Ahead of the Curve – Emerging CF Therapies 2009: Ongoing Research into CFTR Modulation

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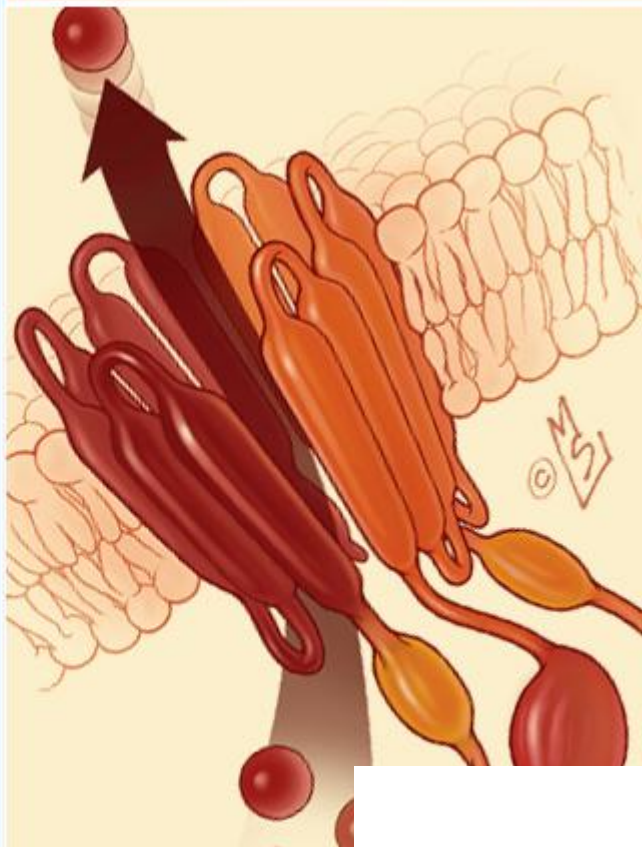
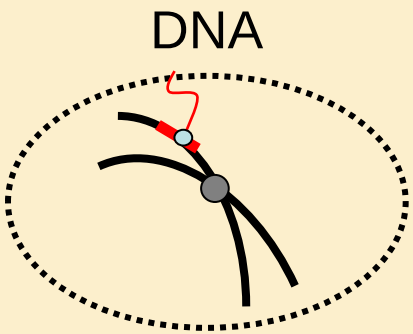


Disclosures

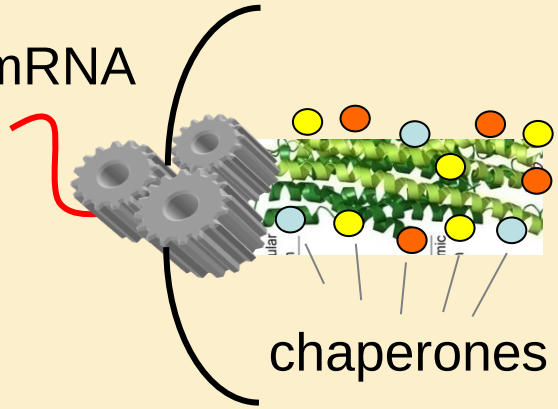
- CFFT-TDN Translational Center
  - CFFT funding through center grant
- Vertex Pharmaceuticals
  - Study PI: A Randomized, Double-Blind, Placebo-Controlled, Multiple Dose Study of VX-809 to Evaluate Safety, Pharmacokinetics, and Pharmacodynamics of VX-809 in Cystic Fibrosis Subjects Homozygous for the ΔF508-CFTR Gene Mutation
  - Nasal PD Central Reading Services for study entitled a phase 2a, randomized, double-blind, placebo-controlled study of VX-770 to evaluate safety, pharmacokinetics, and biomarkers of CFTR activity in cystic fibrosis (CF) subjects with genotype G551D
    - VX-770-02 (co-investigator)
    - VX-770-03 (co-investigator)
    - VX-770-04 (co-investigator)
- PTC Therapeutics
  - Nasal PD central reading services for clinical trial entitled: A Phase 3 Efficacy and Safety Study of PTC124 as an Oral Treatment for Nonsense-Mutation-Mediated Cystic Fibrosis
- Grant reviewer
  - CFF, CFFT, NIH (Rare Disease Network DSMB)

The steps from CF gene to CFTR protein

- CF gene (chromosome #7)
  - Codes for…
- CFTR (cystic fibrosis transmembrane conductance regulator)
  - Membrane protein that regulates salt transport

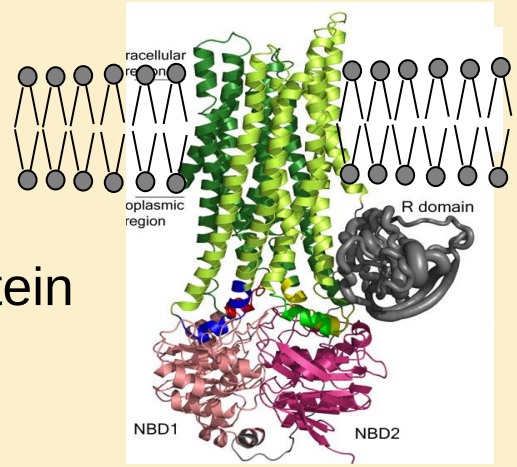


Nucleus –
Gene/chromosome



ER/Golgi –
Translation/protein maturation

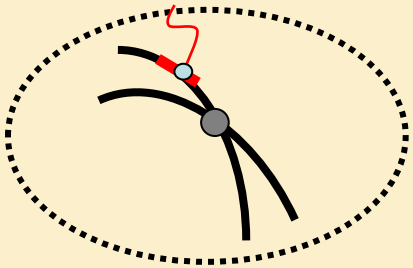
protein



Plasma membrane –
Pore open/closed

What is a CFTR modulator, and why is it different from other CF therapies?

