Phenotypic Characterization of CFTR Drugs, VX-770 and VX-809, in BioMAP® Human Primary Cell Systems Connects Target Biology to Disease Mechanisms

Ellen L. Berg, Sharlene Velichko, Alexandra Folias, Daniel Bassoni, and Alison O'Mahony
DiscoveRx Corporation, Fremont, CA 94538

Abstract

Cystic fibrosis (CF) patients have mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel that result in reduced membrane expression and function. CF patients have impaired lung function due to chronic airway inflammation driven by dysregulated Th17 and Th2 immune responses. These responses lead to overproduction of mucous and increased susceptibility to *Pseudomonas aeruginosa* infection, key hallmarks of the disease.

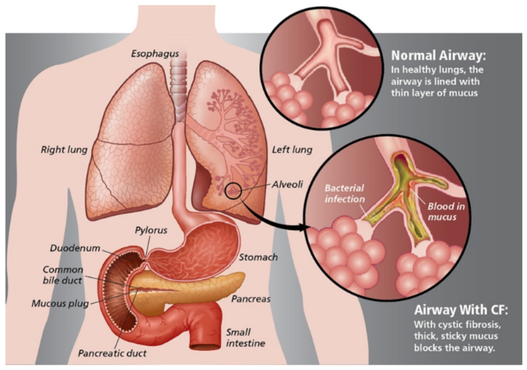
VX-770 and **VX-809** are small molecule drugs designed to increase CFTR function. VX-770 is a potentiator of CFTR activity and VX-809 acts as a pharmacochaperone that has been shown to rescue expression of the CFTR-ΔF508 mutant that is susceptible to misfolding. VX-809 is active in an enzyme fragment complementation (EFC)-based assay for pharmacotrafficking that detects increased cell surface levels of CFTR-ΔF508.

In order to characterize biological activities of VX-770 and VX-809 that might be relevant for clinical efficacy, we profiled VX-770 and VX-809 across a standardized panel of human primary cell based disease models (**BioMAP Diversity PLUS®** panel). Several bioactivities that are directly associated with disease mechanisms in cystic fibrosis were identified. These include inhibition of PGE_2 in a monocyte-driven model of inflammation, decreased IL-17F in a model of T cell-dependent B cell activation, and decreased Eotaxin-3 in a model of epithelial cell responses. PGE_2 is known to drive overproduction of mucous in CF patients; IL-17F is a Th17 cytokine associated with epithelial inflammation; and Eotaxin-3 is a chemokine associated with helper Th2-type immune responses. This pattern of activities is consistent with inhibition of Th17 and Th2-type responses, and is also associated with protection against *Pseudomonas aeruginosa*.

Interestingly, analysis of a large reference database for drugs that are phenotypically similar to VX-809 across the BioMAP assay panel identified ibuprofen, a non-steroidal anti-inflammatory drug (NSAID) that slows disease in CF patients. Both compounds decrease levels of PGE_2 in a monocyte inflammation model. These results show how profiling across a standardized panel of human primary cell systems leads to increased understanding of drug mechanisms relevant to pathologic disease processes. The assays and bioactivities identified may be useful for the characterization and comparison of new drugs. These *in vitro* assays are also highly suitable for testing of drug combinations.

Cystic Fibrosis

- Cystic fibrosis is an inherited chronic disease that affects lungs & digestive system
- Caused by defective CFTR protein - chloride ion channel
- Afflicts ~30,000 children and adults in the US
- Median life expectancy mid 30s

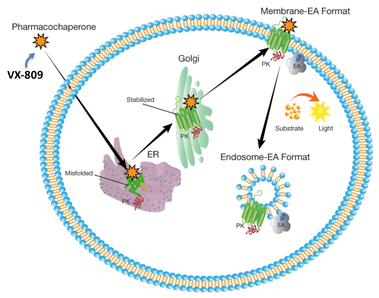


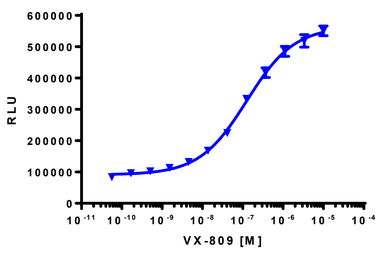
Discover Magazine http://discovermagazine.com/2013/september/14-doorway-to-a-cure

VX-770 and VX-809

- VX-770 (ivacaftor) and VX-809 (lumacaftor) are modulators of CFTR function with different mechanisms
- VX-770 (ivacaftor) – approved in 2012
  - Small molecule CFTR potentiator that binds to the CFTR channel (including mutated forms)
  - Induces a non-conventional mode of gating that keeps the channel open
- VX-809 (lumacaftor) – experimental compound in Phase II
  - Small molecule CFTR corrector (pharmacochaperone) that binds to a specific mutated form of CFTR (ΔF508)
  - Suppresses protein mis-folding

