Discovery of CFTR modulators for the treatment of Cystic Fibrosis

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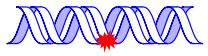
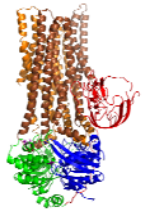
Disclosure

- Employee of Vertex Pharmaceuticals Incorporated
- Has stock or stock options in Vertex
- The content and opinions expressed are mine and do not necessarily reflect those of my employer

Mutant proteins cause disease



Gene mutation



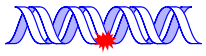
Mutant protein

- Loss of normal function
- Gain of toxic function

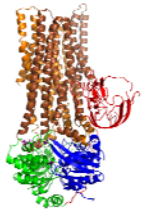
Disease

Gavrin et al.; *J. Med. Chem.* **2012**, 55, 10823-10843

CFTR gene mutations lead to Cystic Fibrosis



Mutant *CFTR* gene



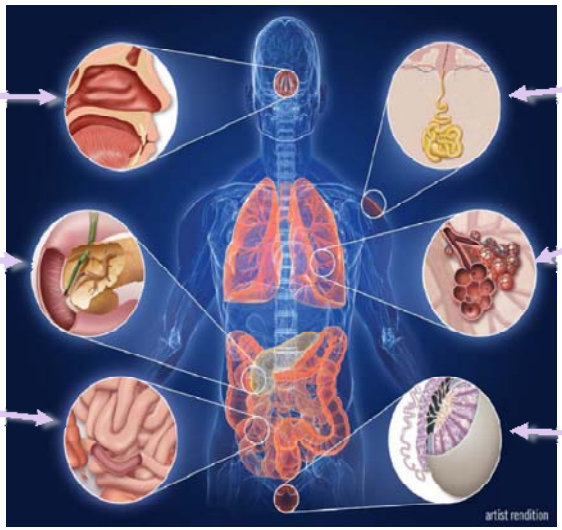
Mutant CFTR protein

Cystic Fibrosis (CF)

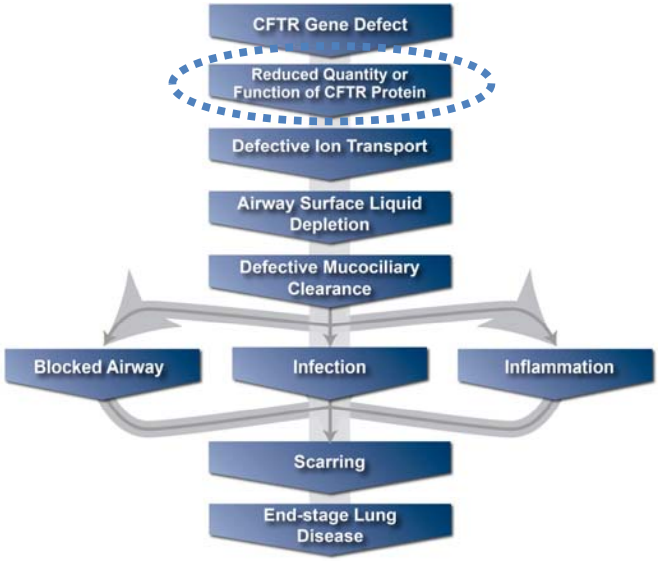
- **C**ystic **F**ibrosis **T**ransmembrane conductance **R**egulator
- Loss of chloride transport
- Most common life-threatening autosomal recessive disorder in Caucasian populations
- ~75,000 children and adults

