Fixing ΔF508-CFTR: Bringing New Correctors into the Fold

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Presenter Disclosure

- Andrew Kolodziej, Ph.D.
- No conflicts to disclose
- Flatley Discovery Labs is a not-for-profit company wholly supported by the Flatley Foundation
About FDL

- Independent not-for-profit biotech
- Focused on small molecule drug discovery & development of CFTR modulators
- Supported by The Flatley Foundation
- Located in Charlestown, MA
- Lab operation started August 2009
- HTS initiated August 2010
- ~6,000 sq. ft.
- Currently 24 FTE & 4 volunteers
- Current Capabilities: HTS, electrophysiology, molecular biology, Chem/Bioinformatics
CF Lung Disease

normal

CF

Courtesy Alan Verkman, UCSF
Cystic Fibrosis: A Lethal Orphan Genetic Disorder

- Autosomal recessive mutations of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene.
- 70,000 patients WW; 30,000 in U.S.
- Most CF patients (>70%) carry the ΔF508 mutation
- Widely expressed in epithelia
- Many affected organs: lung, skin, pancreas, small intestine, vas deferens.
- Cystic Fibrosis patients are prone to chronic infections and inflammation that cause gradual lung damage and reduce life expectancy to the mid-30s.
- Disease severity is genotype dependent
- Different therapies are needed to address different mutations.
ΔF508-CFTR: Structure and Dysfunction

- Membrane bound glycoprotein, 1480 aa
- An ABCC type anion channel: Cl⁻, HCO₃⁻
- cAMP, ATP dependent
- Regulatory “R” domain is a unique feature not present in other ABC transporters
- ΔF508 located within Nucleotide Binding Domain-1

Serohijos et al. PNAS, 105, 9, 3257.
Pathophysiology & Treatments of Cystic Fibrosis

- Underlying Cause of CF
  - Defective CF gene
  - Deficient CFTR protein
  - Decreased chloride secretion
  - Altered ionic transport
  - Increased water absorption
  - Abnormal mucus composition
  - Bronchial obstruction
  - Bacterial infections
  - Inflammation
  - Bronchiectasis and lung insufficiency

Most approved drugs treat CF symptoms
- Need to treat underlying cause of CF

- GENE CORRECTION
- PROTEIN THERAPY
- CFTR MODULATORS
- POTENTIATORS KALYDECO
- CORRECTORS VX-809
- ASL RESTORATION
- ALTERNATIVE ION CHANNELS
- OSMOLYTES
- MUCUS MODIFICATION
- MUCOLYTICS PULMOZYME
- ANTIMICROBIALS
- INHALED TOBRAMYCIN
- ANTI-INFLAMMATORY
- IBUPROFEN
- LUNG TRANSPLANT
- IMMUNOSUPPRESSANTS
Correctors and Potentiators

$\Delta F_{508}$-CFTR

**Low Cell Surface $\Delta F_{508}$-CFTR**

- VX-809
- VX-661

**CFTR Corrector**

- Increased Cell Surface $\Delta F_{508}$-CFTR

- VX-770
- Ivacaftor

**CFTR Potentiator**

- Increased $\Delta F_{508}$-CFTR Expression and Activity

$G_{551D}$-CFTR

- VX-770
- Ivacaftor

http://www.cftr.info

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Gene Specific Therapy: 1977 CF-Causing Alleles

North American Patients by Mutation

- F508del Homozygotes: 14,400
- F508del Heterozygotes with no Gating Mutation: 10,500
- F508del Heterozygotes with a Gating Mutation: 2,970
- Gating Mutation with no F508del: 1,500
- Other: 510

Bonnie W Ramsey, MD "Roadmap to a Cure (Part 2) Clinical Research Pathway to Ensure That All Patients With CF Benefit From Novel Therapies"
Cystic Fibrosis Demands Combination Therapies

- Mutations result in insufficient and/or ineffective CFTR
- ΔF508 mutation results in both, necessitating a corrector to increase channel number and a potentiator to improve channel gating
- Corrector VX809 and Potentiator VX-770 (Ivacaftor) had no clinical efficacy as monotherapy in ΔF508 homozygous patients
  - VX-770 effective in G551D and R117H patients
- Phase 3 Combination Trials of VX809 plus Ivacaftor yielded 2.6-4% mean FEV1 improvement and mean relative improvements of 4.3 percent to 6.7 percent
CFTR Modulators: Safety Pharmacology

Issues

- Correctors are designed to reset CFTR activity to normal
- Selectivity and specificity for modulating CFTR folding and trafficking is important
  - Mechanism of efficacy: increased channel number or channel activity?
  - Are increases in chloride current mediated through CFTR or are other channels involved?
  - Is “folding” generally targeted?
- Chronic vs. acute effects on CFTR maturation
- Combination therapy: DDI concerns
CFBE41o- HTS YFP-Quench Assay

