del CFTR

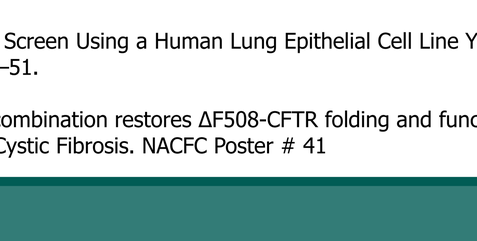


The combination of two correctors and one potentiator results in synergistic restoration of CFTR function

Corrector Cpd A and B exhibit channel opening activity in primary cells derived from F508del CFTR homozygous patients as measured by trans-epithelial clamp circuit (TECC); Our lead potentiator (GLPG1837/ABBV-974) was used in these assays to increase opening of corrected F508del CFTR channel. Primary F508del CFTR cells differentiated for 21 days in ALI culture were treated for 24 hours with Cpd A, B alone or in combination with C18. Rescued F508del CFTR channel activity was measured after addition of forskolin and GLPG1837/ABBV-974.

F508del/F508del primary cell data using GLPG1837/ABBV-974 as potentiator (TECC data, 2 donors)





Conclusions

- Novel mechanism correctors have been identified using two different cellular systems detecting F508del CFTR cell surface expression. Mechanistic studies of two series suggest that the enhanced CFTR activity is due to increased CFTR surface expression with synergism when co-treated with the corrector C18.
- Equivalent to superior restoration of CFTR function was observed in primary patient cells with the Compounds A or B alone. Combination studies with C18 and GLPG1837/ABBV-974, our lead potentiator, demonstrate that restoration of CFTR function can be further enhanced 3-5 fold over the effects observed with C18 with GLPG1837/ABBV-974 alone.

Summary

The utilization of two distinct cellular systems has identified several novel corrector series with activity alone and in combination with C18, a VX-809 analog. Compounds from two of the series exhibit a 3-5 fold improvement in CFTR function in primary patient F508del cells in combination with C18 + GLPG1837/ABBV-974 compared to C18 + GLP1837/ABBV-974 alone. Taken together, these studies led to the identification of novel, complementary corrector series that when combined with existing CFTR modulators, restore >40% of WT CFTR function in F508del homozygous patient cells. Hits from these screens serve as the foundation of our novel corrector drug discovery program.

Acknowledgements
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