Ratjen et al.; *Lancet* **2003**, 361, 681-689

CF is a multi-organ disease

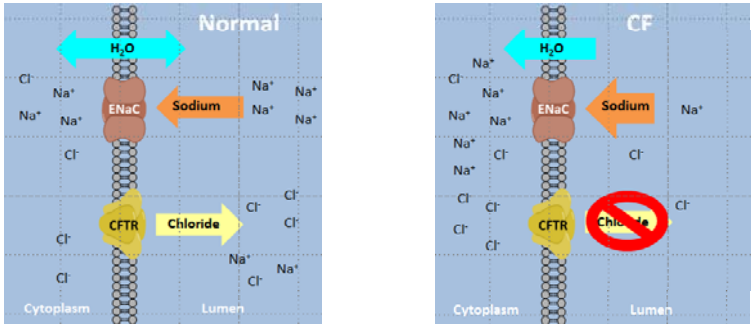


Pathophysiologic cascade in CF lung disease



The Target - CFTR

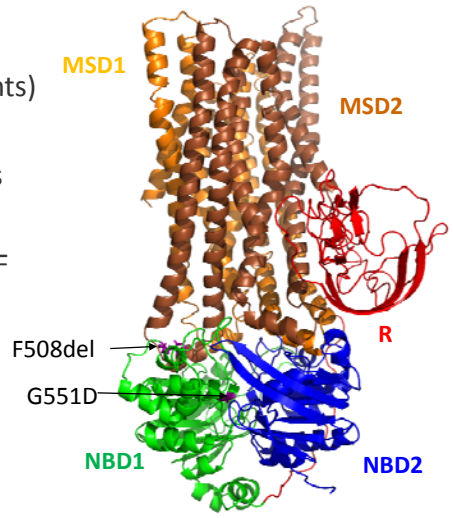
- 1480 amino-acids ATP binding cassette (ABC) transporter protein
- Regulated by cAMP-dependent protein kinase A and ATP
- Expressed at the apical membrane of epithelial cells
- CFTR functions as a chloride channel



There are >1900 mutations[1] in the CFTR protein

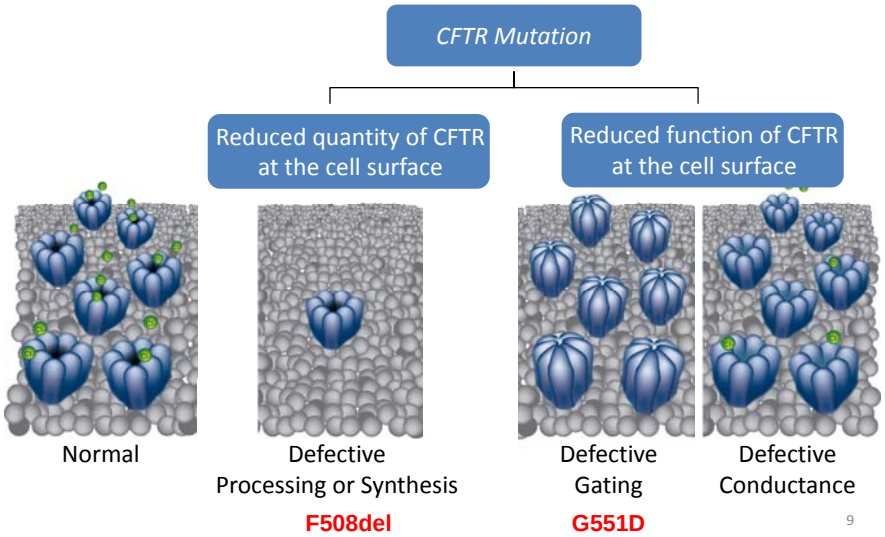
- F508del is most common mutation (~90% of CF patients)
- F508del is a mutation with quantity and function defects
- G551D is a mutation with function defects (~ 4-5% of CF patients*)

- Clear link between *CFTR* mutations, CFTR activity, and disease severity

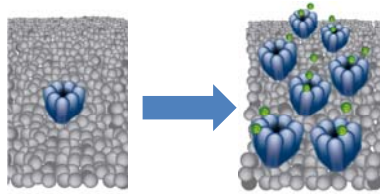


[1]http://genet.sickkids.on.ca/StatisticsPage.html
*http://www.cff.org/UploadedFiles/research/ClinicalResearch/PatientRegistryReport/2012-CFF-Patient-Registry.pdf

CFTR mutations can reduce the quantity or function of the CFTR protein at the cell surface

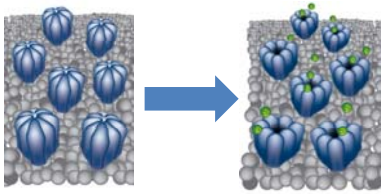


CFTR modulators increase the quantity and function of CFTR at the cell surface



CFTR Correctors

Increase the **delivery** and **amount** of functional CFTR protein to the cell surface, resulting in enhanced ion transport

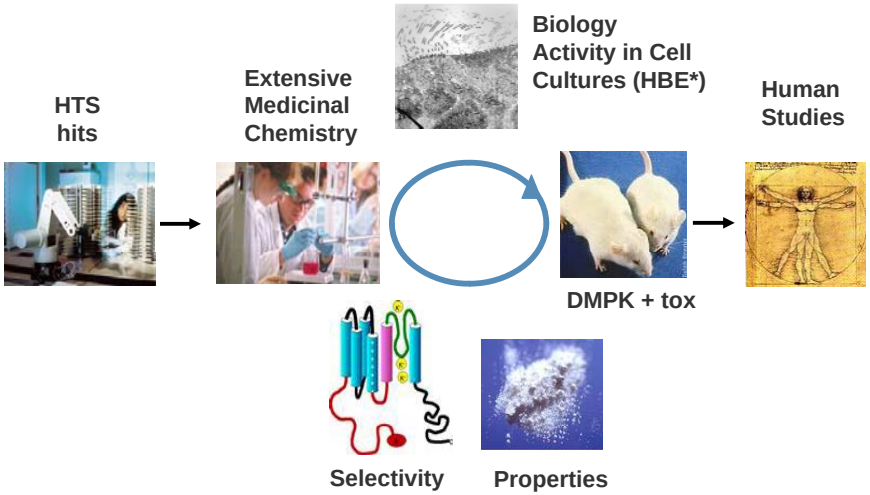


CFTR Potentiators

Increase **channel activity** of CFTR protein located at the cell surface, resulting in enhanced ion transport

