EFFECT OF NOVEL F508DEL-CFTR MODULATOR COMBINATION FDL169 AND FDL176 ON EXPRESSION AND FUNCTIONAL ACTIVITY

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Abstract

Introduction: Reduced CFTR activity has been observed in primary CF cells upon prolonged treatment with lumacaftor + ivacaftor, which may decrease clinical benefit. Flatley Discovery Lab is developing a novel CFTR corrector, FDL169, and potentiator, FDL176, which in combination show greater in-vitro activity following chronic treatment compared to the lumacaftor + ivacaftor combination.

Method and Results: F508del-CFTR cell surface expression and functional activity were determined in cells treated with FDL169 + FDL176 or lumacaftor + ivacaftor. CFBE41o- cells transfected with the overexpressing F508del CFTR plasmid were used for western blot experiments and cells with HRP-tagged F508del CFTR plasmids were used for HRP cell surface assays. Homozygous F508del CF human bronchial cells (hBE) from lung transplants were used to determine functional activity in electrophysiological assays and for western blot experiments.

In western blot experiments and HRP assays, FDL169 increased CFTR maturation and cell surface expression in homozygous F508del-CFBE cells. In the equivalent current functional assay, homozygous F508del-CFTR hBE cells corrected with FDL169 (3 µM) and acutely exposed to FDL176 (1 µM) had a similar maximum chloride current response to cells treated with lumacaftor (3 µM) and acute ivacaftor (1 µM).

In HRP assays, a 60% reduction in CFTR surface expression was observed following 24 hours exposure of CFBE cells to lumacaftor (3 µM) + ivacaftor (2 µM), whereas a 30% reduction was seen in CFBE cells exposed to FDL169 (3 µM) and FDL176 (2 µM) (see Figure). Chronic exposure (24 hours) to FDL169 (3 µM) and FDL176 (1 µM) reduced the chloride current by 15% compared to exposure to both compounds under acute conditions; 24 hour exposure of cells to lumacaftor (3 µM) and ivacaftor (1 µM) decreased the chloride current by 55%.

Conclusion: FDL169 promotes CFTR maturation and cell surface expression with equivalent potency and efficacy to lumacaftor. Upon chronic treatment of primary F508del-CFTR hBE cells, the combination of CFTR corrector FDL169 and CFTR potentiator FDL176 had greater in vitro efficacy than the lumacaftor and ivacaftor combination

