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Properties of a Novel F508del-CFTR Corrector FDL169

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Abstract

Objectives: Flatley Discovery Lab is pursuing the discovery and development of small molecules that restore F508del-CFTR mediated chloride ion transport. Clinical candidate FDL169 is a F508del-CFTR corrector with distinct *in vitro* and *in vivo* properties. FDL169 is currently in phase 1 clinical trials.

Results: In Primary hBE cells FDL169 has potency and efficacy that is comparable to lumacaftor, but combining FDL169 and lumacaftor does not further increase F508del-CFTR activity. However, several differences between lumacaftor and FDL169 have been found. Chronic exposure (≥ 24 hr) of cells to FDL169 + ivacaftor leads to mild inhibition (15%) whereas 55% inhibition is observed with lumacaftor + ivacaftor. Comparison of short circuit currents before and after potentiation with ivacaftor and forskolin indicates that FDL169 increases the amount of F508del CFTR on the surface membrane, but does not impact open probability of F508del-CFTR as lumacaftor does. This is supported by HRP cell surface assay results in CFBE41o- cells showing similar F508del-CFTR expression with FDL169. FDL169 has a higher free fraction in human plasma proteins than lumacaftor, and correction of F508del-CFTR by lumacaftor is more sensitive than FDL169 to a potency shift when human serum is added to the correcting media. FDL169 demonstrates enhanced biodistribution to the lung showing a >4 fold increase in lung:plasma partitioning in rat biodistribution studies compared to lumacaftor.

Conclusion: FDL169 is a novel F508del-CFTR corrector that increases the amount of F508del-CFTR at the cell surface, has low risk of reduced activity upon chronic treatment and distributes well into lung tissue.

FDL169 Equivalent In-Vitro Efficacy and Potency to Lumacaftor

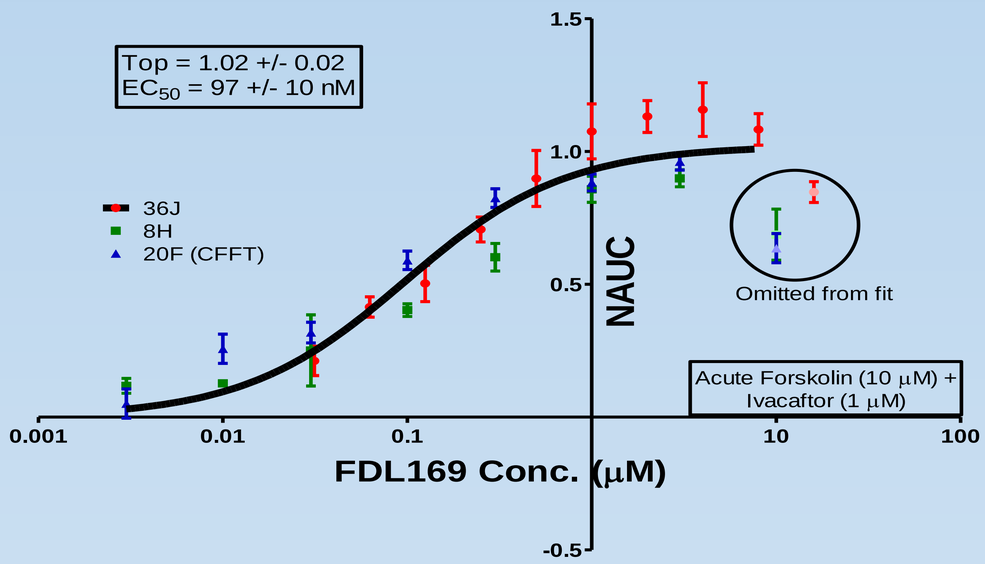
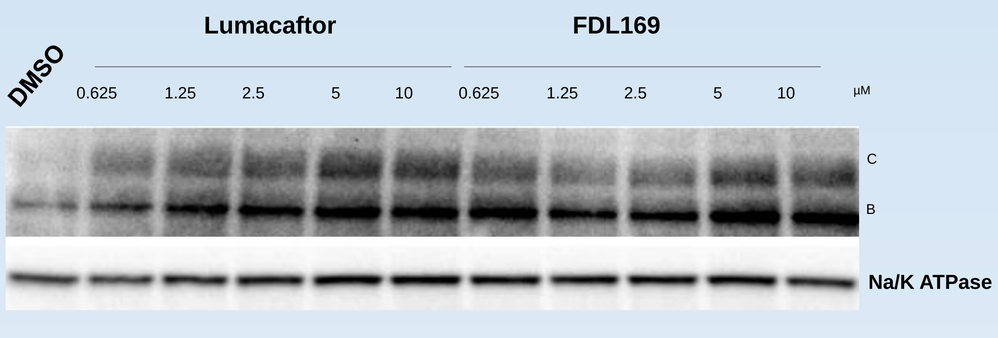


Figure 1: Acute Stimulation with Forskolin and Ivacaftor.
Methods: Dose response data obtained using homozygous F508del CF human bronchial cells from lung transplants (UNC) from 3 different patients and performed in 2 different labs. NAUC (y-axis) is the increase in chloride current after treatment with FDL169 normalized to the maximum response produced by lumacaftor (3 μM). The EC_{50} of lumacaftor is 83 nM in the FDL assay. Error bars are SDs. Note: The 2 correctors FDL169 and lumacaftor are not additive (not shown).

