THE PATH TO 30% AND BEYOND: DISCOVERY AND DEVELOPMENT OF SECOND GENERATION CFTR MODULATORS

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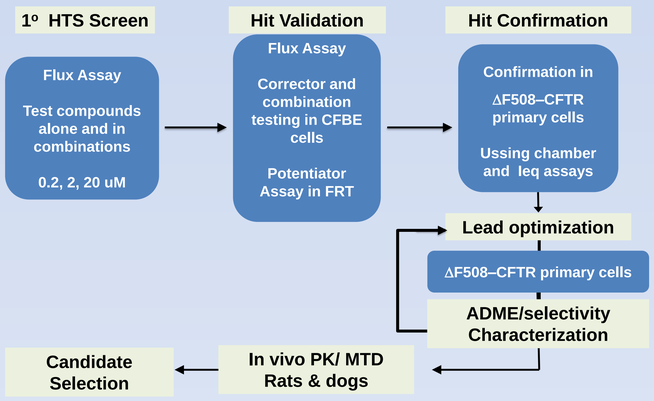
Abstract: Over recent years, the CF research community has made great strides in bringing promising new therapies, which treat the underlying cause of this devastating genetic disease, into clinical trials. The FDA approval of Kalydeco for the treatment of patients with the G551D mutation demonstrates that CFTR modulation by a small molecule drug can produce significant clinical benefit. Additionally, recent publications indicate that the combination of a potentiator and a corrector promotes modest clinical efficacy in treating the most prevalent mutation ΔF508-CFTR. It is widely believed that the path to a cure for ΔF508-CFTR patients will require a combination of two or more compounds, of complementary mechanism of action (MOA), which improves chloride ion current to ≥30% of wild-type CFTR with sustained efficacy upon chronic treatment.

Flatley Discovery Lab (FDL) is pursuing the discovery and development of small molecule modulators that restore ΔF508-CFTR-mediated chloride ion transport to ≥30% of wild-type CFTR levels upon chronic treatment. FDL high throughput screening campaigns and additional lead identification efforts delivered two chemically distinct hits that each work in combination with first generation corrector FDL304 (or VX809) in primary cultures of ΔF508-CFTR human bronchial epithelial cells to modestly enhance chloride ion flux 1.2-2 fold over positive control, cells corrected with VX809 alone. Lead optimization of each series using primary ΔF508-CFTR hBE cells corrected with a first generation corrector has delivered multiple compounds that work in combination treatments to increase chloride ion current 2-3.5 fold over the positive control, approaching 30-50% of wild-type CFTR activity at test concentrations ≤ 10 μM. Preliminary data suggest that each of the FDL second generation correctors works directly on CFTR through different, complementary MOAs.

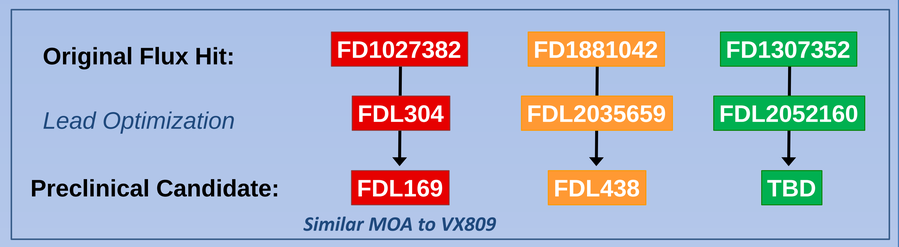
FDL Lead Optimization Platform

Development of ΔF508 Modulators

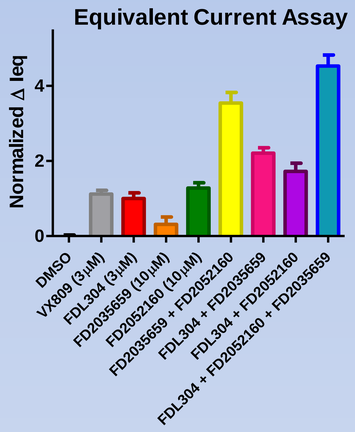


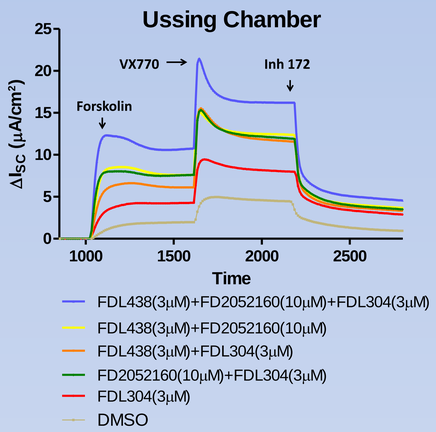
Acknowledgements: This work is supported by the Flatley Foundation.