Total CFTR Activity = **Surface density** * **Open probability** * Conductance

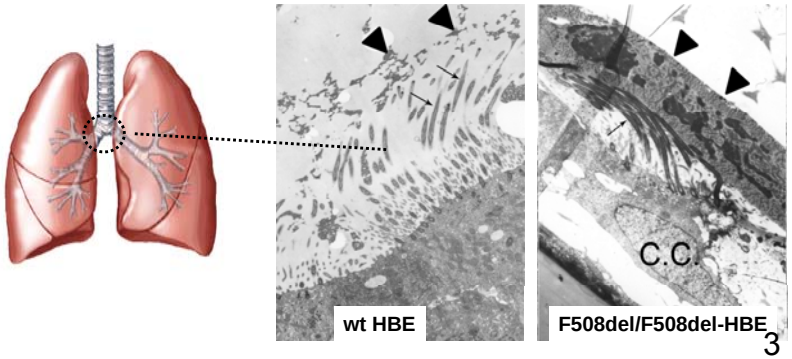
CFTR modulator drug discovery process at Vertex



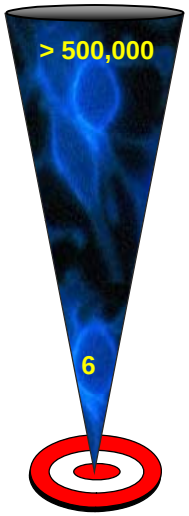
*HBE: Human bronchial epithelial cells

Human bronchial epithelial cells in cultures

- Cultured bronchial epithelia isolated from human tissue
- Differentiated epithelia show the same defective ion transport
- Used as the pharmacology model for Vertex CFTR modulators



Vertex high throughput screen
Multiple potentiator and corrector hits

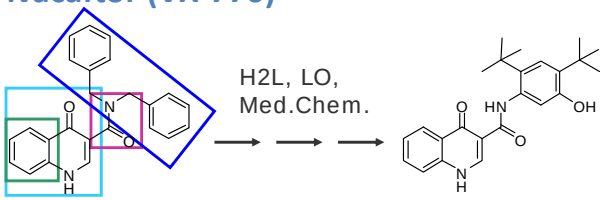


- Hit rates:
  - <<0.1% for correctors
  - ~0.1% for potentiators
  - Many inactive in primary human bronchial epithelia (HBE)
- 6 scaffolds selected for hit-to-lead followed by extensive medicinal chemistry & SAR effort

Van Goor et al. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2006**; *290*: L1117.

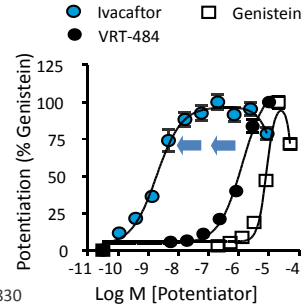
Hit to ivacaftor (VX-770)

EC_{50} for F508del mutation



VRT-484 (Hit)
3T3 EC_{50} 1.8 μM
HBE EC_{50} 1.5 μM
MW = 368, ClogP = 2.9

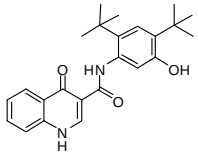
Ivacaftor (VX-770)
3T3 EC_{50} 0.003 μM
HBE EC_{50} 0.022 μM



[1]Standard potentiator reference compound

Van Goor et al. *PNAS* **2009**, *106*, 18825-18830

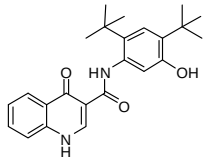
Ivacaftor preclinical profile



- Potentiator, not activator
- In vitro activity against multiple genotypes [1,2]
  - On residual CFTR in F508del/F508del HBE: 22 nM
  - G551D/F508del HBE: 236 nM
- In vitro selectivity
- >99% plasma protein binding
- Favorable oral pharmacokinetics in rodents and non-rodents

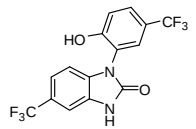
[1]Van Goor et al. *PNAS* **2009**, *106*, 18825-18830; [2] Yu, H et al. *J. Cyst. Fibros.* **2012**, *11*(3), 237-245

