Are We Close to Curing Genetic Diseases Like CF?

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Cystic Fibrosis

- Caused by an abnormality of chloride channels in epithelial cells
- Clinical signs are caused by obstruction in tissues with tubular epithelial structures:
  - mucus in lungs and sinuses
  - pancreatic insufficiency in ~ 90% of pts.
  - hepatobiliary problems
  - bowel obstructions
  - male infertility, relative female infertility
- Median age ~ 38 years

Notable History

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1838</td>
<td>Carl von Rokitansky’s autopsy of infant with Meconium peritonitis</td>
</tr>
<tr>
<td>1905</td>
<td>Meconium ileus described by Austrian Karl Landsteiner</td>
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<tr>
<td>1938</td>
<td>Cystic Fibrosis disease identified by American Dorothy H. Andersen</td>
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Things that have Contributed to Improved Outcomes

- Newborn Screening
- Improved Nutrition
- Improved airway clearance and new medicines
Every state in the U.S. will have a CF NBS Program by the beginning of 2009… …as long as you don’t consider Texas part of the U.S.!

What is a “Positive NBS” for CF?
(NY State IRT/DNA Method)

- Two CFTR mutations detected
- 1 CFTR mutation detected
- "Ultrahigh" IRT measured

- All need to be referred for sweat test
  - 2 mutations likely diagnostic of CF
  - Other groups are at increased risk of CF

The frequency varies in different populations

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence</th>
<th>Carriers</th>
<th>F508del</th>
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<tbody>
<tr>
<td>Caucasians</td>
<td>1 / 3,300</td>
<td>1 / 29</td>
<td>70%</td>
</tr>
<tr>
<td>Hispanics</td>
<td>1 / 9,000</td>
<td>1 / 48</td>
<td>48%</td>
</tr>
<tr>
<td>African-Americans</td>
<td>1 / 15,300</td>
<td>1 / 60</td>
<td>48%</td>
</tr>
<tr>
<td>Asian-Americans</td>
<td>1 / 32,100</td>
<td>1 / 90</td>
<td>30%</td>
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F508del accounts for just 70% of CF cases

CFTR – Cystic Fibrosis Transmembrane Regulator
the “CF gene” product is an ion channel that regulates other ion channels

What should you say when a NBS is Positive?

- A positive screen DOES NOT mean that the infant has CF
- Most screen positive babies DO NOT have CF
- CF is a serious diagnosis; we need to be sure your baby does not have CF

Sweat Testing Technique

Can be done at any time > 48 hours of age
Pilocarpine is iontophoresed into sweat glands
Sweat is collected for 30 minutes
Must be able to collect 75 mg or 15 µL of sweat
Manifestations of CF

- **Endocrinologic**: Insulin resistance, diabetes
- **Gastrointestinal**: Meconium ileus, distal intestinal obstruction syndrome
- **Hepatic**: Cirrhosis, portal hypertension, cholelithiasis, steatosis
- **Pancreatic**: Exocrine PI, pancreatitis
- **Reproductive**: CBAVD, reduced fertility
- **Sinopulmonary**: Chronic bacterial/fungal infections, air trapping, mucus plugging, bronchiectasis, atelectasis, bronchial cysts, pneumothorax, opacification of sinuses
- **Other**: Digital clubbing, metabolic alkalosis

Management of complications of steatorrhea can be done by “conservative measures, including strapping of the buttocks, defecating in a reclining position, and measures designed to reduce the frequency and bulk of stool”...[such as]... “withholding butter, ice cream, peanut butter, potato chips, french fried potatoes, and mayonnaise....”