Dose Response of FDL169 and Lumacaftor on F508del-CFTR Expression

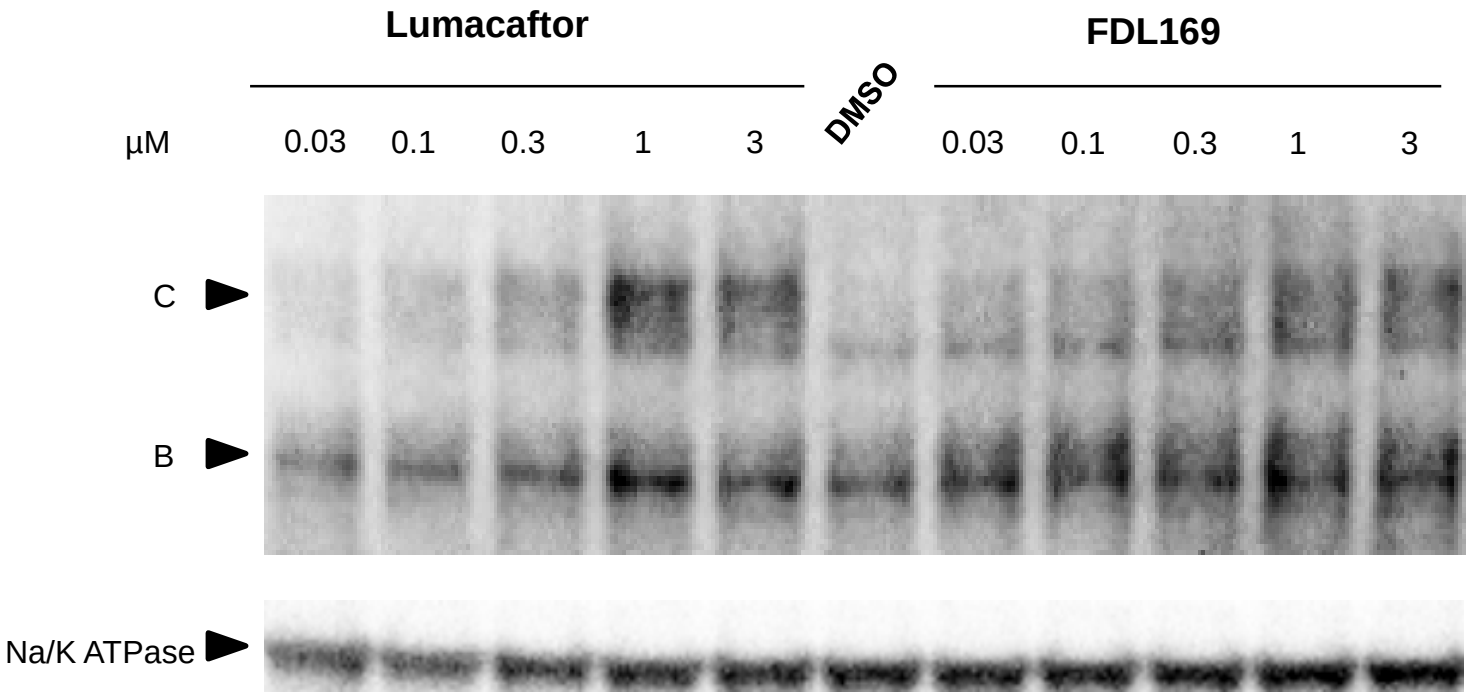


Figure 1. Western blot analysis with anti-CFTR antibody 596 and Na/K ATPase in primary homozygous F508del-CFTR hBE cells (patient code 036J) treated with lumacaftor and FDL169 (0.03, 0.1, 0.3, 1.0 and 3.0 µM). Test compounds were incubated for 48 hours prior to analysis. Lumacaftor and FDL169 showed equivalent efficacy and potency on F508del-CFTR maturation.

FDL169 Demonstrates Similar Efficacy and Potency to Lumacaftor

		Test Concentration (μM)						
		DMSO	0.03*	0.1*	0.3	1	3	10[#]
LUMACAFTOR	C	6.09 ± 2.08	7.30	9.19	11.02 ± 2.65	14.61 ± 4.21	14.19 ± 0.54	12.77 ± 0.66
	B	4.90 ± 2.76	5.97	7.38	4.51 ± 1.87	6.26 ± 2.42	5.95 ± 1.20	3.19 ± 0.04
	C/B	1.59 ± 1.02	1.22	1.24	2.65 ± 0.87	2.39 ± 0.34	2.46 ± 0.53	4.01 ± 0.16
FDL169	C		7.69	6.90	10.70 ± 1.89	11.24 ± 1.97	11.30 ± 0.39	10.04 ± 4.76
	B		7.22	5.43	5.58 ± 0.35	5.12 ± 1.12	5.01 ± 1.74	4.16 ± 0.84
	C/B		1.06	1.27	1.92 ± 0.37	2.22 ± 0.31	2.27 ± 0.25	2.35 ± 0.67

** Only tested in a single experiment with patient code 036J; [#] Tested in two experiments with different patient codes 036J and 020K*

Table 1: Summary of densitometric measurements of band C, band B and calculated C/B ratios in primary F508del-CFTR hBE cells treated with FDL169 and lumacaftor (or vehicle DMSO). Data (mean ± SD) are averaged across three independent experiments using two patient codes (except where noted).

