**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 assay (lec). 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**

**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 EC50 for FDL169 increased 3.5-fold whereas the EC50 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 cells corrected 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 FDL169 doses.

**Conclusion**

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

**Summary of pre-clinical results**

- FDL169 is more efficacious than Tezacaftor
- FDL169 is less sensitive to protein binding (20% human serum)
- Chronic treatment with FDL169 + FDL176 is more efficacious than TEZ + IV

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**Figure 1: Dose-response curve (left) and maximum response bar graph (right) of FDL169 vs Tezacaftor of primary CFBE cells with homogenous F508del-CFTR. Data generated from equivalent current assay (TECC-24 platform) after treatment of test compounds was done for 24 hours followed by acute stimulation with FSK (10µM) + Ivacaftor (1µM).**

**Figure 2: Graphs above demonstrate dose-response curves of FDL169 (left) and Tezacaftor (right) in the presence (blue curves) and absence (red curves) of 20% Human Serum. Shift in potency of Tezacaftor due to protein binding is ~20 fold higher whereas FDL169 potency shift is ~3.5 fold higher. Data above is reported as Ieq normalized with vehicle control were reported.**

**Figure 3: The effect of Chronic treatment on efficacy of FDL169/FDL176 in comparison to Tezacaftor/Ivacaftor. Blue bars are cells corrected with Tezacaftor (3µM) exposed for 24 hrs to different concentrations of FDL169. Red bars are cells corrected with Tezacaftor (3µM) exposed for 24 hrs to different concentrations of ivacaftor. For comparison, light blue and pink bars are cells treated with FDL169 and Tezacaftor and acutely stimulated with FDL176 and Ivacaftor at Cmax respectively. Data above is reported as AUC of increase in Ieq current normalized with vehicle control. Homozygous primary F508del CFBE cells were incubated for 24 hrs with corrector ± 24 hrs with potentiator and stimulated with FSK (10µM) + Ivacaftor (1µM).**

**Figure 4: Data above demonstrates the effect of chronic treatment on cell membrane expression of F508del-CFTR with FDL169/FDL176 in comparison to Tezacaftor/Ivacaftor. FBE41o expressing F508del-CFTR labelled with HRP were treated with Corrector ± Potentiator for 24 hrs. Plasma membrane CFTR is measured by chemiluminescence due to HRP exposed on cell surface (as RLU = relative luminescence unit).**

**Figure 5: Chloride transport evaluated in concentration matrix experiment. Primary F508del homozygous CFBE cells were treated for 24 hours with corrector-potentiator combination at various concentrations as shown on 3D graph. Cells were stimulated with FSK (10µM) + Potentiator and leq AUC values normalized to positive control were reported. Bar graphs on the right show the dose-responsive increase (with FDL176) versus its decrease (with ivacaftor) in chloride current at highest dose of corrector.**