In vitro Properties of F508del-CFTR Second Site Corrector FD2052160

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(NACFC 2017)

Introduction: Flatley Discovery Lab is developing a corrector that increases CFTR activity in combination with corrector FDL169 and potentiator FDL176. The *in vitro* activity of lead FD2052160 in F508del-CFTR expression and chloride transport assays is described.

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 correction by FD2052160 increased chloride transport in primary hBE cells with similar maximum efficacy as correctors FDL169, Lumacaftor and Tezacaftor. The effect of combination FD2052160 + FDL169 on chloride transport was approximately 2-fold higher than FDL169 alone; which was similar to the effect of FD2052160 in combination with Lumcaftor or Tezacaftor compared to Lumacaftor or Tezacaftor alone. A concentration matrix examining 24-hour exposure of primary F508del-CFTR cells to increasing doses of FDL169 and FD2052160 shows that peak efficacy of the combination is observed at the concentration of each corrector that gives its maximum response alone. In the HRP cell surface assay, FD2052160 increased the amount of F508del-CFTR in the plasma membrane by >2-fold compared to vehicle control. Combination FD2052160 + FDL169 further enhanced F508del-CFTR expression by >4-fold over vehicle control cells. In Western blot analyses, FD2052160 increased band C expression in primary hBE cells compared to vehicle control cells, and triple combination FD2052160 + FDL169 + FDL176 further increased band C expression.

Conclusion: FD2052160 is a F508del-CFTR corrector that increases protein expression and chloride current both alone and in combination with other correctors. Triple combination of FD2052160 with corrector FDL169 and potentiator FDL176 increases chloride current and F508del-CFTR expression >4-fold over vehicle treated cells. The FD2052160 series has the potential to increase efficacy of corrector/potentiator combinations currently in clinical development.