**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-115. A compound's potency 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-1 cells labeled with horseradish peroxidase (HRP) on the fourth extracellular loop. Cells were treated with test compound(s) for 24 hours and HRP exposure 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 2) was determined in primary F508del-CFTR cells following 48-hour exposure to test compounds.

**Results:** F508del-CFTR correction by FD2052160 increased chloride transport in primary NSBE cells with similar maximum efficacy as correctors FDL169, Lumacaftor and Tezacaftor. The effect of combination FD2052160 + FDL169 or chloroquine on transport was approximately 2-fold higher than FDL169 alone, which was similar to the effect of FD2052160 in combination with Lumacaftor or Tezacaftor compared to Lumacaftor or Tezacaftor alone. A concentration matrix using varying 24-hour exposure of primary NSBE 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 analysis, FD2052160 increased band C expression in primary NSBE 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.

**Fig. 1** Typical traces

**Fig. 2** FD2052160 increases correction to greater than corrector FDL169 alone

**Fig. 3** FD2052160 series increases correction to greater than Lumacaftor alone

**Fig. 4** Most Efficacious combination of 2160 and FDL169

**Fig. 5** Potency of FD2052160 is unaffected by other correctors or potentiators

**Fig. 6** Band C increases with co-correction

**Summary:**

1. FD2052160 increases current/protein when used in combination with FDL169, Lumacaftor, or Tezacaftor
2. Increase is additive.
3. Potency is not changed by other correctors
4. Maximum efficacy of combination is seen at maximum efficacy of each corrector separately

**Acknowledgements**

This work is supported by the Flatley Foundation.

**Flatley Discovery Lab**

Seeking a Cure for Cystic Fibrosis