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47th EUROPEAN CYSTIC FIBROSIS CONFERENCE

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

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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 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

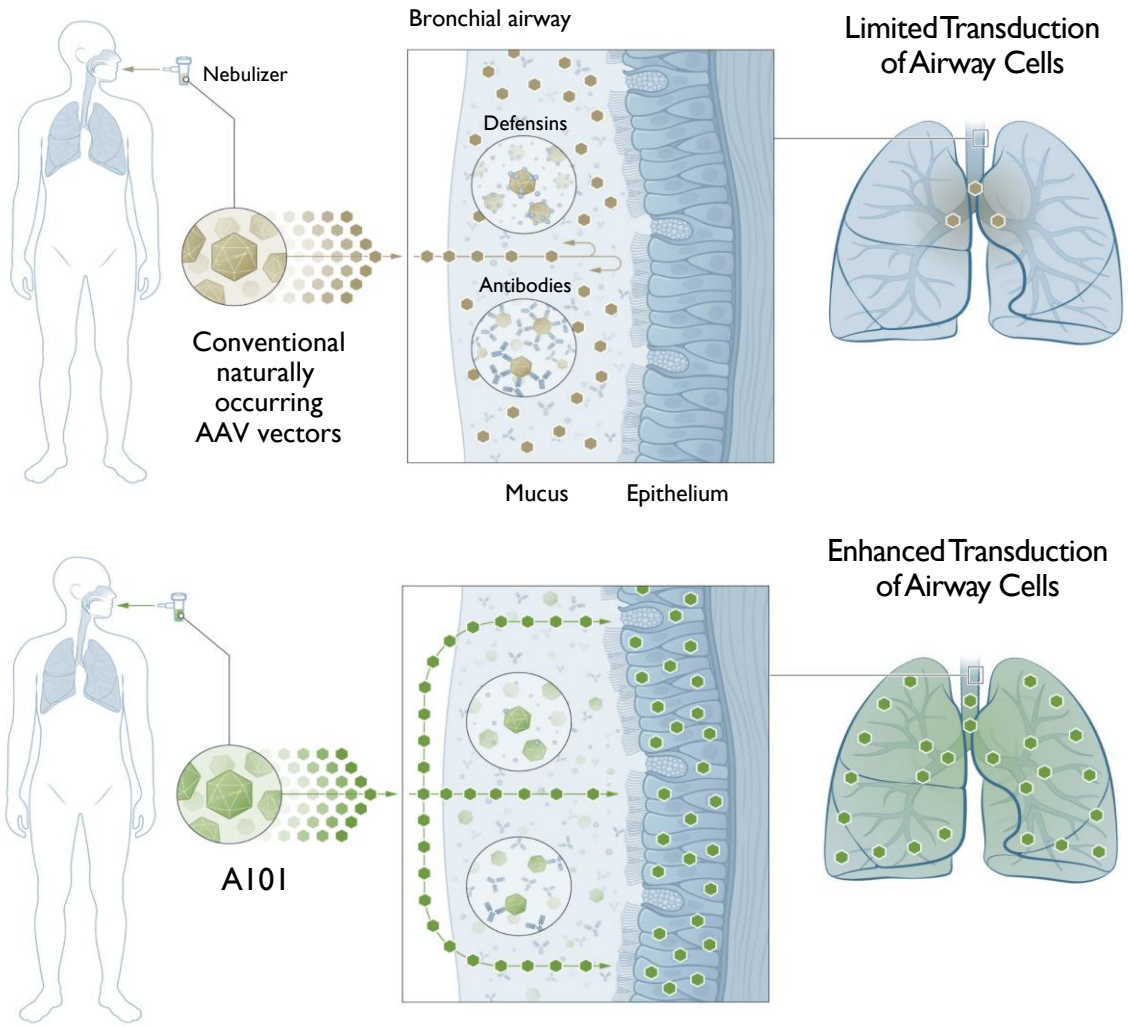
6 Clinical Trials Evaluating AAV2-based Gene Therapy (tgAAVCF) in Upper and Lower Airways[1–8]

- 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 FEV_1: No change vs controls

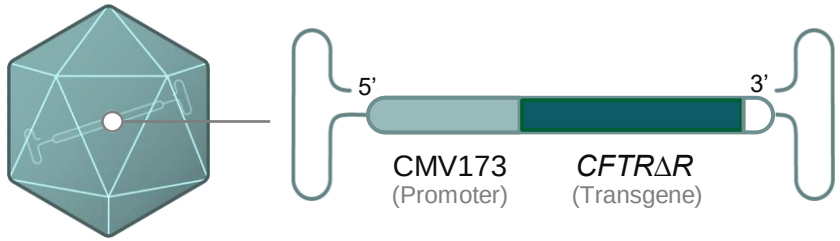
1. Wagner JA et al. *Hum Gene Ther* 1998; 9: 889-909. 2. Wagner JA et al. *Lancet* 1998;351:1702-3. 3. Wagner JA et al. *Laryngoscope* 1999;109:266-74. 4. Wagner JA et al. *Hum Gene* Ther 2002;13:1349-59. 5. Flotte TR et al. *Hum Gene Ther* 2003;14:1079-88. 6. Flotte TR et al. *Hum Gene Ther* 2005;16:921-8. 7. Aitken ML et al. *Hum Gene Ther* 2001;12:1907–16. 8. Moss RB et al. *Chest* 2004;125:509-21. 9. Moss RB et al. *Hum Gene Ther* 2007;18:726-32.