- Modulator – targets underlying defects in CFTR
  - Restore function (not replace)
- Addresses primary cause of CF
  - Not downstream symptoms
- Different defects – different targets – different strategies
  - Generally based on CF mutation class



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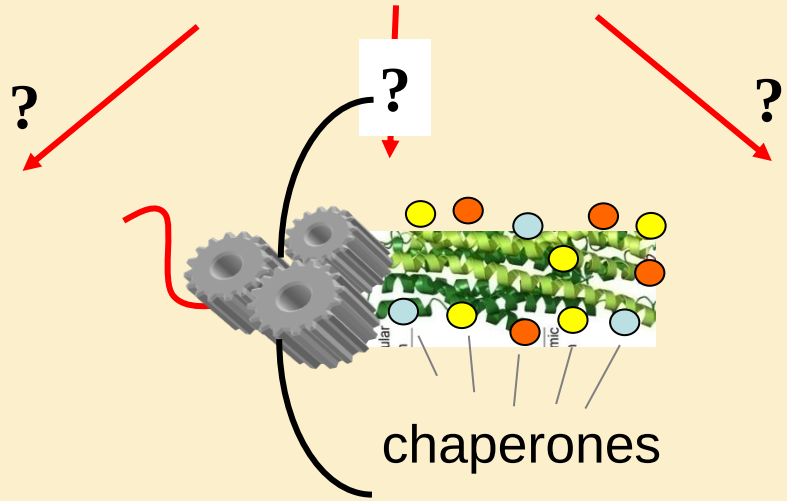
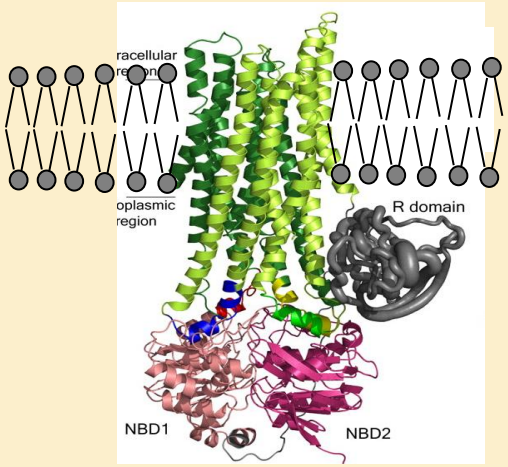
Gene/chromosome



ER/Golgi –
Translation/protein maturation



Plasma membrane –
Pore open/closed

Defining modulator strategies

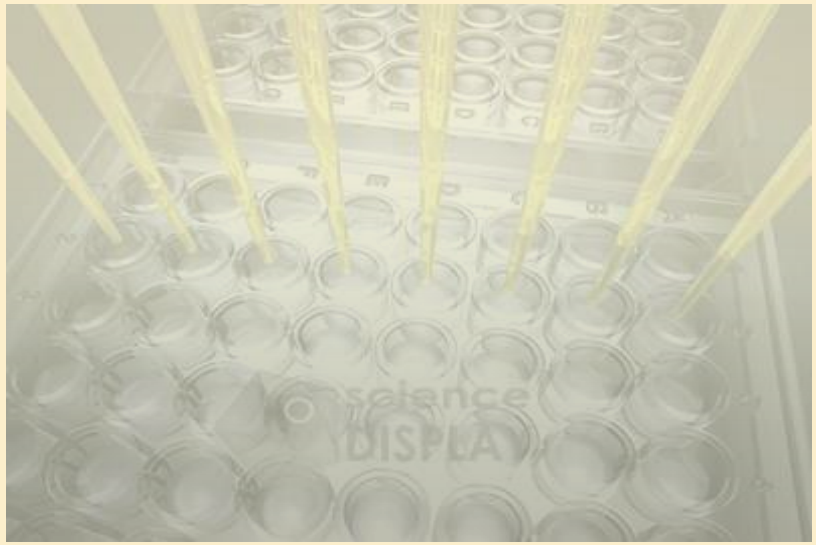
- Structural drug design from mutant protein
  - Requires detailed structural ‘map’ (ie, crystallized protein)
- Bench observations
  - Dissect the pathway/problem that causes defect
  - Identify targets to exploit
  - **Adapt approved therapies** based on Mechanism of Action
- High Throughput Screening (HTS)
  - Bulk screening of compound libraries
  - Validation of hits, optimization
  - Testing in preclinical model systems

HTS – identification of small molecule modulators

- Use of cell lines (stably expressing CFTR or reporter gene)
  - Luciferase gene with stop mutation
  - CFTR (wt, ΔF508, G551D)

- PTC Therapeutics
  - **Suppressors (PTC124)**

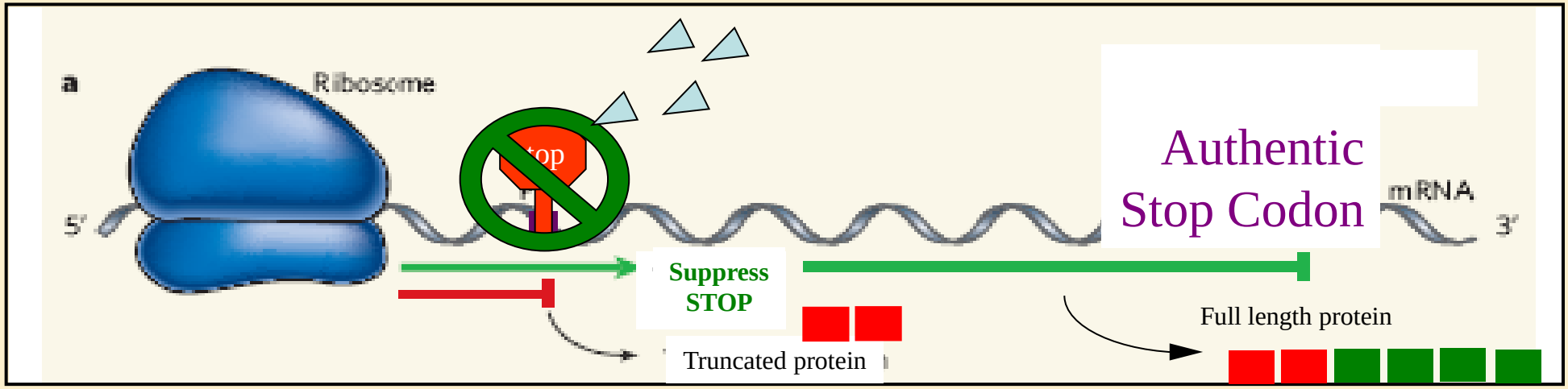
- Vertex Pharmaceuticals
  - **Potentiators (VX-770)**
  - **Correctors (VX-809)**



Suppressors of stop mutations

- 'Class I': Defective early step(s) in CFTR production
  - One example = Premature Termination Codons (PTCs)
  - Characterized by 'X' (stop signal) in gene
    - Translation stops
    - Short (truncated) protein
    - Degradation of mRNA

- Suppression of PTC ► production of full length CFTR protein

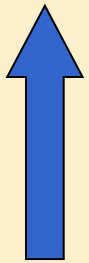


Nature: News and Views (2007)

Suppression of PTCs with aminoglycosides (gentamicin)