Three Chemically Distinct and Synergistic Mechanisms

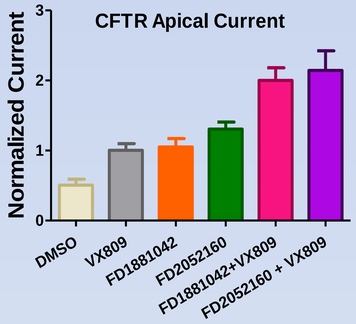


FDL Combinations in Primary CF Cells:





FDL Correctors Directly Modulate CFTR



Experimental Conditions:

- ENaC blocked with Benzamil
- Driving force blocked with Barium (block K+ channels)
- Ouabain used to block any chemical gradients
- Basolateral membrane permeabilized with Amphotericn B
- Set 10/1 chloride ion gradient

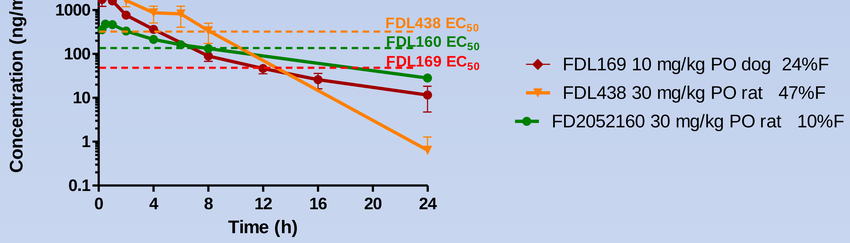
EPY Results and Conclusions:

- FDL identified three complementary mechanisms for enhancing CFTR Cl^- current
- FDL438/FD2052160 treatment is 2 fold the efficacy of Vx809 or FDL304
- FDL438 doubles the efficacy of first generation correctors VX809 & FDL304
- FD2052160 doubles the efficacy of first generation correctors VX809 & FDL304
- The triple combination of FDL304/FDL438/FD2052160 is approximately 3-4 fold more efficacious than Vx809 or FDL304
- The triple combination is ~40% WT (WT CFTR gives 45 μA/cm² in the Isc assay)

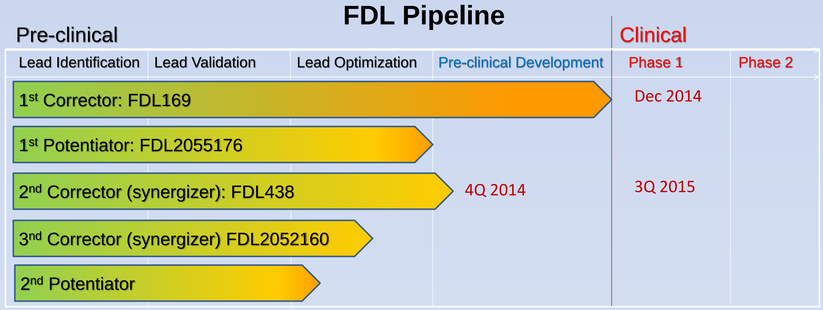
Pre-Clinical Profile of FDL ΔF508-CFTR Modulators

Efficacy and Potency in Primary CF hBE cells			
Criteria	**FDL169**	**FDL438**	**FDL2052160**
Efficacy-alone	Equiv to Vx809	~1/2 of Vx809	Equiv to Vx809
Efficacy w/ 1st gen corr	---	2-fold VX809 alone	2-fold VX809 alone
Efficacy of dual Combination	---	2-fold VX809 alone	
Efficacy-triple Combination	3 to 4-fold Vx809 alone or ~40% of WT CFTR		
Potency (EC_{50})	0.094 μM	~0.75 μM	0.3 μM
Multiple Patient Code Activity	YES	YES	YES
***In vitro* ADME and Safety Pharmacology**			
HLM t½	~15 min	>1 hr	6 min
Human hepatocyte t½	1 h	>>1 hr	TBD
CYP Inhibition: 5 isoforms	IC_{50} > 50x Ieq EC_{50}	IC_{50} > 30x Ieq EC_{50}	IC_{50} > 20x Ieq EC_{50}
CYP3A4 Induction	Modest, IC_{50} ~3 μM	none	TBD
hERG IC_{50}	IC_{50} > 70x	IC_{50} > 100x	IC_{50} > 100x
Off-target selectivity	IC_{50} 50x > EC_{50} across majority of 80 targets		TBD
***In vivo* Pharmacokinetics**			
Oral availability	> 20% F (dog)	> 40% F (rat)	10% F (rat)
Oral Exposure	Exposure ≥ EC_{50} 12 hr	Exposure ≥ EC_{50} 8 hr	Exposure ≥ EC_{50} 8 hr

Oral Exposure of FDL ΔF508-CFTR Modulators



FDL Pipeline



Summary:

- FDL developed three chemically and mechanistically distinct series that work in combination to increase CFTR-mediated Cl^- flux up to ~40% of WT-CFTR.
- Corrector FDL169 works similarly to VX809 and is on track to begin FIH clinical studies late Dec 2014.
- FDL438 doubles the efficacy of FDL304 (or VX809) corrected primary CF cells and has entered preclinical development.
- FD2052160 doubles the efficacy of FDL304 (or VX809) corrected cells
- Dual combination of FD2052160 and FDL438 is 2-fold more efficacious than VX809 (or FDL304) correction.

Flatley Discovery Lab

Seeking A Cure for Cystic Fibrosis