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

Product Design and Characteristics



4D-710 Design



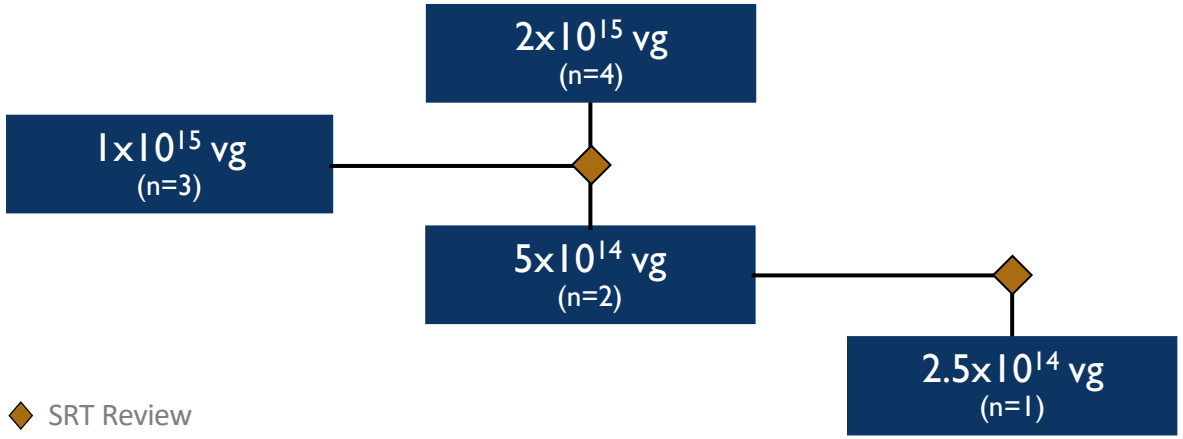
Vector:	Lung-specific evolved A101 capsid
Transgene:	Codon-optimized human *CFTRΔR*
Promoter:	CMV173

- Efficient mucus penetration
- Efficient transgene expression
- Resistance to pre-existing antibodies

4D-710 Phase 1/2 Clinical Trial: Dose Exploration

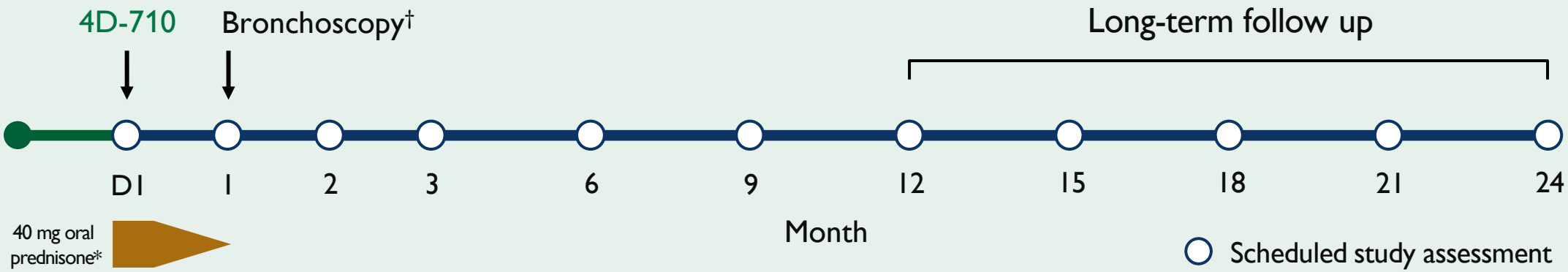
Open-label Trial in CFTR Modulator-ineligible/intolerant Adults with Cystic Fibrosis Lung Disease

Phase 1 Dose Exploration



Key Eligibility Criteria

- Age ≥18 years
- Confirmed diagnosis of CF lung disease
- Ineligible for CFTR modulator therapy *or* discontinued due to adverse effects
- % predicted FEV_1 ≥50% and <100%



*28-day taper. †Endobronchial biopsy (4D-710 transgene and protein expression). CFTR, cystic fibrosis transmembrane conductance regulator; SRT, Safety Review Team.

4D-710 Phase 1/2 Clinical Trial: Dose Exploration

Demographics and Baseline Characteristics

	2×10^{15} vg				1×10^{15} vg			5×10^{14} vg		2.5×10^{14} vg
Age, y	37	27	32	69	36	24	20	42	39	25
Sex	Female	Male	Female	Female	Male	Male	Female	Female	Female	Male
Race	White	White	White	White	White	White	White	White	Black	White
Ethnicity	Non-Hispanic	Non-Hispanic	Non-Hispanic	Non-Hispanic	Non-Hispanic	Non-Hispanic	Non-Hispanic	Non-Hispanic	Non-Hispanic	Non-Hispanic
CFTR modulator status	Ineligible	Ineligible	Ineligible	Intolerant	Intolerant	Ineligible	Ineligible	Intolerant	Ineligible	Ineligible
Sweat chloride, mmol/L*	84	96	103	114	74	103	110	107	134	120
$ppFEV_1$	90	56	80	86	83	69	95	100	77	58
CFQ-R-R score	78	72	89	78	72	61	83	72	78	28
A101 anti-capsid Ab	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Positive	*Pending*	Negative
A101-specific T cells	Positive	Negative	Negative	Negative	Negative	Positive	Positive	*Pending*	*Pending*	*Pending*

*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); FEV_1, forced expiratory volume in 1 second.

4D-710 Safety & Tolerability: 2×10^{15} vg (Highest Studied Dose)

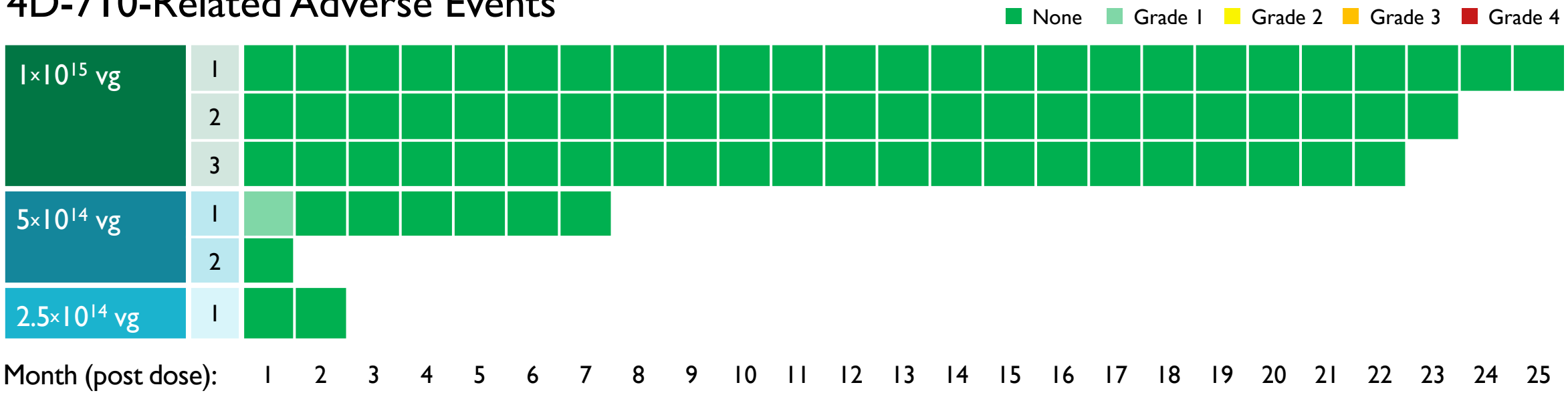
Duration of Follow up: 13–17 Months (n=4)