HRP Cell Surface Assay in CFBE41o- Cells

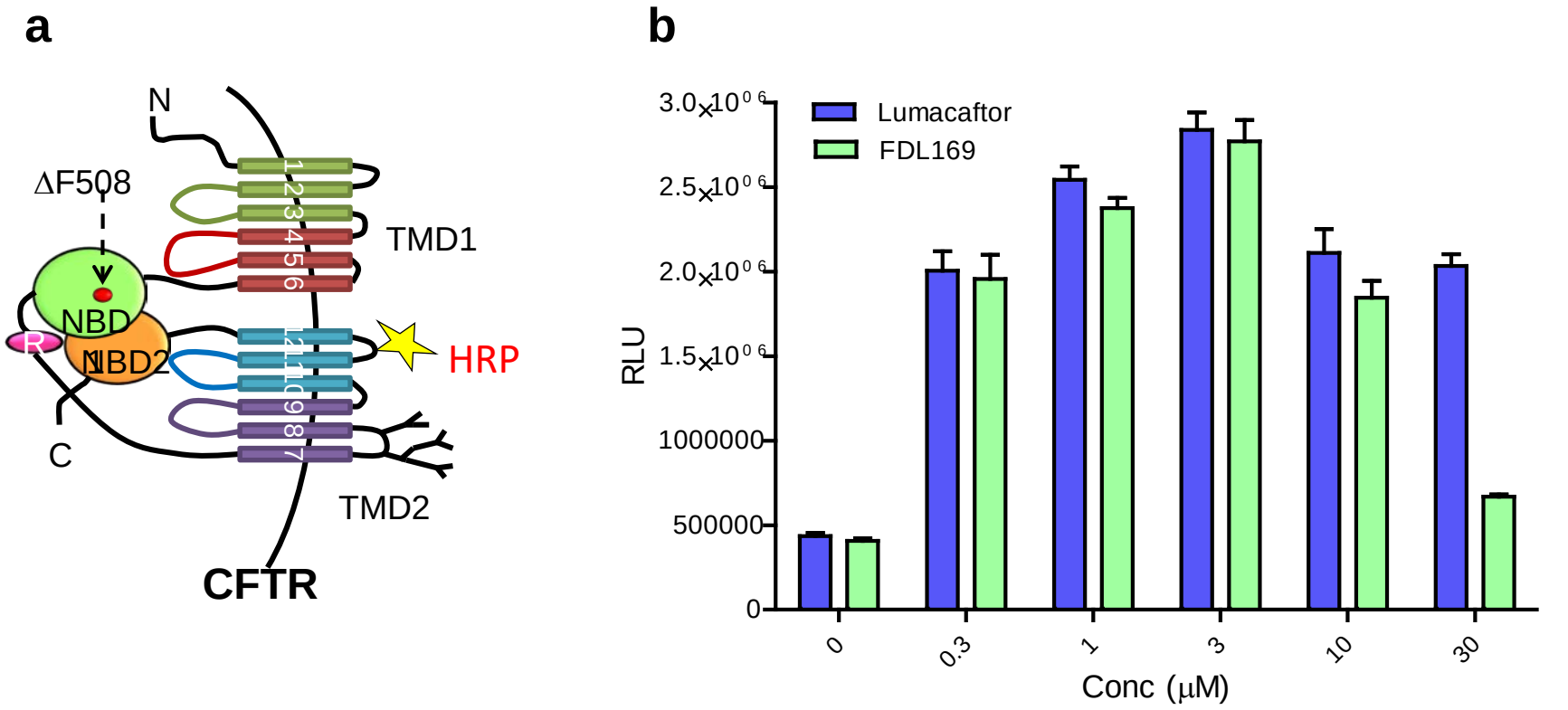


Figure 2. (a) Horseradish peroxidase (HRP) is inserted to the fourth extracellular loop of F508del-CFTR and is exposed on the cell surface when F508del-CFTR is at the plasma membrane. This represents the plasma membrane (PM) density of F508del-CFTR. (b) HRP cell surface assay of CFBE41o- cells treated with FDL169 and lumacaftor at 0, 0.3, 1, 3, 10 and 30 μM for 24 hours. Lumacaftor and FDL169 give a similar potency and efficacy on PM density of F508del-CFTR. (mean ± SEM, *n*=4)

