In vitro Efficacy of Combination FDL169/FDL176 is Greater than Tezacaftor/Ivacaftor

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Introduction: Flatley Discovery Lab is developing CFTR corrector-potentiator combination FDL169-FDL176 for the treatment of cystic fibrosis. *In vitro* evaluation of FDL169-FDL176 and tezacaftor-ivacaftor combinations was conducted to determine the effect of each treatment on F508del-CFTR chloride transport and expression.

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 corrector FDL169 increased chloride current in primary hBE cells with greater potency and approximately 1.2-fold greater maximum efficacy than tezacaftor. In the presence of human serum (20%) the EC_{50} for FDL169 increased 3.5-fold whereas the EC_{50} for tezacaftor increased 20-fold, indicating FDL169 is less sensitive than tezacaftor to potency shifts due to plasma protein binding. F508del-CFTR hBE cells exposed to combination FDL169-FDL176 for 24 hours demonstrated 2-fold higher chloride transport compared to cells exposed to combination tezacaftor-ivacaftor for 24 hours. Similar trends were observed in F508del-CFTR expression assays. In the HRP cell surface assay, F508del-CFTR expressed in the plasma membrane was approximately 2-fold higher in CFBE41o- cells treated with FDL169-FDL176 compared to cells treated with tezacaftor-ivacaftor. In a concentration matrix examining 24-hour exposure of primary F508del-CFTR cells to increasing doses of each corrector and potentiator, escalating doses FDL176 had a dose responsive increase of chloride current for all FDL169 doses whereas ivacaftor had a dose responsive reduction of chloride current for all tezacaftor doses.

Conclusion: Combination of FDL169-FDL176 increased chloride transport and F508del-CFTR expression with greater *in vitro* efficacy than tezacaftor-ivacaftor.