- 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
- 2×10^{15} vg dose will not be further evaluated; 1×10^{15} vg defined as the MTD

4D-710 Safety & Tolerability: 2.5×10^{14} to 1×10^{15} vg

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

4D-710-Related Adverse Events



- 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

SAE, serious adverse event.

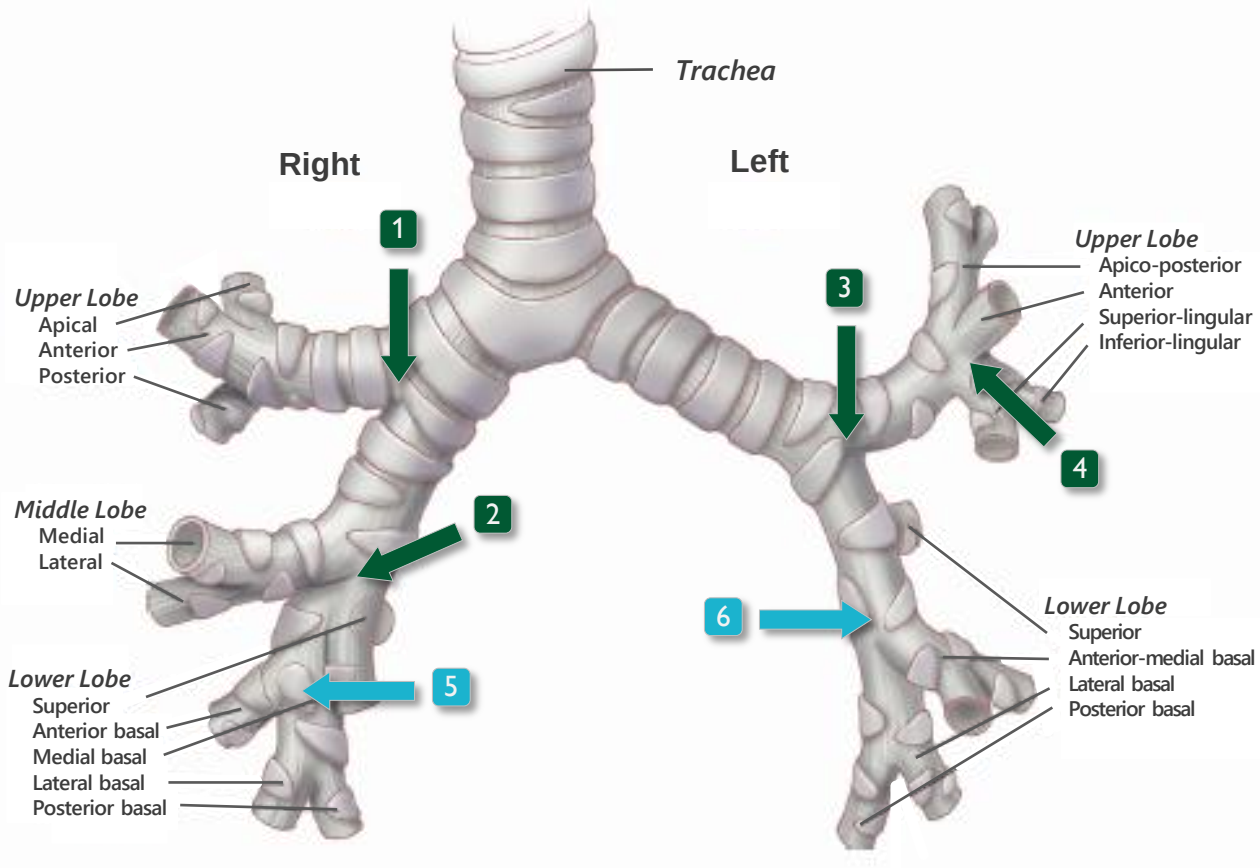
4D-710 Phase 1/2 Clinical Trial

Bronchoscopic Sampling Plan

Bronchoscopy: Week 4*

Bronchoscopic Sampling Sites			Biomarker	
			RNA† Protein‡	DNA¶
Endobronchial biopsy				
	1	Right secondary carina		X
	2	Right middle lobe carina	X	
	3	Left secondary carina	X	
	4	Left upper lobe/lingula carina		X
Endobronchial brushing				
	5	Right lower lobe basal seg x 2	X	
	6	Left lower lobe basal seg x 2	X	