Ivacaftor is potent *in vitro* compared with other CFTR potentiators

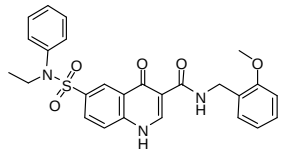


EC_{50} F508del 3T3 / HBE

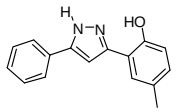
Ivacaftor (VX-770)
0.003 μM / 0.022 μM



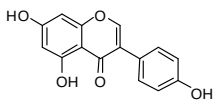
NS1619
0.3 μM / 0.4 μM



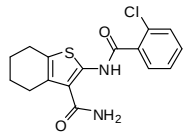
Verkman SF-03
0.3 μM / 0.2 μM
[*BioorgMedChemLet* 16 (2006) 537]



VRT-532
2.4 μM / 2.7 μM
[*AJPLCMP* 290 (2006) L1117]



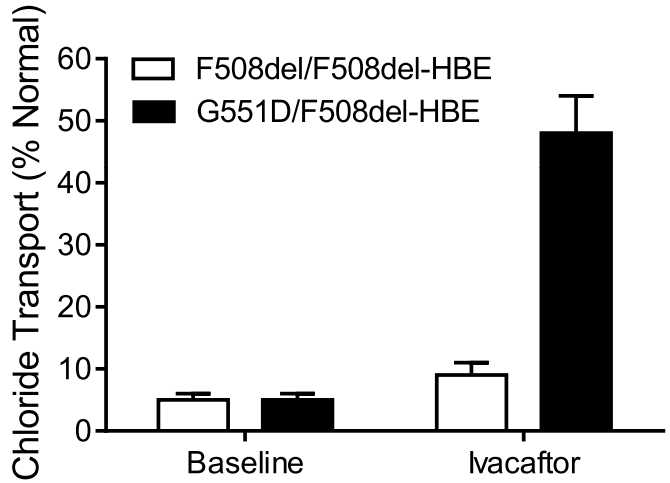
Genistein
7.9 μM / 8.3 μM



Verkman $\Delta F508_{act}$-02
3.8 μM / 0.7 μM
[*JBC* 278 (2003) 35079]

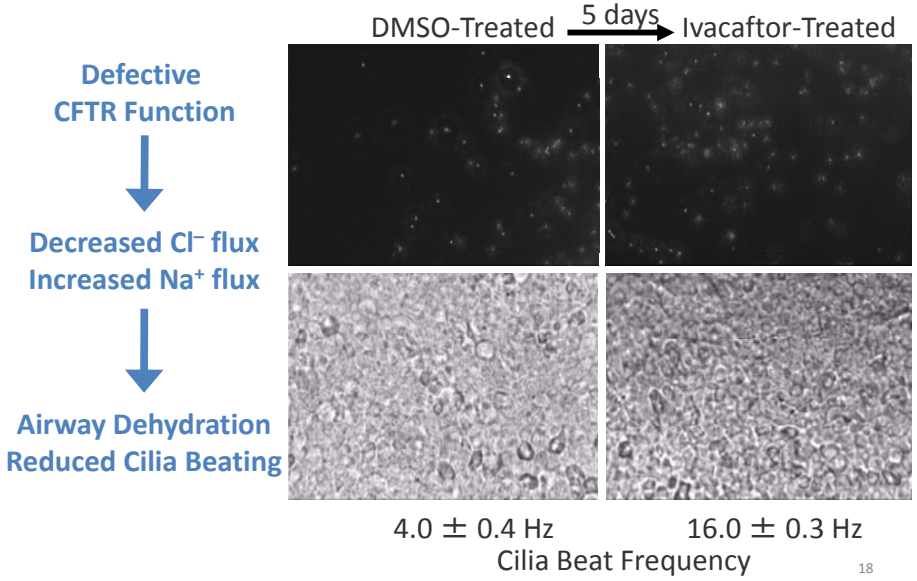
Ivacaftor increased G551D-CFTR function in vitro

- Ussing chamber studies

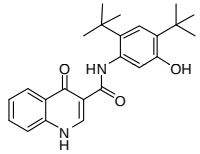


Van Goor et al. *PNAS* **2009**, *106*, 18825-18830; [2] Yu, H et al. *J. Cyst. Fibros.* **2012**, *11*(3), 237-245

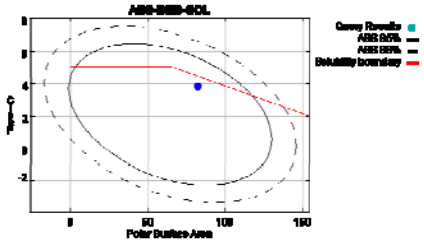
Ivacaftor increased cilia beating in G551D/F508del HBE