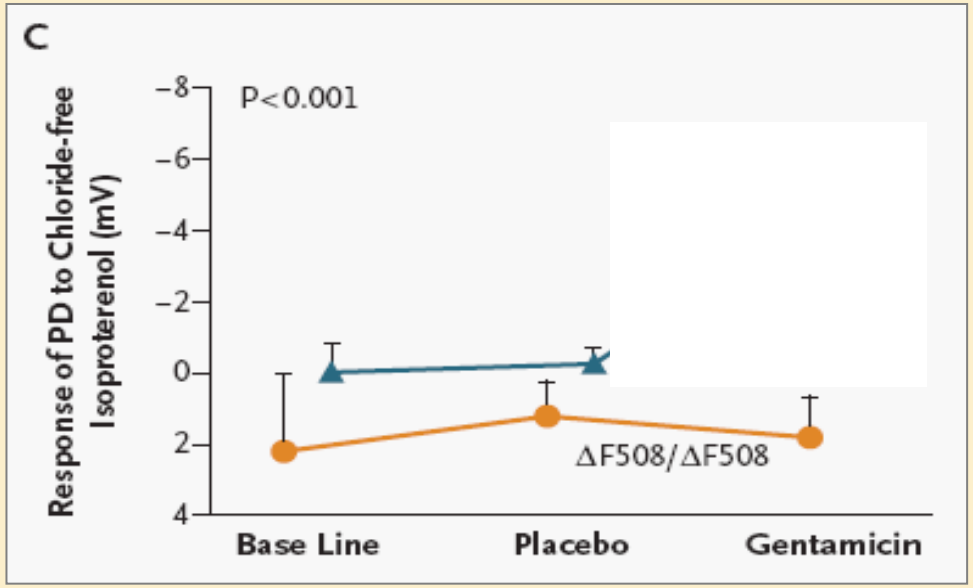
- 1st described in bacteria and yeast
- Applied principle to CF-causing mutations
  - Single cells - transgenic mice - CF patients

CFTR activity as measured by NPD (Cl^- transport)



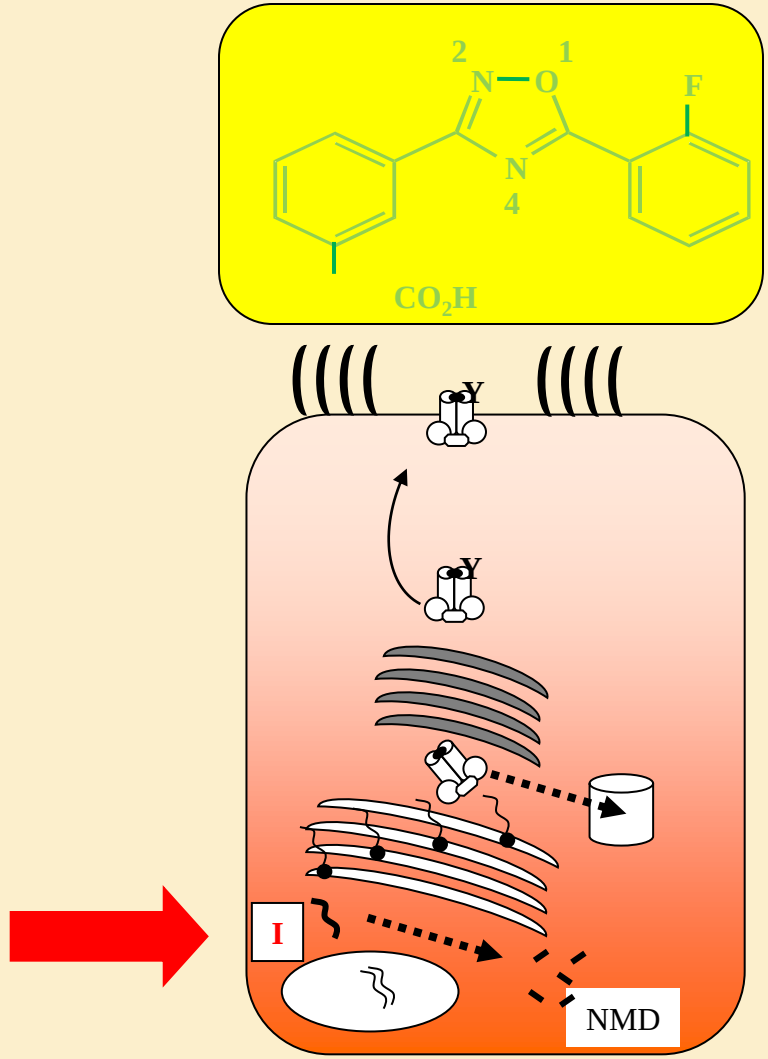
More negative
= improvement

Cl^- transport



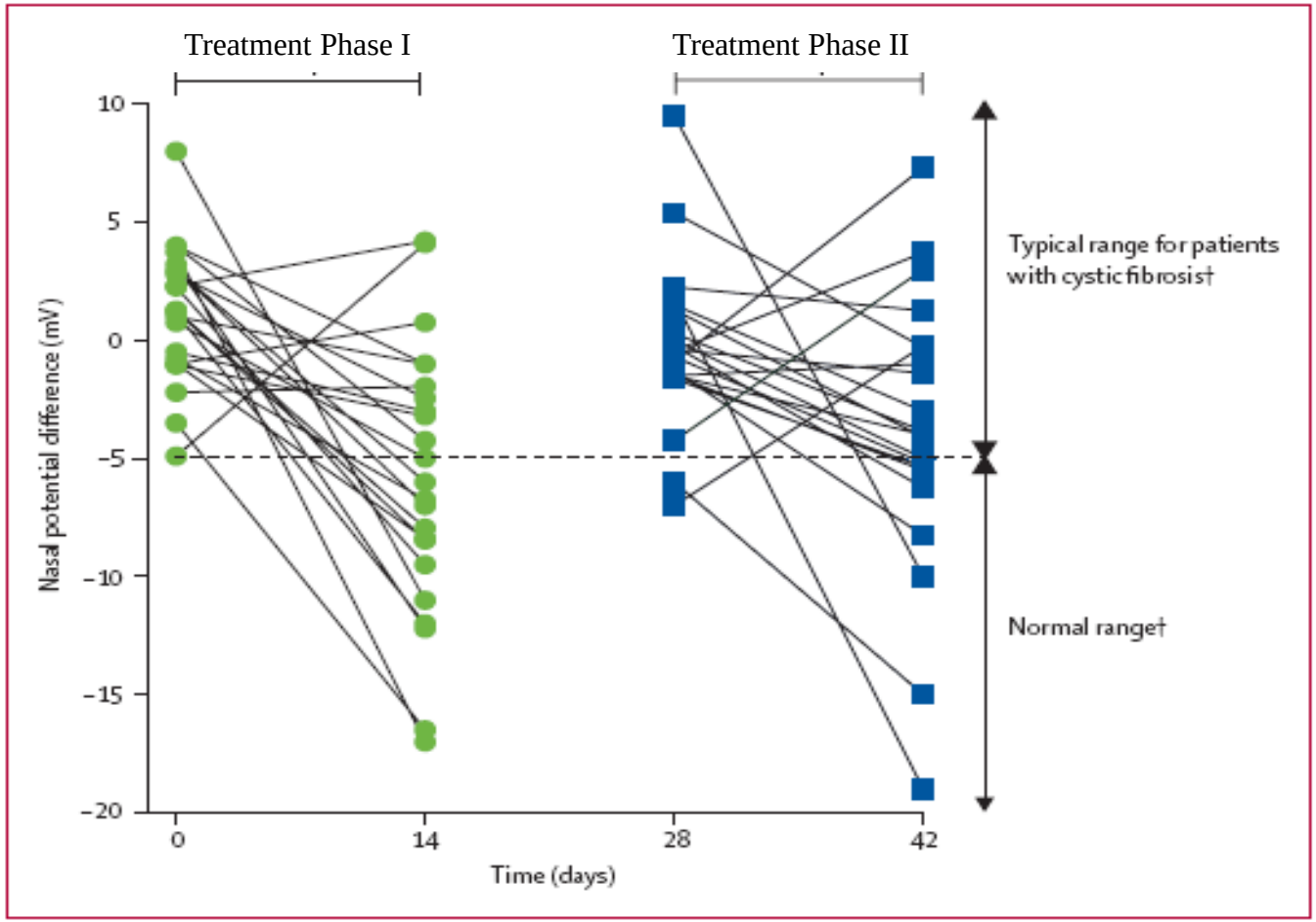
PTC124 (ataluren®)