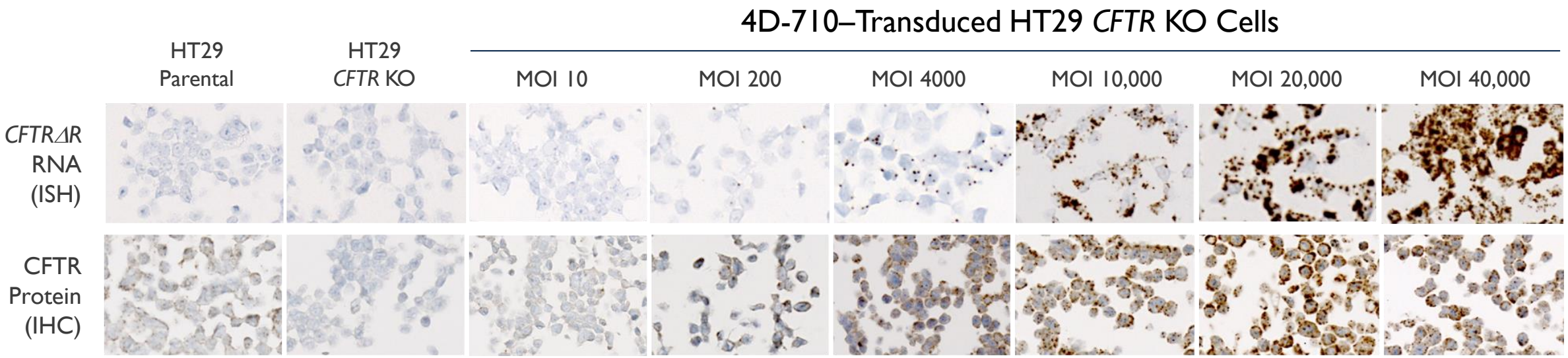
*One bronchoscopy conducted at Week 8 due to pulmonary exacerbation (unrelated to 4D-710).
†Assessed by in situ hybridization. ‡Assessed by immunohistochemistry. ¶Assessed by qPCR.



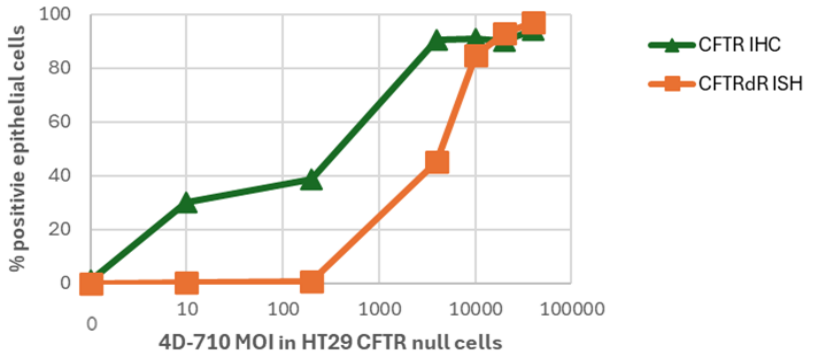
Minnich DJ, Mathisen DJ. Anatomy of the trachea, carina, and bronchi. *Thorac Surg Clin* 2007;17:571-85.

IHC & ISH Assay Specificity and Sensitivity

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



CFTR IHC and ISH % (+) vs 710 MOI in HT29 KO Cells



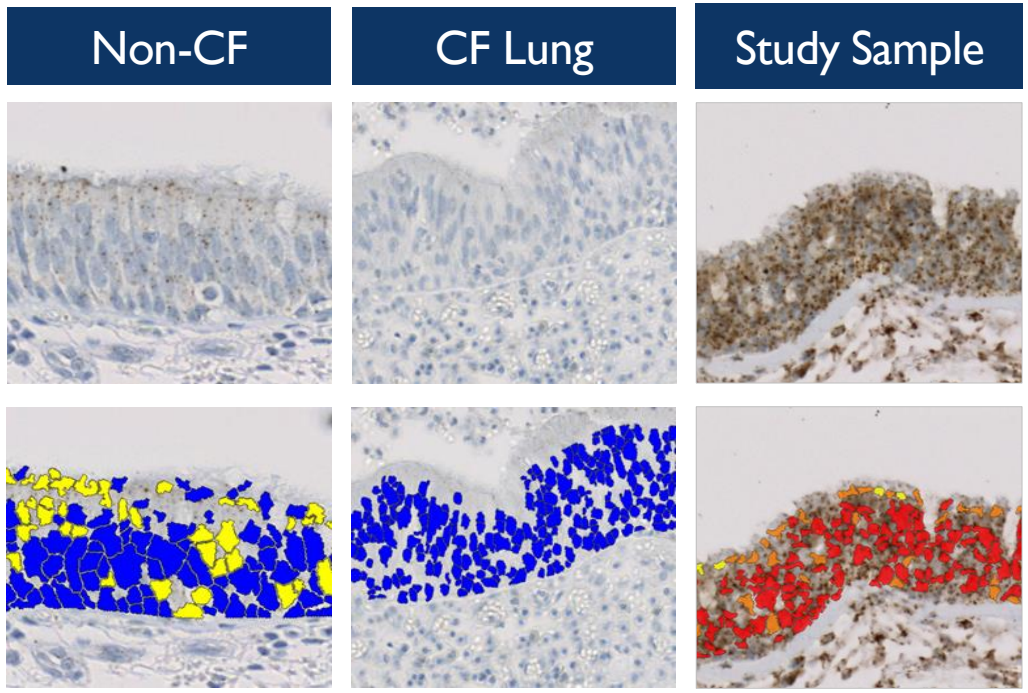
- IHC is more sensitive and has a different dynamic range compared to ISH

HT29 cells: human-derived intestinal epithelial cells expressing endogenous CFTR protein [1]. 1. Sood et al. *EMBO J* 1992;11:2487–94.