Ivacaftor: development challenges



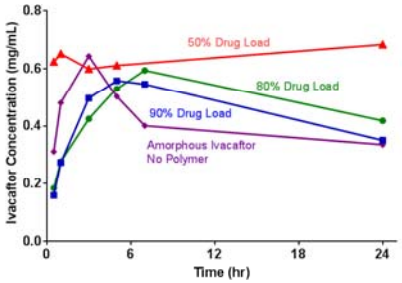
- Ivacaftor meets Lipinski rules ….
  - MW 392
  - Calculated logP: 3.82
  - HBA: 5
  - HBD: 3



- **But actual properties indicate that traditional development would be challenging**
  - Mp: 292°C, aqueous solubility: <0.05 µg/mL
  - Measured log P: 5.68

Spray-dried dispersion of ivacaftor is stable *in vitro* and on the shelf

- Kinetic Solubility in Fed Simulated Intestinal Fluid (FeSSIF) demonstrates that solid dispersion of ivacaftor and polymer stabilizes the amorphous forms in aqueous media

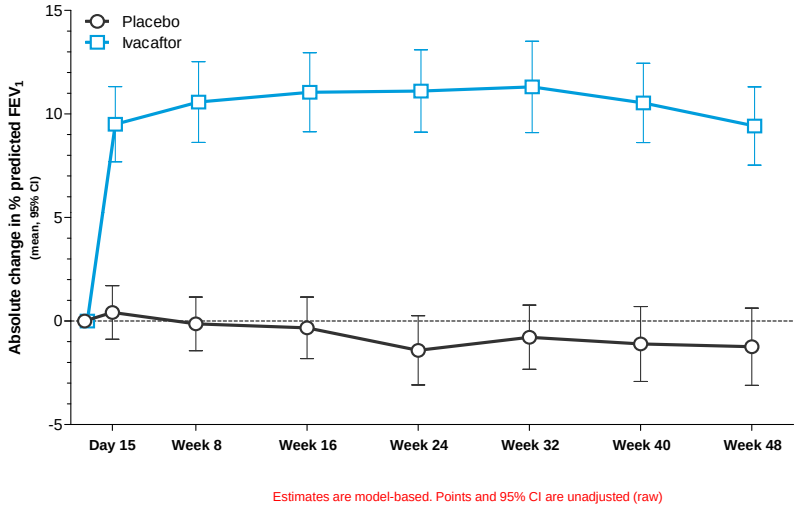


- High Tg of ivacaftor results in SDD that is highly resistant to crystallization upon storage
  - Estimated time to 10% crystallization: >10 years at 25°C/100% RH

Ivacaftor SDD Open Dish Stress Study at 8 weeks Storage

Stress Condition	75% RH	100% RH
40°C	Amorphous	Amorphous
50°C	Amorphous	Amorphous
60°C	Amorphous	Trace Crystallinity
80°C	Amorphous	Trace Crystallinity

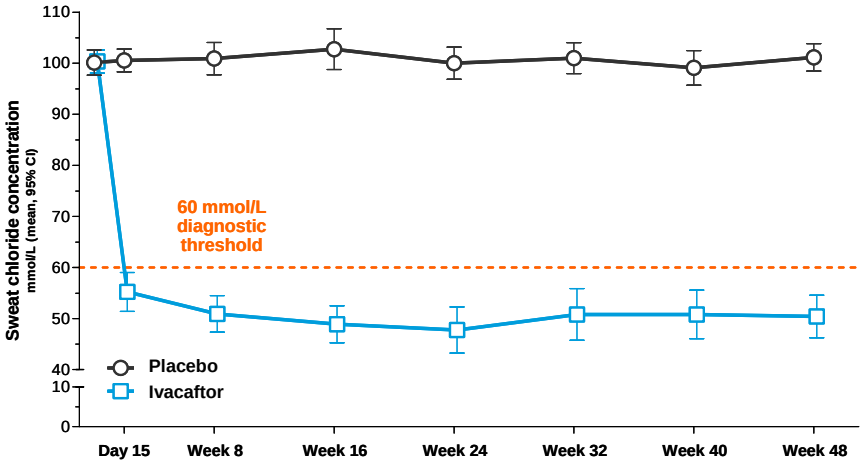
Ivacaftor increased FEV_1 (% predicted) in people with CF 12 years of age and older who have the *G551D* gating mutation



Estimates are model-based. Points and 95% CI are unadjusted (raw)

