In vitro Properties of F508del-CFTR Second Site Corrector FD2052160

I. Kwok, P. Bhatt, V. Bailey, J. Chin, C. Bresilla, A. Dasgupta, A. Deshpande, M. Zawistoski, B. Cole, M. E. Krouse

Flatley Discovery Lab Charlestown, MA 02129

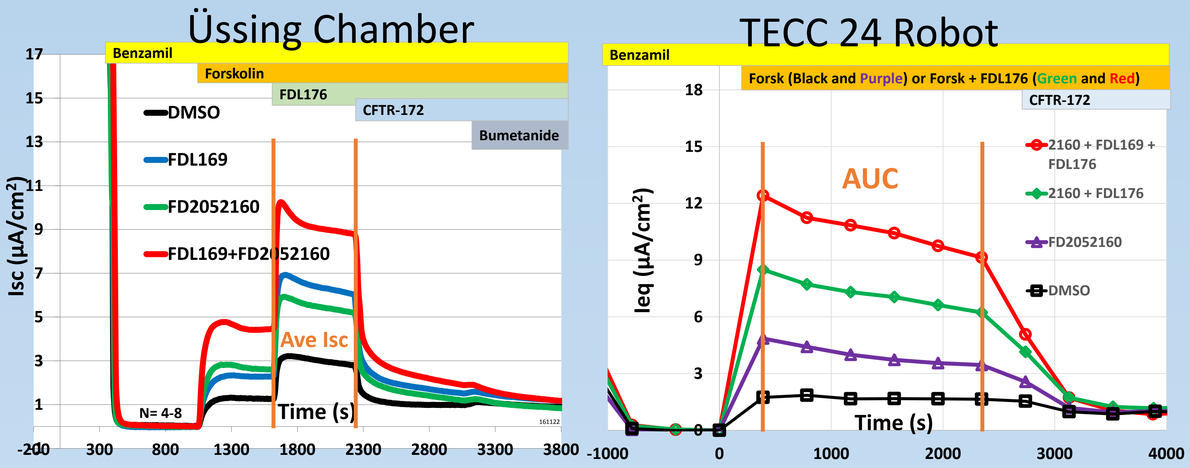
Introduction: Flatley Discovery Lab is developing a corrector that increases CFTR activity in combination with corrector FDL169 and potentiator FDL176. The *in vitro* activity of lead FD2052160 in F508del-CFTR expression and chloride transport assays is described.

Methods: Chloride transport experiments were performed in the TECC-24 equivalent current assay (Ieq). Primary F508del-CFTR airway cells exposed to test compound(s) for 24 hours were stimulated with forskolin + potentiator after sodium current was eliminated with benzamil. Area under the curve (AUC) was calculated from the average Ieq for ~30 minutes after stimulation minus the current before stimulation and after CFTR inhibition with CFTR-172. A compound's Cl^- transport response was measured as the AUC from cells treated by test compound minus AUC of vehicle treated control cells. F508del-CFTR cell surface expression was measured in F508del-CFTR CFBE41o- cells labeled with horseradish peroxidase (HRP) on the fourth extracellular loop. Cells were treated with test compound(s) for 24 hours and HRP exposed on the cell surface was measured by chemiluminescence, representing the plasma membrane expression of F508del-CFTR. Western blot analysis of mature, fully glycosylated F508del-CFTR (band C) was determined in primary F508del-CFTR cells following 48-hour exposure to test compound(s).

Results: F508del-CFTR correction by FD2052160 increased chloride transport in primary hBE cells with similar maximum efficacy as correctors FDL169, Lumacaftor and Tezacaftor. The effect of combination FD2052160 + FDL169 on chloride transport was approximately 2-fold higher than FDL169 alone; which was similar to the effect of FD2052160 in combination with Lumacaftor or Tezacaftor compared to Lumacaftor or Tezacaftor alone. A concentration matrix examining 24-hour exposure of primary F508del-CFTR cells to increasing doses of FDL169 and FD2052160 shows that peak efficacy of the combination is observed at the concentration of each corrector that gives its maximum response alone. In the HRP cell surface assay, FD2052160 increased the amount of F508del-CFTR in the plasma membrane by >2-fold compared to vehicle control. Combination FD2052160 + FDL169 further enhanced F508del-CFTR expression by >4-fold over vehicle control cells. In Western blot analyses, FD2052160 increased band C expression in primary hBE cells compared to vehicle control cells, and triple combination FD2052160 + FDL169 + FDL176 further increased band C expression.

