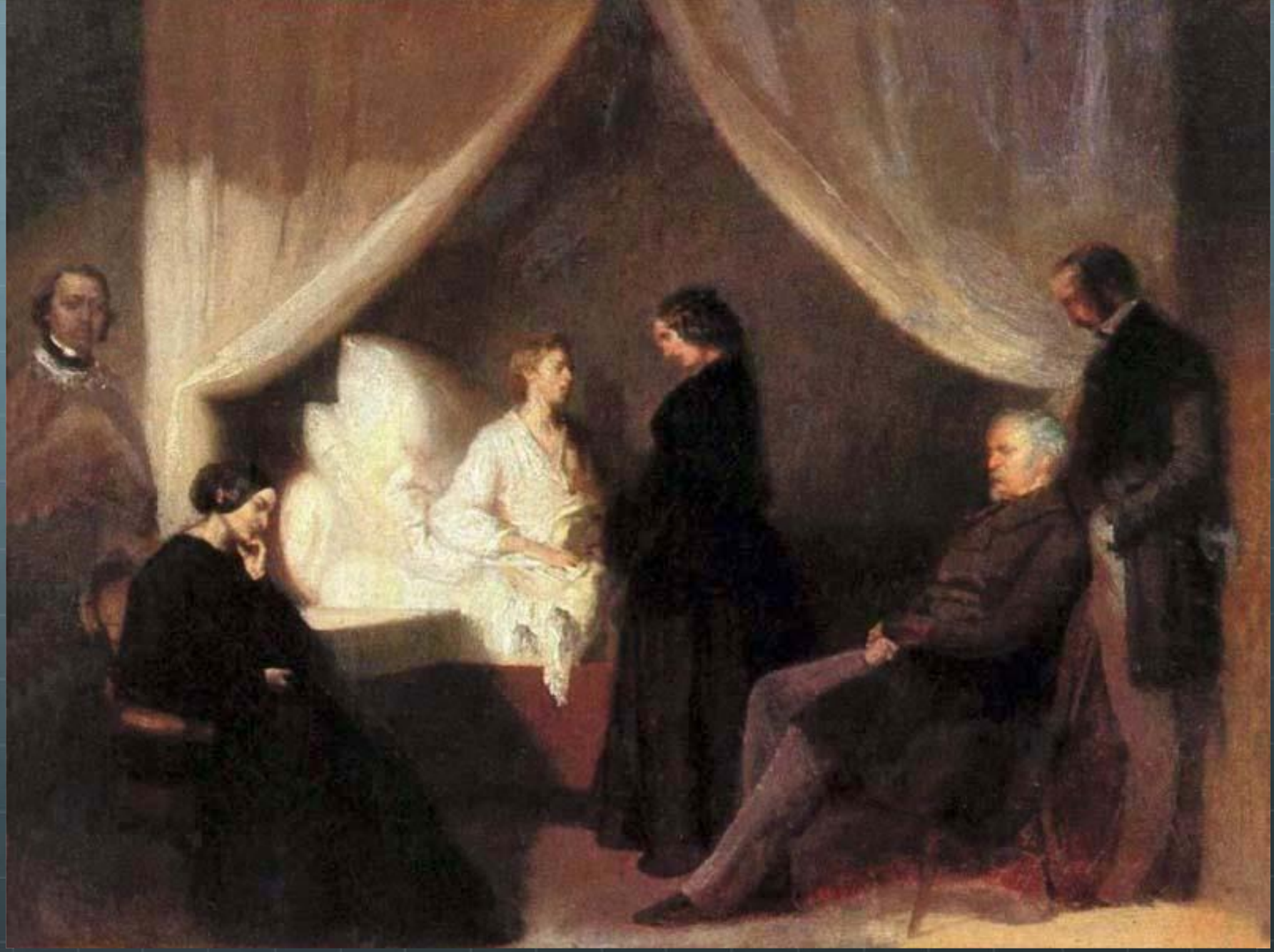
Cystic Fibrosis

Thanks: Eric Olson

"Woe to the child who tastes salty from a kiss on the brow, for he is cursed and soon must die."

-- Swiss "Almanac of Children's Songs and Games" (1857), repeating folk wisdom handed down since the Middle Ages

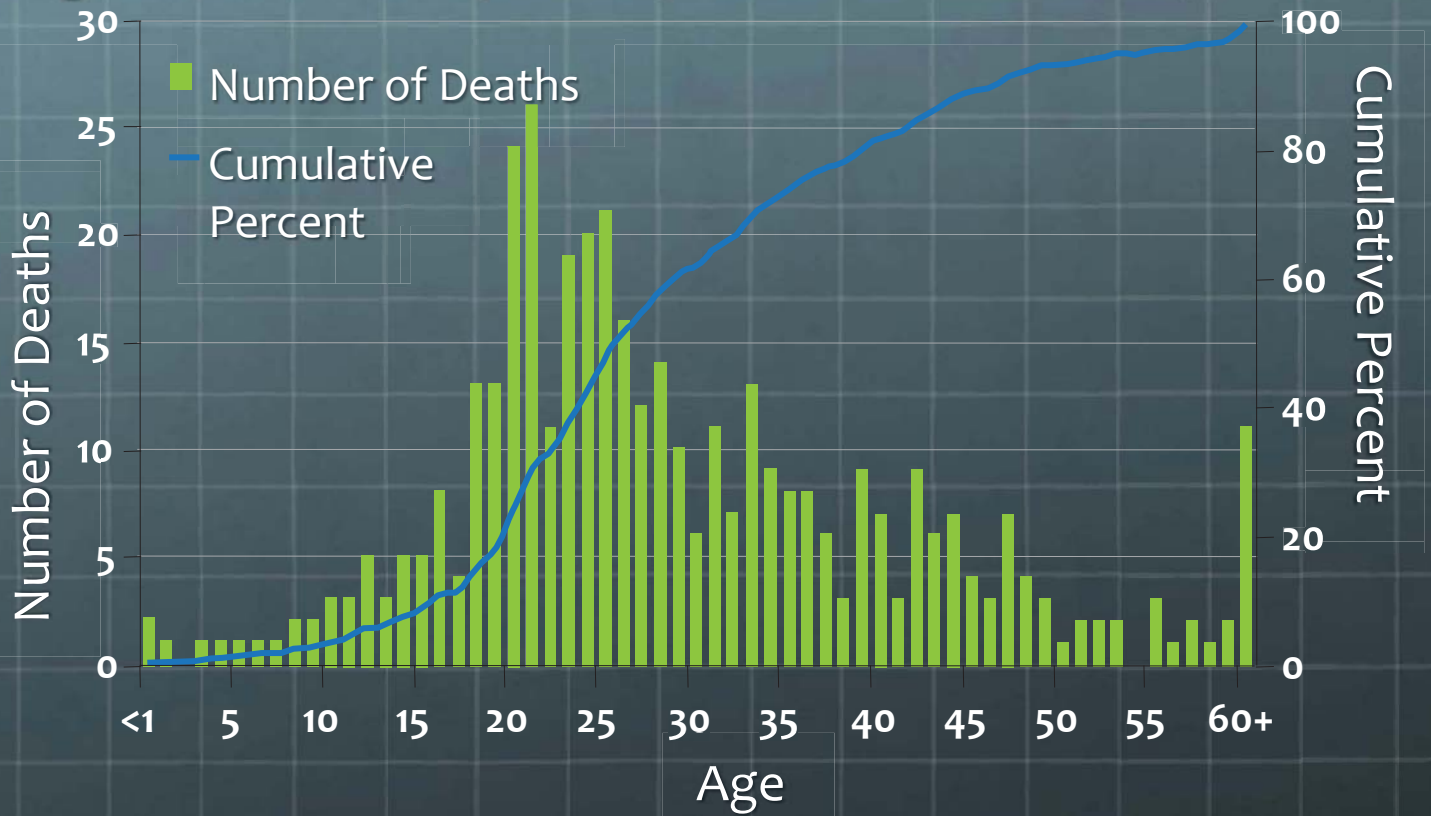


Last moments of Frédéric Chopin by Teofil Kwiatkowski. This image is in the public domain.

Age Distribution of CF Patients (USA)

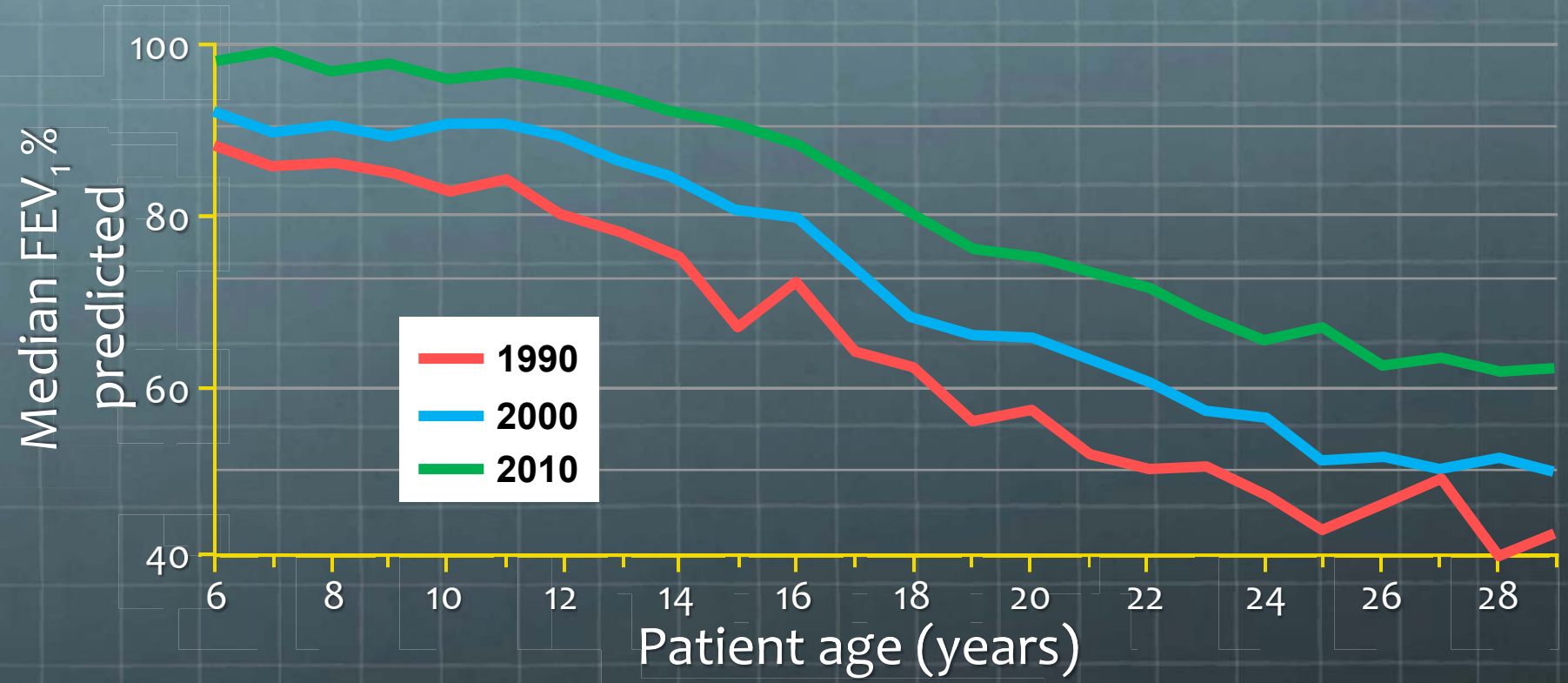
Median age of death due to CF in 2010: 26.3 years

Lung disease is the primary cause of morbidity and mortality



Cystic Fibrosis Foundation Patient Registry; 2010 Annual Data Report. Bethesda, Maryland.