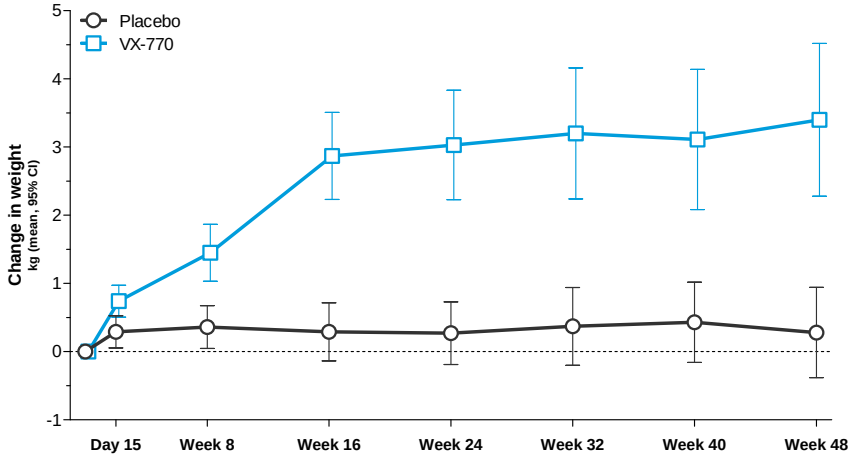
Ramsey et al. *NEJM,* **2011**, *365*, 1663

Ivacaftor reduced sweat chloride concentrations in people with CF 12 years of age and older who have the *G551D* gating mutation



Ramsey et al. *NEJM,* **2011**, *365*, 1663

Ivacaftor increased body weight in people with CF who are 12 years of age and older who have the *G551D* gating mutation

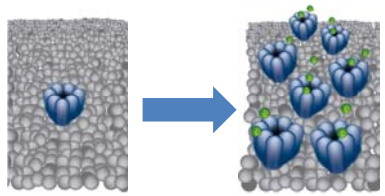


Ramsey et al. *NEJM,* **2011**, *365*, 1663

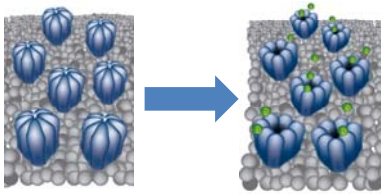
Safety information from ivacaftor clinical trials

- **Transaminase Elevations**
  - Elevated transaminases were reported in patients with CF receiving KALYDECO. ALT and AST should be assessed prior to initiating KALYDECO, every 3 months during the first year of treatment, and annually thereafter. Patients who develop increased transaminase levels should be closely monitored until the abnormalities resolve. Dosing should be interrupted in patients with ALT or AST of greater than 5 times the upper limit of normal (ULN)
- **Concomitant Use with CYP3A Inducers**
  - Use of KALYDECO with strong CYP3A inducers, such as rifampin, substantially decreases the exposure of ivacaftor, which may reduce the therapeutic effectiveness of KALYDECO. Co-administration of KALYDECO with strong CYP3A inducers, such as rifampin, rifabutin, phenobarbital, carbamazepine, phenytoin, and St. John's Wort is not recommended
- **Serious Adverse Reactions**
  - Serious adverse reactions, whether considered drug-related or not by the investigators, which occurred more frequently in patients treated with KALYDECO included abdominal pain, increased hepatic enzymes, and hypoglycemia
- **Adverse Reactions**
  - The most common adverse reactions in patients with a G551D mutation in the CFTR gene (Trials 1 and 2) with an incidence of ≥8% and at a higher incidence for patients treated with KALYDECO (N=109) than for placebo (N=104) were headache (24% vs 16%), oropharyngeal pain (22% vs 18%), upper respiratory tract infection (22% vs 14%), nasal congestion (20% vs 15%), abdominal pain (16% vs 13%), nasopharyngitis (15% vs 12%), diarrhea (13% vs 10%), rash (13% vs 7%), nausea (12% vs 11%), and dizziness (9% vs 1%)
  - The safety profile for patients with a G1244E, G1349D, G178R, G551S, G970R, S1251N, S1255P, S549N, or S549R mutation enrolled in Trial 4 was similar to that observed in Trials 1 and 2

Vertex corrector program



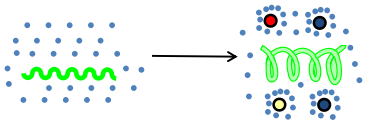
CFTR Correctors
Increase the **delivery** and **amount** of functional CFTR protein to the cell surface, resulting in enhanced ion transport



CFTR Potentiators
Increase **channel activity** of CFTR protein located at the cell surface, resulting in enhanced ion transport