Dose Response of FDL169 and Lumacaftor on F508del-CFTR Function

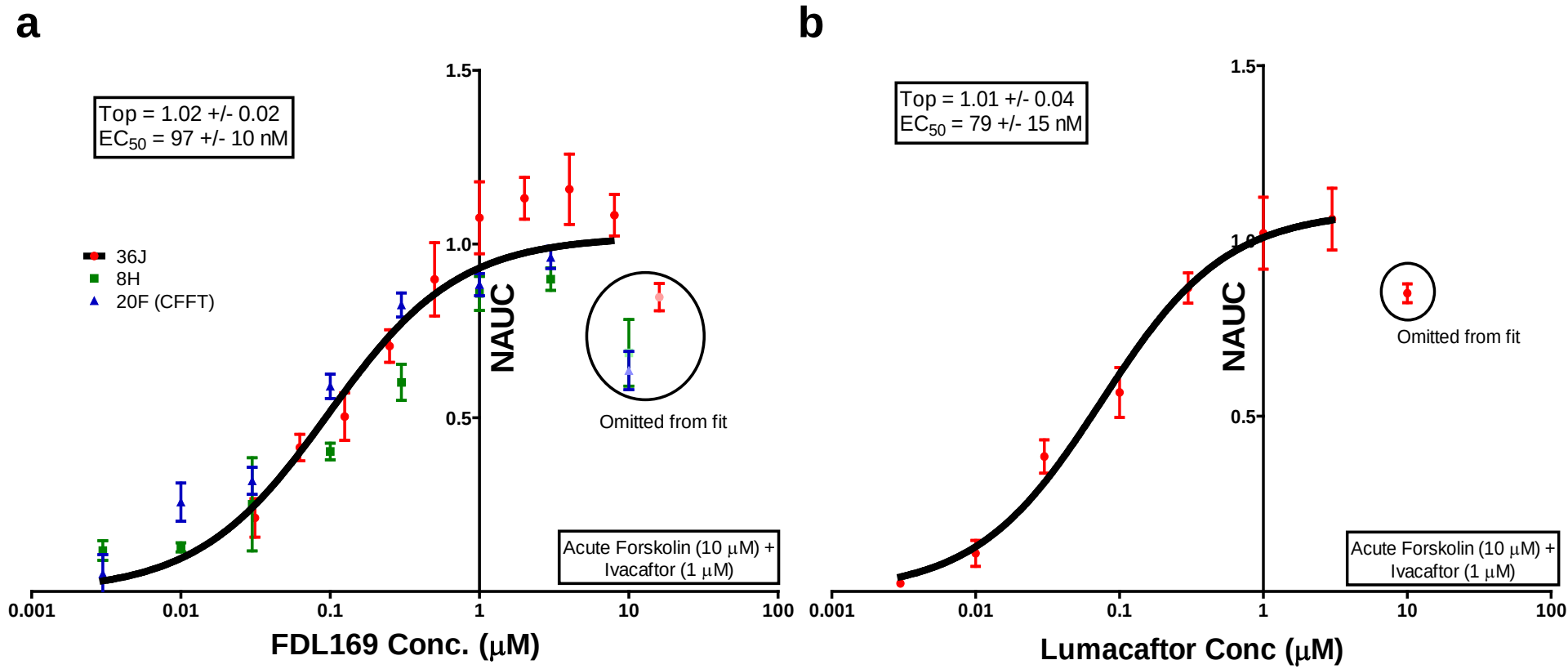


Figure 3. Functional activity of primary hBE cells treated with (a) FDL169 and (b) lumacaftor for 24 hours and acute addition of forskolin (1 μM) and ivacaftor (1 μM). NAUC represents the increase in chloride current after normalization to the maximum response produced by lumacaftor at 3 μM. FDL169 at 3 μM shows equivalent maximum response (NAUC~1) to lumacaftor.