FEV1 in the CF Population (USA)

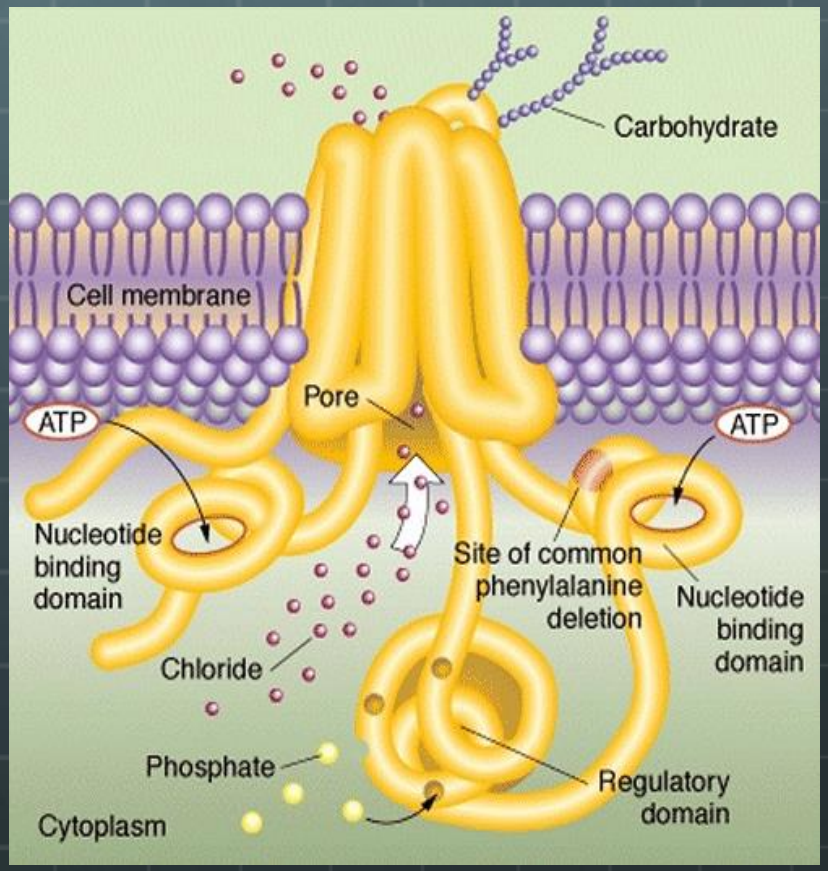


CFF Patient Registry Annual Data Report 2009. Bethesda, MD. Cystic Fibrosis Foundation.
Pellegrino et al. *Eur Respir J.* 2005;26:48-968
Davies et al. *Respir Care.* 2009;54:606-615
Kerem E, et al. *N Engl J Med.* 1992;362:1187-1891

CFTR (Cystic fibrosis transmembrane conductance regulator): An Epithelial Ion Channel

Mutant CFTR does not flux chloride ions, causing viscous mucus to build up around the cells.

- 1480 amino acid trans-membrane protein
- ABC family transporter of Cl- and HCO3- ions
- Activated by cAMP-dependent phosphorylation
- Regulates salt and fluid transport in fluid-secreting / absorbing tissues

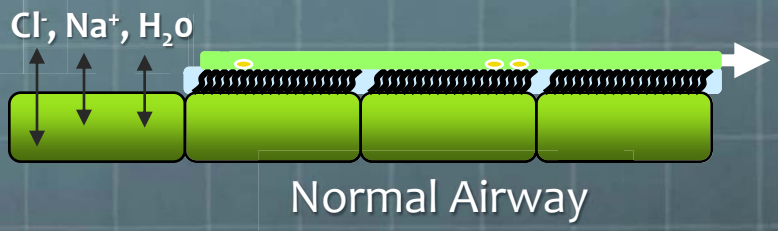


CFTR: Early Mechanistic Hypothesis

"Our results raise the possibility that the activity of mutant CFTRs in epithelial cells might, by appropriate pharmacological intervention, be increased sufficiently to ameliorate disease symptoms that appear to be largely related to insufficient Cl^- secretion."

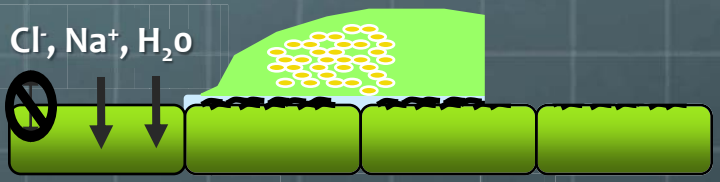
Drumm et al., 1991. Chloride Conductance Expressed by DF508 and Other Mutant CFTRs in Xenopus Oocytes. Science 254:1797

How is CFTR Function Linked to Lung Pathophysiology?



Normal Airway

Mucus layer moves bacteria, viruses and particles out of the airway



CF Airway

- Reduced fluid
- Mucus accumulates
- Blocks small airway
- Traps bacteria
- Inflammation
- Bronchiectasis
- Fibrosis, scarring

CFTR Mutations in the US CF Population

Mutation	Percent of Patients
F508del	88.5
G542X	4.6
G551D	4.4
R117H	2.7
N1303K	2.5
W1282X	2.4
R553X	1.8
621+1G->T	1.8
1717-1G->A	1.7
3849+10kbC->T	1.6
2789+5G->A	1.3
3120+1G->A	1.0

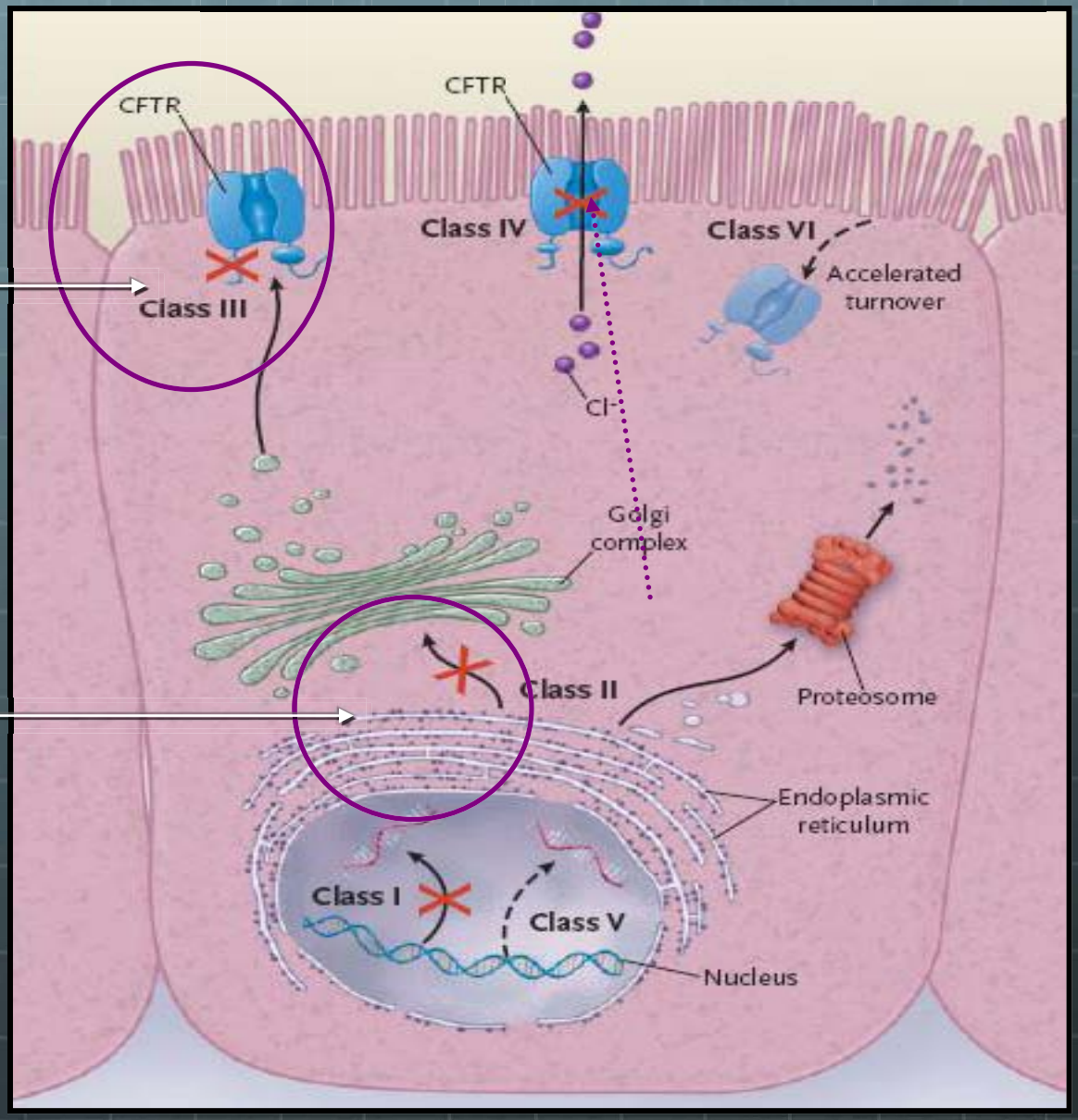
- Autosomal recessive
- Several hundred different *CFTR* mutations can cause CF
- ΔF508 is most common

