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DISCOVERY AND DEVELOPMENT OF NOVEL ΔF508-CFTR CORRECTORS

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Abstract:

Cystic fibrosis is a devastating genetic disease that is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Until recently, most CF drugs focused on treating symptoms of the disease but not the underlying cause of non-functioning CFTR protein. Small molecule modulators that correct the most prevalent mutation, ΔF508-CFTR, have shown some clinical efficacy and present an exciting opportunity to deliver new breakthrough therapeutics to CF patients. Flatley Discovery Lab (FDL) is dedicated to the discovery and development of small molecule modulators that correct ΔF508-CFTR misfolding to enhance protein trafficking and restore CFTR-mediated chloride ion transport.

The FDL high throughput screening campaign identified FD1027382, which is a novel ΔF508-CFTR corrector that demonstrates modest activity in promoting iodide influx in CFBE cells transfected with ΔF508-CFTR. Subsequent screening in primary cultures of ΔF508-CFTR human bronchial epithelial cells (hBE cells) confirmed the ability of FD1027382 (20 μM) to restore approximately 20% of the chloride ion flux response as compared to positive controls, Vx-809 and CF-C18. Hit to lead optimization of this FDL HTS hit has delivered multiple compounds with maximum efficacy equivalent to positive controls at test concentrations ≤ 1 μM. The efficacy of FDL lead compounds in primary ΔF508-CFTR hBE cells and their preclinical evaluation for safety, selectivity and oral bioavailability will be presented.
(Supported by the Flatley Foundation)

Flatley Discovery Lab: Our Mission

FDL is a non profit research organization dedicated to finding a cure for cystic fibrosis

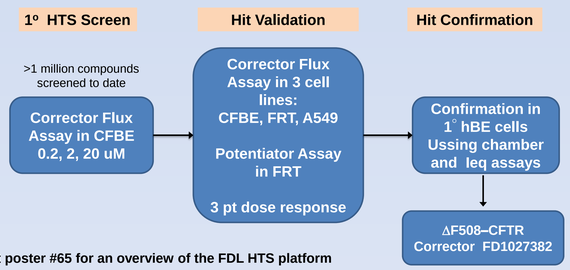
Our goal is to identify small molecule modulators of ΔF508-CFTR that restore CFTR-mediated chloride ion transport

The FDL pipeline is strong with compounds being developed in multiple synergistic mechanisms

FDL Core Capabilities:

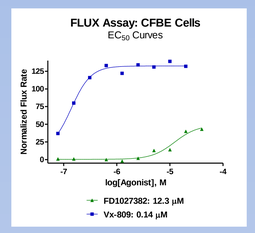
- HTS facility operating whole-cell screening
- Molecular biology lab to conduct mechanism of action studies and develop novel corrector assays
- Electrophysiology lab to determine ion channel activity in human primary ΔF508-CFTR homozygous bronchial epithelial cells
- Medicinal chemistry expertise to develop HTS hits into clinical candidates
- Computational and in-silico modeling capabilities

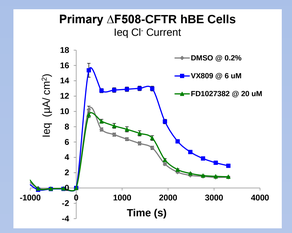
Identification of ΔF508-CFTR Corrector FD1027382



Please visit poster #65 for an overview of the FDL HTS platform

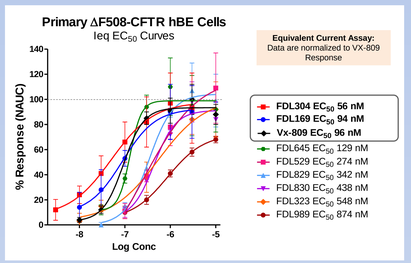
Activity of FD1027382: FDL Starting Point





HTS hit FD1027382 elicits ~20% maximum response in primary ΔF508-CFTR human bronchial epithelial cells (hBE) compared to positive control Vx-809 (AUC normalized to Vx-809 response, NAUC = 0.2)

Lead Optimization: Improved Potency & Efficacy



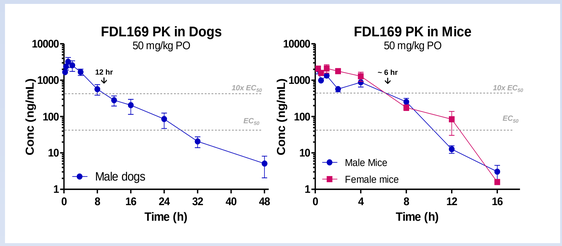
- The FDL lead optimization effort developed multiple analogs that achieve efficacy equivalent to Vx-809 with good potency (EC_{50} <1 μM)
- FDL169 & FDL304 are the most promising compounds developed
- Compounds in this series are not synergistic with Vx-809