Corrector-Potentiator Combination Treatment in CFBE41o- Cells

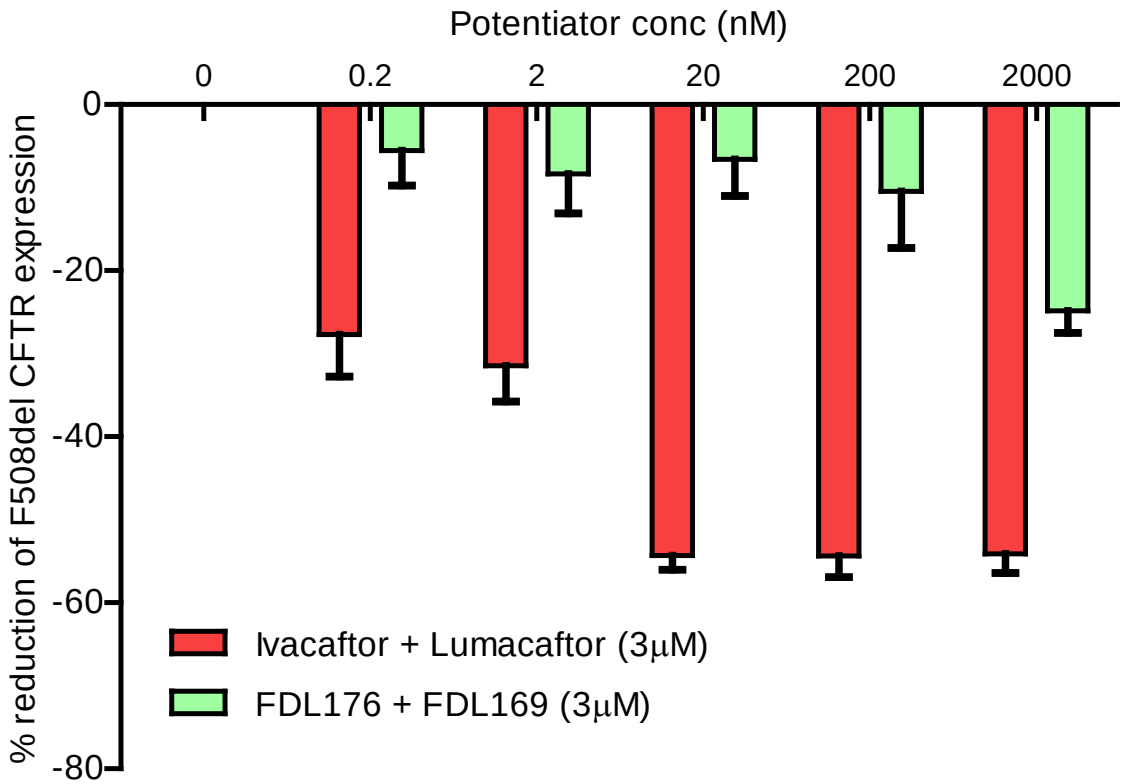


Figure 4. HRP cell surface assay showing the percentage reduction of F508del-CFTR PM density after treatment of ivacaftor/lumacaftor (3 μM) and FDL176/FDL169 (3 μM) for 24 hours in CFBE41o- cells. Ivacaftor reduced F508del-CFTR PM density in lumacaftor treated cells in a dose responsive manner achieving a maximum inhibition of ~55% at ≥ 20 nM. FDL176 demonstrates mild inhibition of F508del-CFTR PM density in FDL169 treated cells achieving a maximum inhibition of about 25% at 2 μM. (mean ± SEM, *n*=4)

F508del-CFTR Function in Primary F508del-CFTR Cells Following Combination Treatment