All reduce either the level or function of the CFTR protein

Potentiators & Correctors

Potentiators
(Increase channel gating)

Correctors
(Improve folding of F508del)

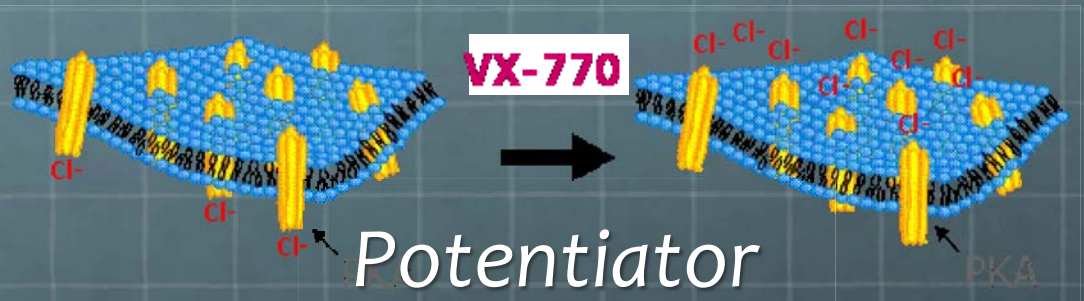


Adapted from Rowe et al. NEJM 2005

Cystic Fibrosis Program Goal: Target Underlying Cause of CF by Modulating CFTR Function to Enhance Ion Transport

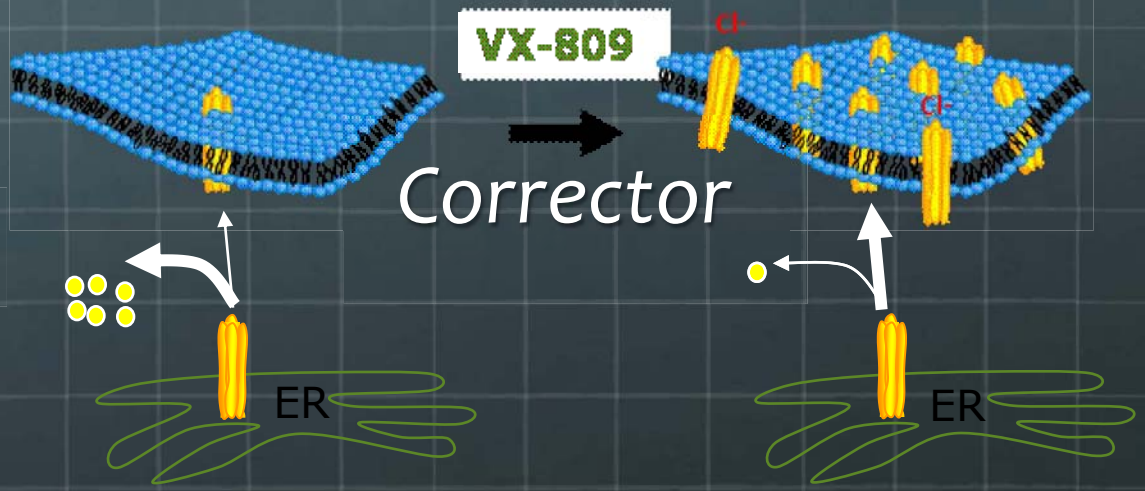
CFTR Potentiators:
Increase channel activity of CFTR protein located at the cell surface, resulting in enhanced ion transport.

Example: VX-770 (Marketed)



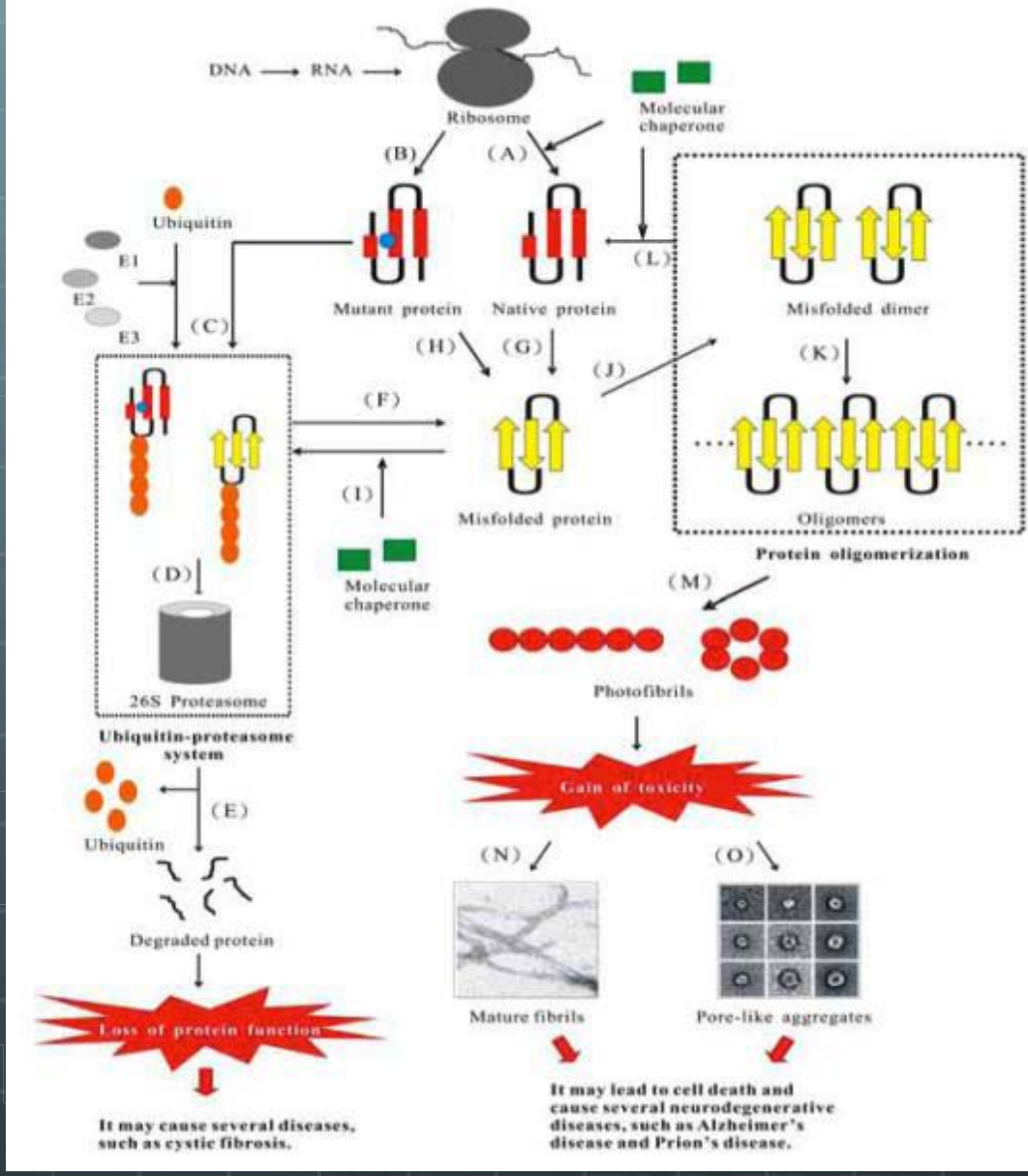
CFTR Correctors:
Increase amount of functional CFTR protein trafficked to the cell surface, resulting in enhanced ion transport.

Example: VX-809 (Phase II)

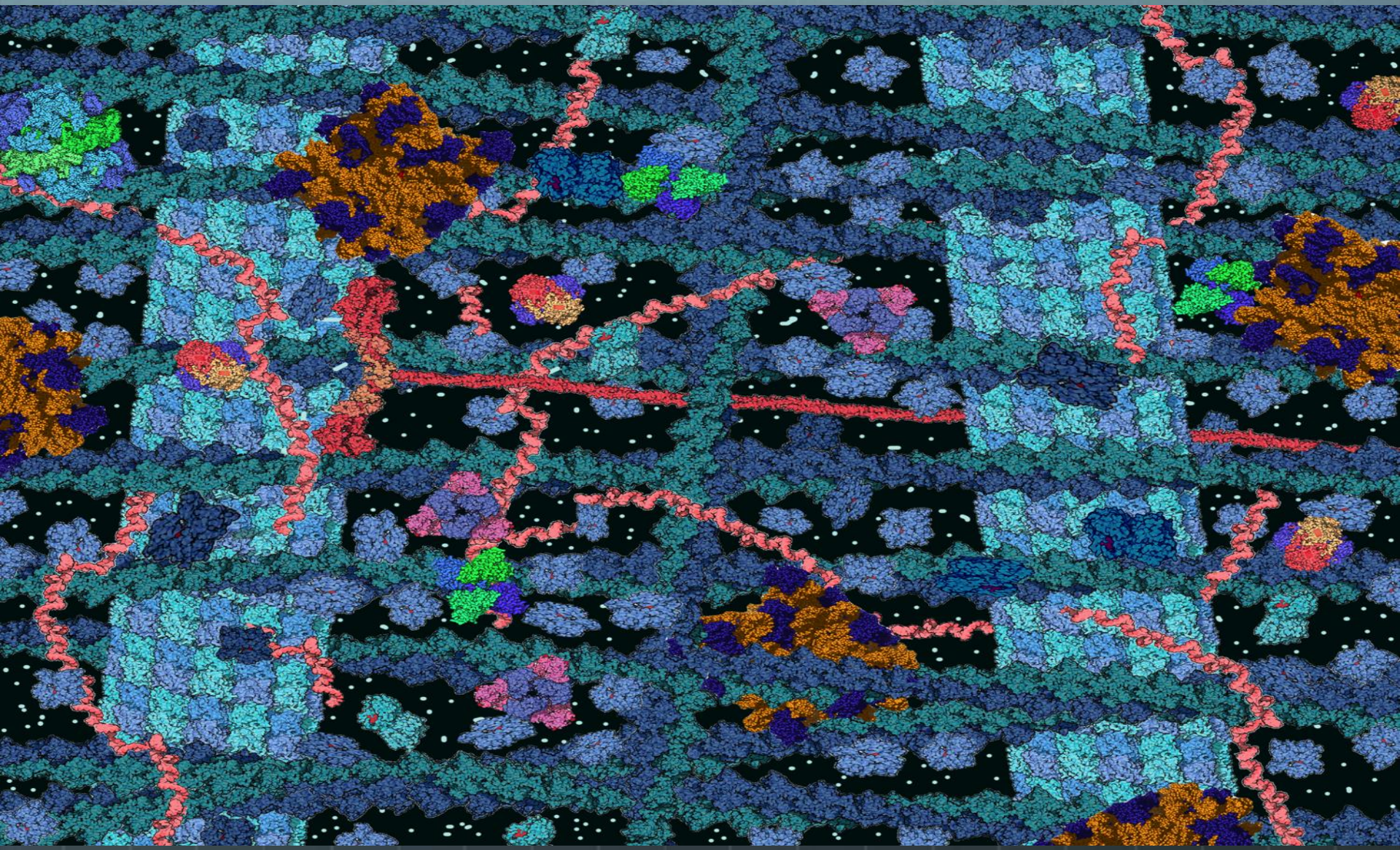


PROTEOSTASIS: Protein Homeostasis

- Process by which unfolded translated proteins arrive at their native structure(s) and how these structures are maintained and turned over.
- Proteins must fold, traffic, localize, and function in a variety of distinct environments defined by the cell's compartmentalized organization.
- Proteins cycle between inactive and active conformations in response to posttranslational modification(s) and engage in protein-protein interac-tions that enable their biology.
- These competing biological pathways comprising hundreds of components controlled by numerous integrated signaling pathways.



