The following relationship(s) exist(s) related to this presentation:

- To advance drug development and a search for a cure, the Cystic Fibrosis Foundation has contractual agreements with several companies to receive royalties related to drugs that are developed as a result of CF Foundation funding. Any royalties we receive are used in support of our mission. More information about our model is available at cff.org/industry.
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PATH TO A CURE

MANY ROUTES, ONE MISSION
Path to a Cure

- An ambitious agenda to treat the underlying cause of CF and deliver a cure
- $500 million to be allocated through 2025
- A challenge to accelerate the pace of progress
MUTATED CFTR GENE

WHAT DO WE NEED TO FIGURE OUT?

Repair CFTR protein

Restore CFTR protein

Fix or Replace CFTR gene
PATH TO A CURE
MANY ROUTES, ONE MISSION
Path to a Cure – Five Mission Critical Tasks

1. **PRECLINICAL MODEL SYSTEMS** for nonsense mutations and genetic-based therapies

2. **VALIDATED TARGET CELLS** for genetic-based therapies

3. **DELIVERY SYSTEMS** to target genetic cargo to the correct cells in the correct tissue

4. **CFTR GENE EDITING** methods that are amenable to clinical development

5. **CLINICAL STUDIES** to correct mutations that are not responsive to CFTR modulators
Marie Egan, MD
Yale School of Medicine
Professor of Pediatrics (Respiratory) and of Cellular And Molecular Physiology
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Vice Chair for Research, Department of Pediatrics
Presenter Disclosure

Marie E. Egan, MD

The following relationships exist related to this presentation:

• Trucode Gene Repair Inc., consultant
• NIH, research funding
• Cystic Fibrosis Foundation, research funding
Emerging Technologies for CFTR Restoration in all People With CF
Will C regarding life as a patient with premature stop mutation and a second mutation that does not respond to known small molecules.
A Lifelong Cure for all CF Patients

Complications → Protein → RNA → Gene (DNA)

Continuous Therapy → Daily Therapy → Periodic Therapy → Permanent Repair

lungs, liver, pancreas, gut

Gene

CF Mutation

Protein
What's in the Therapeutic Toolbox?
Screening for Nonsense Therapies

CFFT Laboratory
- Screened 200,000 compound library
- Developed tools to enable screening programs such as gene-edited cell lines, new assays, NMD inhibitors

Southern Research/University of Alabama (Symposia 17)
- 750,000 compounds screened
- 500,000 compounds were rescreeened with a more sophisticated assay
- High level of correction in FRT cells, modest correction in primary cells

Icagen
- Screening of the 2M+ compound library is in process (8M compound virtual)
- 400,000 compounds screened in pilot assay

Eloxx Pharmaceuticals
- Modified aminoglycoside with activity in intestinal organoids
- Subcutaneous delivery in mice with G542X
- CF Foundation funded Phase 2 clinical trial for ELX-02 in the US; Phase 2 trial also underway in Europe
What’s in the Therapeutic Toolbox?
The potential power of genetic-based technologies:
- RNA replacement
- Gene (DNA) transfer
- Gene (DNA) editing
Emerging RNA-Based Therapies

What are the options?

• tRNA
• mRNA
• Antisense oligonucleotide

Who will benefit?

• Nonsense mutations
• Mutation agnostic (everyone)
• A variety of mutations including splicing mutations
Emerging tRNA Therapies

Abstracts: 136, 172, 175

Lueck et al.
Nat Commun 2019; 10: 822

*Anticodon Engineered-tRNA
mRNA Replacement to Restore CFTR Protein and Function

- mRNA needs to be **delivered** to the affected cells which will require a delivery system/carrier
- Once delivered mRNA needs to be able to be **translated** into a protein
Emerging mRNA Therapies: Translate Bio

mRNA (MRT5005) is nebulized

Specialized carrier for mRNA

Data from the Translate Bio website and press release, July 31, 2019
Antisense RNA Oligonucleotide Therapies (ASO)

What are antisense RNA oligonucleotides?
Small pieces of single strand RNA that are designed to:
• Block/interfere with mRNA
• Enable mRNA degradation
• Upregulate/enhance mRNA

FDA-approved therapies
• Retinitis CMV: fomivirsen
• Muscular Dystrophy: eteplirsen
• Hypercholesterolemia: mipomersen
• Spinal Muscular Atrophy: nusinersen
Antisense RNA Oligonucleotide Therapies

Can be designed to block or inhibit splice sites such as 3849 + 10kb C<T CFTR

Treatment with ASO resulted in:
- Correction of the splicing defect
- CFTR-related chloride currents

The lead ASO restores the CFTR function in primary respiratory epithelial cells (HNEs) from patients carrying the 3849 splicing mutation

Example of Ussing Chamber trace showing activation of the CFTR function

Data shared by Dr. Batsheva Kerem
RNA Therapy Programs in CF

**Type**

- tRNA
  - ReCode Therapeutics
- mRNA:
  - Translate Bio
  - Arcturus
  - Acuitas
- Antisense oligonucleotide
  - SpliSense
  - Ionis

**Challenges**

- Delivery
- Stability/Longevity
- Off-target Effects/Toxicity
What’s in the Therapeutic Toolbox?
Gene Therapy and Gene Editing

**GENE THERAPY**
adding a normal CFTR gene into the cells of people with CF

**GENE EDITING**
repairing the mutations in a patient’s own DNA

Diagram:
- **GENE THERAPY**:
  - CFTR MUTATION
  - HEALTHY GENE
  - DNA-CUTTING ENZYME
- **GENE EDITING**:
  - CFTR MUTATION
  - HEALTHY GENE
  - GUIDE MOLECULE

**Gene Therapy** involves adding a normal CFTR gene into the cells of people with CF. **Gene Editing** involves repairing the mutations in a patient's own DNA through the use of DNA-cutting enzymes and guide molecules.
Gene Therapy: Why Is This Time Different?