Human CF Bronchial Epithelial 1\textsuperscript{st} Cells

Robotic Platform: Equivalent Current $I_{eq}$

Ussing Short Circuit $I_{sc}$

$$y = (A_0 - A_1)e^{-tQR} + A_1$$

$QR$ = YFP quenching rate

$NQR = (QR - QR_{NC})/(QR_{PC} - QR_{NC})$
FDL Screening Cascade

Primary HTS
CFBE cell
Corrector protocol (3x2)

Validation HTS
CFBE, FRT, A549
Corrector & potentiator (3x3)

SAR HTS
CFBE, FRT, A549
Corrector, potentiator (10x5)

Primary hits

Validated hits

2nd Assays (EPY & Biochem)

Cytotoxicity
hERG
ADME & PK

Lead series SAR

>1,000,000
HTS Primary

>4,000
HTS Verification

>100
hBE Verification

>30
Verified leads

LEAD

7/12/2017
FDL169: Efficacy and Potency

- FDL169 achieves similar efficacy and potency to Vx-809 in primary ΔF508-CFTR human bronchial epithelial cells
- No synergy with VX-809

Ussing Assay Traces

Equivalent Current Assay

<table>
<thead>
<tr>
<th>Benzamil 3 μM</th>
<th>Forskolin 10 μM</th>
<th>VX770 1 μM</th>
<th>CFTR Inhibitor</th>
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</thead>
</table>

FDL169: EC₅₀ 94 nM
VX-809: EC₅₀ 96 nM
FD1027382: EC₅₀ 12 uM
FDL169 Enhances CFTR Trafficking

- Western blot assay demonstrates maturation of CFTR to core glycosylated form (Band C)
Intestinal Organoid Swelling Assay

Data courtesy Sylvia Boj, Hubrecht Institute University of Utrecht

Rectal biopsy derived cells, treated 24 h in culture, stimulated with 2 µM forskolin, 3 µM VX-770

FD2027304 (1µM) + VX-770 (3µM)

DMSO
Organoids: Theranostic Testing of CFTR Correctors

- Corrector response quantifiable as AUC
- Easily harvested
- Cells provide greater flexibility: expandable and passageable
- Individual patients and genotypes can be evaluated
FDL169 Clinical Candidate

- FDL169 is a CFTR corrector with equivalent in vitro efficacy and potency to lumacaftor (Vx-809).
- FDL169 has excellent biodistribution.
- FDL169 has an excellent preclinical safety profile.
- FDL169’s pharmacokinetic profile supports t.i.d. or b.i.d. dosing.
- FDL has completed IND-enabling preclinical studies on FDL169.
- FDL169 exhibits a favorable in vivo pharmacology safety profile at exposure levels well above those expected to be efficacious.
- FDL expects to file a CTA in Q4 2014.
- FDL has filed composition of matter patents to cover FDL169 and related molecules.
FDL Second Generation Correctors
multiple, distinct folding barriers to overcome

VX-809

Soo Jung Kim and Skach
HTS flux assay was tuned to provide a dynamic range ~3-fold higher than the response of Vx-809 alone.
Representative HTS Combination Data
Three CFTR Correctors: Multiple Combinations

Chemically Distinct and Mechanistically Synergistic

MOA 1
Similar MOA to VX809
Original Hit: FD1027382
Lead Optimization: FDL304
Preclinical Candidate: FDL169

MOA 2
Synergizes w VX809
Original Hit: FD1881042
Lead Optimization: FD2035659
Preclinical Candidate: FDL438

MOA 3
Synergizes w VX809
Original Hit: FD1307352
Lead Optimization: FD2052160
Preclinical Candidate: TBD
**FDL Correctors: Equivalent Current Assay**

- **FDL304** efficacy is ~90% of VX809
- EC50: 32 nM
- No synergy/additivity with VX809

- **FD2052160** efficacy is ~90% of VX809, EC50=0.5 µM
- **FD2035659** efficacy is ~40% of VX809, EC50=2.4 µM

**Conditions:** ΔF508-CF-hBE, 24 hr treatment; 3 µM FDL304, 10 µM FD2052160, 10 µM FD2035659
Synergistic behaviors suggest three separate and independent mechanisms.
Substantial correction that exceeds 30% wt current.
FD1881042 and FD2052160 Enhance ΔF508-CFTR Cell Surface Expression
FDL438: Activity & ADME Profile

- More than doubles efficacy of current corrector regimes in primary cells
- Increases cell surface expression, alone or in combination with VX-809

