In vitro Properties of F508del-CFTR Potentiator FDL176

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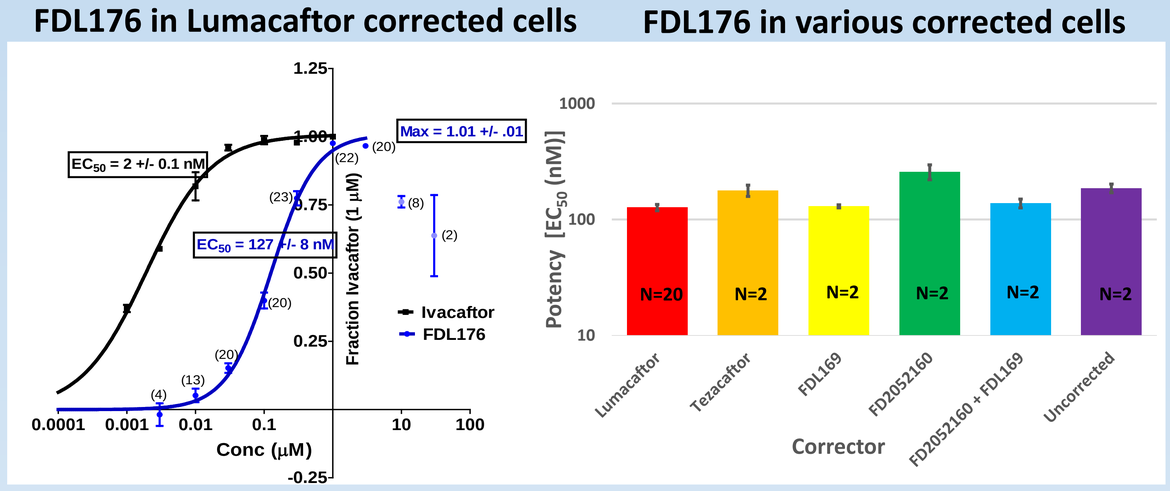
Introduction: CFTR potentiator FDL176 is being developed with corrector FDL169 as a combination treatment for cystic fibrosis. *In vitro* evaluation of FDL176 was conducted to determine its effect on F508del-CFTR chloride transport and expression.

Methods: Chloride transport experiments were performed in the Ussing chamber short-circuit current assay (Isc) or TECC-24 equivalent current assay (Ieq) in primary F508del-CFTR airway cell exposed to F508del-CFTR correctors FDL169, FD2052160, Lumacaftor or Tezacaftor for 24 hours. In these experiments, sodium current was eliminated with benzamil and chloride current was measured as area under the curve (AUC) calculated from the average response after CFTR stimulation and before CFTR inhibition with CFTR-172. 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: In chloride transport experiments, acute stimulation with potentiator FDL176 gave a dose-responsive increase in chloride current with a maximum chloride transport response equivalent to Ivacaftor in F508del-CFTR hBE cells corrected with FDL169, FD2052160, Lumacaftor or Tezacaftor. Prolonged exposure of F508del-CFTR hBE cells corrected with FDL169, FD2052160, Lumacaftor or Tezacaftor (≥ 24 hr) to FDL176 results in a minimal reduction of chloride current response compared to its acute potentiation response (0-20%); prolonged exposure to Ivacaftor results in a substantial reduction of chloride current response compared to its acute potentiation response (30-70%). Matrix experiments measuring chloride current response to various concentrations of potentiator and corrector show that prolonged exposure of FDL176 gives a dose-responsive increase of chloride current whereas increasing doses of Ivacaftor give a dose-responsive decrease. In the HRP cell surface assay, prolonged exposure of CFBE41o- cells corrected with FDL169, FD2052160, Lumacaftor or Tezacaftor to FDL176 results in <30% reduction of F508del-CFTR expression in the plasma membrane compared to cells treated with corrector only; prolonged exposure to Ivacaftor results in >30% reduction of F508del-CFTR expression in the plasma membrane compared to cells treated with corrector only. Treatment of primary F508del-CFTR cells with FDL176 and corrector FDL169 does not affect band C expression compared to cells treated with FDL169 only.

