WS0501: AAV-mediated Gene Therapy for Cystic Fibrosis: Interim Results from a Phase 1/2 Clinical Trial

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Disclosures

- Personal financial relationships with commercial interests relevant to medicine, within the past year:
  - As faculty at an institution that is part of the CF TDN, I am/have been site/national PI on studies for 4DMT, Vertex, and Eloxx.
  - I have done clinical trial consulting for Vertex.
  - I serve on a DMC for AbbVie.

- Personal financial support from a non-commercial source relevant to medicine, within the past year:
  - I have received grant funding from the CF Foundation.
  - I have no personal relationships with tobacco industry entities.
  - I serve as the adult patient care representative to the CFF Board of Trustees, and on the CF Foundation’s Clinical Research Executive Committee, Clinical Research Advisory Board, and as immediate past chair of the CF TDN’s Sexual Health, Reproduction and Gender Research-Working Group, on the scientific advisory board for Emily’s Entourage, and on the ATS International Conference (Chair-elect) and Respiratory Heath Award Committees.
Failure of Conventional AAV Gene Therapy in CF Lung

PREVIOUS CLINICAL TRIAL EXPERIENCE WITH TGAAVCF

- Six trials with AAV2-based gene therapy (tgAAVCF) in upper\(^1-6\) and lower \(^5-8\) airways

- Nasal and sinus administration (n=3 trials):
  - Participants dosed: 34
  - Safe and well tolerated
  - DNA: detected
  - Transgene expression: detected
  - CFTR function: detected (vs contralateral control)

- Aerosol to lung (n=3 trials):
  - Participants dosed: 84 (mild to moderate)
  - Safe and well tolerated
  - DNA: detected
  - Transgene expression: Not detected
  - ppFEV\(_1\): No change vs controls

4D-710: Next-Gen Aerosolized Gene Therapy for Cystic Fibrosis Lung Disease

A101 TARGET VECTOR PROFILE AND 4D-710 PRODUCT DESIGN AND KEY ATTRIBUTES

Potential Barriers
Bronchial airway
Defensins
Antibodies
Mucus
Epithelium

Limited Transduction of Airway Cells

Conventional naturally occurring vectors

Enhanced Transduction of Airway Cells

Overcome Potential Barriers

A101

Mucus penetration efficient
Resistance to pre-existing human AAV antibodies
Transgene expression efficient
Specificity for lung (>99.9%)

A101 KEY ATTRIBUTES
CFTR Function Assays: 4D-710 Function Comparable to Trikafta

DOSE-RELATED 4D-710–MEDIATED CFTR FUNCTION

Dose-dependent CFTR Activity > Lumacaftor

4D-710 CFTR Function = Trikafta

Activated CFTR Function

*Activated CFTR function in CF ΔF508 ALI airway epithelial cultures (n=3 difference experiments). †CFTR activity in CF ΔF508 ALI airway epithelial cultures transduced with 4D-710 (1x10⁶) or Trikafta (2 µM VX-445, 3 µM VX-661, 0.1 µM VX-770); n=3 different experiments; error bars, ±SD. ALI, air–liquid interface; CFTR, cystic fibrosis transmembrane conductance regulator; EGFP, enhanced green fluorescent protein; NT, not treated.
**4D-710 Phase 1/2 Clinical Trial Study Design (4D-710-C001)**

**OPEN-LABEL PHASE 1/2 TRIAL IN MODULATOR-INELIGIBLE ADULTS WITH CYSTIC FIBROSIS**

### Dose Exploration

- **Cohort 1**
  - 1E15 vg
  - (n=3–6)

- **Cohort 2**
  - 2E15 vg
  - (n=3–6)

### Dose Expansion

- **Dose Expansion**
  - Selected dose
  - (n=up to 12)

### 4D-710 Administration

- Pre-dose procedures (Day 1):
  1. Airway clearance technique
  2. Aerosol bronchodilator (albuterol)
  3. Pre- & post-bronchodilator spirometry

- 4D-710 administered via AeroEclipse® II breath-actuated nebulizer

### Observation Period

- **4D-710 single administration**
- **Screen**
- **Bronchoscopy**
- **-D28**
- **D1**
- **D28**
- **Month 24**
- **Month 6**
- **Month 12**

### Long-term Follow-up Period

- **Month 12**

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Vertical bars represent study clinic visits. *28-day taper (Day -1 to Day 27). ACTs, Airway Clearance Techniques; SRT, Safety Review Team.*
4D-710 Phase I/2 Clinical Trial
STUDY OBJECTIVES AND ELIGIBILITY CRITERIA

Study Objectives
- Evaluate a single nebulized dose of 4D-710 (1E15, 2E15 vg)
  - Safety, tolerability, and immunogenicity
  - Transduction/transgene expression in lung (bronchoscopy samples)
  - Impact on pulmonary function (ppFEV1)
  - Impact on health-related quality of life
- Identify recommended Phase 2 dose