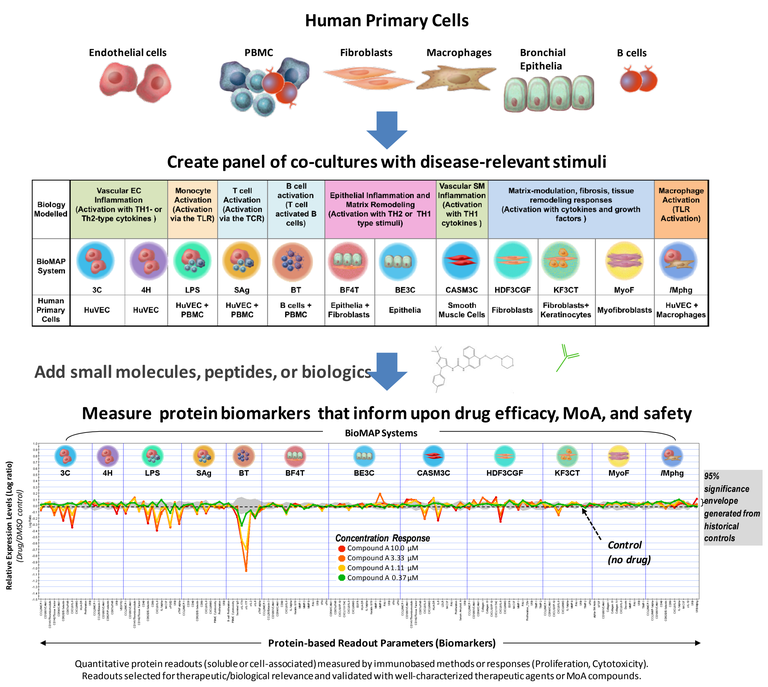
VX-809 (lumacaftor) is active in the PathHunter® CFTR-ΔF508 Pharmacotrafficking assay





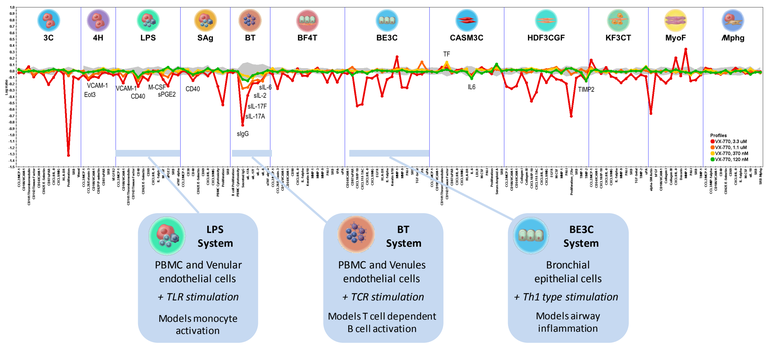
The PathHunter CFTR-ΔF508 assay is based on Enzyme Fragment Complementation (EFC) technology that consists of a β-galactosidase (β-gal) enzyme split into two inactive components, the enzyme donor peptide (ED or ProLink™ (PK)) and enzyme acceptor (EA). When brought together, ED complements with EA forming an active β-gal complex. With the addition of substrate, the active β-gal catalyzes the formation of chemiluminescent products (measured as RLU or relative luminescence units). For the PathHunter CFTR-ΔF508 assay, EA is localized to the plasma membrane through a PM tag, and the N-terminus portion of CFTR ion channel is tagged with ED Prolink (PK). As shown on the right, increased concentrations of VX-809 increases the detectable cell surface levels of CFTR-ΔF508 as measured by RLU.

BioMAP Systems Model Human Disease Biology



Quantitative protein readouts (soluble or cell-associated) measured by immunobased methods or responses (Proliferation, Cytotoxicity). Readouts selected for therapeutic/biological relevance and validated with well-characterized therapeutic agents or MoA compounds.

