New Developments in CF Research

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Surface of a CF Trachea

Simel et al., Ped. Pathol. 2:47-64, 1984
Pathophysiology of Cystic Fibrosis

1. **CFTR gene defect**
2. **Abnormal CFTR protein**
3. **Defective ion transport**
4. **Depleted ASL Abnormal Mucus**
5. **Defective mucociliary clearance**

**Cycle of Destruction**
- **Infection**
- **Inflammation**
- **Mucus Obstruction**
- **Scarring**

**End Stage Lung Disease**
Development of CF Lung Disease and Possible Interventions

Basic Defect

Abnormal Gene

Abnormal Protein

Altered Ion Transport, Mucus Secretion

Infection & Inflammation, Tissue Destruction

Organ Destruction, Respiratory Failure

Gene Therapy

Protein Rescue

MCC, Ion Transport

Anti-Inflammatory, Anti-microbials

Transplantation

Associated Abnormalities
CFTR Mutation Classes

- > 1,900 mutations in CFTR identified

- Divided into six classes

- Class I caused by nonsense mutations (common minority)

- Class II defect most common (F508del CFTR)

- Class III include G551D (target for VX-770)

Therapeutic Approaches by Class

- **F508del CFTR Processing Corrector**
  - F508del, possibly others

- **CFTR Potentiators**
  - G551D, R117H, R1070W...

- **Translational Readthrough**
  - G542X, W1282X, R1162X, ...

Ultimate Goal:
Slow the Rate of Decline in FEV$_1$

Increase FEV$_1$ with same rate of decline = little effect on survival

Slow FEV$_1$ rate of decline = improves survival
FEV$_1$ % Predicted Absolute Change from Baseline

Treatment effect through Week 24
+ 10.6 %
$P < 0.0001$

Treatment effect through Week 48
+ 10.5 %
$P < 0.0001$

Time-to-First Pulmonary Exacerbation

Modified Fuchs’ criteria

Week 24
Hazard Ratio
0.40
P = 0.0016

Week 48
Hazard Ratio
0.46
P = 0.0012

Event-Free Rate At Week 48
0.41

Placebo
VX-770

Proportion of event-free subjects

Study day

Key secondary endpoint in gold

Ivacaftor Results in G551D CF Subjects Present a Road Map for Success

Adapted from Wilschanski et al., AJRCCM 2006 Oct 1;174(7):787-94
GOAL Study

Core Study Measures
- Clinical outcome
- Sweat chloride
- Quality of life
  - CFQ-R
  - SNOT-20
  - CFRSD
- Biomarker collection
  - Serum
  - Plasma
  - DNA
  - Urine
  - Sputum

Additional Sub-Study Measures
- MCC/Rheology – visits 2, 3, 4
  - Radionuclear mucociliary clearance
  - Micro-rheology
  - Bulk rheology
- Sweat Rate – visits 1 to 4
  - Sweat evaporimetry
  - Exploratory sweat outcomes
- Intestinal pH – visits 2, 3
  - Intestinal pH by radiofrequency transmitter
- Sputum Inflammation & Microbiome – visits 2, 5
  - Induced sputum
  - Inflammatory mediators
  - Sputum microbiome

Visit 1
- Decision made to start ivacaftor? (before end of study enrollment)
  - yes
    - Visit 2 → Day 1
      - Pre-Dose
      - First dose of ivacaftor
    - Visit 3
      - 1 month after Day 1
  - no
    - Ivacaftor not prescribed
    - Visit 1b

Visit 2 → Day 1 → Visit 3 → Visit 4 → Visit 5 → Visit 1b
Change in FEV$_1$% with Ivacaftor

Rowe et al., Am J Resp Crit Care Med, 2014
Change in *P. aeruginosa* Culture Rate

**Percent with *Pseudomonas Aeruginosa* & 95% CI**

- [-12, -6) months: 55% (N: 126)
- [-6, 0) months: 52% (N: 143)
- [0, 6) months: 34% (N: 122)
- [6, 12) months: 35%

*Change in *P. aeruginosa* Culture Rate*

- *p < 0.01*
- **p < 0.001 Wilcoxon sign test*

Beneficial effect of Ivacaftor on Sputum Microbiology

Heltshe S et al., Clin Infect Dis. 2015 Mar 1;60(5):703-12
MCC Imaging

Pre-drug

Post-drug

Scott Donaldson, Sub-study PI; Images courtesy Tim Corcorran, U Pittsburgh
Correction of F508del is an Important but Challenging Therapeutic Target

- F508del CFTR exhibits multiple defects:
  - Impaired cellular processing due to improper protein folding, resulting in degradation
  - Small amounts of F508del-CFTR that are delivered to membrane exhibit defective gating
  - Poor membrane half-life and increased turnover
Increased Activity of F508del Homozygous CFTR in Cell Cultures with Lumacaftor in Combination with Ivacaftor

F508del-CFTR Chloride Transport (% of normal CFTR)

- No drug
- Ivacaftor Alone (3 μM)
- Lumacaftor Alone (3 μM)
- Lumacaftor + Ivacaftor (3μM + 3μM)