- Developed through HTS
- Non-antibiotic
- Human studies:
  - CF patients
    - Israel
    - US
    - Belgium/France
  - Duchennes MD patients



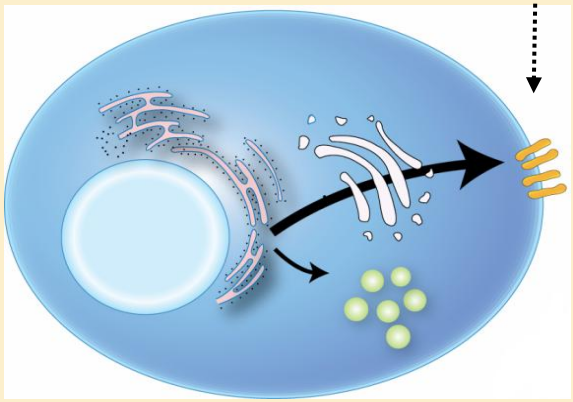
PTC124 – evidence for suppression of PTCs in CF patients

More negative
= improvement

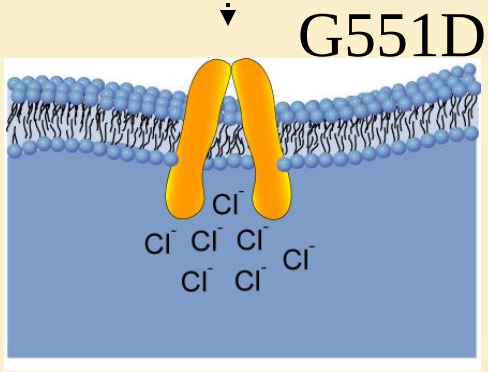


Potentiators of CFTR – open the channel

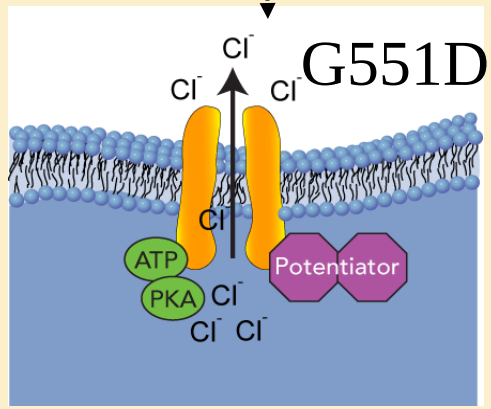
Normal Levels of Surface CFTR



Gating Defect Channel 'locked'



'Unlock' channel

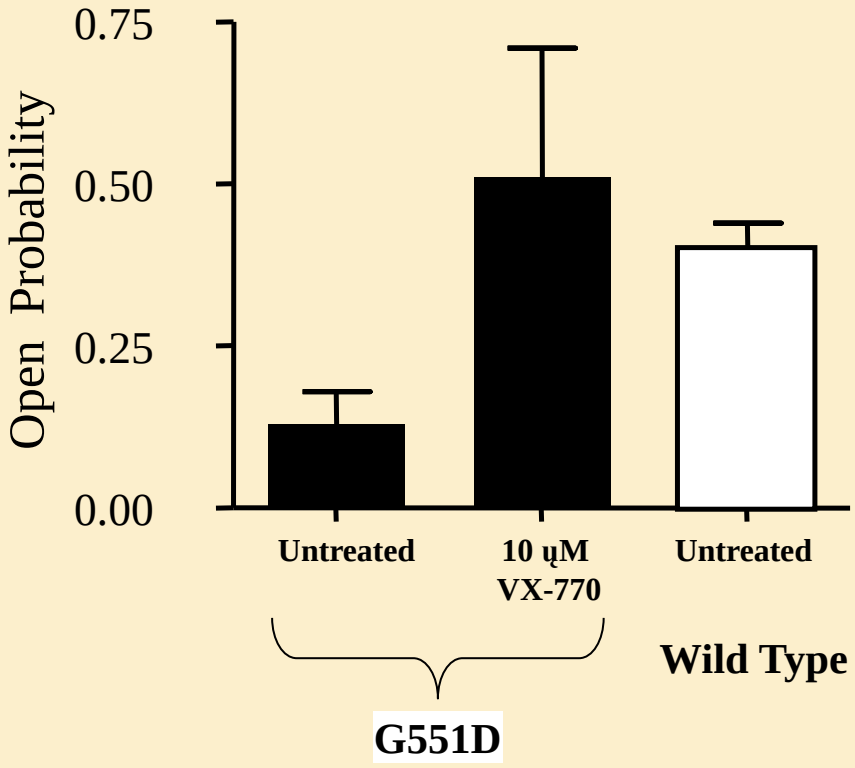


Logical targets:

- G551D CFTR (3-4% CF patients)
- Normal amounts of G551D CFTR at the plasma membrane
- Other mutations?

