Cystic Fibrosis
“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

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FEV1 in the CF Population (USA)

Median FEV1 % predicted

Patient age (years)

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Pellegrino et al. Eur Respir J. 2005;26:48-968
Davies et al. Respir Care. 2009;54:606-615
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 transmembrane protein
- ABC family transporter of Cl- and HCO3- ions
- Activated by cAMP-dependent phosphorylation
- Regulates salt and fluid transport in fluid-secreting / absorbing tissues
“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?

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

• Reduced fluid
• Mucus accumulates
• Blocks small airway
• Traps bacteria
• Inflammation
• Bronchiectasis
• Fibrosis, scarring
CFTR Mutations in the US CF Population

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Percent of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>F508del</td>
<td>88.5</td>
</tr>
<tr>
<td>G542X</td>
<td>4.6</td>
</tr>
<tr>
<td>G551D</td>
<td>4.4</td>
</tr>
<tr>
<td>R117H</td>
<td>2.7</td>
</tr>
<tr>
<td>N1303K</td>
<td>2.5</td>
</tr>
<tr>
<td>W1282X</td>
<td>2.4</td>
</tr>
<tr>
<td>R553X</td>
<td>1.8</td>
</tr>
<tr>
<td>621+1G-&gt;T</td>
<td>1.8</td>
</tr>
<tr>
<td>1717-1G-&gt;A</td>
<td>1.7</td>
</tr>
<tr>
<td>3849+10kbC-&gt;T</td>
<td>1.6</td>
</tr>
<tr>
<td>2789+5G-&gt;A</td>
<td>1.3</td>
</tr>
<tr>
<td>3120+1G-&gt;A</td>
<td>1.0</td>
</tr>
</tbody>
</table>

- 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)

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Adapted from Rowe et al. NEJM 2005
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 interactions that enable their biology.

These competing biological pathways comprising hundreds of components controlled by numerous integrated signaling pathways.

Courtesy of the authors. License: CC-BY.
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

Behaviors

- 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.”

“Cathedral Thinking”

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.

Framework For Thinking About CF

The target and its modulation

Preclinical pharmacology

Therapeutic goal

Clinical proof-of-concept
Natural History Data Provides Clue to Drug Requirements

CFTR Activity vs. Disease Severity:
- ~50% CFTR Activity: Severe CF
- ~30% CFTR Activity: Milder CF
- ~10% CFTR Activity: No phenotype

No phenotype: Heterozygote carriers
CF-related phenotypes
Milder CF
Severe CF: “null” mutations
Phenotypic Screening’s Track Record

First-in-class

- 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

Followers

Isolation of Primary Cells From CF Airway


(2) Rescue of airway epithelial cell function in vitro by a CFTR potentiator. PNAS, 2009, 106, 18825.
Isolation of Primary Cells From CF Airway:

CFTR Pharmacology in Cultured Human Bronchial Epithelia

Differentiated CF epithelia show defective ion & fluid transport


(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

Potentiation (% Genistein)

Log M [Potentiator]

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Van Goor et al. PNAS 2009;106:18825-30
What is the Cl-flux across the CFTRs of the cell layer?
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)

Courtesy of Minh Vuong. Used with permission.
MUsE-24 With Automated Pipetting

24-well Ussing chamber

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

Preclinical pharmacology

Therapeutic goal

Clinical proof-of-concept
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

Therapeutic goal: **Pulmonary function, weight gain, and decreased exacerbations**

Clinical proof-of-concept: **Ion transport in upper airway & sweat gland; improved pulmonary function**

- Pulmonary function
- Chloride ion transport
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