+ CFTR corrector

+ CFTR potentiator

P=0.0189
P=0.0033
P=0.0288
P=0.0119
P=0.0189

Modified from Van Goor et al. PNAS 2011
Lumacaftor/Ivacaftor Combination Therapy: FEV$_1$ % predicted in F508del homozygous patients

![Graph showing change in absolute FEV$_1$ % predicted (mean ± 95%CI) for monotherapy and combination therapy over days 1 to 56.](image)

- **Monotherapy**
- **Combination**

- * $P<0.05$ within-group
- ** $P \leq 0.01$ within-group
- † $P<0.05$ vs placebo
- †† $P<0.01$ vs placebo

Study Design

- Two Phase 3, randomized, double-blind, placebo-controlled, parallel-group study. Patients who completed TRAFFIC/TRANSPORT were able to enter the PROGRESS (105) rollover study.
  - Conducted at 187 sites in North America, Europe, and Australia
  - TRAFFIC: Ambulatory ECG in a subset; TRANSPORT: Adolescent PK in a subset
- Key eligibility criteria:
  - Age $\geq 12$ years, confirmed CF diagnosis
  - Homozygous for $F508del-CFTR$
  - Percent predicted FEV$_1$ $\geq 40$ to $\leq 90$ at screening
Percent Predicted FEV$_1$: Pooled TRAFFIC & TRANSPORT

Absolute Change from Baseline in Percent Predicted FEV$_1$

<table>
<thead>
<tr>
<th>Treatment Description</th>
<th>Treatment Difference vs Placebo (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUM 600 mg qd + IVA 250 mg q12h</td>
<td>3.3 (P&lt;0.0001)</td>
</tr>
<tr>
<td>LUM 400 mg q12h + IVA 250 mg q12h</td>
<td>2.8 (P&lt;0.0001)</td>
</tr>
</tbody>
</table>

*As assessed by the average absolute change from baseline at Weeks 16 and 24 according to the prespecified statistical analysis plan.
Analysis of Pulmonary Exacerbations: Pooled

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Events (rate/ 48 weeks)</th>
<th>Rate Ratio vs Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>251 (1.14)</td>
<td>--</td>
</tr>
<tr>
<td>LUM 600 mg qd + IVA 250 mg q12h</td>
<td>173 (0.80)</td>
<td>0.70, P=0.0014</td>
</tr>
<tr>
<td>LUM 400 mg q12h + IVA 250 mg q12h</td>
<td>152 (0.70)</td>
<td>0.61, P&lt;0.0001</td>
</tr>
</tbody>
</table>
PROSPECT PART B

Cohort 3 (DF508) Part A
Visit 1 Visit 2 Visit 3
Day 0 Day 14 Month 3
Note: V3 would be skipped if Day 1 (and V4 are scheduled prior to V3 (Month 3) of Part A

Additional DF508 homozygous subjects allowed to enroll in PART B only

Decision made to start 809/770? (Anytime after V2)

Visit 4 Day 1 Visit 5 Visit 6 Visit 7 Visit 8
First dose of lumacaftor/ivacaftor 1 months after Day 1 3 months after Day 1 6 months after Day 1 12 months after Day 1

Part B Core Procedures
- Specimen Collection for Banking:
  - Serum/Plasma/Buffy Coat
  - Urine
- Nasal Epithelial Cell Procurement (at selected sites)
- Sweat Chloride
- Clinical Labs:
  - CBC w/diff
- Spirometry
- Sputum induction:
  - Inflammatory mediators
  - Microbiome
  - Bank

Part B Sub-Studies
- MBW/FENO Sub Sites (N= 10)
  - MBW (V 4,5,6,7,8)
  - FENO (V4,5,7)
- MBW/FENO Sub Subjects (N= 68)
- MCC Sub Sites (N= 4)
  - MCC Sub Subjects (N=44)*
    - Sub-set of MBW/FENO subjects
  - MCC (V4,5)
- GIFT Sites (N= 30)
  - GIFT Subjects (N=75)
    - Fecal Collection (V 5,6)
    - Breath Test (V 4,5)
    - HbA1C (V4,7,8)
    - 2 HR OGTT (V4,5,7,8)
      - glucose/insulin/c-peptide (0, 30, 60, 90, 120 min)
- pH Pill Subjects (N=20)*
  - Sub-set of GIFT subjects
    - V (4, 5)
Mapping Pharmacological CFTR Response Onto Genotype-Phenotype Relationship

Adapted from Wilschanski et al., AJRCCM 2006
Solving the F508del Problem Will Likely Require Multi-agent Therapy

- CFTR correction of F508del for single agents is still inherently inefficient
- The next challenge is to develop corrector and potentiator combinations that achieve near normal processing and function
- New discoveries pinpointing F508del-induced defects are providing precise molecular targets and a new path for discovery
- However, there are challenges:
  - Agents may interact in negative fashion
  - Need means to predict efficacy on an individual level
Major ‘Shots on Goal’ for CF therapy in the next few years