Source: Zhao, Jian-Hua, Hsuan-Liang Liu, et al. "Chemical Chaperone and Inhibitor Discovery: Potential Treatments for Protein Conformational Diseases." *Perspectives in Medicinal Chemistry* 1 (2007): 39-48.



Microtubules (light blue), actin filaments (dark blue), ribosomes (yellow & purple), soluble proteins (light blue), kinesin (red), small molecules (white) and RNA (pink)

Behaviors of Successful Pharma Teams

- Urgency
- Focus on patient needs; have a TPP early
- Solve high-value problems
- Curate relevant knowledge
- Interpret complex data
- Pay attention to details
- Develop validated readouts
- Generate PK data early/often
- Validate targets
- Challenge assumptions
- Resilient
- Communicate in all directions
- Have a senior champion
- Take chances
- Be practical

The Four Pillars of Effective Drug Research

- Teamwork (ad hoc) & lack of hierarchy
- Feedback from practice (tracking performance)
- Fundamental research
- Freedom to take risks

"We must make sure these qualities are not stifled. There is such an immense need for new drugs that it would be consummate folly to cripple modern drug research."

Paul Janssen, Clinical Research Reviews 1981, 1, 87-89; Susan Hughes, Scrip Magazine, December 1992; Paul Janssen, "The effect of choice on research", March, 1980; Reyntjens & Van Reet, DN&P 4(3), April 1991

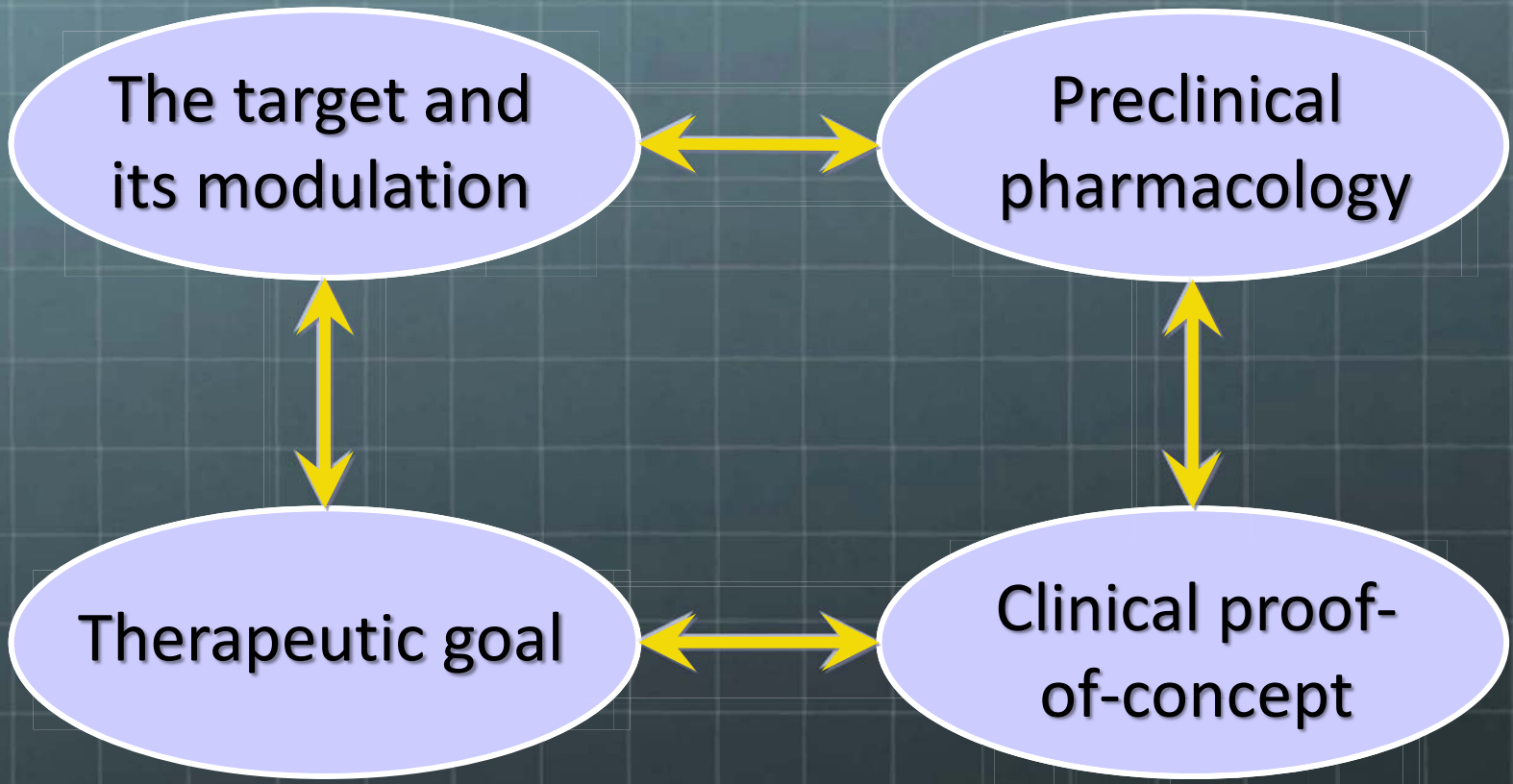
"Cathedral Thinking"

"Construction of the Tower of Babel" from the Morgan Bible is in the public domain.

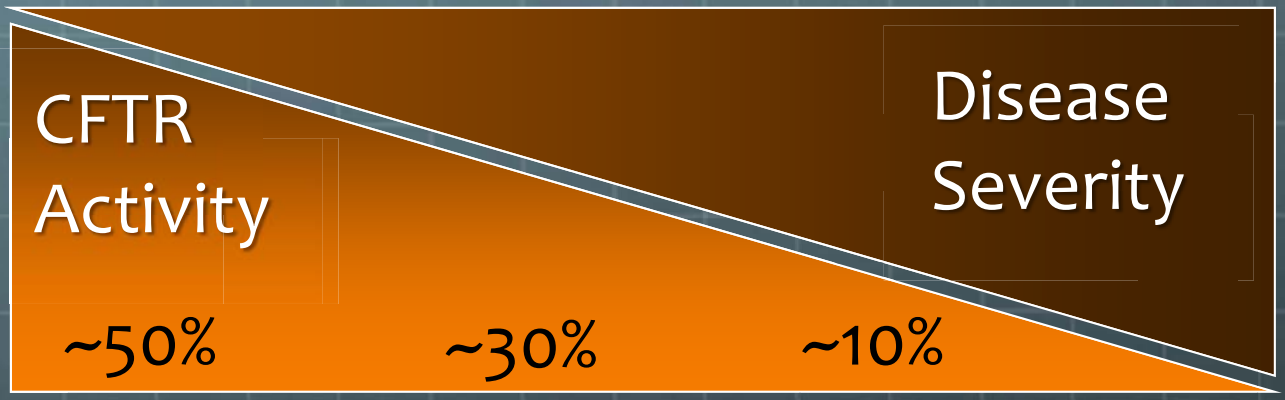
It is awe-inspiring; you are part of a larger team; many different skills are required; the work really matters; it is bigger than you are; it will outlast you; it is challenging; sometimes the building collapses but you just have to keep going

Quarryman – Stone cutter – Sculptor – Mason – Mortar maker – Carpenter – Blacksmith – Roofer – Glazier – Architect – Engineer

Framework For Thinking About CF



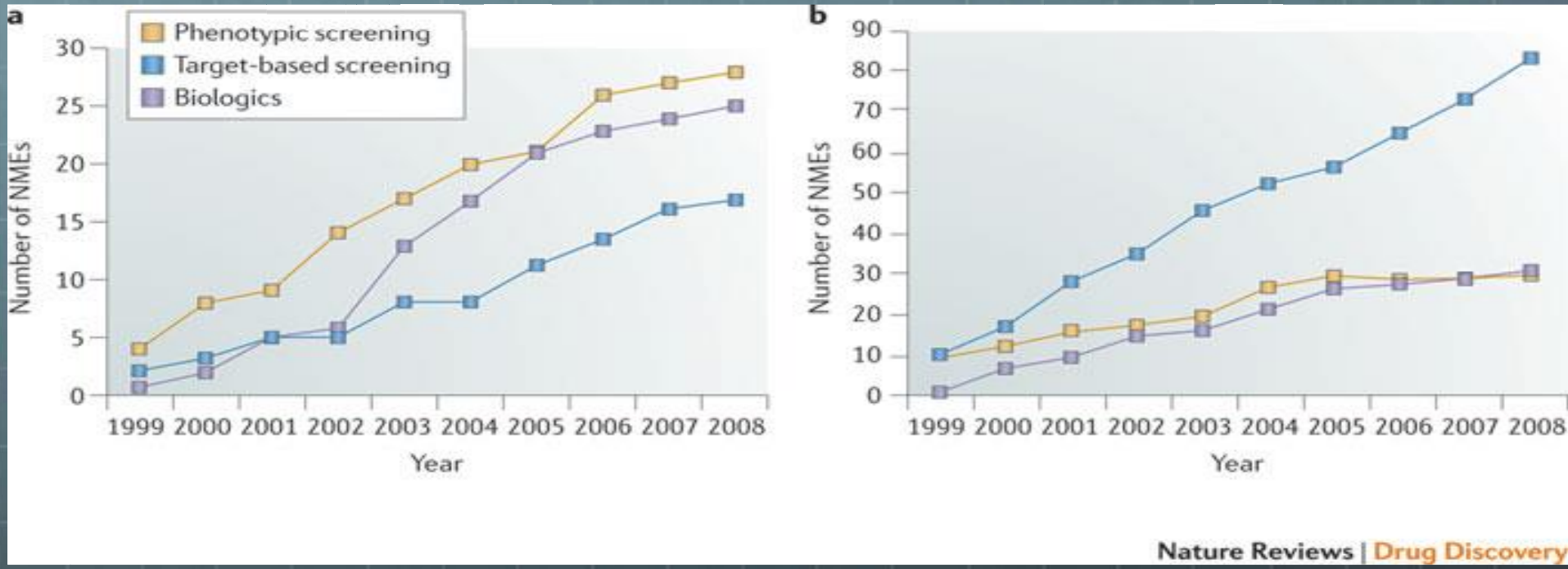
Natural History Data Provides Clue to Drug Requirements