30 Years of Experience

410 trials: Gene-modified cell therapy, including CAR-T

290 trials: Gene delivery vectors (viral and nonviral)

220 trials: Cell therapy, including stem cell therapy

27 trials: Gene editing

900+ companies dedicated to regenerative medicine
59,000 patient participants worldwide
Current FDA-Approved Gene Therapy

- Oncology (2 agents):
  - 40-60% of patients have a complete response

- Beta Thalassemia:
  - 75% of patients achieved transfusion independence

- Retinal disease/blindness:
  - 55% of patients had a complete response

- Spinal Muscular Atrophy:
  - 93% of infants treated with SMA1 were alive without ventilatory support at 24 months

from Nationwide Children’s Hospital
Gene Therapy for CF: Delivery

• AAV vectors
  • Safety profile is excellent
  • Designer viral capsids based on evolutionary biology

• Lentivirus vectors
  • Integration
  • Designer lentiviral envelopes

• Nonviral vectors
  • Polymer-based nanoparticles
  • Lipid-based nanoparticles

Steines et al., JCI Insight 2016 Sep 8 1(14)
Gene Therapy: Current Major CF Programs

4D Molecular Therapeutics
- Develop AAV specific for lung
- 500M variants (preclinical)

Spirovant Sciences (formerly Talee Bio)
- AAV program (preclinical)
  - High level expression (cells, animal)
  - Lentivirus program (preclinical)

Abeona
- AAV program

UK Gene Therapy Consortium
- Pseudotyped Sendai virus
Gene Editing Technologies

**Approaches**
- CRISPR/Cas9
- Zinc Finger Nucleases
- Transcription activator-like effector nucleases (TALENs)
- Triplex-forming Peptide Nucleic Acids (PNA)/DNA

**Clinical trials**
- CAR T
- Sickle cell
- Blindness
- HIV
Gene Editing in Cystic Fibrosis

**Zinc Finger Nucleases:**
- CFTR was corrected in a CF airway cell line in 8% of cells.
  (Lee et al, Biores Open Access 2012 Jun 1 (13) 99-108.)
- CFTR was corrected and its function restored in induced pluripotent stem cells (iPSC).
  (Crane et al. Stem Cell Reports, Vol 4 Issue 4, April 2015 569-577)

**CRISPR-Cas9:**
- CFTR was corrected and its function restored in intestinal organoids from CF patients.

**TALENs:**
- CFTR was corrected and its function was restored in a CF airway cell line.
  (Xia et al, Genes 2019 Jan 11 10 (1)).

**PNA/DNA Nanoparticles:**
- CFTR was corrected in human airway epithelium and in airway epithelium from CF mice.
  (McNeer et al. Nat Commun. 2015 Apr 27;6:6952.)
Triplex forming PNA/DNA Nanoparticles

- Genomic DNA
- Donor DNA
- PNA

"Donor DNA" and PNA targeting the genome

Nanoparticles (PNA/DNA)

Airway Epithelial Cells
- F508del
- W1282X
- G542X

Gene-specific Reverse primer

Allele-specific Forward primer

Recombination and repair

Abstracts 161, 164 (Piotrowski-Daspit et al)
Triplex forming PNA/DNA Nanoparticles

Delivery approaches

- Topical/inhalation
- Systemic

Increases in FSK/IBMX-stimulated chloride current

+ Respiratory
- GI tract
+ 
+ 

Abstracts 161, 164 (Piotrowski-Daspit et al)
What’s in the Therapeutic Toolbox?
Cell-Based Therapies

Correct the gene defect ex vivo and then return the corrected cells to the patient

Primary airway basal cells

CRISPR-CAS9 /AAV vectors

Purify cells to enrich for corrected cells

CAR T-cell therapy: to treat cancer

Vaidyanathan et al Scientific Session IV: Therapeutic Strategies for correcting the CFTR Gene June 24, 2019, CFF Research conference Pushing the Frontiers Stowe Vt

adapted from Al-Sayed et al LIO Dec 2017
Recap

- Many novel approaches that get to the heart of the problem
- Many advances in the field that make some therapies much more feasible
- Some of these novel therapies are now being transitioned to the clinic
Challenges in CF

- Multisystem Disease
  - Respiratory tract
  - Gastrointestinal tract
  - Pancreas
  - Sweat gland
  - Vas deferens
  - Immune cells

And then there’s the mucus…
Cell Types of the Airways

Airway Atlas CFTR expression
- Ionocytes ++++++++ 
- Ciliated cells + 
- Goblet cells + 
- Basal cells + 

Animal models that let us know which cells we have actually targeted and that mirror human anatomy

Better human model systems (spheres/chips/3D) that mirror human airways

Better readouts for ion transport/fluid/mucus

Better ways to screen many delivery vectors quickly: high throughput

Addressing the Challenges as a Community

CF FOUNDATION RESEARCH DEVELOPMENT CENTERS

CFF BIOREPOSITORY

CELL MODEL RESOURCES

EPITHELIAL STEM CELL CONSORTIUM

GENE THERAPY WORKING GROUP

GENE EDITING WORKING GROUP
100%

Goal: Everyone has a therapy to prevent disease
Thank You

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Yale Cystic Fibrosis Center
Our Amazing Team
Our Families and Patients
Will Corcoran and his Family
Yale Cystic Fibrosis Research Center

The CF Foundation
Genevieve Maul
Jenna Vince
Chris Penland
Bill Skach
Please join us downstairs in Hall A2 for the Welcome Reception.