Key Inclusion Criteria
- Age ≥18 years
- Confirmed diagnosis: CF lung disease
- Ineligible for CFTR modulator therapy (per USPI) OR discontinued due to adverse effects
- % predicted FEV1 ≥50% and <100%
- Resting O2 sat ≥92% on room air

CFTR, cystic fibrosis transmembrane conductance regulator; ppFEV1, forced expiratory volume in 1 second.
4D-710 Phase 1/2 Clinical Trial: Major Study Endpoints

- **Primary endpoint:**
  - Incidence and severity of adverse events

- **Key secondary endpoints:**
  - Transgene transfer and expression in bronchoscopy samples (biopsies, brushings)
  - Change in ppFEV₁ from baseline (through Month 12)
  - Change in Cystic Fibrosis Questionnaire-revised (CFQ-R) scores (through Month 12)

ppFEV₁, percent predicted expiratory volume in 1 second.
4D-710 Phase 1/2 Clinical Trial: Cohort 1

DEMOGRAPHICS AND BASELINE CHARACTERISTICS

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cohort 1 (1E15 vg dose)</th>
<th>Participant 1</th>
<th>Participant 2</th>
<th>Participant 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td>36</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td>Non-Hispanic white</td>
<td>Non-Hispanic white</td>
<td>Non-Hispanic white</td>
</tr>
<tr>
<td>CFTR modulator eligibility</td>
<td>Tolerability</td>
<td>Ineligible variant</td>
<td>Ineligible variant</td>
<td></td>
</tr>
<tr>
<td>CFTR variant (class)</td>
<td>II/V</td>
<td>I/I*</td>
<td>I/II</td>
<td></td>
</tr>
<tr>
<td>Historical sweat chloride, mmol/L</td>
<td>74</td>
<td>103</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>Percent predicted FEV₁</td>
<td>83</td>
<td>69</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Pre-dose NAb to A101 capsid†</td>
<td>Low</td>
<td>Negative</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Pre-dose anti-drug antibody titer†</td>
<td>Low</td>
<td>Negative</td>
<td>Moderate</td>
<td></td>
</tr>
</tbody>
</table>

*Large gene deletion projected to result in a null variant profile. †Nab and antibody titer categories defined as negative (0), low (1:1–1:999), moderate (1:1000–1:14,999) and high (≥1:15,000).

**4D-710 Phase 1/2 Clinical Trial: Bronchoscopic Sampling Plan**

### Bronchoscopy: Week 4*

<table>
<thead>
<tr>
<th>Bronchoscopic Sampling Sites</th>
<th>Biomarker</th>
<th>RNA† Protein‡ DNA¶</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endobronchial biopsy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Right secondary carina</td>
<td>RNA†</td>
<td>X</td>
</tr>
<tr>
<td>2 Right middle lobe carina</td>
<td>RNA†</td>
<td>X</td>
</tr>
<tr>
<td>3 Left secondary carina</td>
<td>RNA†</td>
<td>X</td>
</tr>
<tr>
<td>4 Left upper lobe/lingula carina</td>
<td>RNA†</td>
<td>X</td>
</tr>
<tr>
<td><strong>Endobronchial brushing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Right lower lobe basal seg x 2</td>
<td>Protein‡</td>
<td>X</td>
</tr>
<tr>
<td>6 Left lower lobe basal seg x 2</td>
<td>Protein‡</td>
<td>X</td>
</tr>
</tbody>
</table>

*Participant 3 bronchoscopy conducted at Week 8 due to pulmonary exacerbation (unrelated to 4D-710).
†Assessed by in situ hybridization. ‡Assessed by immunohistochemistry. ¶Assessed by qPCR.

Widespread Transgene Delivery and Expression: Cohort 1

CONSISTENT TRANSDUCTION ACROSS PARTICIPANTS AND LUNG REGIONS

4D-710 DNA (+) Lung Biopsies

<table>
<thead>
<tr>
<th>Participant</th>
<th>Left Upper Lobe/ Lingula Carina</th>
<th>Right Secondary Carina</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>n/a</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

4D-710 RNA Expression (+) Lung Biopsies

<table>
<thead>
<tr>
<th>Participant</th>
<th>LSC</th>
<th>RML</th>
<th>Mean</th>
<th>% Positive Epithelial Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41</td>
<td>47</td>
<td>40</td>
<td>36–47% (+)</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36–47% (+)</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td></td>
<td>40</td>
<td>36–47% (+)</td>
</tr>
</tbody>
</table>

*qPCR assay range: 25 - 25,000,000 copies.
†Participant 2 LSC not sampled. Quantification by Visiopharm AI Machine Learning Analysis. ISH, in situ hybridization; LSC, left secondary carina endobronchial biopsy; RML, right middle lobe endobronchial biopsy.
CFTR Protein Expression
QUALITATIVE AND QUANTITATIVE ANALYSES

- Immunohistochemistry (IHC)
  - Tissue samples tested for CFTR protein
  - Control: normal lung (n=7) and CF lung (n=10)