Maturation of F508del-CFTR is Similarly Enhanced by FDL169 and Lumacaftor



C	0.10	0.93	0.91	0.97	1.04	0.97	0.86	0.68	0.80	1.02	1.09
B	0.40	0.80	0.82	0.81	0.80	0.80	0.90	0.76	0.87	0.98	1.11
C/B	0.25	1.16	1.10	1.19	1.29	1.22	0.95	0.91	0.91	1.03	0.98

Figure 2: Methods: Representative Western blot assay from CFBE41o- cells exposed to various concentrations of lumacaftor or FDL169 for 24 hours. Densitometer readings were normalized to the density of the Na/K ATPase. Band densities from 3 assays were quantified, averaged and shown in the table below the Western blot. Results: band C, the fully mature form of CFTR, was not significantly different between lumacaftor and FDL169.

FDL169 Differs from Lumacaftor as Revealed by Potentiation

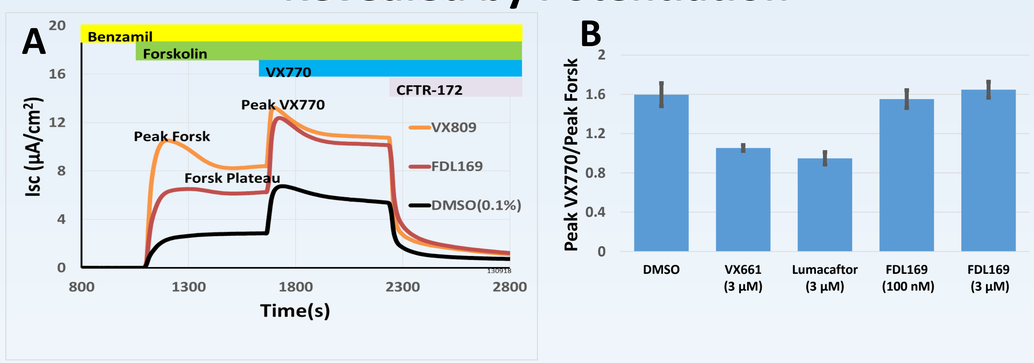


Figure 3 A: Ussing chamber traces of the change in Cl^- current after addition of forskolin and ivacaftor. After stimulation with forskolin and ivacaftor the responses to both lumacaftor and FDL169 are the same as shown in the figure 1.

B: FDL169 has a larger potentiator response than lumacaftor. The ratio of the peak ivacaftor response to the forskolin plateau (~1.6) for FDL169 is the same as that seen with DMSO. N is between 2-5, with 4 wells per experiment. Error bars are SEM.

FDL169 Protects F508del CFTR from Chronic Inhibition by Ivacaftor

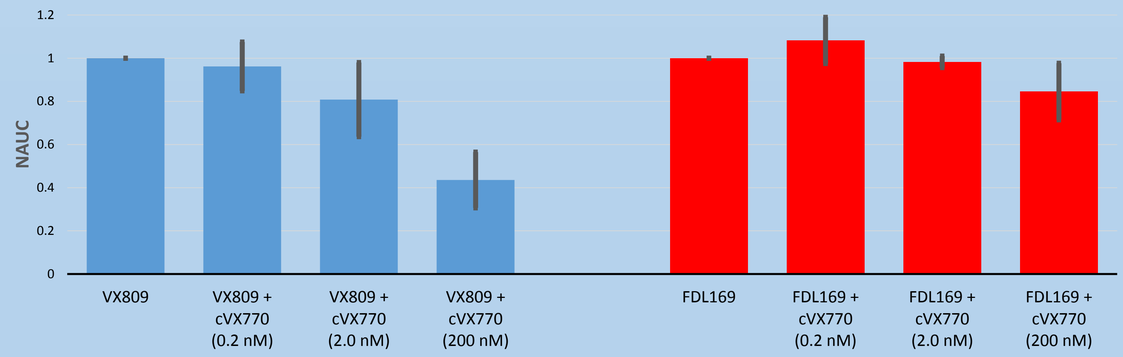


Figure 4: Bar graph of normalized chloride current amplitude after treatment with lumacaftor and FDL169 correctors and various concentrations of ivacaftor for 24 hrs (cVX770). Cells corrected with lumacaftor (blue bars) show a larger inhibition of current by ivacaftor than cells corrected with FDL169 (red bars). Data from 2 experiments, a total of 20 wells for each bar. Controls labeled VX809 (lumacaftor) and FDL169 are the chloride current responses with acute VX770 treatment (no inhibition). Error bars are SDs.