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

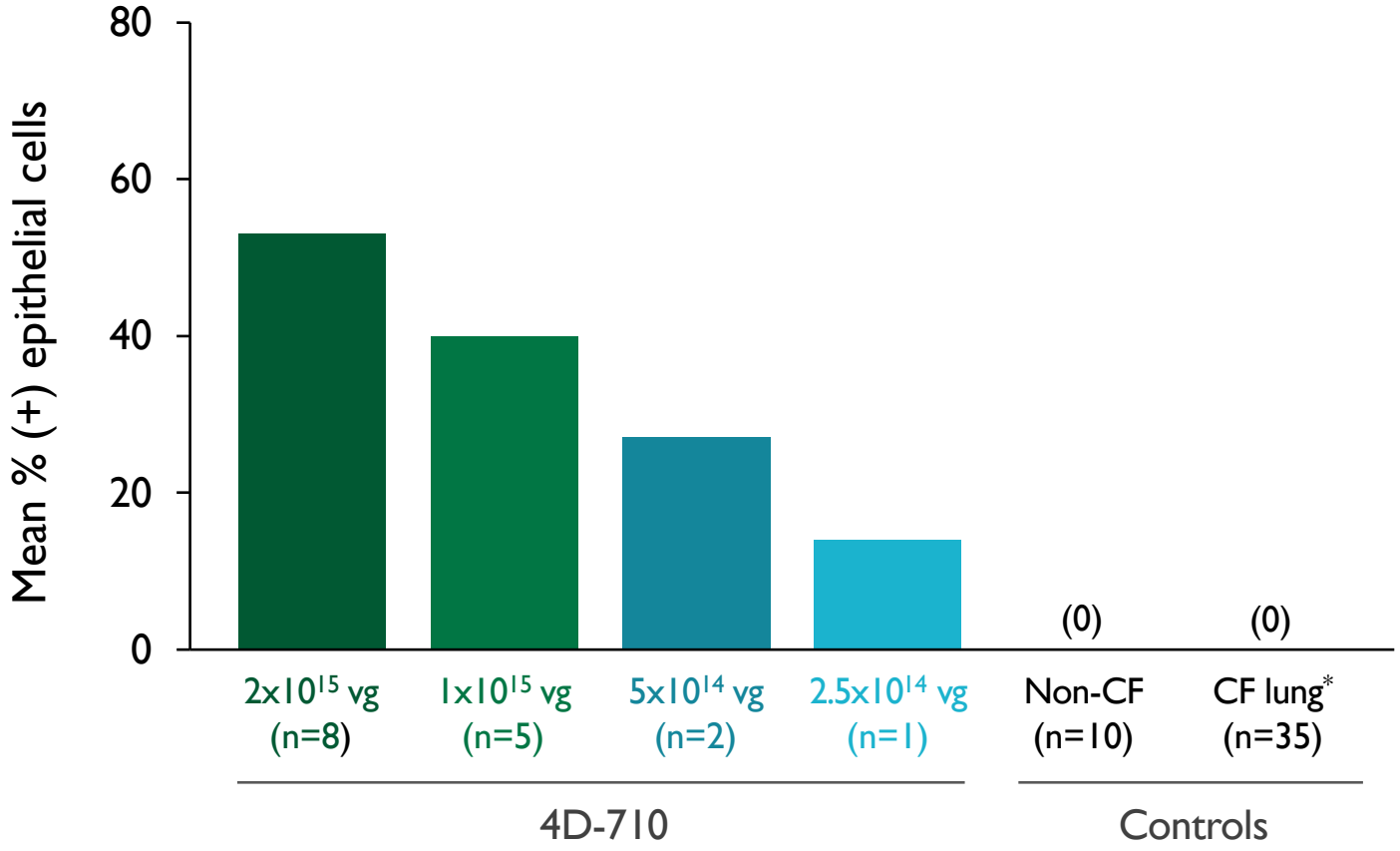


*Visiopharm® image analysis software. CFTR, cystic fibrosis transmembrane conductance regulator; IHC, immunohistochemistry.

4D-710 Transgene Delivery and RNA Expression

Dose-dependent *CFTRΔR* RNA Expression

CFTRΔR RNA (ISH)



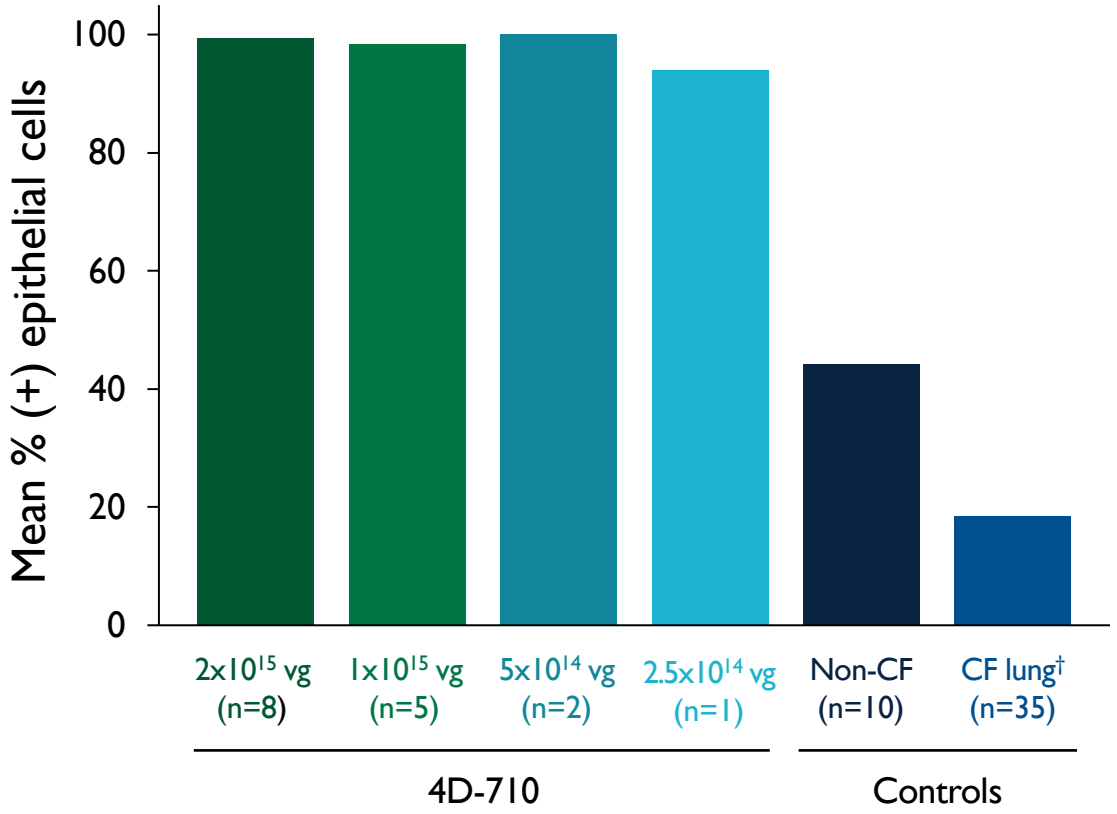
- Dose-dependent *CFTRΔR* mRNA expression in bronchial epithelial cells
- No *CFTRΔR* mRNA expression observed in commercial non-CF and CF lung samples
- Commercial non-CF samples positive for endogenous *CFTR* mRNA expression

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.