Pharmacokinetic Profile of FDL169

	Male SD Rat PK Dose : 1 mg/kg IV			Male Beagle Dog PK Dose : 1 mg/kg IV		
ID	t 1/2 (h)	AUC (h*ng/mL)	Vz_F_ (L/kg)	t 1/2 (h)	AUC (h*ng/mL)	Vz_F_ (L/kg)
FDL169	1.3	693	2.8	9.1	1009	15.3
Vx-809	3.3	14,789	0.3			

Unlike Vx-809, FDL169 distributes well into peripheral tissue as evidenced by a large apparent volume of distribution in rats and dogs

Oral PK: Exposure of FDL169 in Dogs & Mice



FDL169 demonstrates good exposure with plasma levels ≥ 10x EC_{50} for ≥ 6 hr after a single oral dose in male dogs and mice

Pre-Clinical Profile of FDL Lead Correctors

Efficacy and Potency in Primary CF hBE cells			
Criteria	**Desired Endpoint**	**FDL304**	**FDL169**
Corrector Efficacy	≥VX-809 & VX-661	Equiv to Vx	Equiv to Vx
Corrector Potency	< 1 μM	0.056 μM	0.094 μM
Patient Code Activity	Independent of patient code	YES	YES
Potentiation w/Vx-770	Acts in combination	YES	YES

In vitro ADME and Safety Pharmacology			
Criteria	**Desired Properties**	**FDL304**	**FDL169**
CYP Inhibition: 5 isoforms	CYP IC_{50} 30x > Ieq EC_{50}	IC_{50} > 50x	IC_{50} > 50x
CYP Induction	No/minimal induction at >100x EC_{50}	2-fold	2-fold
Cardio safety	hERG, Na1.5, Ca-L IC_{50} 30x > EC_{50}	IC_{50} > 100x	IC_{50} > 70x
Off-target receptor	IC_{50} 10x > EC_{50} across 80 targets	IC_{50} > 50x for majority of targets	

In vivo Pharmacokinetics			
Criteria	**Desired Properties**	**FDL304**	**FDL169**
Oral availability	≥ 20% of Dose	18%F	38%F
Half life	Supports tid oral dosing or better	Supports tid	Supports bid
Exposure	Plasma levels >10 x EC_{50} for ≥4 hours	4 hr in dogs	10 hr in dogs

FDL169 was selected for preclinical development based on its superior potency, efficacy, safety and pharmacokinetic profile

FDL Progression Strategy & Timeline

4Q2010 — **Primary HTS**
FLUX assay in CFBE cells
↓ *Emax >0.4, no cytotoxicity*

1Q2011 — **Hit Validation**
Flux assay in CFBE, FRT & A549 cells; Active in 1° CF-hBE cells
↓ *Corrector activity in ≥ 2 cell lines; CF hBE Emax ≥0.2*

2-3Q2011 — **Hit follow-up**
Flux assay, CF-hBE Ieq and Ussing Chamber assays
↓ *≥ 3 analogs active in primary CF hBE cells*

4Q2011 — **Lead Series Identification**
↓ *Improve potency, efficacy & drug-like properties*
Med Chem Optimization
↓ *Potency/efficacy ≥ Vx809, feasible path to IND*

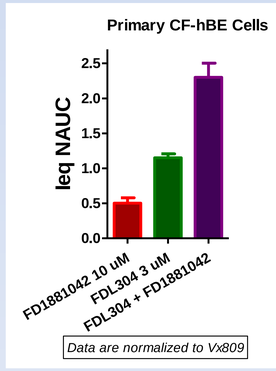
3Q2013 — **Pre-IND candidate:**
ΔF508-CFTR Corrector FDL169

Summary

- Developed ΔF508-CFTR correctors with efficacy similar to Vx-809 and EC_{50} <1μM
- FDL169 was selected for preclinical development
  - Excellent efficacy and potency in CF-hBE cells
  - Very good safety pharmacology profile
  - Pharmacokinetics that support bid or tid dosing in man
- FDL169 is not synergistic with Vx-809

Future Research at FDL

- FDL is pursuing leads that work in synergy with FDL169
- Promising compounds such as FD1881042 work in combination with FDL169 and FDL304 to restore chloride ion transport >2-fold that of positive control (3 μM Vx-809) in primary CF-ΔF508-CFTR human bronchial epithelial cells



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Flatley Discovery Lab

Seeking A Cure for Cystic Fibrosis