In vitro Properties of F508del-CFTR Potentiator FDL176

Flatley Discovery Lab Charlestown, MA 02129

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 TECC24 Current Ieq HRP assay. Chloride transport experiments were performed in the Ussing chamber short-circuit current assay (Isc) or TECC24 Current Ieq HRP assay. Fibrosis. In all 6 cases the potency of FDL176 is consistent with a single potency of FDL69 (~130 nM) for F508del 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 potentiator. (compare green dots in Fig C).

Conclusions: Potentiator FDL176 increases F508del-CFTR current with maximum chloride transport response greater than ivacaftor under acute 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.

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

Seeking a Cure for Cystic Fibrosis