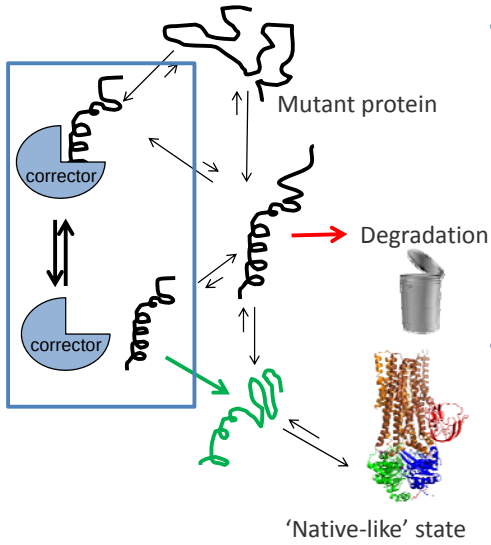
Several ways to correct mutant CFTR

- **Mutations**
  - 'Revertant' or 'suppressor' mutations
- **Temperature**
  - Lower temperature partially rescues F508del CFTR
- **Osmolytes** (chemical chaperones)
  - E.g. glycerol, TMAO



- **Chaperones**
  - Large molecules (molecular chaperones)
  - Small molecules (**correctors** or pharmacological chaperones)

Hypothesis: correctors mimic chaperones during protein biogenesis

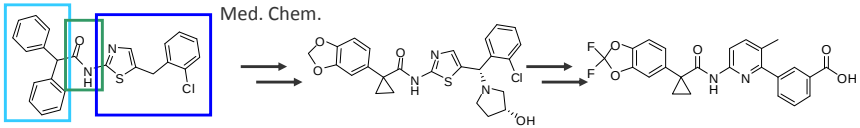


- Folding intermediates expose hydrophobic surfaces prone to aggregation
  - Correctors stabilize nascent hydrophobic surfaces or cavities
  - Correctors need to 'let go' of the protein
- Implications:
  - Folding intermediates represent soft, transient binding sites for correctors

Hit to VX-809 (lumacaftor)

EC_{50} for F508del mutation

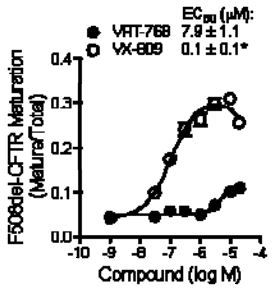
H2L,LO, Med. Chem.



VRT-768 (Hit)
MW = 419
cLogP = 6.9
FRT EC_{50} 7.9 µM

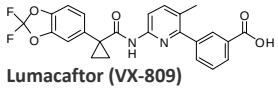
VRT-534 (C18)[1]

VX-809
MW = 452
cLogP = 5.8; cLogD = 3.5
FRT EC_{50} 0.10 µM
HBE EC_{50} 0.08 µM



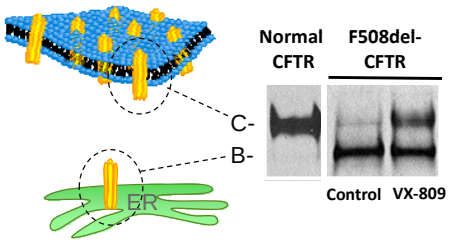
Van Goor et al. ; *Proc. Nat. Ac. Sci.* **2011**, *108*, 18843-18848
[1] http://www.cff.org/research/ForResearchers/ResearchTools/CFTRAntibodiesModulators/

In vitro lumacaftor allowed more mature CFTR protein to reach the cell surface

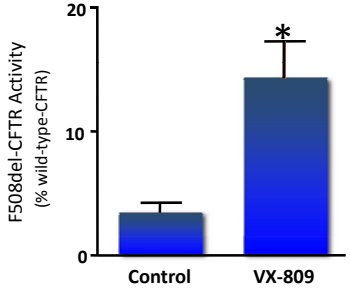


Lumacaftor (VX-809)

Cellular Processing of F508del-CFTR



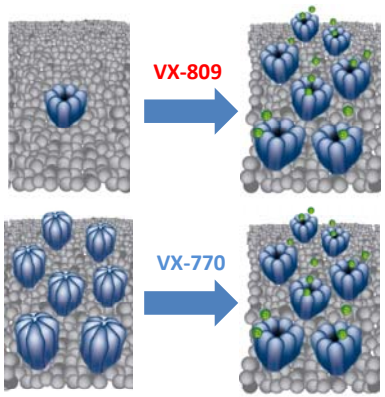
CFTR-mediated Cl^- Flux

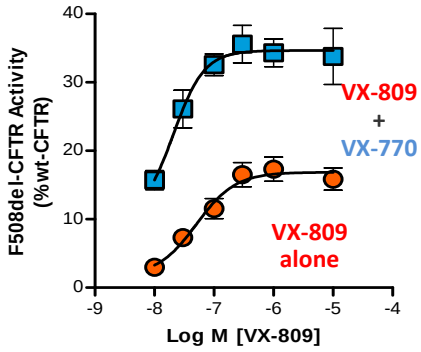


~15% increase

Van Goor et al. *Pediatr Pulmonol* 2009;44(S32):154absS9.4

In vitro VX-770 doubled the activity of VX-809





Total CFTR activity= Surface density * Open probability * Conductance

Lumacaftor (VX-809) / Ivacaftor Phase 3

Statistically significant mean absolute and relative improvements in lung function were observed for all four treatment groups