- New companies with potentially advantageous CFTR modulators
  - Potentiators, correctors, **translational readthrough agents**
- Multi-agent corrector therapy
- Circumventing CFTR by other ion transport agents
  - ENaC blockers, TMEM agonists
- **Tools for individualizing CFTR-directed therapies**
- Targeting CF mucus itself
Premature Termination Codons (PTCs) Frequently Cause Human Disease

Transcription

mRNA

Translation

Truncated Protein (non-functional, unstable)
Molecular Mechanism of Translation Termination

Translation Termination: ~99.9%

Translation Elongation: ~0.1%
(Readthrough)

Polypeptide Release Factors

Near-Cognate tRNA

UAG

AUA

Truncated Protein
1600 clinically approved compounds

- TECC assay
  - FRT G542X cells (10 μM)
  - 58 hits
    - 30 removed (Specificity)
    - 28 hits
      - 29 removed (Repeat, n=3, Gt)
      - 8 Lead hits
        - 6 removed (Specificity, Gt)
        - 5 removed (Poor efficacy)
        - Dose Response in TECC Dual Luciferase HRP assay
          - Isc on HBE ΔF508/G542X cells
            - Herbal agent ESCIN

- Dual Luciferase assay
  - QXN UGAC & CFTR G542X (10 point dose response)
  - 115 hits
    - 90 removed (Repeat & medicinal properties)
    - 25 hits
Hits identified from the primary high throughput screens

**Hits identified from Luciferase assay (SR)**

**Hits identified from TECC assay**
Major ‘Shots on Goal’ for CF therapy in the next few years

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- **Tools for individualizing CFTR-directed therapies**
- Targeting CF mucus itself
Nasal Cells from a CF Donor
μOCT capabilities ex vivo

Available metrics:

- Airway surface liquid thickness
- Periciliary liquid thickness
- Ciliary beat frequency
- Mucociliary transport rate

Functional Consequences of Cystic Fibrosis

WT

Non-CF HBE cells 0.0000 sec

CF HBE cells

WT

CF

n=8 derived from 4 donors each
μOCT Imaging of G551D/F508 HBE

N=10 measures

Forskolin + VX-770

Forskolin

10μm  G551D  Frame rate: 32 fps  Time: 0.03 s
Effect of Ivacaftor on G551D/F508del HBE Viscosity
Effect of CFTR modulators on mucus viscosity
In vitro ion transport predicts in vivo sweat chloride whereas in vitro MCT predicts clinical response.
ASL \[\mu m\]

frequency [lines]

filter

cells

MCT \[\mu m/\text{second}\]

frequency [images]

HAE normal  Frame rate: 32 fps  Time: 0.83 s

ASL [microns]

MCT [microns/second]
μOCT probe (outer tube removed)
Swine \textit{in vivo} 50 \mu m

- Adult swine trachea
- 5-10 second balloon inflation time
- 40 fps ASL

Time

CBF

PCL

MCT

ASL
Development of Nasal Cell Organoids as a Tool for Precision Medicine

J Guimbellot
An inherent mucus abnormality contributes to CF Pathogenesis

- A hypothesis regarding abnormal CF mucus has gained significant traction since it links the mucus defect in the respiratory and other organs, such as the pancreas or GI tract.

- This could also explain CF severity as opposed to other diseases of mucociliary clearance.

- A new treatment for abnormal mucus would address serious unmet medical need.
CF: The ‘Mucoviscidosis’
Mucin Reactions Post-Release: An electrostatically driven reaction?

\[ \text{Ca}^{2+}\text{-Mucin} + 2\text{Na}^+ \rightleftharpoons 2\text{Na}^+\text{-Mucin} + \text{Ca}^{2+} \]
PAAG may be a Mucolytic and ‘Adhesiolytic’ by electrostatic interactions

\[
\text{Ca}^{2+}\text{-Mucin} + \text{PAAG} + 2\text{Na}^+ \leftrightarrow \text{PAAG-Mucin} + 2\text{Na}^+ + \text{Ca}^{2+}
\]
PAAG Reduces CF Sputum Viscosity and Elasticity

![Graph showing the reduction of sputum viscosity and elasticity with PAAG treatment compared to PBS.](image-url)
PAAG Increases Particle Diffusion

PBS Control

PAAG (500 µg/ml)

500 nm PEG coated fluorescent nanoparticle

Viscosity at Frequency 0.6 Hz

- PBS
- PAAG (250 µl/ml)
- DNase (250 µl/ml)
- PAAG+DNase
PAAG In Situ: Effects on Viscosity and Functional Microanatomy

*P<0.05, ****P<0.0001
A Personalized Era of CF Therapeutics

- From discovery to proof of concept to clinical approval and wide use in select patients
- Ivacaftor is effective at treating a variety of CF individuals with ‘responsive’ CFTR mutations
- Combination corrector-potentiatior therapy improves CFTR function and clinical outcomes even to the relatively challenging F508del CFTR mutation
- Future multi-agent corrector or other combination therapies may improve outcomes further
- New tools are emerging to test drug responsiveness and improve biomarkers of responsiveness
- New concepts including addressing abnormal CF mucus itself
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