VX-770: restores gating of G551D CFTR *in vitro*

Single channel patch clamp studies in Fisher Rat Thyroid cells expressing G551D CFTR



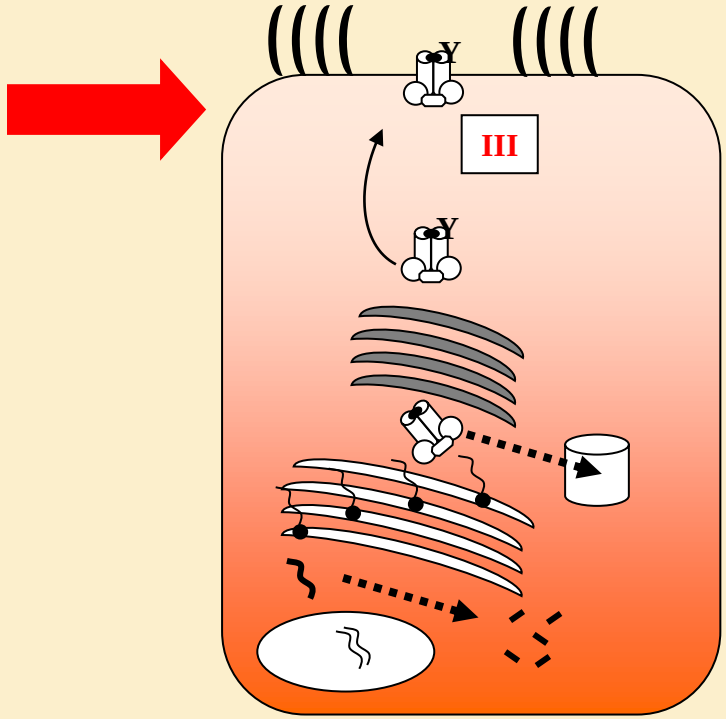
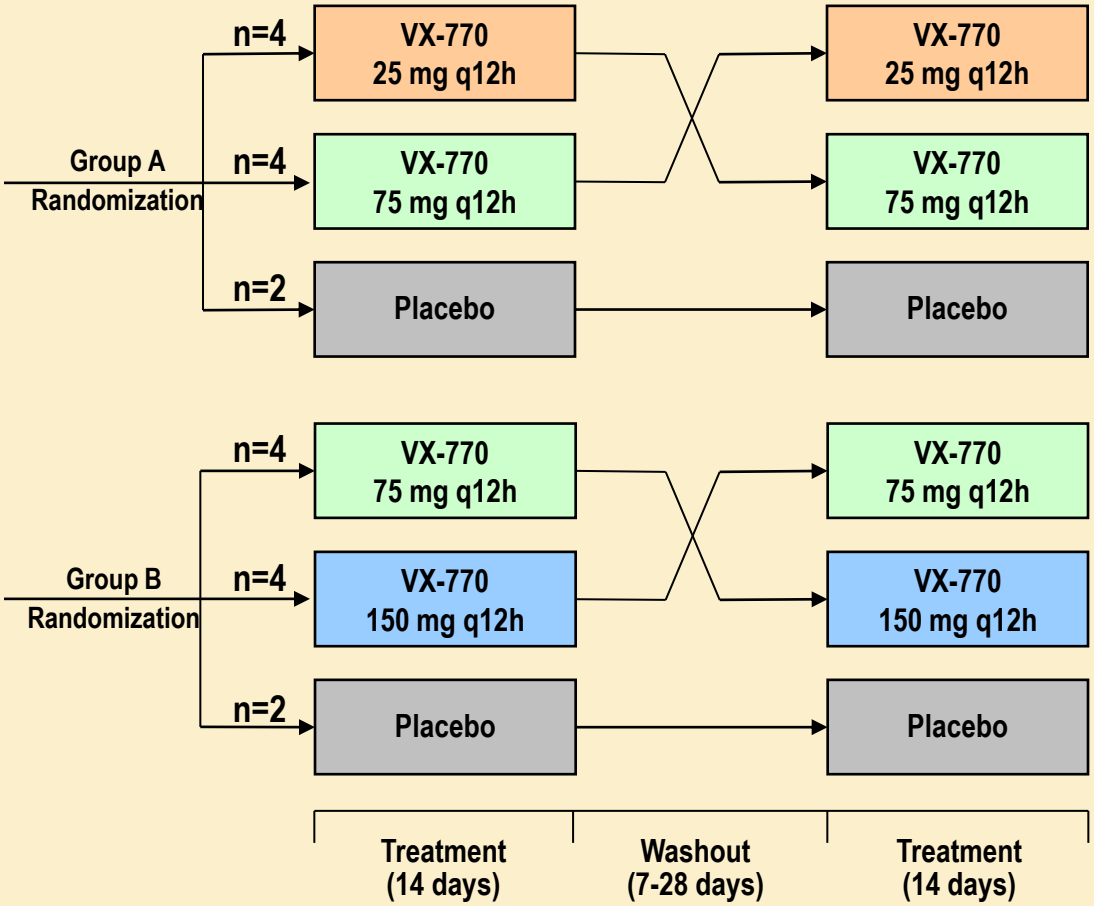