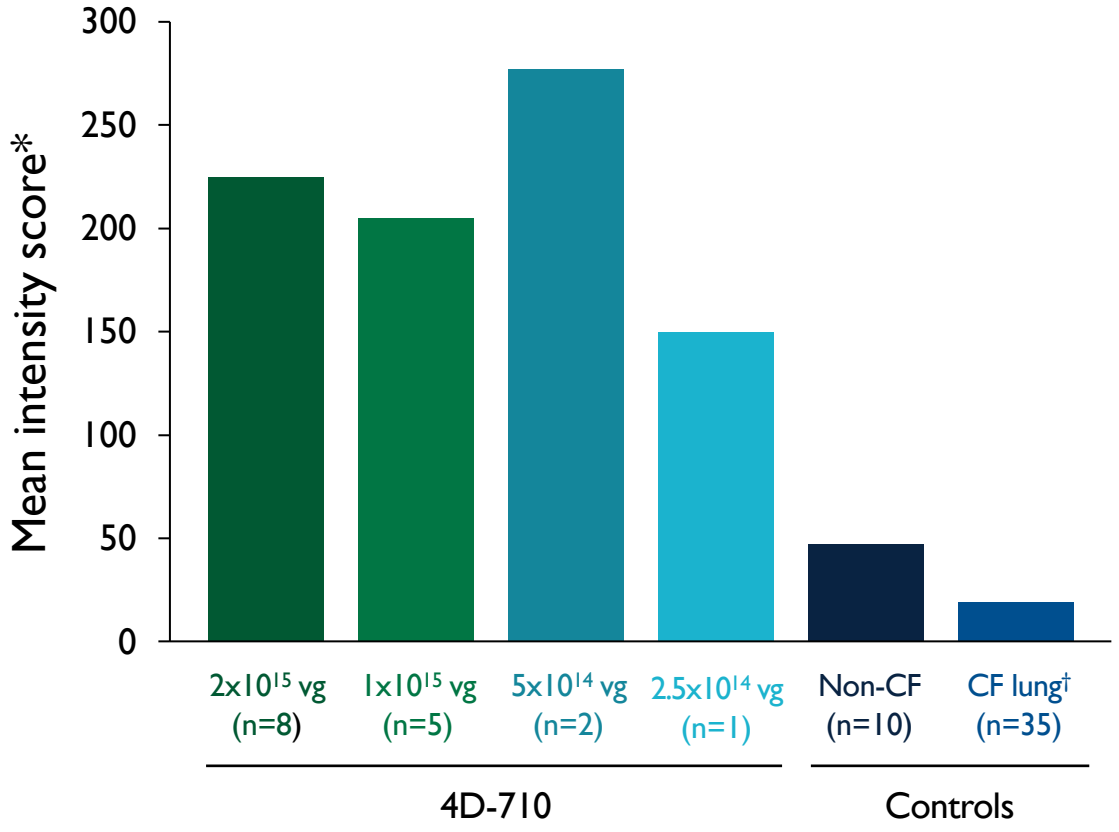
4D-710–Mediated CFTR Protein Expression by IHC

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

CFTR (+) Epithelial Cells (IHC)



CFTR Staining Intensity (IHC)*

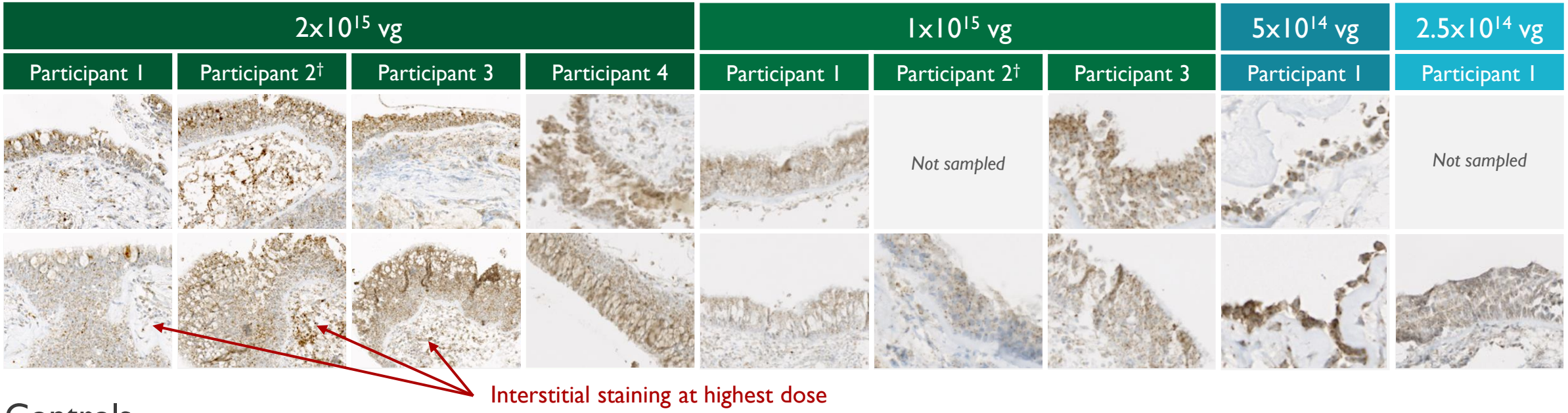


*H-score. Quantification by Visiopharm AI Machine Learning Analysis. †Genotyping of commercial CF samples yielded results for 13/35 samples; of these, a majority were *ΔF508* homozygous mutations. IHC, immunohistochemistry.