No phenotype: Heterozygote carriers	CF-related phenotypes	Milder CF	Severe CF: "null" mutations

Phenotypic Screening's Track Record

First-in-class *Followers*

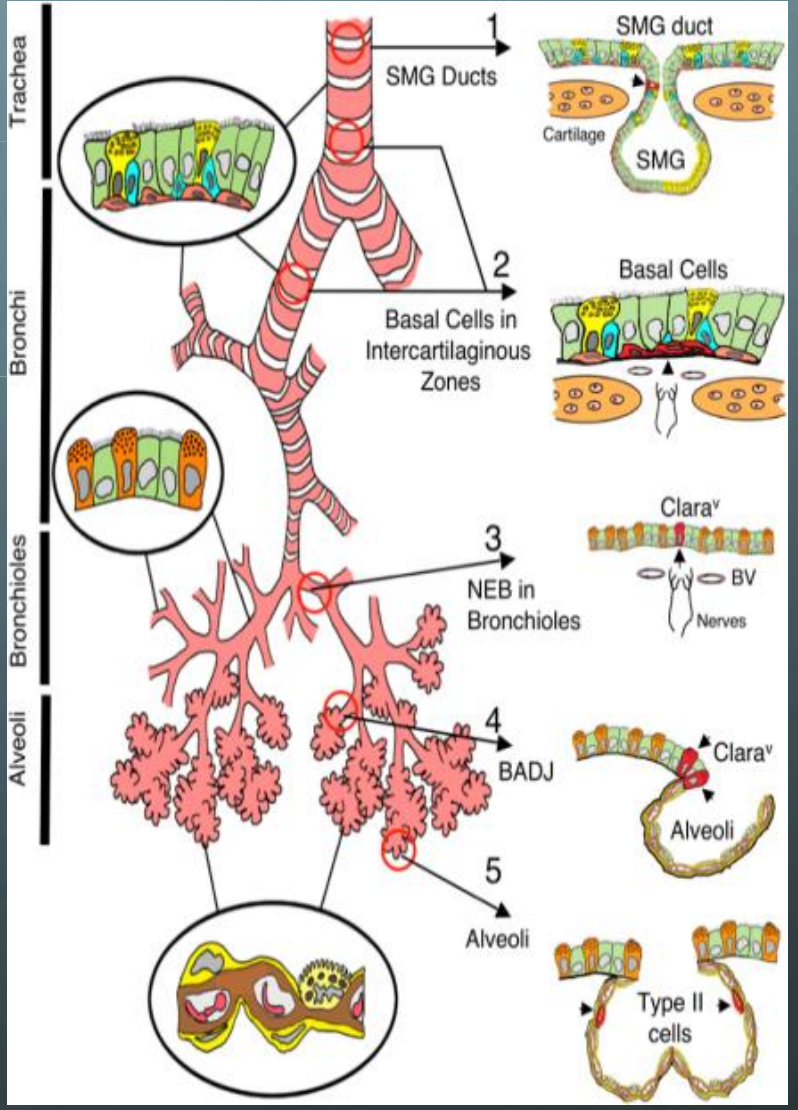


- Probes entire pathway(s) - can be multiple classes of hits
- Hits are excellent tools
- Encourages clear thinking about screening collection & assays
- Focuses chemistry on phenotype, pharmacology, tox

Isolation of Primary Cells From CF Airway

(1) *Neuberger, Van Goor, et al. Chapter 4, Use of 1˚ Cultures of Human Bronchial Epithelial Cells Isolated from Cystic Fibrosis Patients for Pre-clinical Testing of CFTR Modulators. In Cystic Fibrosis, Methods in Molecular Biology 741, M.D. Amaral, K. Kunzelmann (eds.), 2011.*

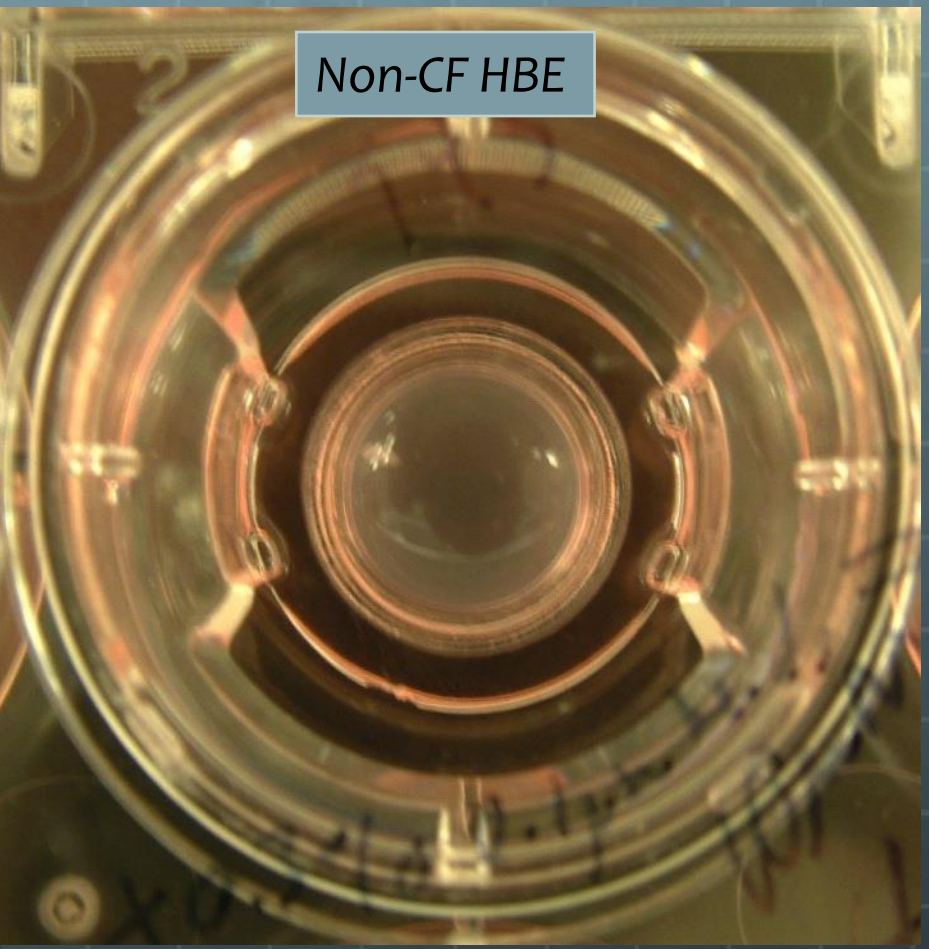
(2) *Rescue of airway epithelial cell function in vitro by a CFTR potentiator. PNAS, 2009, 106, 18825.*

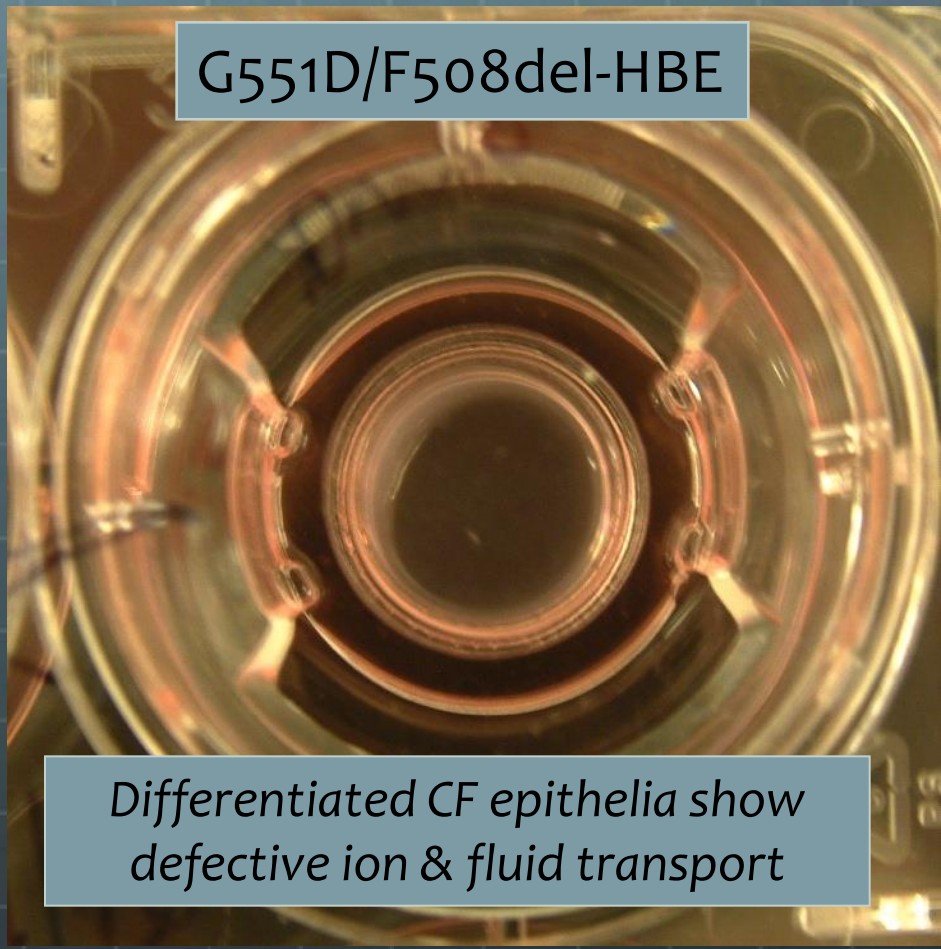


Source: Liu, Xiaoming, and John F. Engelhardt. "The Glandular Stem/progenitor Cell niche in Airway Development and Repair." *Proceedings of the American Thoracic Society* 5, no. 6 (2008): 682.