Dr. Erika Bruck
Director of Metabolic Diseases (including CF)
Children’s Hospital of Buffalo
April 4, 1908 - September 2008

FEV₁ Predicted vs BMI Percentile Patients 6 to 20
Wang & Hankinson Equations

Median CDC Weight Percentile by Gender, 2006

FEV₁ in childhood in CF-PI pts stratified by weight-for-length achieved at age 2 years
Currently Available Enzymes

- **Microspheres**
  - Creon 5, 10 & 20
  - Pancreacarb 4 & 8
  - Pancrease (not MT)
  - Cotazyme-S
  - Lipram

- **Microtablets**
  - Ultrase 12, 18 & 20
  - Pancrease MT 4, 10, 16 & 20

- **Unknown**
  - Ku-Zyme
  - Kutase
  - Panokase
  - Plaretase

**Altus Crystalomomics™ Technology**

- **Soluble Protein**
- **Protein Crystal**
- **Protein Products**
  - High stability
  - High purity
  - Highly concentrated
  - Multiple delivery options

- **Low stability**
- **Low purity**
- **Dilute**
- **Poor delivery options**

**TRIZYTEK™ (liprotamase, ALTU-135)**

- **Lipase**:
  - Crystal-CLEC (Cross-Linked Enzyme Crystal)
  - Broad positional specificity to hydrolyze the ester bonds of triglycerides at the sn-1, sn-2, and sn-3 positions
  - Stable and active with or without bile salts or co-lipase

- **Protease**:
  - Crystalline
  - Broad substrate specificity
  - Hydrolyzes the peptide bonds nonspecifically to produce amino acids from protein

- **Amylase**:
  - Amorphous
  - Broad substrate specificity
  - Catalyzes the hydrolysis of α-1,4-glucosidic linkages of starch, glycogen and related polysaccharides

**Trizytek Phase III Program**

- 6-Week efficacy study in CF
  - 163 patients in the U.S. and ex-US
  - 138 randomized to Trizytek vs placebo
  - One capsule per meal/snack

- 12-Month safety study in CF
  - 215 CF patients (including 89 from the efficacy study)

- 12-Month safety study in CP
  - ∼40 Chronic Pancreatitis patients
    - enrollment ongoing

**Study Design**

- SCREENING: 3-4 Weeks
  - Out-patient
- BASELINE: 6 Days
  - In-patient
- TREATMENT OPEN-LABEL: 25-27 Days
  - Out-patient
- DOUBLE-BLIND: 6 Days
  - In-patient
- RESUME OPEN-LABEL: 7 Days
  - Out-patient
- FOLLOW-UP: 2 Weeks
  - Out-patient
Change in CFA vs Baseline CFA

Note wide test-retest variability in placebo subjects performed under same conditions ~ 1 month apart

Conclusions

- Trizytek improved fat and protein absorption and stool wt and # in subjects with CF
  - Changes in CFA, CNA and stool weight were significantly greater in Trizytek group vs placebo in all pre-specified sub-groups
  - Acid suppression improves response to Trizytek in CFA <40% subgroup

- No clinically significant adverse events or laboratory abnormalities related to study drug

Next Steps

- A year-long, open label safety study is underway
- Key safety factors are growth and micronutrient status
- These crystalline enzymes can be made into a pediatric formulation

Current Hypothesis

From Mutation to Disease

The mutant form of CFTR prevents chloride transport, causing mucus build-up

Mucus clogs the airways and disrupts the function of the pancreas & intestines.

How is CFTR Protein Synthesized and inserted into the Apical Membrane?
5 Classes of CFTR Mutations

I. Defective Production
II. Defective Processing
III. Defective Regulation
IV. Defective Conductance
V. Reduced Amounts

How Could we fix the Abnormal Protein in the Different Classes of CFTR Mutations?

Modulators of CFTR Function

Normal Levels of Surface CFTR

Gating Defect
Low Chloride Flux

Increased Chloride Flux

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Over 1,500 mutations in CFTR have been found. F508del accounts for just 70% of CF cases.

Things that have Improved Patients’ Clinical Status

- Newborn Screening
- Improved Nutrition
- Improved airway clearance and new chronic medicines – especially drugs to fix abnormal CFTR!