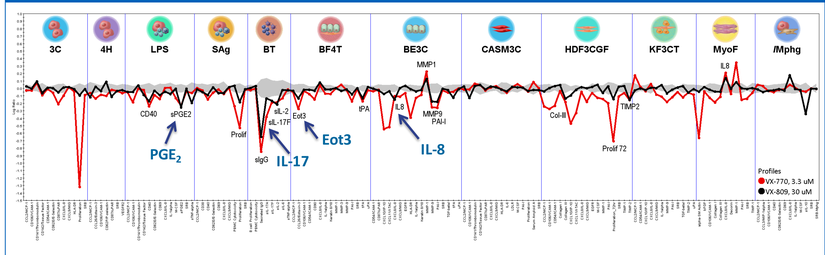
BioMAP Profile of VX-770 in Diversity PLUS



Key activities of VX-770 (ivacaftor), a CFTR potentiator

- Anti-proliferative to endothelial cells, T cells, fibroblasts.
- Inflammation-related activities: decreased Eotaxin-3, VCAM-1, $sPGE_2$, IL6
- Immunomodulatory activities: decreased CD40, M-CSF, sIgG, sIL-17A, sIL-17F, sIL-2, sIL-6
- Tissue remodeling activities: decreased TIMP2
- Hemostasis-related activities: increased TF
- Clinical exposure: Cmax is 2-5 µM

Comparison of VX-770 and VX-809



Overlay of VX-770 (3.3 µM) and VX-809 (30 µM)

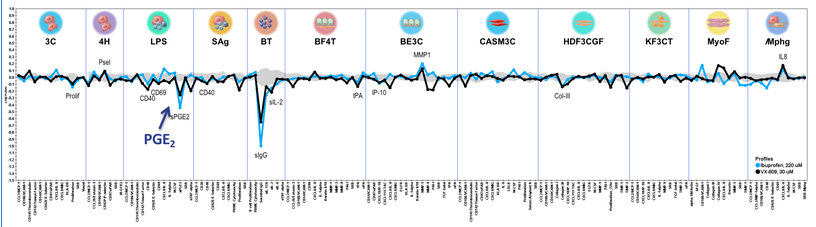
Common activities annotated – these are likely related to the target pathway mechanism (promotion of CFTR ion channel function)

PGE_2, IL-17 and **IL-8** are human translational biomarkers associated with the neutrophilic inflammation at epithelial surfaces found in CF patients

IL-17, and **Eotaxin-3** are associated with Th17 and Th2 responses

CFTR potentiator **VX-770** and CFTR corrector **VX-809** decrease levels of these biomarkers

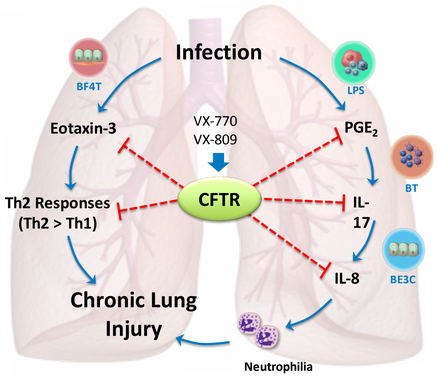
Similarity of VX-809 and Ibuprofen



Similarity of VX-809 (lumacaftor) and Ibuprofen

- A search of the BioMAP profiles of > 3,000 reference compounds revealed similarity of **VX-809** to **Ibuprofen** (Pearson correlation of r = 0.705)
- Note that both compounds reduce the levels of PGE_2 in a vascular inflammation system (BioMAP LPS System)

Clinical Mechanisms of CFTR Drugs



Infection leads to increased PGE_2 (**LPS System** modeling infection mediated vascular inflammation)

PGE_2 promotes mucous secretion and IL-17 production (**BT System** modeling T cell dependent B cell activation)

IL-17 promotes neutrophilic inflammation, and increases IL-8 (**BE3C System** modeling lung inflammation)

IL-8 promotes neutrophil recruitment, a hallmark of CF lung disease

Infection increases chemokines, such as Eotaxin-3 (**BF4T System** modeling lung inflammation), which promotes Th2 immune responses

Susceptibility to chronic *Pseudomonas aeruginosa* infection is associated with Th17 and Th2 immune responses

- Cystic Fibrosis patients have dysregulated Th17 and Th2 responses (Tiringer, 2013; Hector, 2015; Mulcahy, 2015).
- Inhibition of Th17 and Th2 biomarkers by VX-770 and VX-809 suggests that they mediate clinical efficacy in cystic fibrosis, in part, through immunomodulatory effects

Summary

Ivacaftor (VX-770) and lumacaftor (VX-809), CFTR modulators with different mechanisms of action, are active in human primary cell systems (BioMAP Diversity PLUS panel). Shared activities include decreased PGE_2, IL-17, IL-8 and Eotaxin-3. These biomarkers are associated with disease processes in the lung of CF patients: overproduction of mucous and susceptibility to *Pseudomonas aeruginosa* infection. Interestingly, VX-809 shows similarity with ibuprofen, an NSAID (COX inhibitor) that is efficacious in cystic fibrosis patients. Profiling in primary human cell systems can connect diverse targets (CFTR and COX) to the disease processes in complex diseases such as cystic fibrosis.

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tel | 1.866.448.4864 tel | +44.121.260.6142 e | info@discoverx.com

DiscoveRx
www.discoverx.com