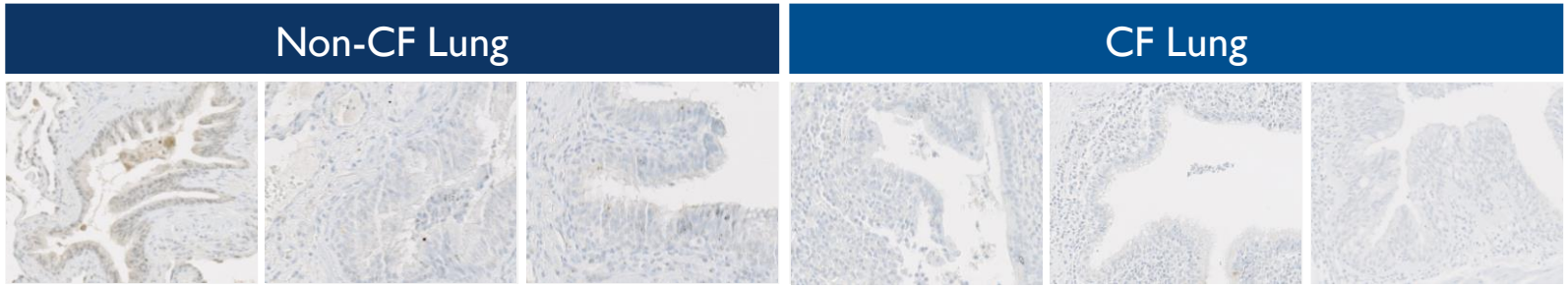
Widespread Consistent CFTR Protein Expression: 100% of Samples

16 of 16 Endobronchial Biopsy Samples Positive for CFTR Protein by IHC 4–8 Weeks After 4D-710 Dosing*

4D-710



Controls

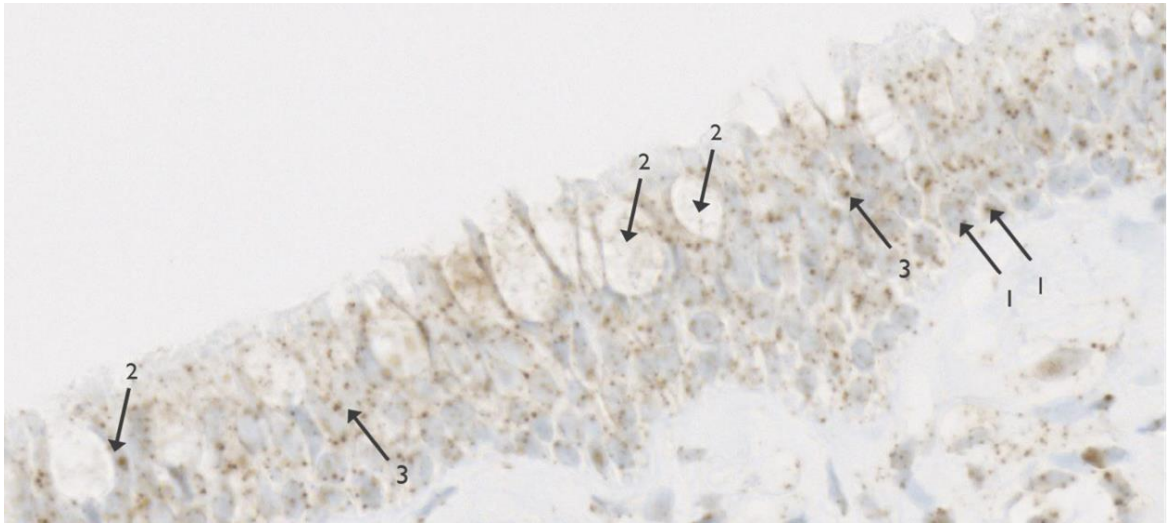


*Representative images, endobronchial biopsy samples obtained from the left secondary carina (row 1) and right middle lobe (row 2). †Endobronchial biopsy performed at Week 8.

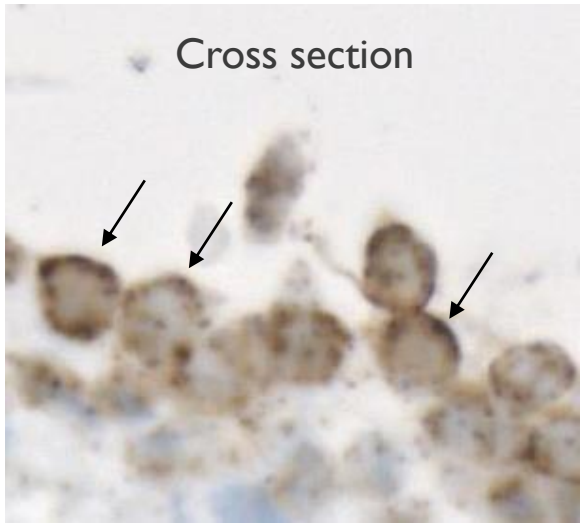
CFTR Protein Expression Observed in Multiple Airway Cell Types

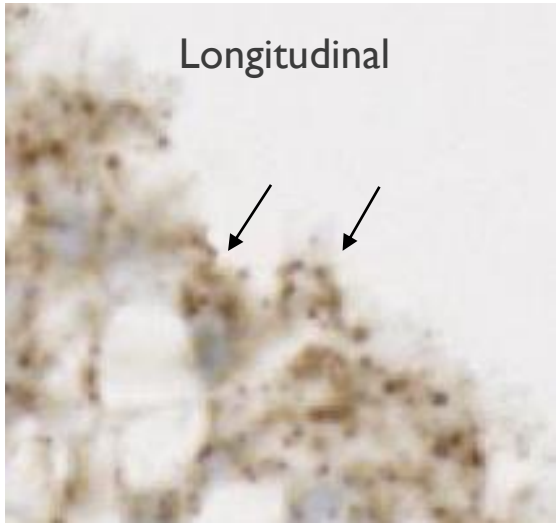
CFTR Protein Localization (IHC) Following Administration of 4D-710: secretory, ciliated & basal cells

