CFTR Transgene Expression in Airway Epithelial Cells Following Aerosolized Administration of the AAV-based Gene Therapy 4D-710 to Adults with Cystic Fibrosis Lung Disease

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 served 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 and NIH.
  - 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, as immediate past chair of the CF TDN’s Sexual Health, Reproduction and Gender Research-Working Group and Chair of the Health Equity Team Science Awards Study Section, on the Scientific Advisory Board for Emily’s Entourage, on the NIH Clinical Trials Study Section and as the ATS International Conference Committee Chair-elect.
Conventional AAV-based Gene Therapy in CF Lung Disease

6 Clinical Trials Evaluating AAV2-based Gene Therapy (tgAAVCF) in Upper and Lower Airways

- Nasal and sinus administration (3 trials; N=34)
  - Safe and well tolerated
  - DNA: Detected
  - Transgene expression: Detected
  - CFTR function: Demonstrated (vs contralateral control)

- Aerosol delivery to lung (3 trials; N=84)
  - Safe and well tolerated
  - DNA: Detected
  - Transgene expression: Not detected
  - Percent predicted FEV₁: No change vs controls

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

**Product Design and Characteristics**

**4D-710 Design**

- **Vector:** Lung-specific evolved A101 capsid
- **Transgene:** Codon-optimized human CFTRΔR
- **Promoter:** CMV173

- Efficient mucus penetration
- Efficient transgene expression
- Resistance to pre-existing antibodies
4D-710 Phase 1/2 Clinical Trial: Dose Exploration
Open-label Trial in CFTR Modulator-ineligible/intolerant Adults with Cystic Fibrosis Lung Disease

**Phase I Dose Exploration**

- 1x10^{15} vg (n=3)
- 5x10^{14} vg (n=2)
- 2x10^{15} vg (n=4)
- 2.5x10^{14} vg (n=1)

**Key Eligibility Criteria**

- Age ≥18 years
- Confirmed diagnosis of CF lung disease
- Ineligible for CFTR modulator therapy or discontinued due to adverse effects
- % predicted FEV₁ ≥50% and <100%

**SRT Review**

**Long-term follow up**

- 40 mg oral prednisone
- 28-day taper
- Endobronchial biopsy (4D-710 transgene and protein expression)
- SRT, Safety Review Team

* CFTR, cystic fibrosis transmembrane conductance regulator; SRT, Safety Review Team.
### Demographics and Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>2×10^{15} vg</th>
<th>1×10^{15} vg</th>
<th>5×10^{14} vg</th>
<th>2.5×10^{14} vg</th>
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<td><strong>Age, y</strong></td>
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<td>27</td>
<td>32</td>
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<td><strong>Sex</strong></td>
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<td><strong>CFTR modulator status</strong></td>
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<td>Ineligible</td>
<td>Intolerant</td>
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<tr>
<td><strong>Sweat chloride, mmol/L</strong></td>
<td>84</td>
<td>96</td>
<td>103</td>
<td>114</td>
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<tr>
<td><strong>ppFEV₁</strong></td>
<td>90</td>
<td>56</td>
<td>80</td>
<td>86</td>
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<td><strong>CFQ-R-R score</strong></td>
<td>78</td>
<td>72</td>
<td>89</td>
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<td><strong>A101 anti-capsid Ab</strong></td>
<td>Negative</td>
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<tr>
<td><strong>A101-specific T cells</strong></td>
<td>Positive</td>
<td>Negative</td>
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<td>Negative</td>
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</table>

*Sweat chloride normal range ≤29 mmol/L. Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation (2017). Ab, antibody; CFTR, cystic fibrosis transmembrane conductance regulator; CFQ-R-R, Cystic Fibrosis Questionnaire–revised (respiratory domain); FEV₁, forced expiratory volume in 1 second.
4D-710 Safety & Tolerability: $2 \times 10^{15}$ vg (Highest Studied Dose)
Duration of Follow up: 13–17 Months (n=4)

- Treatment-related adverse events
  - Pneumonitis and FEV$_1$ decline (n=1 participant); resolved
  - Previously reported SAE, pneumonitis NOS (n=1 participant); resolved
    - ppFEV$_1$ at last assessment (month 12) +6% compared to baseline

- Analysis of tissue samples from lung biopsies obtained at weeks 4–8:
  - No evidence of inflammation or toxicity
  - CFTR protein expression
    - ~400% higher in epithelium compared to normal (non-CF) lung samples
    - Widespread expression observed in interstitium

- $2 \times 10^{15}$ vg dose will not be further evaluated; $1 \times 10^{15}$ vg defined as the MTD
4D-710 Safety & Tolerability: $2.5 \times 10^{14}$ to $1 \times 10^{15}$ vg

Duration of Follow up: 1–25 Months (n=6)