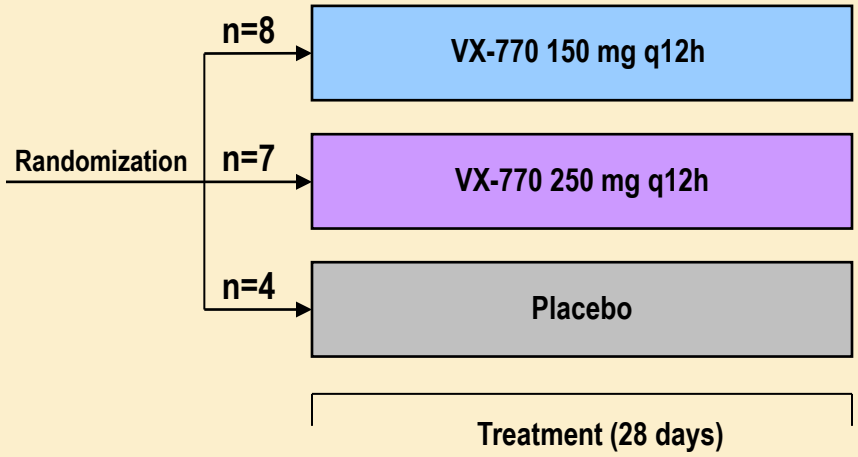
Figure compliment of F. Van Goor

Study schematic

Part 1

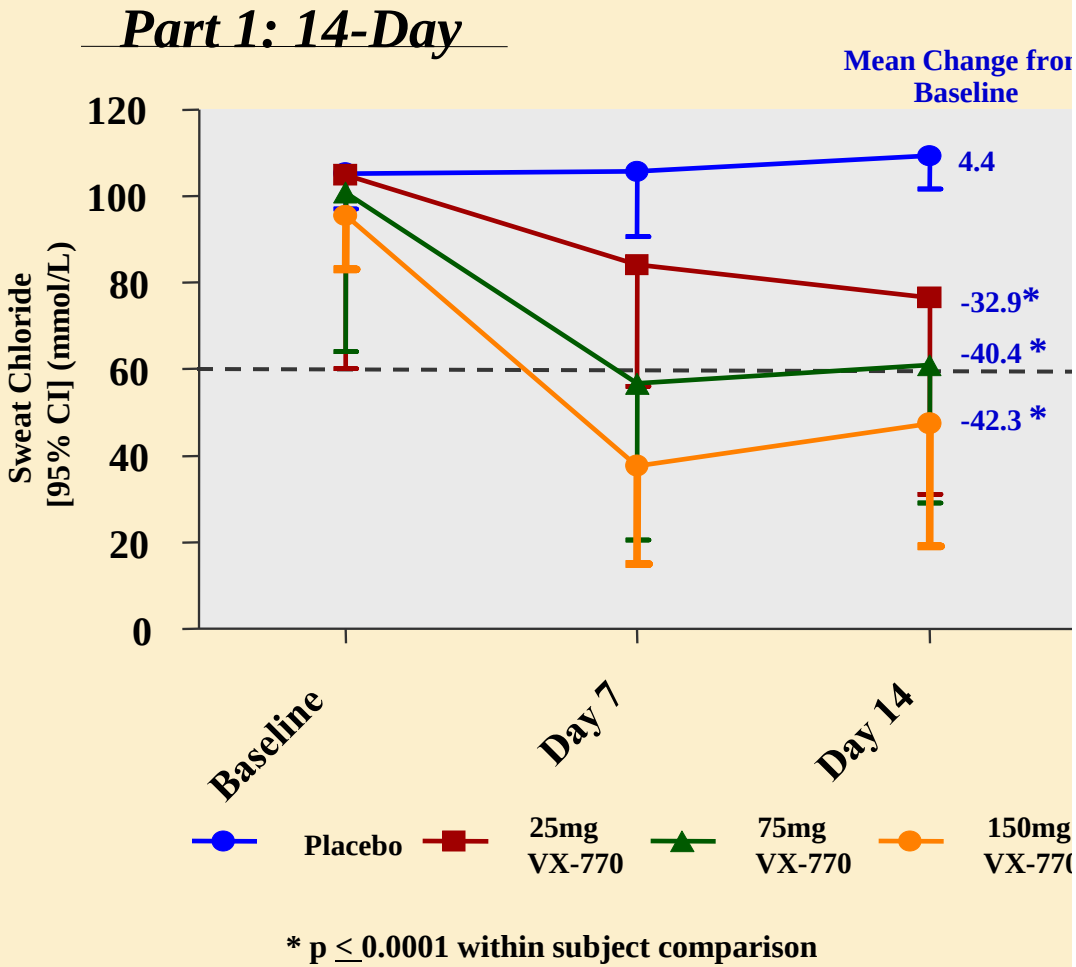


Part 2



- Part 1:
  - **55% female**
  - **Median age 30 y**
- Part 2:
  - **47% female**
  - **Median age 21 y**
- Genotype
  - **All with G551D**
  - **80% G551D/ΔF508**