Potency of FDL169 is Less Sensitive to Human Serum

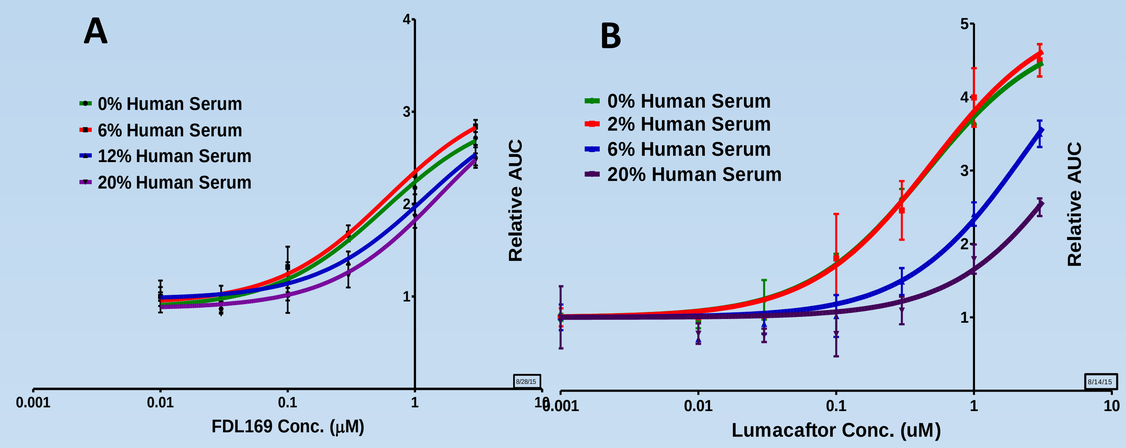
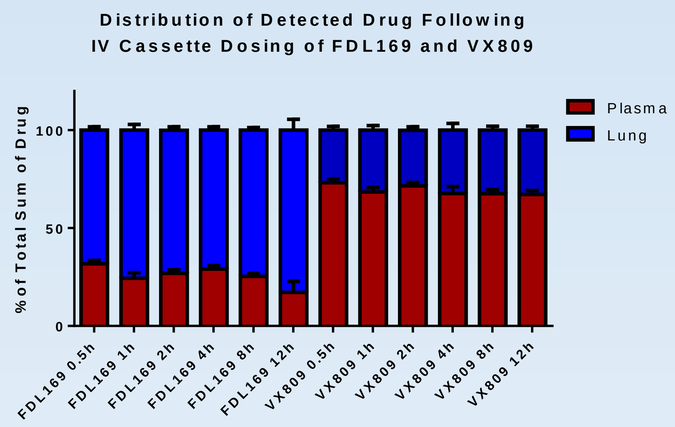


Figure 5: Shift of the dose response curves for FDL169 (A) compared to Lumacaftor (B) with increasing human serum in the media. The y-axis is labeled relative AUC as all AUCs for a particular human serum concentration are normalized to the AUC of DMSO. As more serum protein is present more corrector binds to the increased protein, thus reducing the free concentration of corrector and shifting the dose response curve to the right. At serum concentrations higher than 30% the cells tended to die after 24 hour exposure.
Note: The middle human serum concentrations differ in A and B.

FDL169 has Enhanced Biodistribution to Lung

Figure 6:
Methods: Sprague Dawley rats (n= 5) were Injected intravenously with a solution Containing FDL169 and lumacaftor at a dose of 1 mg/kg each. Plasma and lung samples were collected at 6 time points over 12 hours post administration. Concentrations of FDL169 and lumacaftor were determined in lung and plasma samples, and the data were used to estimate a lung to plasma ratio at each time point.
Results: lung to plasma ratio for FDL169 ranged from 2.2 to 3.7 and lumacaftor from 0.37 to 0.5
Data suggests that at the same concentration of FDL169 or lumacaftor in blood, the concentration of FDL169 in the lung will be higher than that of lumacaftor.



Summary and Conclusions

- **FDL169 is a corrector with potency and efficacy equivalent to lumacaftor *in vitro.***
- ***In vitro*, FDL169 increased the levels of mature F508del CFTR protein (Band C) to a similar extent as lumacaftor.**
- **Cells corrected with FDL169 are less sensitive to inhibition by prolonged exposure to ivacaftor.**
- **In human serum FDL169 is less protein bound than lumacaftor.**
- **FDL169 distributes better to the lungs than lumacaftor.**
- **FDL169 has favorable properties *in vitro* and *in vivo* that differentiate it from the lumacaftor.**
- **FDL169 is currently in Phase 1 clinical trials.**