Change in $ppFEV_1$		Pooled TRAFFIC and TRANSPORT		
		Placebo (n=371)	Lumacaftor (600 mg once daily) + Ivacaftor (250 mg q12h) (n=368)	Lumacaftor (400 mg q12h) + Ivacaftor (250 mg q12h) (n=369)
Mean Absolute Change (percentage points)	Treatment Difference	N/A	3.3 (p < 0.0001)	2.8 (p < 0.0001)
	Within Group	-0.32 (p=0.3983)	3.0 (p < 0.0001)	2.5 (p < 0.0001)
Mean Relative Change (%)	Treatment Difference	N/A	5.6% (p < 0.0001)	4.8% (p < 0.0001)
	Within Group	-0.17% (p=0.8030)	5.4% (p < 0.0001)	4.6% (p < 0.0001)

p≤ 0.0250 required for statistical significance (vs. placebo)

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Lumacaftor (VX-809) / Ivacaftor Phase 3

Significant improvement in secondary endpoints including pulmonary exacerbations, associated hospitalizations, and improvement in BMI

Key Secondary Endpoints		Pooled TRAFFIC and TRANSPORT		
		Placebo (n=371)	Lumacaftor (600 mg once daily) + Ivacaftor (250 mg q12h) (n=368)	Lumacaftor (400 mg q12h) + Ivacaftor (250 mg q12h) (n=369)
Number of Pulmonary Exacerbations	Number of Events (rate per 48 weeks)	251 (1.14)	173 (0.80)	152 (0.70)
	Rate Ratio	N/A	0.70 (p=0.0014)	0.61 (p < 0.0001)
Change in Body Mass Index	Treatment Difference	N/A	+0.28 (p < 0.0001)	+0.24 (p=0.0004)
	Within Group	+0.13 p=0.0066	+0.41 (p < 0.0001)	+0.37 (p < 0.0001)
Patients with 5% or Greater Relative Improvement in $ppFEV_1$	%	22%	46%	39%
	Odds Ratio	N/A	2.95 (p < 0.0001)	2.22 (p < 0.0001)
Change in CFQ-R	Treatment Difference	N/A	+3.1 (p=0.0071)	+2.2 (p=0.0512)
	Within Group	+1.9 (p=0.0213)	+4.9 (p < 0.0001)	+4.1 (p < 0.0001)

p≤0.0250 required for statistical significance (vs. placebo)

http://investors.vrtx.com/releasedetail.cfm?ReleaseID=856185

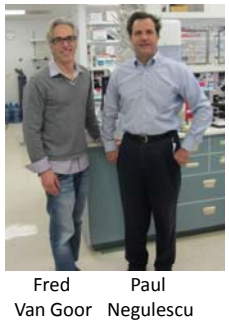
Safety information from Phase 3 Lumacaftor / Ivacaftor trials

- Safety results from these studies were reported on a pooled basis for each dosing arm across the studies.
- The most common adverse events, regardless of treatment group, were infective pulmonary exacerbation, cough, headache and increased sputum, and adverse events that occurred more frequently in patients who received the combination regimens than those who received placebo were generally respiratory in nature and included dyspnea and respiration abnormal.
- 4.2 percent of all patients who received combination therapy, regardless of dosing group, discontinued treatment because of adverse events compared to 1.6 percent of those who received placebo.
- Across the two studies, elevated liver enzymes (greater than three times the upper limit of normal) were observed in 5.2 percent of patients who received combination therapy compared to 5.1 percent of those who received placebo. Seven patients who received combination therapy experienced serious adverse events related to abnormal liver function tests, compared to zero patients who received placebo. Following discontinuation or interruption of the combination treatment, liver function tests returned to baseline for six of the seven patients and the seventh patient's liver function tests improved substantially.

Summary & perspective

- HTS followed by extensive medicinal chemistry and SAR efforts resulted in the discovery of orally active 'drug-like' CFTR modulators
- In the US, KALYDECO (ivacaftor) is approved for the treatment of CF in patients 6 years of age and older who have one of the following mutations in the CFTR gene: [*G551D, G178R*, *S549N*, *S549R*, *G551S*, *G1244E*, *S1251N*, *S1255P* & *G1349D*]
  - Additional studies are underway to determine if ivacaftor alone or in combination with a CFTR corrector can provide clinical benefit to other patients with CF
- Cell-based functional membrane potential assays were important to drive medicinal chemistry optimization
  - Can we can move beyond the paradigm of cellular assays?
  - Is the biology too complex for a simple biochemical or binding assay?

Acknowledgements



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