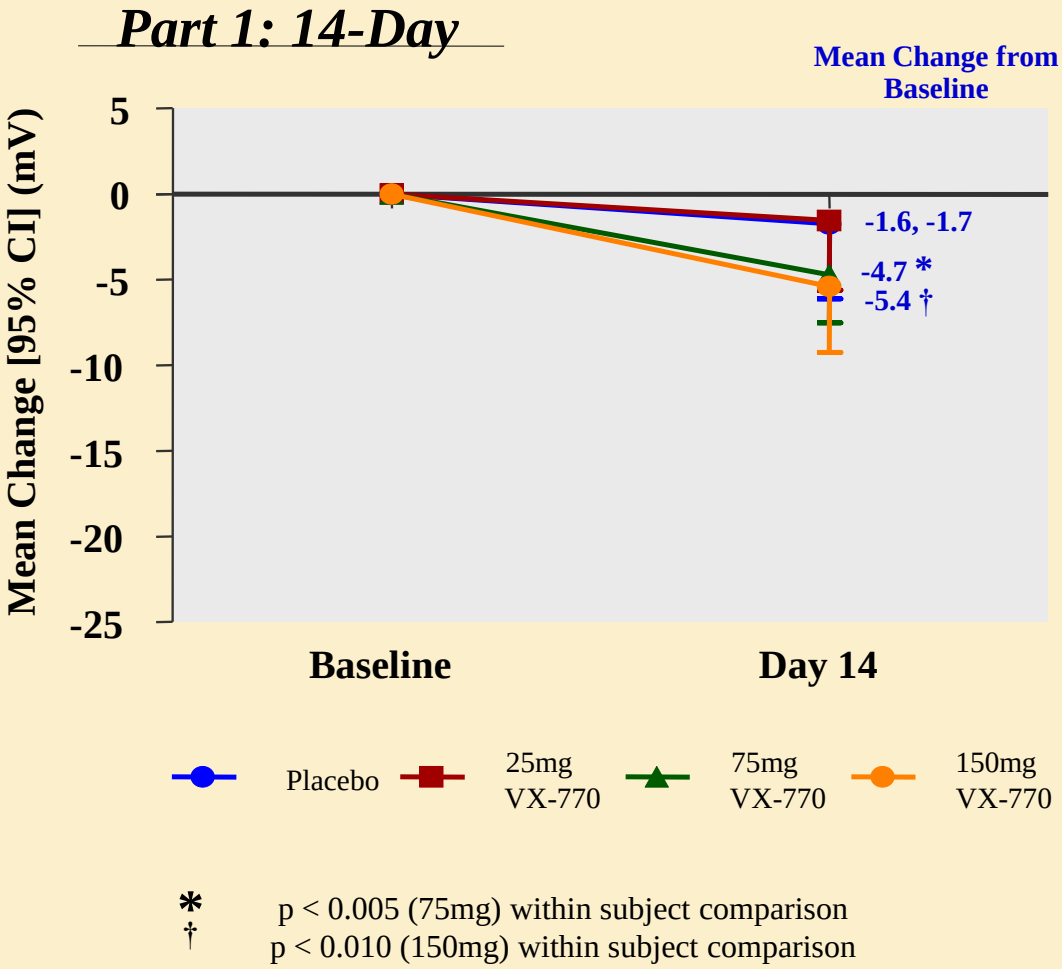
Change in sweat [Cl^-] after VX-770



* p ≤ 0.0001 within subject comparison

Lower value
= improvement

Change in NPD CFTR activity after VX-770: NPD: 0[Cl^-]/Isoproterenol Response

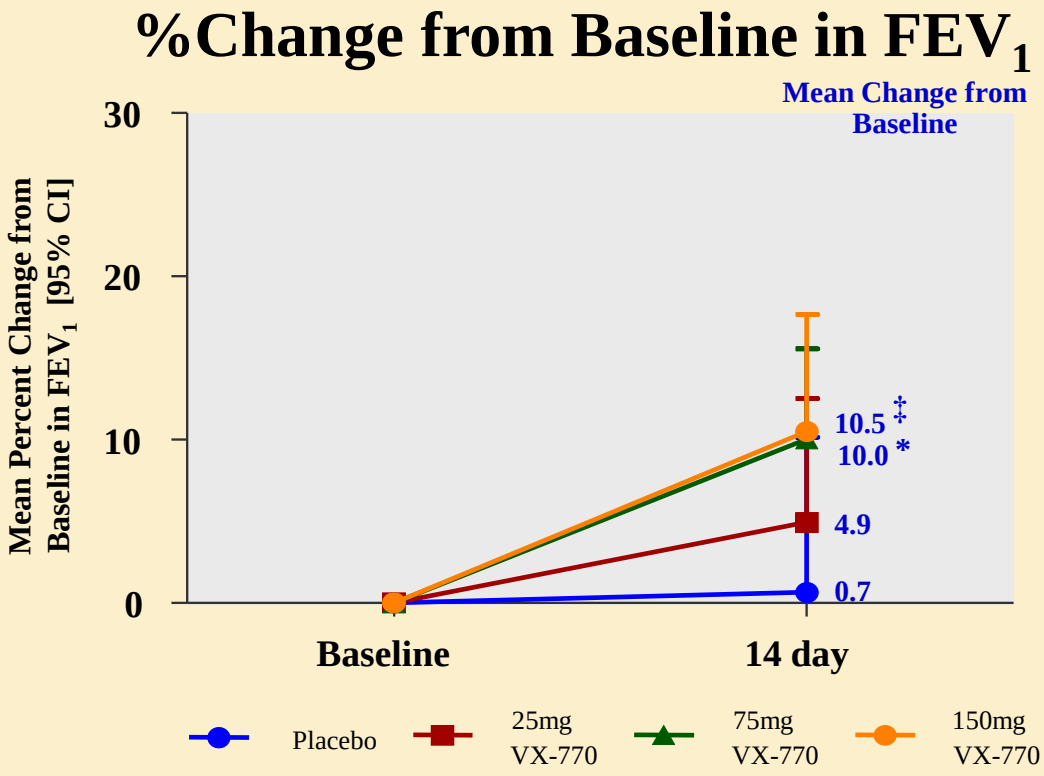


* $p < 0.005$ (75mg) within subject comparison
† $p < 0.010$ (150mg) within subject comparison

Lower value
= improvement

% change in FEV_1 from baseline after VX-770

Part 1: 14-Day

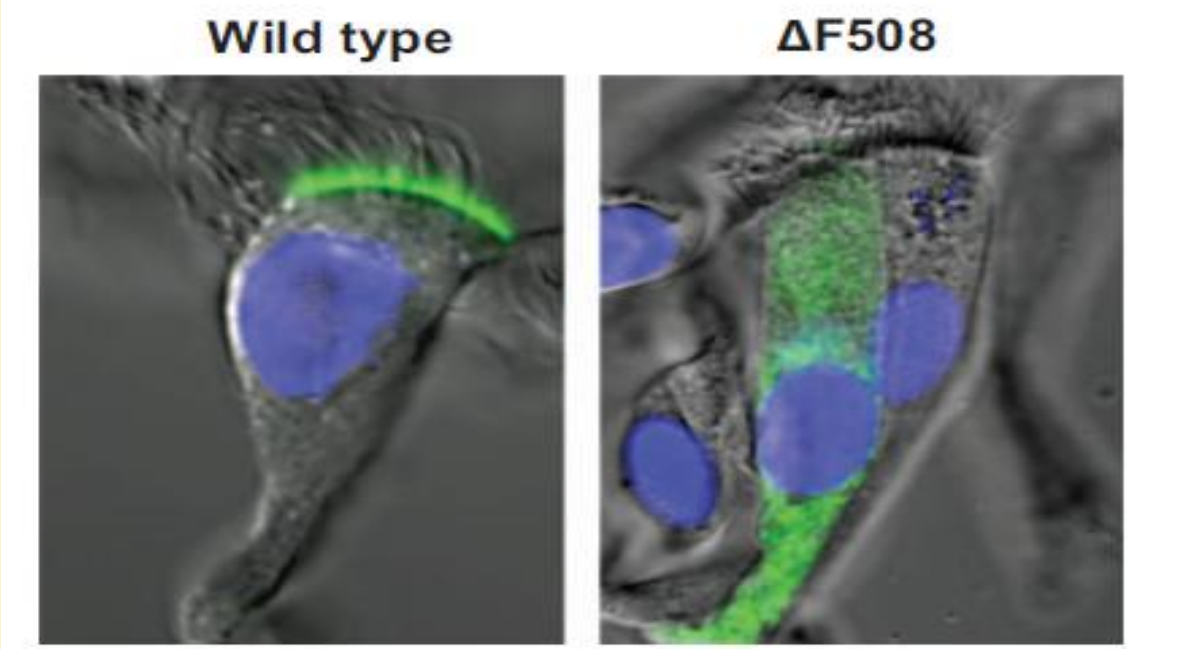


* p = 0.002 within subject comparison
‡p = 0.008 within subject comparison

Higher value
= improvement

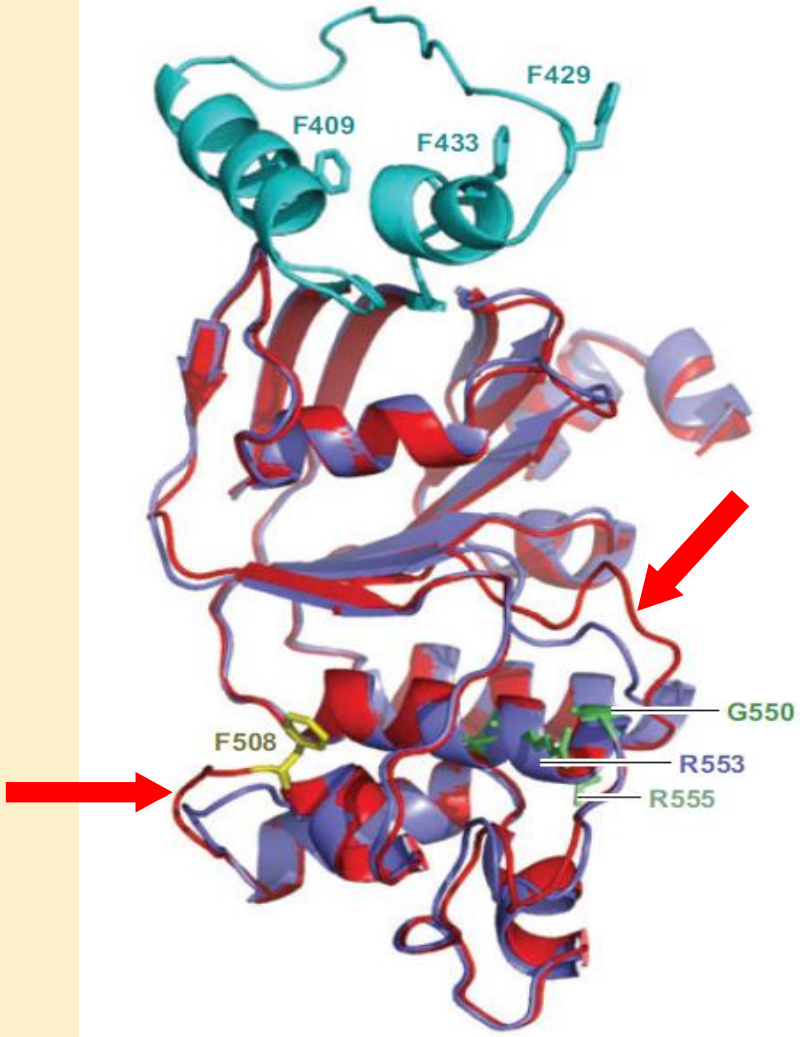
Correction of ΔF508 CFTR maturation and folding