**leq Dose Response**
VX809 corrected hBEs

- Emax 2.2
- EC50 0.75 µM

**Cell Surface Expression**

ΔF508-CFTR

**HRP Activity**

- FDL438
- FDL438+VX809

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FDL438: *in vitro* ADME & Safety Pharmacology

- Excellent *in vitro* ADME & cardiac safety profile
- No CYP3A4 induction
- Off target: clean vs. kinase & GPCR panels
- Moving forward to development

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<th>Cardiac Safety and CYP Profile</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (%inhib @ 30)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;/EC&lt;sub&gt;50&lt;/sub&gt;</th>
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<td>hERG IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>&gt; 100 µM</td>
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<td>CYP 3A4 induction</td>
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<th>Metabolic Stability (37 °C)</th>
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<td>HLM t&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>&gt; 60 min</td>
<td></td>
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<tr>
<td>Hepatocytes</td>
<td>&gt; &gt;60 min</td>
<td></td>
</tr>
<tr>
<td>Human Plasma</td>
<td>100% @ 2 hr</td>
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Correctors Modulate Apical Current

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 Amphotericin B
- Set 10/1 chloride ion gradient
FDL Correctors Do Not Induce Heat Shock Response

- **Hsp70**
  - **37°C**: Low
  - **42°C**: High
- **Hsp40**
  - **37°C**: Low
  - **42°C**: Low
CFTR N-Terminal Fragment Stabilization

- **FDL304**
- **FD2052160**
- **FD1881042**
- **VX809**
- **DMSO**

**Data:** Hongyu Ren and Doug Cyr, University of North Carolina

- **380X**
- **653X**
- **837X**
- **1172X**

**N-Terminus**

- **FD1881042:** stabilizes 380X and synergizes with VX809
- **FD2052160:** no stabilization or synergy
CFTR C-Terminal Fragment Stabilization

C-terminus
- FD1881042 stabilizes
- FD2052160 destabilizes

FD2052160
FD1881042
FDL304
DMSO

837-1480

% Control: 100 71 170 24 160 150 230 34 130 23

Data: Hongyu Ren and Doug Cyr, University of North Carolina
FDL Second Generation Correctors

multiple, distinct folding barriers to overcome
FDL Potentiators: Potency & Efficacy in ΔF508–CFTR hBEs.
“Potentiator Ivacaftor Impedes Pharmacological Correction of ΔF508 CFTR” (Poster#53 ; 2013 NACFC) (Cholon, et.al.)
Chronic exposure to Ivacaftor (Vx770) inhibits correction of ∆F508 primary cells.
VX770 Chronic Effect is Related to Potentiator Activity

**VX770 Conc. (µM)**

<table>
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<tr>
<th>VX770</th>
<th>VX809 (3 µM)</th>
<th>IC$_{50}$ = 3 +/- 1 nM</th>
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<tbody>
<tr>
<td></td>
<td>+VX770</td>
<td>IC$_{50}$ = 2 +/- 1 nM</td>
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**Vehicle Corrected**

**Relative CFTR Activation**

EC$_{50}$ = 5 nM

**VX770 Conc. (µM)**

**NAUC**

**[VX-770] µM**
Chronic FD2033129 Does Not Inhibit ΔF508-CFTR Trafficking
Potentiator Effects are Independent of Correction

Normalized Current Response (NAUC)
FD2033129 Does Not Reduce Surface Expression of ΔF508 CFTR
Two new mechanistically distinct pathways to CFTR correction now identified
- **FDL169, FDL304, VX809**
- **FDL438**
- **FD2160**

Corrector combinations elicit robust synergistic correction
- Two way combinations: FDL304 + FD5659 & FDL304 + FD2160
- Promising clinical development path: **FDL438**

**SPS: Synergies in trafficking effects need better surveyance**

**Target destabilization through agonism: CFTR specific?**

New correctors and respective pathways represent potential new routes to address non-\(\Delta F508\) associated folding defects

50% correction is in sight!
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R1070W-ΔF508-CFTR

I539T-ΔF508-CFTR
Stabilization of CFTR folding mutations reflects corrector mechanistic differences

- **FDL304** corrects all mutants tested: R117H, V232D, R1070W, 1172X, 1282X, and N1303K.
- **FD1881042** may increase Band B but requires FDL304 to overcome folding defects & increase trafficking.
- **FD2052160** increases C/B ratio of CFTR folding mutations R117H and R1070W.

Data courtesy Hongyu Ren and Doug Cyr, University of North Carolina
Mutant CFTR Correction: C/B

- **FDL304**
- **FD1881042**
- **FD2052160**
FD1881042 Promotes Band B Expression