### 4D-710-Related Adverse Events

<table>
<thead>
<tr>
<th>Dose (vg)</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1 \times 10^{15}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$5 \times 10^{14}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$2.5 \times 10^{14}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Month (post dose): 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

- Administration of aerosolized 4D-710 well tolerated
  - No dose-limiting toxicities
  - No 4D-710–related SAEs
  - No clinically significant 4D-710-related adverse events after administration

- No inflammation or toxicity in tissue samples from lung biopsies
**4D-710 Phase 1/2 Clinical Trial**

**Bronchoscopic Sampling Plan**

**Bronchoscopy: Week 4**

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

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

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**Diagram:**

- **Trachea**
- **Upper Lobe**
  - Apico-posterior
  - Superior-lingular
  - Inferior-lingular
- **Middle Lobe**
  - Medial
  - Lateral
- **Lower Lobe**
  - Superior
  - Anterior basal
  - Medial basal
  - Lateral basal
  - Posterior basal

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IHC & ISH Assay Specificity and Sensitivity

Superior Sensitivity of IHC Compared to ISH Confirmed in 4D-710–Transduced HT29 CFTR CRISPR KO Cells

- IHC is more sensitive and has a different dynamic range compared to ISH

CFTR Expression: Machine Learning-Assisted Image Analysis
Qualitative and Semi-quantitative Analyses

- Reliable diagnostic-grade image analysis software*
- Holistic and objective whole-slide analysis
  - 100% of airway epithelial cells analyzed
  - 100% manual QC to confirm accuracy of cell classification and exclusion of sectioning/staining artifacts
- Percent positive cells & H-score calculated by software algorithm
  - H-score (range, 0–300): measure of staining intensity and distribution; higher scores indicate increased signal intensity and distribution

*Visiopharm® image analysis software. CFTR, cystic fibrosis transmembrane conductance regulator; IHC, immunohistochemistry.
4D-710 Transgene Delivery and RNA Expression

Dose-dependent CFTRΔR RNA Expression

**CFTRΔR RNA (ISH)**

- Dose-dependent CFTRΔR mRNA expression in bronchial epithelial cells
- No CFTRΔR mRNA expression observed in commercial non-CF and CF lung samples
- Commercial non-CF samples positive for endogenous CFTR mRNA expression

Quantification by Visiopharm® AI Machine Learning Analysis. Number shown below each group indicates the number of lung samples.

*Genotyping of commercial CF samples yielded results for 13/35 samples; of these, a majority were ΔF508 homozygous mutations. CFTR, cystic fibrosis transmembrane conductance regulator; ISH, in situ hybridization.
**4D-710–Mediated CFTR Protein Expression by IHC**

**Dose-independent CFTR Protein Expression Following 4D-710 Administration**

**CFTR (+) Epithelial Cells (IHC)**

**CFTR Staining Intensity (IHC)**

*H-score. Quantification by Visiopharm AI Machine Learning Analysis. †Genotyping of commercial CF samples yielded results for 13/35 samples; of these, a majority were ΔF508 homozygous mutations. IHC, immunohistochemistry.
Widespread Consistent CFTR Protein Expression: 100% of Samples
16 of 16 Endobronchial Biopsy Samples Positive for CFTR Protein by IHC 4–8 Weeks After 4D-710 Dosing*

### 4D-710

<table>
<thead>
<tr>
<th>Dose (vg)</th>
<th>Participant 1</th>
<th>Participant 2†</th>
<th>Participant 3</th>
<th>Participant 4</th>
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<tbody>
<tr>
<td>2x10¹⁵</td>
<td>![Image]</td>
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<tr>
<td>1x10¹⁵</td>
<td>![Image]</td>
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<tr>
<td>5x10¹⁴</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>2.5x10¹⁴</td>
<td>![Image]</td>
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</tbody>
</table>

†Endobronchial biopsy performed at Week 8.

**Interstitial staining at highest dose**

### Controls

<table>
<thead>
<tr>
<th>Control</th>
<th>Non-CF Lung</th>
<th>CF Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Image]</td>
<td>![Image]</td>
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<td>![Image]</td>
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</tbody>
</table>

*Representative images, endobronchial biopsy samples obtained from the left secondary carina (row 1) and right middle lobe (row 2).
CFTR Protein Expression Observed in Multiple Airway Cell Types

CFTR Protein Localization (IHC) Following Administration of 4D-710: secretory, ciliated & basal cells

CFTR Protein Expressed in Multiple Cell Types*

1) Basal cells  2) Goblet cells  3) Columnar ciliated cells

Localization to Apical Region†

*Image from Cohort 1 (1x10^{13} vg) participant. †Images from Cohort 2 (2x10^{13} vg) participants. CFTR, cystic fibrosis transmembrane conductance regulator. IHC, immunohistochemistry.
Immunogenicity Analyses