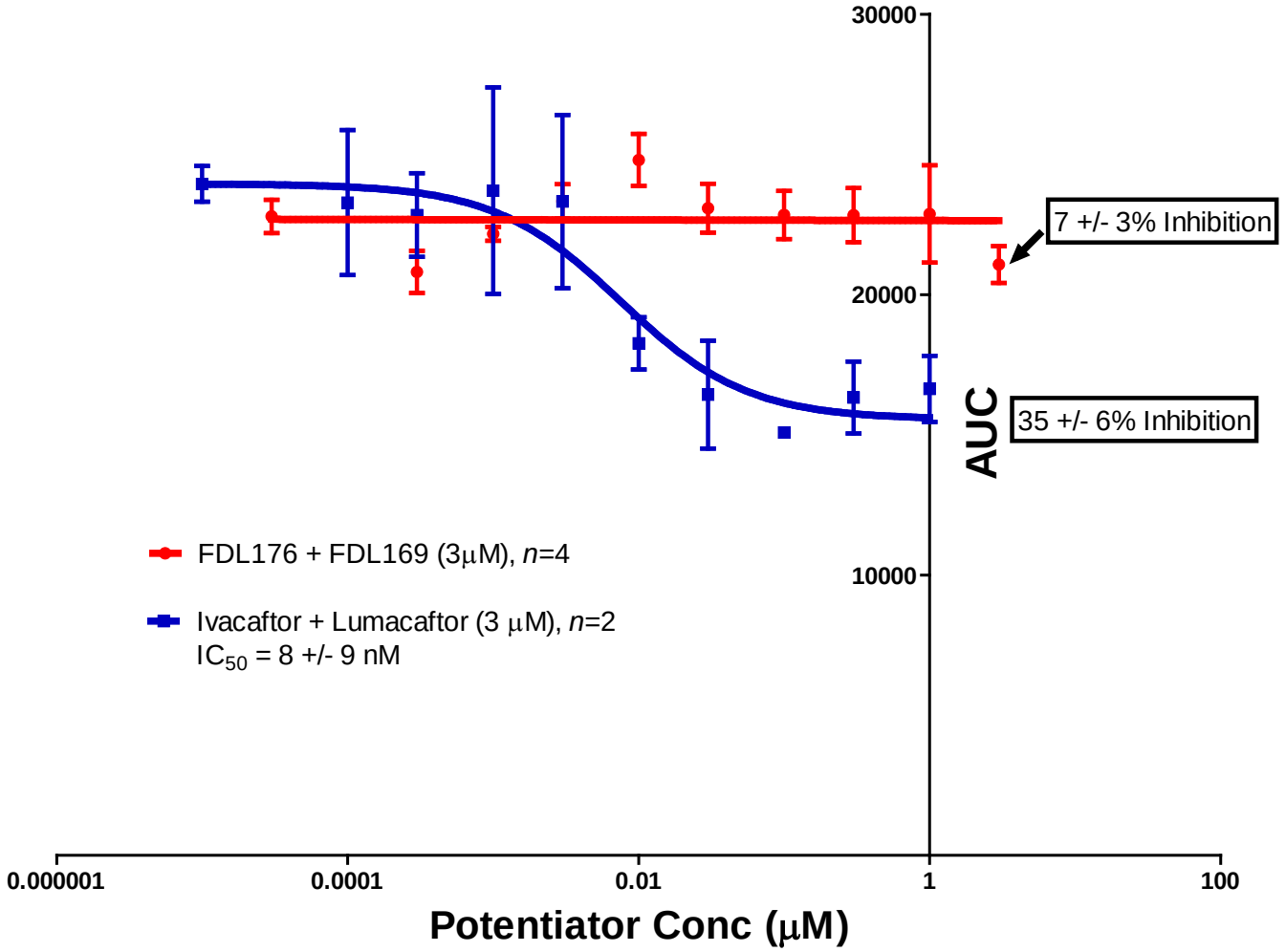
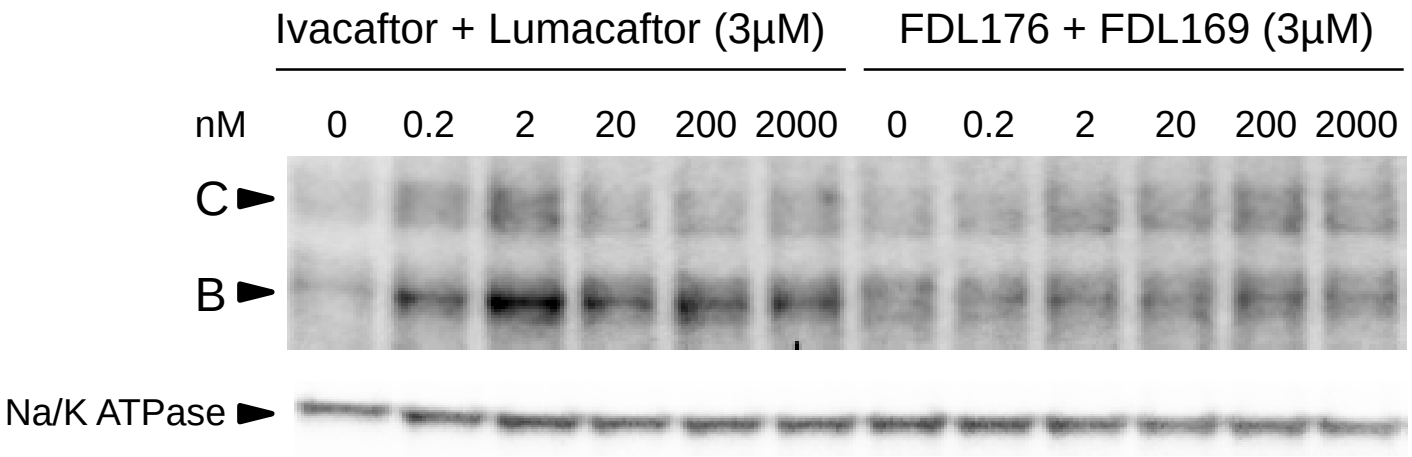