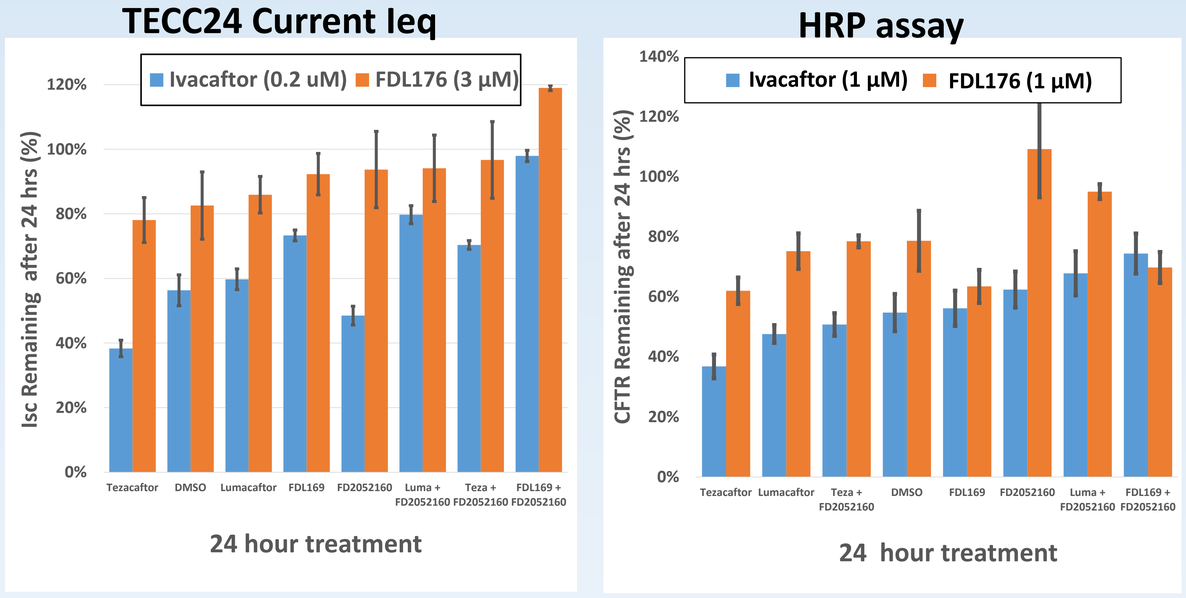
Conclusions: Potentiator FDL176 increases F508del-CFTR current with maximum chloride transport response greater than Ivacaftor under chronic treatment conditions and equivalent to Ivacaftor under acute treatment conditions. Results of HRP experiments suggest reduction in chloride current following chronic exposure to potentiators is due to loss of CFTR in the plasma membrane and that F508del-CFTR expression is higher in cells exposed to FDL176 compared to Ivacaftor under chronic treatment conditions.

Fig. 1 Potency of potentiator FDL176 is similar with all correctors tested



Dose response curves (left) for ivacaftor (black) and FDL176 (blue) in Lumacaftor corrected F508del primary CFhBEs from Üssing chamber experiments. Bar graph (right) of potency of FDL176 in cells corrected with 6 different correctors. In all 6 cases the potency of FDL176 is consistent with a single potency of FDL169 (~130 nM) for F508del CFTR. (Note log scale on y axis of bar chart)