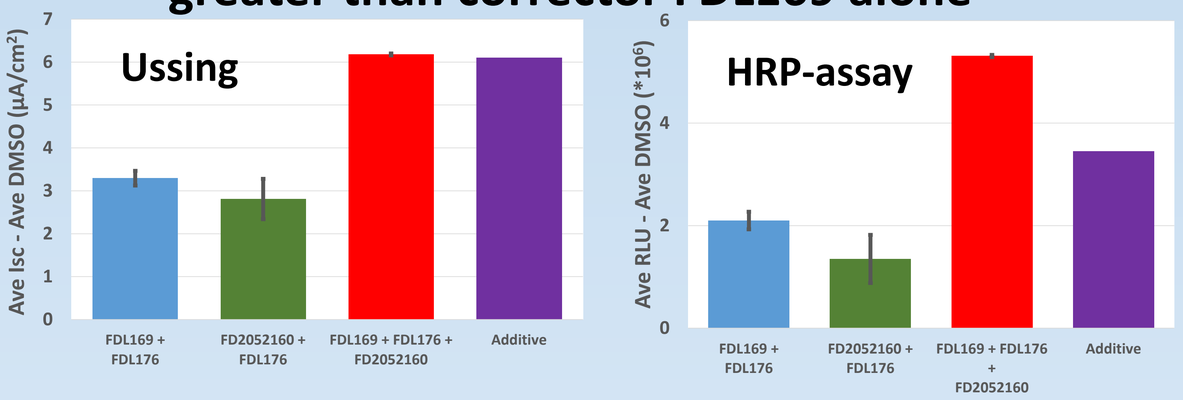
Conclusion: FD2052160 is a F508del-CFTR corrector that increases protein expression and chloride current both alone and in combination with other correctors. Triple combination of FD2052160 with corrector FDL169 and potentiator FDL176 increases chloride current and F508del-CFTR expression >4-fold over vehicle treated cells. The FD2052160 series has the potential to increase efficacy of corrector/potentiator combinations currently in clinical development.

Fig. 1 Typical traces



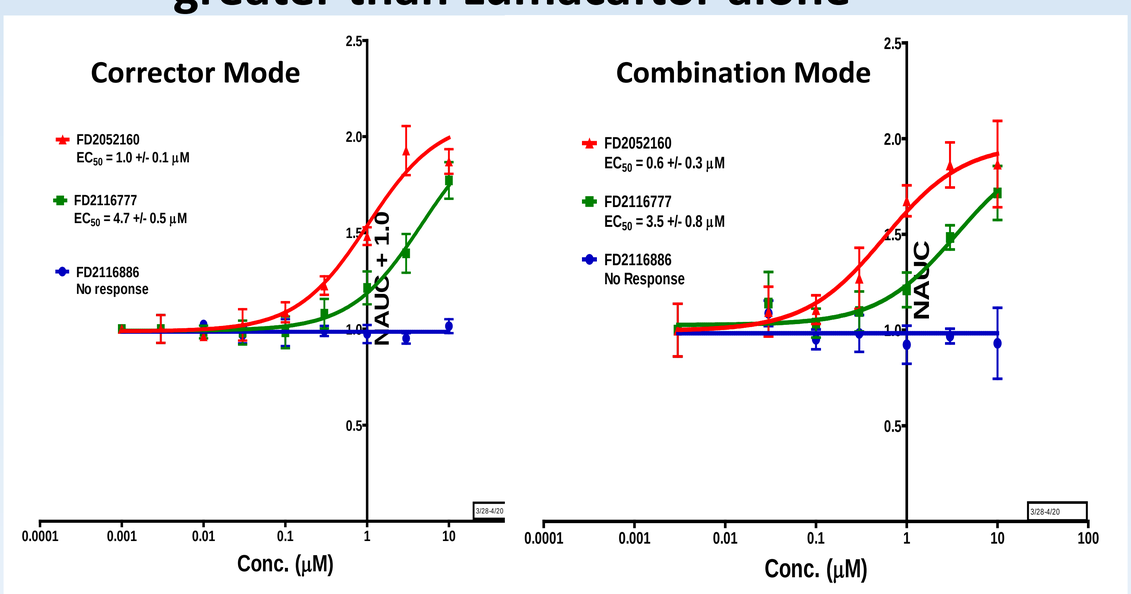
Averaged traces from Ussing chamber or TECC 24 robot. Responses are either the Ave Isc (average current between addition of forskolin + FDL176 to CFTR inhibitor) or the AUC (area under curve between addition of CFTR activators and CFTR inhibitors). Both figures show that the combination of FD2052160 +FDL169 is greater than FD2052160 alone.