Figure 5. Chronic inhibition on cholride current in primary F508del-CFTR hBE cells treated with ivacaftor/lumacaftor (3 μM) and FDL176/FDL169 (3 μM) for 24 hours with acute addition of forskolin (1 μM) and ivacaftor (1 μM). AUC is the increase in chloride current after the treatment. Ivacaftor/lumacaftor shows 35% reduction in CFTR function while FDL176/FDL169 only shows 7%. (mean ± SD)

Corrector-Potentiator Combination Treatment in Primary F508del-CFTR hBE Cells



C	5.73	6.60	7.13	6.34	6.20	6.56	5.76	6.01	6.89	7.03	7.47	7.46
B	6.71	4.51	6.85	6.36	6.53	6.37	5.34	5.28	5.75	5.86	6.14	6.12
C/B	0.85	1.46	1.04	1.00	0.95	1.03	1.08	1.14	1.20	1.20	1.22	1.22

Figure 6. Western blot analysis with anti-CFTR antibody 596 and Na/K ATPase in primary F508del-CFTR hBE cells (patient code 036J) treated with ivacaftor/lumacaftor (3 μM) and FDL176/FDL169 (3 μM) for 48 hours. Expression levels of bands C and B were determined using densitometric analysis and data were normalized to Na/K ATPase control. The resulting data were use to calculate the C/B ratio. In this experiment, there is no significant reduction of band C in both combination treatments.

Conclusions

- FDL169 promotes F508del-CFTR maturation, plasma membrane density and function with equivalent potency and efficacy of lumacaftor in primary F508-del-CFTR hBE cells and CFBE41o- cells .
- HRP cell surface results indicate that FDL176 demonstrates mild inhibition of 25% at 2 µM on F508del-CFTR in FDL169 corrected CFBE41o- cells, whereas ivacaftor inhibited F508del-CFTR in lumcaftor corrected CFBE41o- cells >50% at concentrations of ≥ 20nM.
- F508del-CFTR function was reduced by 7% following chronic treatment of primary hBE cells with FDL176/FDL169 and 35% by ivacaftor/lumacaftor.
- FDL176/FDL169 and ivacaftor/lumacaftor combination treatments did not show significant reduction of band C expression in Western blot analysis of primary F508del-CFTR hBE cells.