_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.