Isolation of Primary Cells From CF Airway:

CFTR Pharmacology in Cultured Human Bronchial Epithelia





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(2) Rescue of airway epithelial cell function in vitro by a CFTR potentiator. PNAS, 2009, 106, 18825.

Ivacaftor (VX-770): 1st Potentiator Development Candidate

High-throughput screening

↓

Prioritize hits

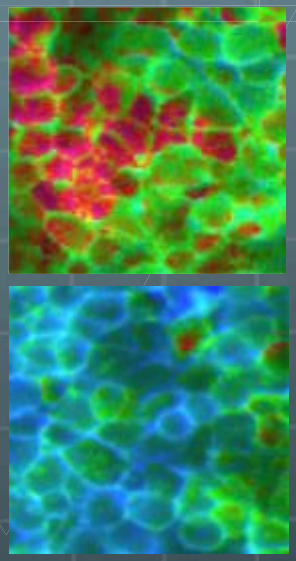
↓

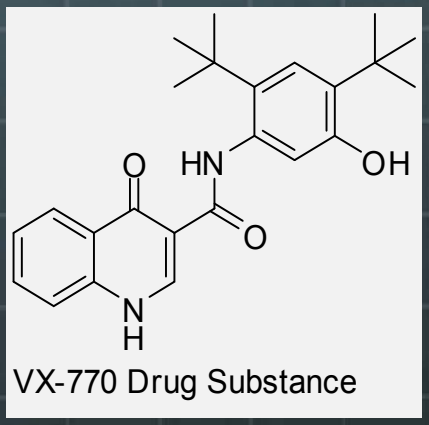
Medicinal Chemistry

↓

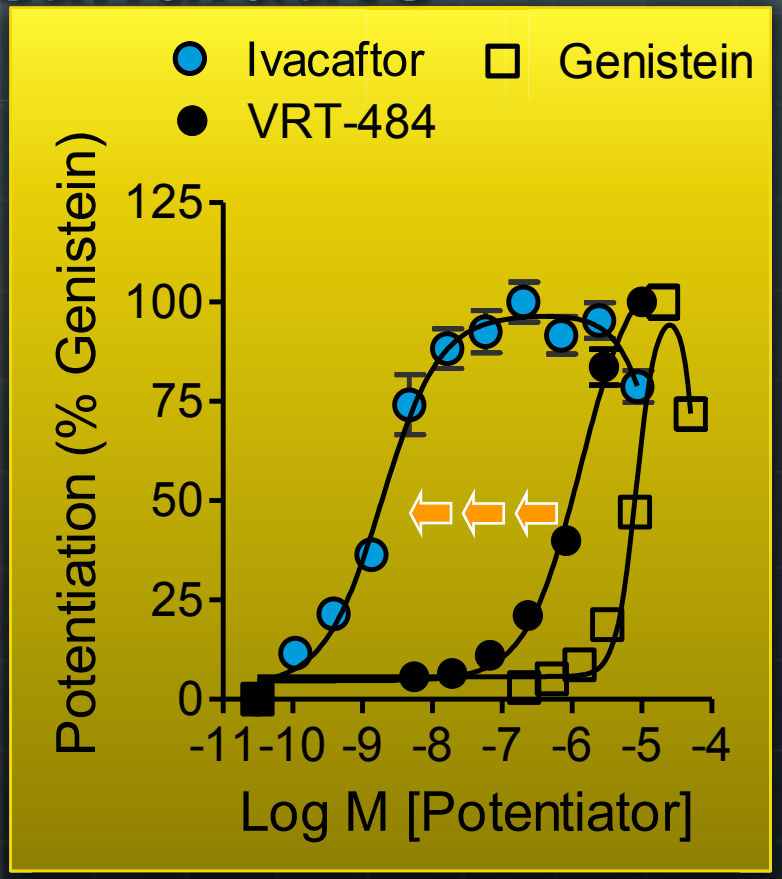
Ivacaftor

Screening Assay



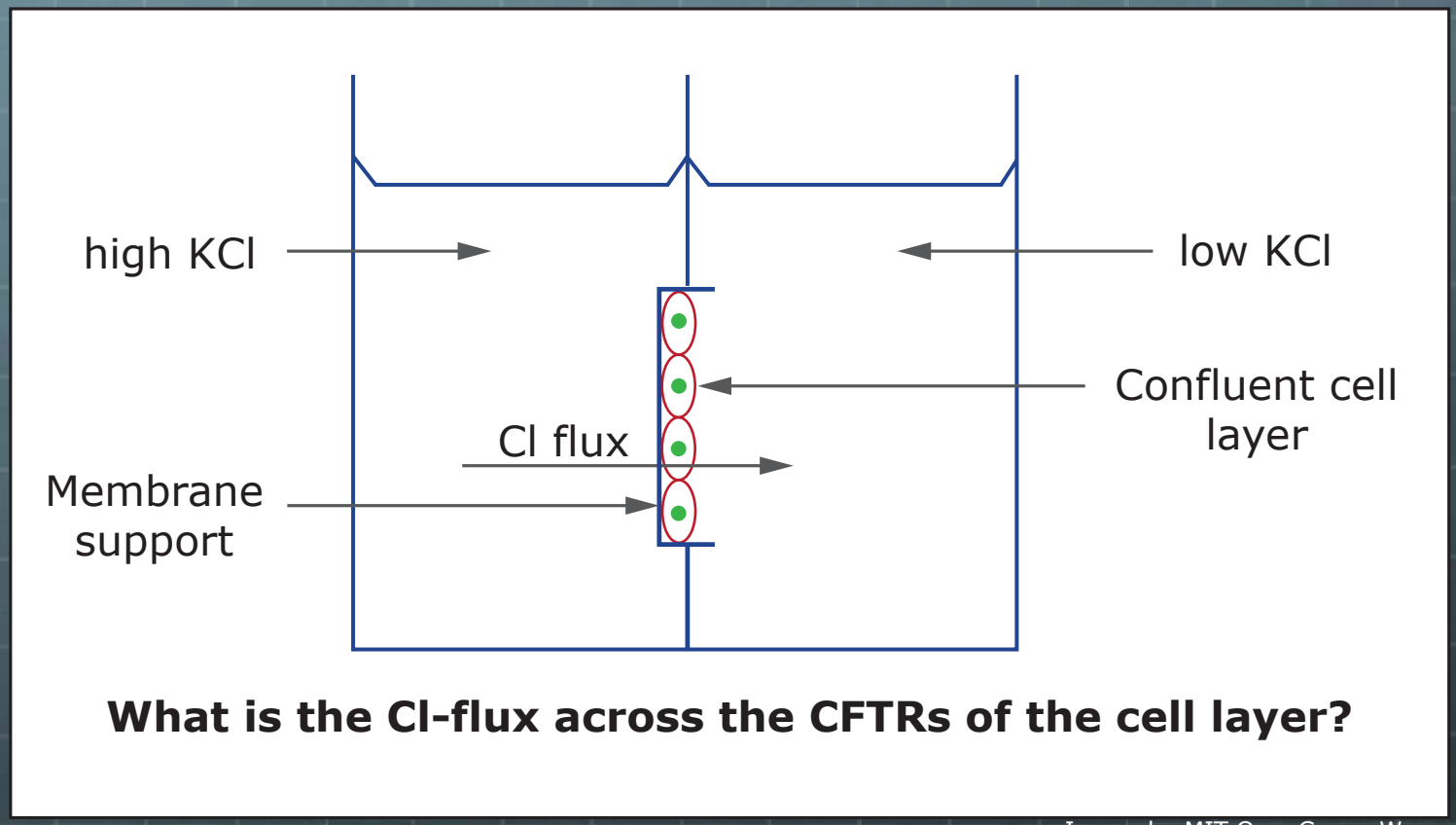


VX-770 Drug Substance



Van Goor et al. PNAS 2009;106:18825-30

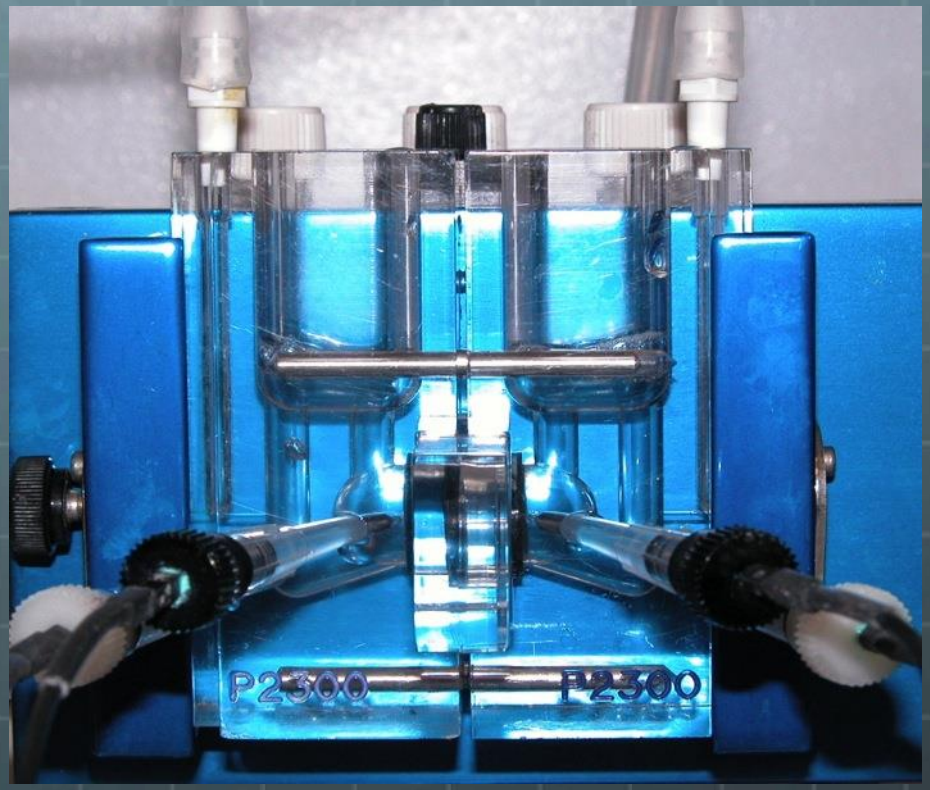
Ussing Chamber Assay



What is the Cl-flux across the CFTRs of the cell layer?