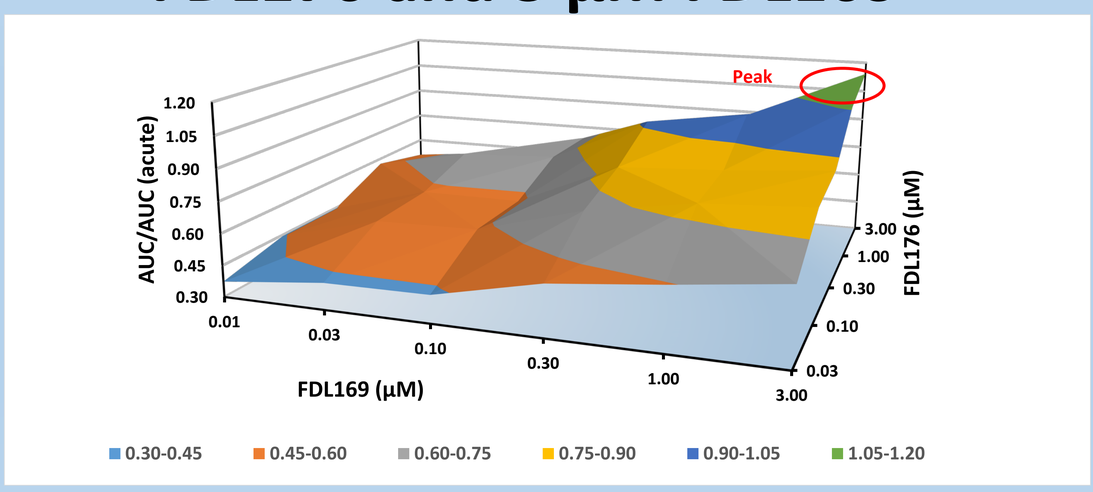
Fig. 2 FDL176 produces less chronic inhibition of CFTR



Bar graph (left) of the average current response after treatment with either 0.2 µM Ivacaftor or 3 µM FDL176 + corrector noted for 24 hours normalized to the response with no chronic exposure to potentiator. The concentration of potentiator used was chosen to reflect the difference of potency of the potentiator activity. Responses for ivacaftor ranged from 40 to 100%, with the least inhibition of corrector occurring with FDL correctors. Responses for FDL176 ranged from 80 to 120% with the largest responses occurring with FDL correctors.

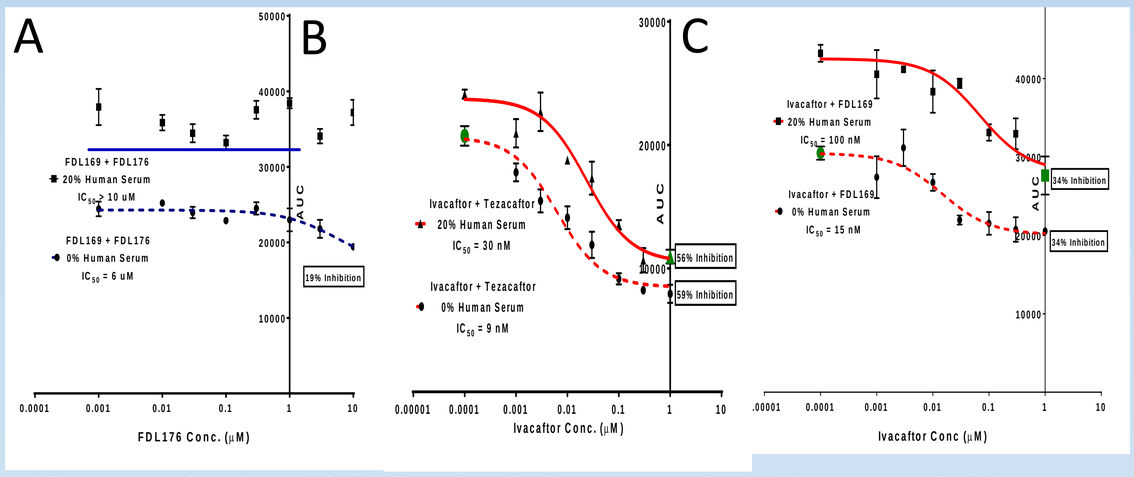
Bar graph (right) of the percent of F508del CFTR on the cell surface as determined with the HRP method. Similar protocol of exposing cells to correctors and potentiator for 24 hours (as above). In this case both potentiator concentrations we set to 1 µM. The 24 response for Ivacaftor ranged from 40 to 70%, with the largest response occurring with FDL correctors. The 24 response for FDL176 ranged from 80 to 100% with the largest response occurring with FDL correctors.