Pre-existing A101 Immunity Did Not Affect CFTRΔR RNA or CFTR Protein Expression

CFTR Expression According to Baseline Anti-A101 Antibodies

Pre-existing Anti-A101 Capsid Antibodies
- 3/9 positive for pre-existing A101 capsid antibodies\(^*\)
- No significant difference in bronchoscopy results between participants with (n=3) and without (n=6) pre-existing A101 antibodies
- No observed effect of pre-existing antibodies on safety

Pre-existing A101-specific T cells
- 3/7 positive for pre-existing A101-specific T cells\(^†\)
- No significant difference in bronchoscopy results between participants with (n=3) and without (n=4) pre-existing A101-specific T cells

\(^*\)Results pending for n=1 participant (5x10^{14} vg group). \(^†\)Results pending for n=2 and n=1 in the 5x10^{14} vg and 2.5x10^{14} vg groups, respectively.
4D-710 Phase 1/2 Clinical Trial
Percent Predicted FEV₁ (12 Months)

Respiratory-related adverse events*:  
- Pulmonary exacerbation
- Viral respiratory infection
- Pneumonitis
- Hemoptysis

*Occurring within 21 days of pulmonary function assessment.
4D-710 Phase 1/2 Clinical Trial
Percent Predicted FEV₁ in Participants with a Baseline Value ≤80% (N=3)

- Three participants had a baseline ppFEV₁ ≤80% and ≥6 months of follow up
- Two showed improvement in ppFEV₁ at 12 months
  - 2x10^{15} vg (n=1): +6%
  - 1x10^{15} vg (n=1): +5%

Respiratory-related adverse events*:
- Pulmonary exacerbation
- Viral respiratory infection
- Pneumonitis

*Occurring within 21 days of pulmonary function assessment.
4D-710 (1×10^{15} vg): Durable Improvement in CFQ-R-R Score
Mean Increase Over 12 Months Consistently Above MCID

CFQ-R Respiratory Symptom Score

Mean Change in CFQ-R Score

*Respiratory-related adverse event within 21 days of assessment. †All enrolled participants (n=3). ‡Excludes participants with a respiratory-related event within 21 days of assessment. CFQ-RD, Cystic Fibrosis Questionnaire-Revised (respiratory symptoms scale). Scores range from 0 to 100, with higher scores indicating better health. MCID=4 points [1]. 1. Quittner AL et al. Chest 2009;135:1610–18.
Administration of a single aerosolized dose of 4D-710 to adults with CF lung disease was generally well tolerated at doses up to $1 \times 10^{15}$ vg (n=6; follow up, 1–25 months)

100% of lung samples positive for CFTR transgene mRNA and protein expression

- **Dose-dependent** CFTRΔR transgene RNA expression
  - Target expression levels achieved across all tested doses

- **Robust, consistent, and widespread** CFTR protein expression
  - CFTR protein levels in 4D-710-treated participants 2–4x higher than non-CF and CF lung samples
  - CFTR protein expression observed in **multiple airway epithelial cell types**, including basal cells

Pre-existing AAV immunity **did not prevent** transgene expression/biological activity

Enrollment in $2.5 \times 10^{14}$ and $5 \times 10^{14}$ vg cohorts ongoing

- Biological activity (CFQR-R QOL and ppFEV₁) to be reported at 12 months
Acknowledgments
Participants and Their Families, Principal Investigators and Study Staff, CFF

Christopher Goss, MD
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Nationwide Children's Hospital

Scott Donaldson, MD
University of North Carolina at Chapel Hill
# 4DMT CFTR IHC Assay Development

Validated by Extensive Control Testing to Ensure Specificity to CFTR Epitope

<table>
<thead>
<tr>
<th>Test</th>
<th>Control Cell/Tissue</th>
<th>Result</th>
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<tbody>
<tr>
<td>Specificity and Signal Differential</td>
<td>Transfected vs. un-transfected HEK293T cells</td>
<td>Confirmed</td>
</tr>
<tr>
<td></td>
<td>Untreated HT29 vs. CFTR CRISPR-modified knockout HT29 cell lines</td>
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<tr>
<td></td>
<td>Vehicle-treated vs. 4D-710–treated NHP lung tissue</td>
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<tr>
<td></td>
<td>Commercial lung samples: normal lung (n=10); genotyped CF lung (n=35)</td>
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<td>Transduced CRISPR-modified knockout HT29 cell lines</td>
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<td>Western blotting using IHC antibody (M3A7)</td>
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<td>Sensitivity</td>
<td>Transduced CRISPR-modified knockout HT29 cell lines (transduction across range of MOIs)</td>
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<td>Negative Control</td>
<td>CFTR null lung samples (CF Foundation)</td>
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<td>NHP lung tissue treated with vehicle &amp; A101 carrying alternate transgene</td>
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<td>Mouse IgG1-matched isotype controls (all tested lung samples)</td>
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