- Quantitative Analyses
  - Visiopharm® machine learning image analysis
  - Quantifies intensity and distribution (% cells) of staining
  - Cell evaluation: assigned value of 1+, 2+, 3+ based on CFTR IHC signal intensity
  - H-score (range, 0–300) higher scores indicate increased signal intensity and distribution

<table>
<thead>
<tr>
<th>Normal Lung</th>
<th>CF Lung</th>
<th>Study Sample</th>
</tr>
</thead>
</table>

Staining intensity: 0 1+ 2+ 3+
**Widespread CFTR Protein Expression**

**CFTR PROTEIN EXPRESSION BY IHC 4–8 WEEKS AFTER 4D-710 DOSING**

- 100% (3/3) of participants positive for CFTR protein in the lung
- 100% (11/11) of lung samples positive for CFTR protein

*Endobronchial biopsy samples collected at Week 4 (Participants 1 and 2) or Week 8 (Participant 3). 3. IHC, immunohistochemistry.
CFTR Protein Localization (IHC)

CFTR PROTEIN EXPRESSED IN MULTIPLE AIRWAY EPITHELIAL CELL TYPES

CFTR Protein Expressed in Multiple Cell Types
1) Basal cells
2) Goblet cells
3) Columnar ciliated cells

Localization to Apical Membrane

Images from Participants 1 and 3, respectively. IHC, immunohistochemistry.
Cohort 1 Acute Safety Data

SERIAL SPIROMETRY AND ADVERSE EVENTS DURING NEBULIZATION OF 4D-710

- Full dose administered (1E15 vg)
- No significant adverse events
- No bronchospasm
- Participant 1: mild, self-limited dry throat during nebulized dosing

Serial Spirometry During 4D-710 Dosing: Through 4 Hours Post Nebulization

AE, adverse event; ppFEV₁, percent predicted forced expiratory volume in 1 second.
Cohort 1 Safety and Tolerability

NO 4D-710–RELATED ADVERSE EVENTS DURING UP TO 12 MONTHS POST DOSING*

4D-710–Related Adverse Events

- No 4D-710–related adverse events
- No 4D-710–related serious adverse events
- No dose-limiting toxicities

*Duration of Cohort 1 safety follow-up as of 12 April 2023: 12 months (Participant 1), 9 months (Participants 2 and 3).
Percent Predicted FEV\textsubscript{1}: Cohort 1

IMPROVED IN PARTICIPANT WITH MODERATE LUNG DISEASE, STABLE IN PARTICIPANTS WITH MILD/NORMAL

Pulmonary Exacerbation/Viral Respiratory Infection (not related to 4D-710)

<table>
<thead>
<tr>
<th>Cohort 1</th>
<th>Month 1</th>
<th>Month 3</th>
<th>Month 6</th>
<th>Month 9</th>
<th>Month 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant 1</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Participant 2</td>
<td>D 8: Grade 3 COVID-19, dyspnea</td>
<td>none</td>
<td>D 176: Grade 1 rhinovirus</td>
<td>none</td>
<td>pending</td>
</tr>
<tr>
<td>Participant 3</td>
<td>Day 28: Grade 2</td>
<td>D 88: Grade 1</td>
<td>none</td>
<td>Day 266: Grade 1 COVID-19</td>
<td>pending</td>
</tr>
</tbody>
</table>

*Pre-dose spirometry assessment. ppFEV\textsubscript{1}, percent predicted forced expiratory volume in 1 second
4D-710 AEROW Trial: CFQ-R Improved in All 3 Pts & 6 of 7 Timepoints

CFQ-R RESPIRATORY SYMPTOM SCORE CHANGE SHOWS CONSISTENT IMPROVEMENTS >MCID (4 POINTS)

Baseline Lung Function

**Moderate**

- Up to +22 points (>MCID)

**Mild**

- Up to +11 points (>MCID)

**Normal**

- Up to +6 points (>MCID)

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**CFQ-R, Cystic Fibrosis Questionnaire-revised. Scores range from 0 to 100, with higher scores indicating better health. MCID=4 points (individuals with CF and stable respiratory disease) [1].**

*Respiratory-related adverse event within 21 days of assessment.

†Grade 1 rhinovirus (D176).

¶Grade 1 COVID-19.

Single-dose administration of 4D-710 was well tolerated
  - No 4D-710–related adverse events post-dosing
  - No serious adverse events
  - No dose limiting toxicities

Widespread transgene delivery and robust protein expression in the lung
  - Increased protein expression compared to normal and CF lung samples
  - Feasibility demonstrated in participants with pre-existing serum antibodies to the A101 capsid

Evidence of clinical activity in all 3 participants
  - ppFEV₁ stable or improved
  - Clinically meaningful improvements in CFQ-R respiratory symptom score
Acknowledgments:
Participants and Their Families, Principal Investigators and Study Staff, CFF

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