Fig. 3 Peak response seen with 3 µM FDL176 and 3 µM FDL169



Dose response surface using a protocol to mimic patient exposure to FDL168/FDL176 combination. F508del primary CFhBE cells are exposed to FDL169 (0.01 - 3 µM) and FDL176 (0.03 - 3 µM) for 24 hours prior to stimulation with forskolin. Re-exposure to FDL176 at the same concentration originally used produces a maximum response at 3 µM FDL169 and 3 µM FDL176. The responses were normalized to 3 µM FDL169 with 3 µM FDL176 added acutely. It can be seen the FDL176 in combination with FDL169 produces very little chronic inhibition of correction (peak ~1.0).

Fig. 4 Addition of 20% human serum does not reduce chronic inhibition of CFTR



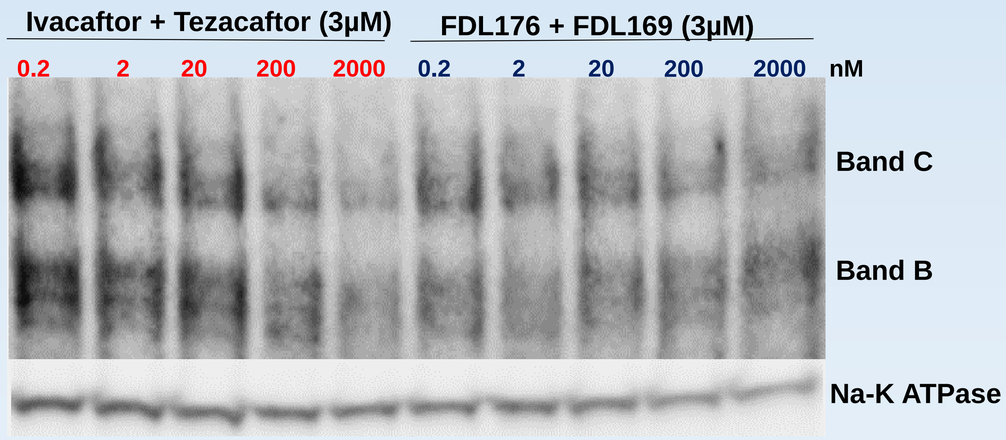
Dose inhibition curves for primary CFhBE cells incubated with test compounds ± 20% human serum for 24 hours and subsequently stimulated with forskolin + 1µM ivacaftor. The addition of human serum 24 hours before the experiment increases the CFTR current. If one uses the incorrect negative control, it may appear there is no chronic inhibition by potentiation. (compare green dots in Figure C)

A: No inhibition measureable in 20% human serum for FDL176.

B: Tezacaftor + Ivacaftor treated cells show ~58% inhibition regardless of the addition of human serum

C: FDL169 + Ivacaftor treated cells show less inhibition than Tezacaftor + Ivacaftor cells (34%).

Fig. 5 Chronic Ivacaftor inhibits both band B and C whereas FDL176 does not



Western blot of primary F508del CFTR CFhBEs.

(**Left**) Cells were exposed to 3 µM Tezacaftor and increasing concentrations of ivacaftor for 24 hours. There is a decrease in the amount of band C and band B with increasing doses of potentiator ivacaftor.

(**Right**) Cells were exposed to 3 µM FDL169 and increasing doses of potentiator FDL176 for 24 hours. There is no decrease in the amount of band C or band B.

Chronic exposure to ivacaftor inhibits correction of CFTR and FDL176 does not.

Summary

1) FDL176 potentiates F508del CFTR current independent of which corrector was used.

2) Chronic (24 hour or longer) exposure to FDL176 inhibits less F508del CFTR than Ivacaftor

3) Maximum response in vitro occurs at 3 µM FDL176 and 3 µM FDL169

4) The presence of human serum does not reduce chronic inhibition of F508del CFTR by Ivacaftor

5) FDL176 does not reduce band B or C of F508del CFTR in primary cultures with 24 hour exposure

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