Fig. 2 FD2052160 increases correction to greater than corrector FDL169 alone



Bar Charts from Ussing chamber and HRP assay from several experiments. Ussing chamber measures functional CFTR on the apical membrane of primary F508del CFhBEs. Whereas the HRP-assay measures the total CFTR on the cell membrane of a CFBE cell line engineered to express F508del CFTR with an extracellular HRP tag. Both bar graphs show that FD2052160 adds correction to FDL169 correction.

Fig. 3 FD2052160 series increases correction to greater than Lumacaftor alone



Dose Response Curves from 3 compounds in the FD2052160 series. In the corrector mode (left graph) the F508del CFhBE were treated for 24 hours with the FD2052160 series compounds only. In the combination mode (right graph) the cells were treated for 24 hours with the FD2052160 series compounds + Lumacaftor (3 µM) Both the corrector and the combination modes are plotted with the same axis for ease of comparison.

Correction by the FD2052160 series adds to Lumacaftor correction.
The compound FD2116886 (blue line) did not cause any correction but shows that there is no inhibition of the Lumacaftor correction in the combination mode.

Acknowledgements

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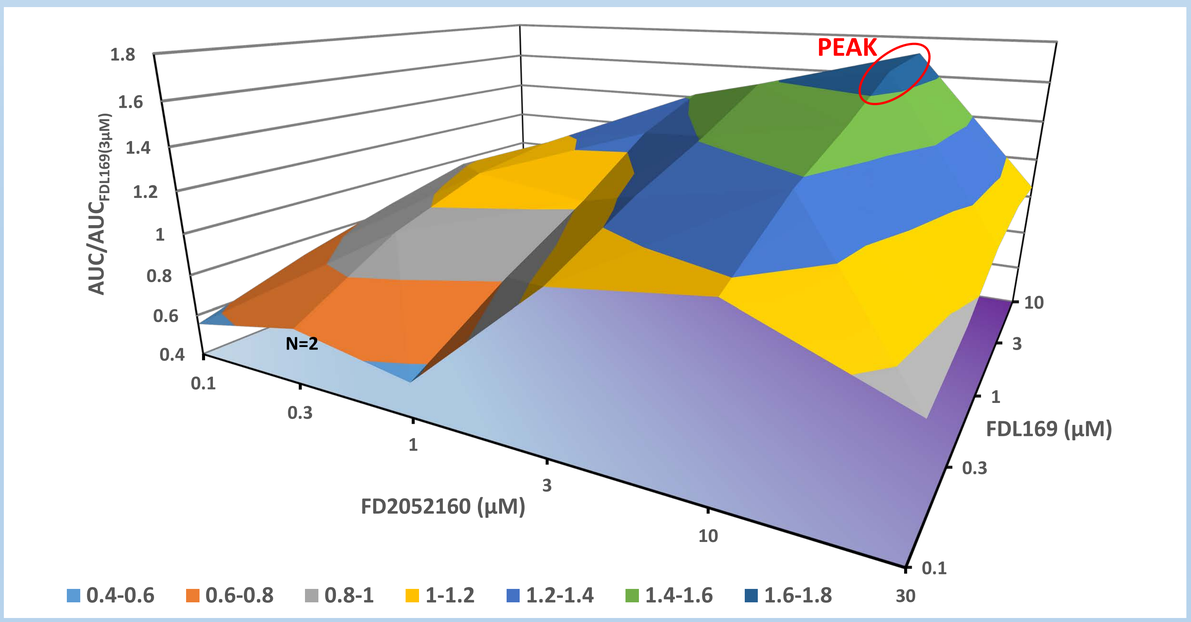
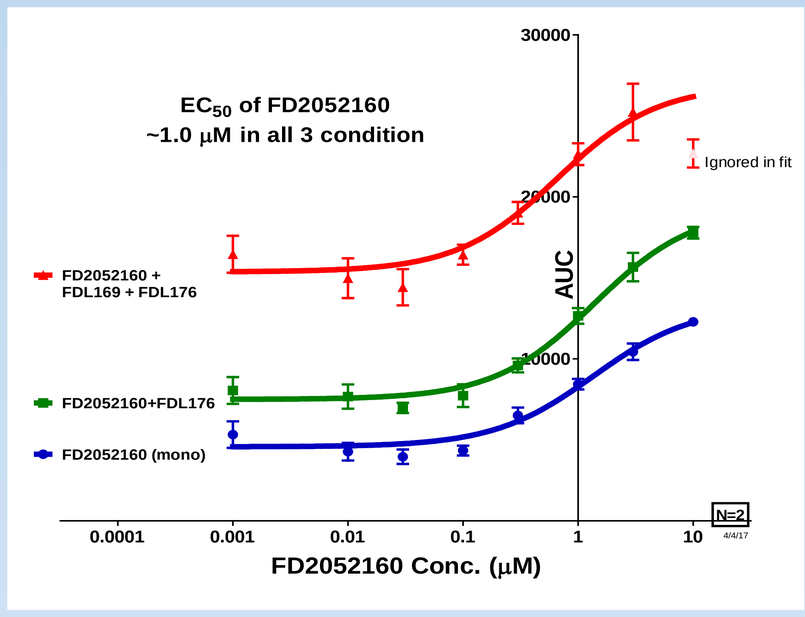
Fig. 4 Most Efficacious combination of 2160 and FDL169