Image by MIT OpenCourseWare.

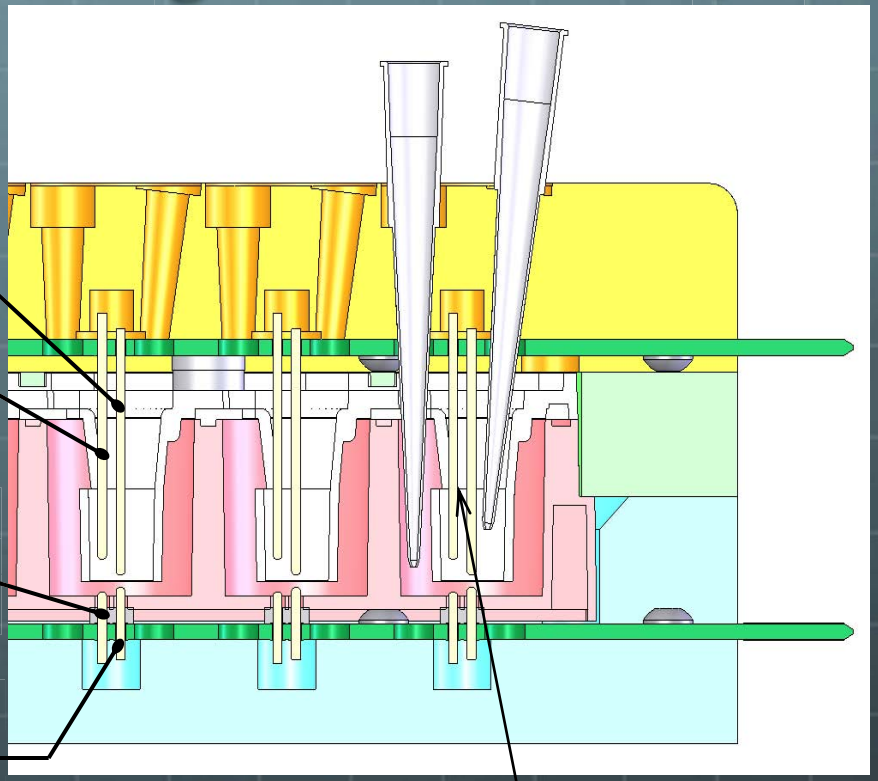
Ussing Chamber Innovation: 1 Well → 24 Wells

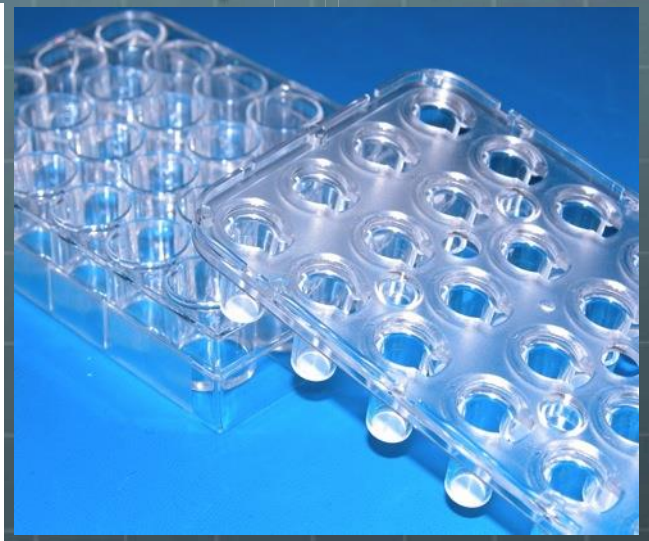


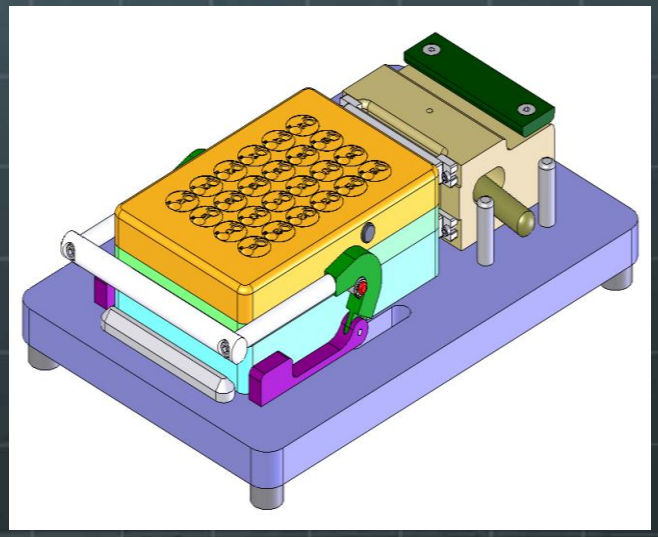
Courtesy of Minh Vuong. Used with permission.

Miniaturized Ussing Engine(MUsE)

24 Ussing chambers in a 24 Transwell® plate

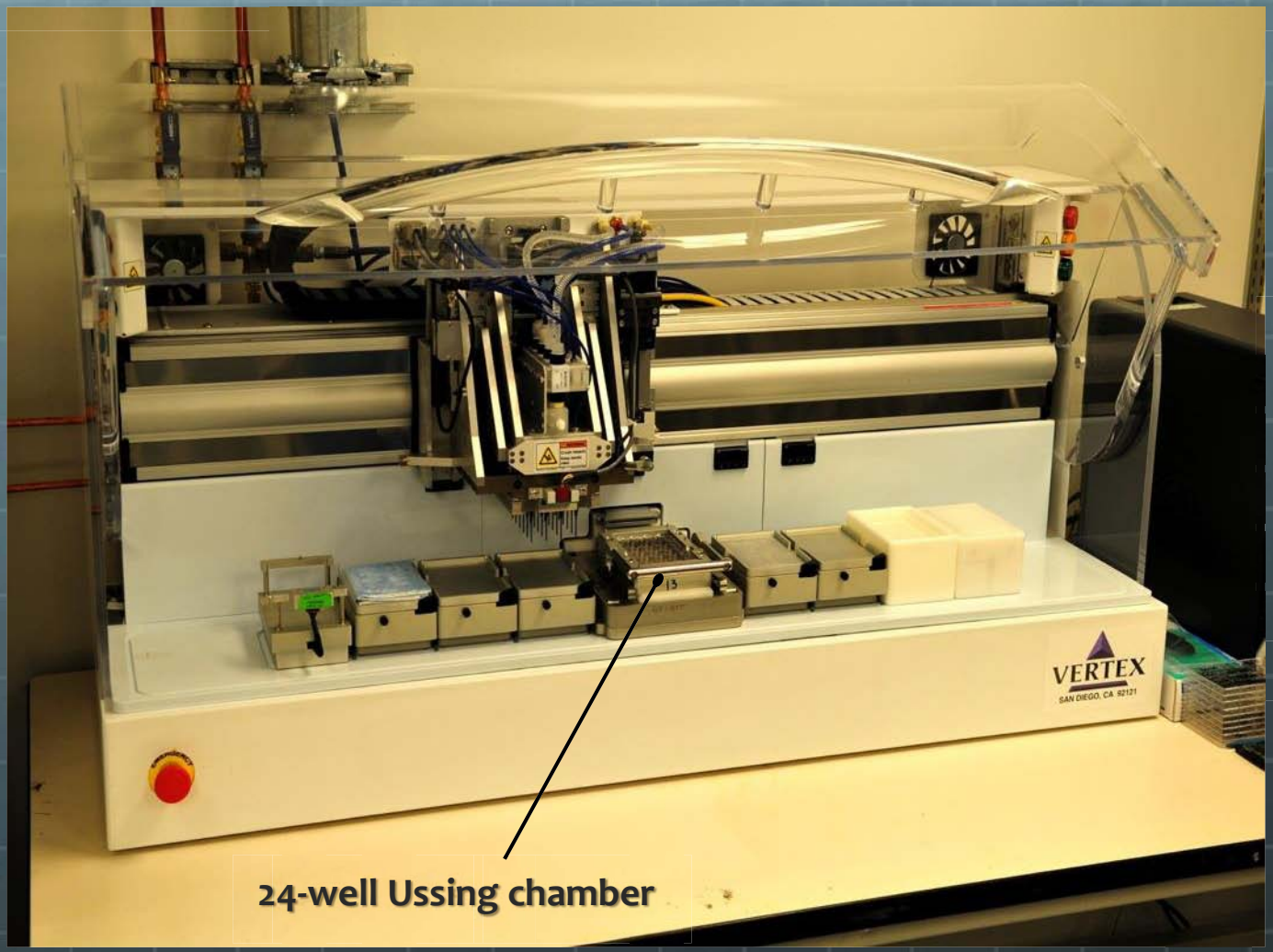






Negulescu, Harootunian, Salzmann, Flores, Sinclair, Vuong, Singh, and Van Goor. US Pat. 7,169,609 B2 (Jan 30, 2007)

MUsE-24 With Automated Pipetting



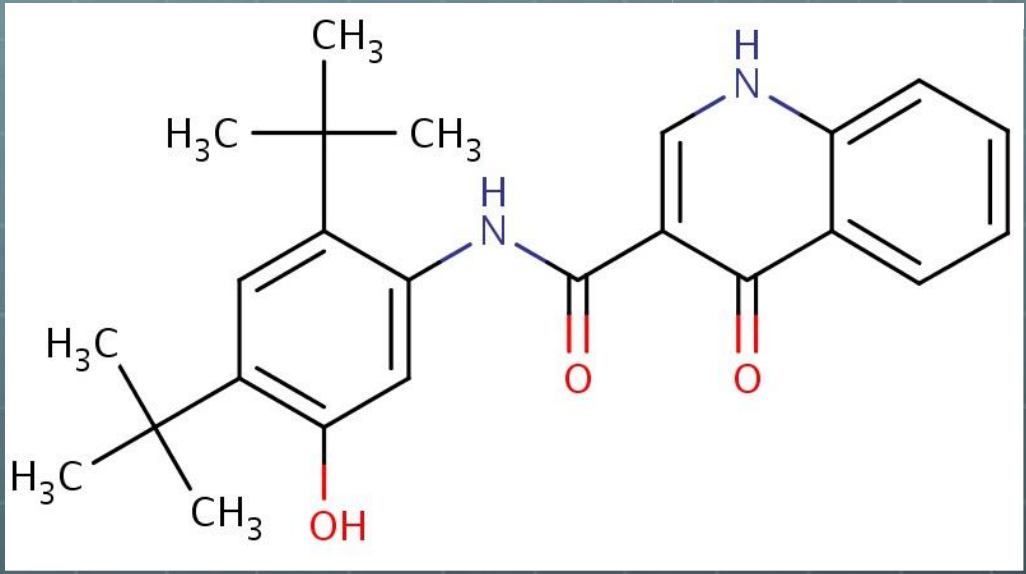
Non-standard pitch 24-channel pipetter

Accommodates 24-Transwell® plate in a MuSE "nest"

Courtesy of Minh Vuong. Used with permission.