- ΔF508 CFTR – most common cause of CF
  - 80-90% of CF patients, 65-70% of CF chromosomes
  - Protein misfolding (ER)
    - Protein marked for degradation
    - Failure to ‘mature’ (reach plasma membrane)



Riordan, J. *Ann Rev. Biochem.* 2008

ΔF508 CFTR – what's the problem?



- Phe 508:
  - External surface of NBD-1
  - Destabilizing
    - Failure of later parts to 'compact'
    - Driven by proximal/distal interactions
  - Failure to mature (full glycosyation)
  - Proteosomal degradation

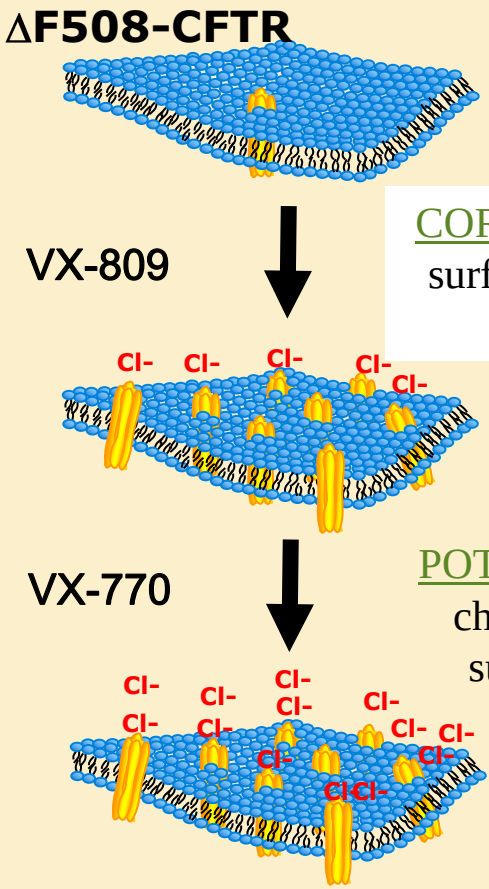
ΔF508 CFTR – the 'triple whammy'

- Folding
  - Protein does not reach the plasma membrane
- Gating
  - After reaching the plasma membrane, Cl^- pore closed >> open
- Membrane recycling
  - After reaching the plasma membrane, rapidly internalized

Denning, G. et al. *Nature*, 1991
Hwang, TC et al. *Am. J. Physiol*, 1997
Wang, W et al. *J. Biol. Chem.* 2005
Swiatecka-Urban et al. *J. Biol. Chem,* 2005
Collawn, J. et al. *Biochem J*, 2008

ΔF508 CFTR 'correctors'

- Developed through HTS (Vertex Pharmaceuticals)



CORRECTOR: Increases cell surface density and function of ΔF508-CFTR

POTENTIATOR: Increases channel opening of cell surface ΔF508-CFTR

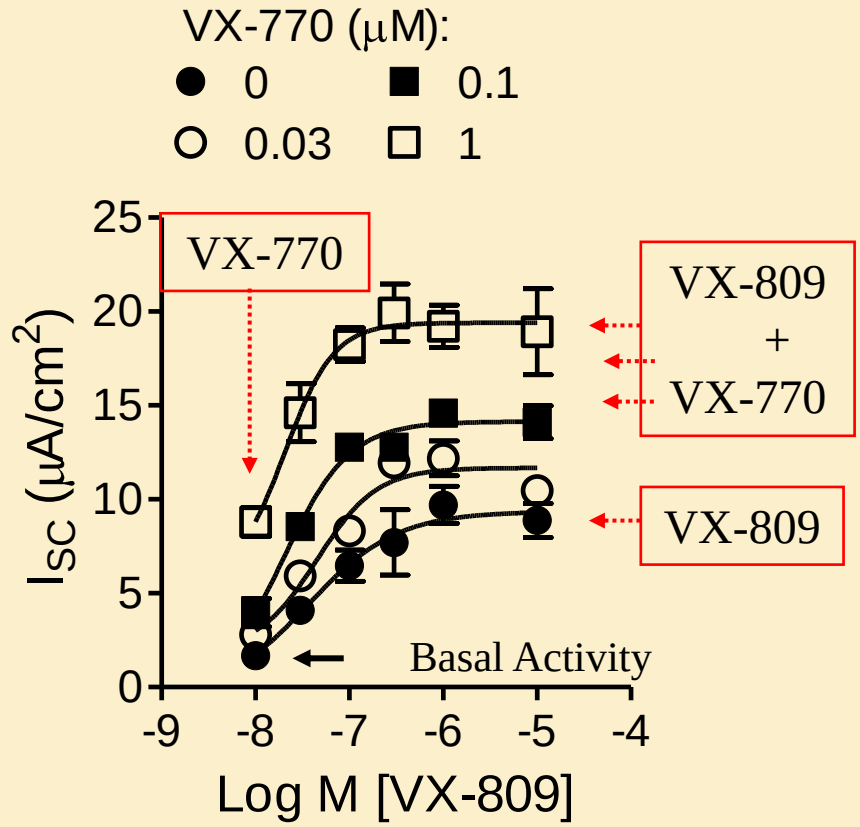


Figure compliment of F. Van Goor

Take home points:

- CFTR modulators:
  - Address underlying causes of CF
- Suppressors
  - Target stop mutations
- Potentiators
  - Open Cl^- channel
- Correctors
  - Target primary defect in ΔF508
- Match genotype to restorative treatment