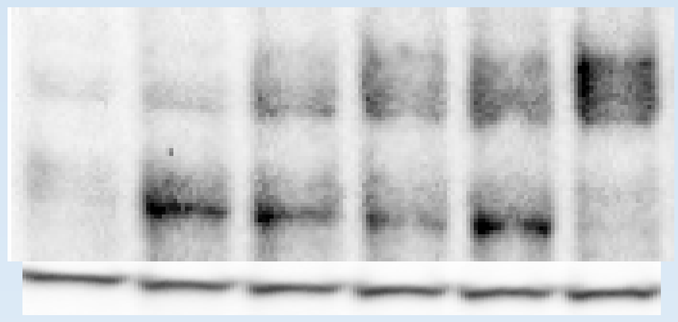
Averaged response of the combination of FD2052160 and FDL169 correctors in a matrix experiment in F508del CFTR primary CFhBE cells. All responses are normalized to the positive control (FDL169 @ 3 µM).
The peak of the combination is at 3-10 µM FDL169 and 10 µM FD2052160.
This is the same peak as noted for each corrector separately (see previous dose response curves).

Fig. 5 Potency of FD2052160 is unaffected by other correctors or potentiators



Averaged dose response curves for FD2052160 either alone (blue), potentiated with FDL176 (green), or in combination with a second corrector (FDL169)and potentiated with FDL176 (red). With each additional compound the response (to either FDL176 {green), or FDL169 + FDL176 (red)) increases (blue->green->red) but the potency of FD2105160 does not change.

Fig. 6 Band C increases with co-correction



No Treatment	+	-	-	-	-	-
FDL176 1µM (potentiator)	-	+	-	-	+	+
FDL169 3 µM (corrector)	-	-	+	-	+	+
FD2052160 3µM	-	-	-	+	-	+

Western blot of CFTR from primary F508del CFTR hBEs. The combination of FD2052160 + FDL169 increases band C (Mature CFTR) more than either corrector alone.

Summary:

1) FD2052160 increases current/protein when used in combination with FDL169, Lumacaftor, or Tezacaftor
2) Increase is additive.
3) Potency is not changed by other correctors
4) Maximum efficacy of combination is seen at maximum efficacy of each corrector separately

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