Instrumentation: Harootunian, Salzmann, Flores, Sinclair, and Vuong

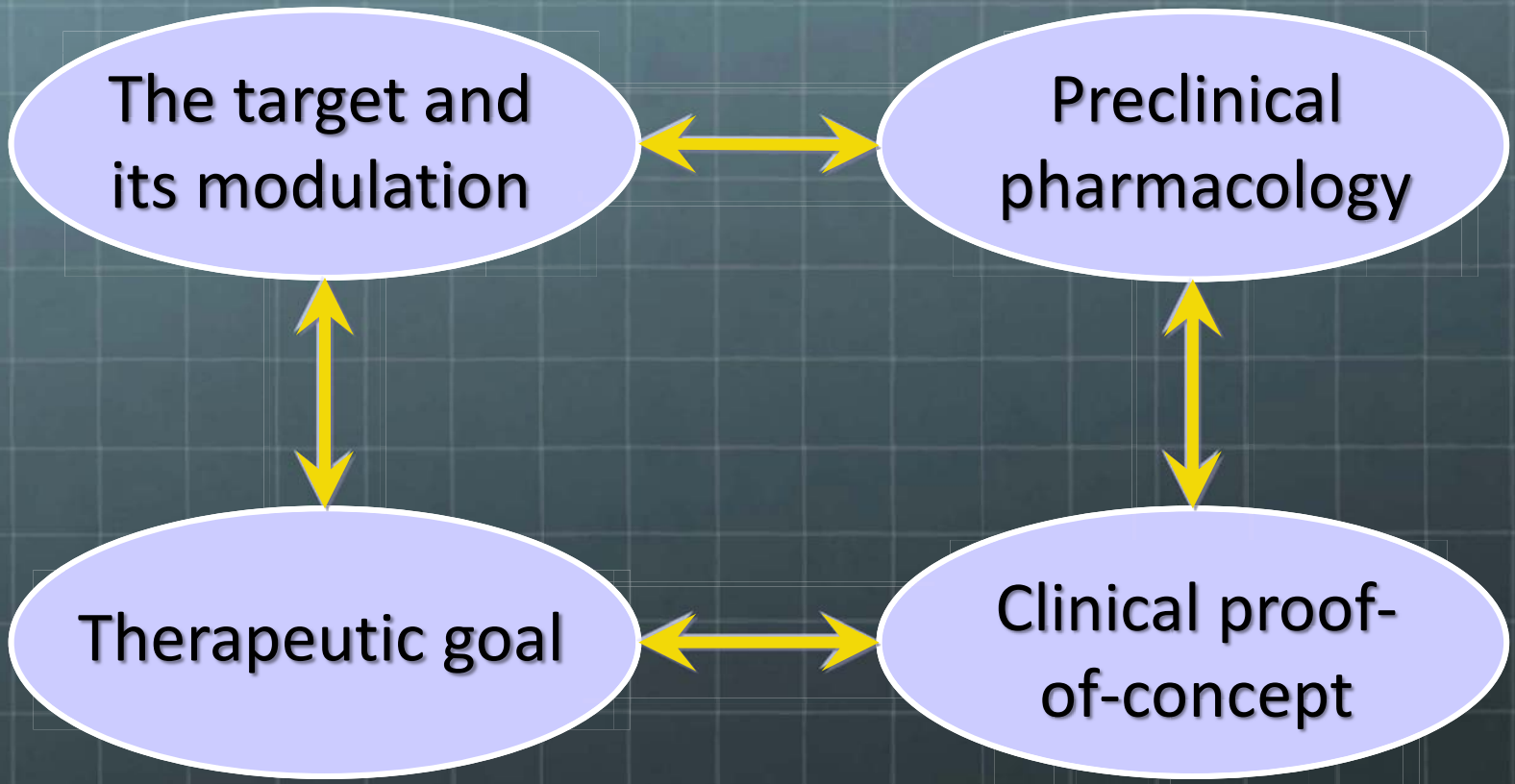
Chemistry of Ivacaftor (Kalydeco)

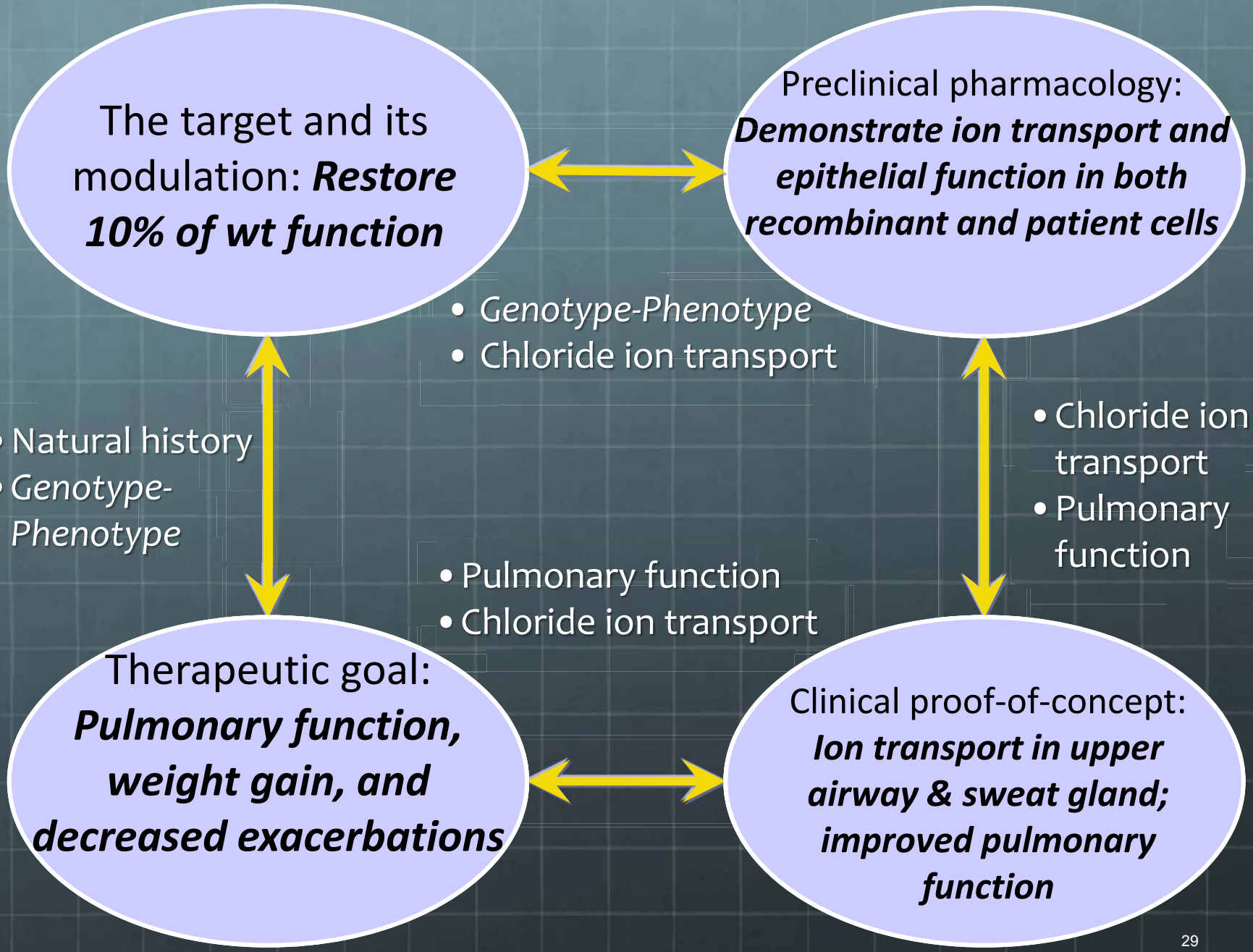


- MW = 392, 3 Hbond donors, 5 Hbond acceptors, PSA ~90, calculated logP ~3.8
- All of these numbers suggest a well-behaved compound.
- However, mp = 292, aqueous solubility < 0.05 µg/ml, and measured logP ~5.7

- One explanation for the poor properties of Ivacaftor may be the extensive crystal packing formed by the molecule.
- A suspension of the spray-dried dispersion was required to achieve reasonable bioavailability.

Framework For Thinking About CF




The target and its modulation: ***Restore 10% of wt function***
Preclinical pharmacology: ***Demonstrate ion transport and epithelial function in both recombinant and patient cells***
• *Genotype-Phenotype*
• Chloride ion transport
• Natural history
• *Genotype-Phenotype*
• Chloride ion transport
• Pulmonary function
• Pulmonary function
• Chloride ion transport
Therapeutic goal: ***Pulmonary function, weight gain, and decreased exacerbations***
Clinical proof-of-concept: ***Ion transport in upper airway & sweat gland; improved pulmonary function***

CF: Lessons

- Genetic diseases can provide a solid link between the target (or pathway) and the therapeutic goal(s)
  - Understand genotype-phenotype relationships and natural history of individuals with a spectrum of mutations
  - Phenotypic programs are great so long as the assays recapitulate disease biology & correlate with clinical outcomes – but require building the right assays and developing new technology when needed (requires time & specialized skills)
  - A proof-of-concept clinical study should connect the molecular mechanism and the therapeutic goal(s)
- Take "rules" about "drug-likeness" with a grain of salt
- Network with disease foundations
- Current clinical, regulatory and payer paradigms are not adequate for CF and other rare genetic diseases

20.201 Mechanisms of Drug Actions

Fall 2013