CFTR Protein Expressed in Multiple Cell Types*



Localization to Apical Region†





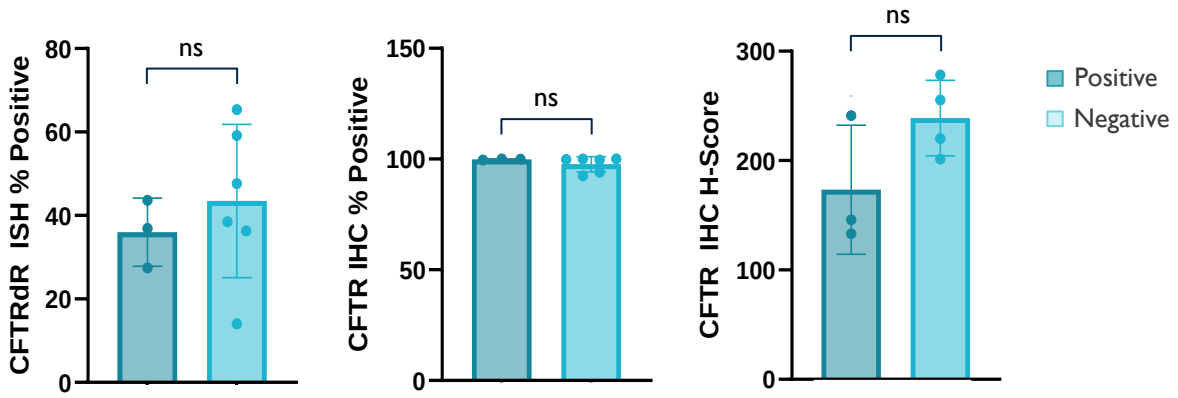
1) Basal cells 2) Goblet cells 3) Columnar ciliated cells

*Image from Cohort 1 ($1x10^{15}$ vg) participant. †Images from Cohort 2 ($2x10^{15}$ vg) participants. CFTR, cystic fibrosis transmembrane conductance regulator. IHC, immunohistochemistry.

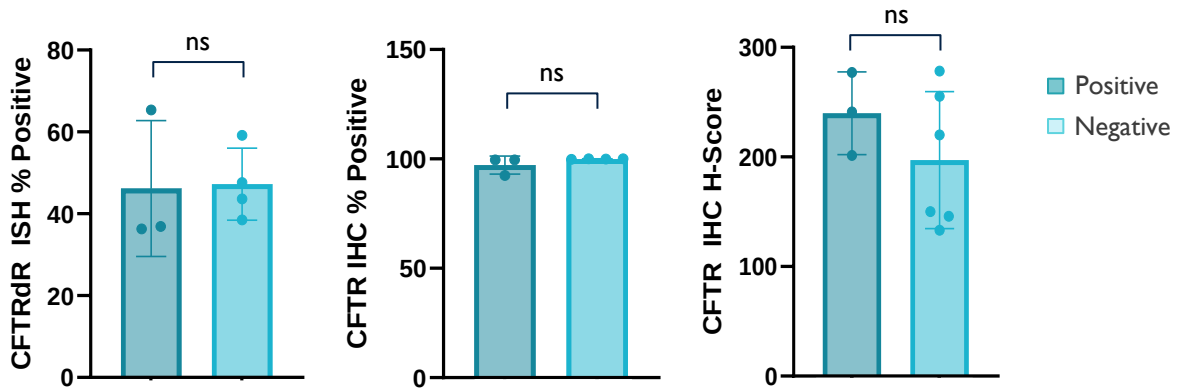
Immunogenicity Analyses

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

CFTR Expression According to Baseline Anti-A101 Antibodies



CFTR Expression According to Baseline A101-specific T Cells



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

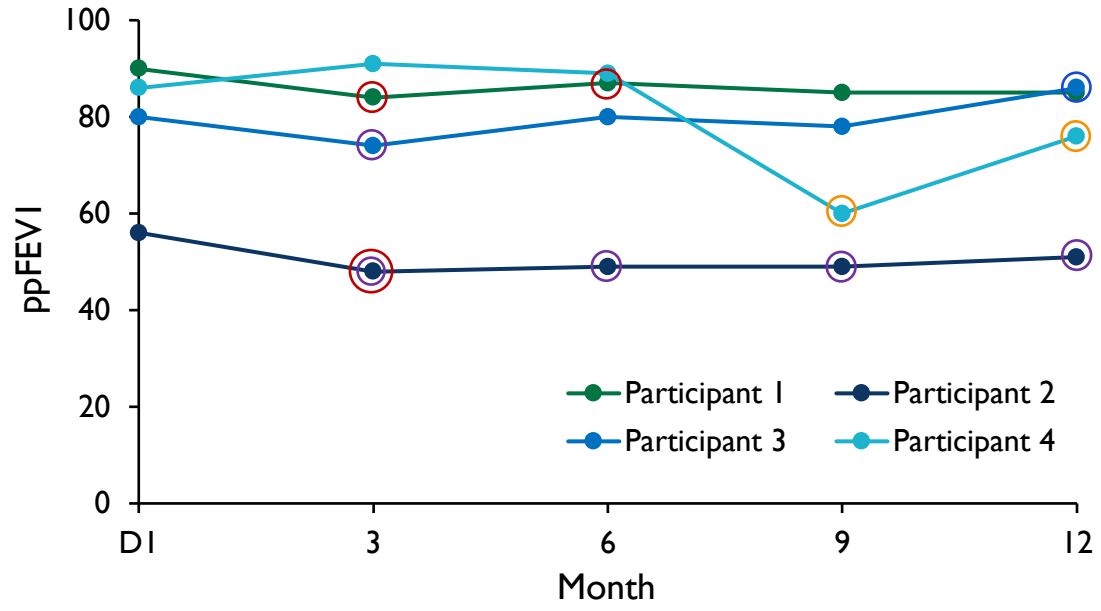
- 3/7 positive for pre-existing A101-specific T cells†
- No significant difference in bronchoscopy results between participants with (n=3) and without (n=4) pre-existing A101-specific T cells

*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 FEV_1 (12 Months)

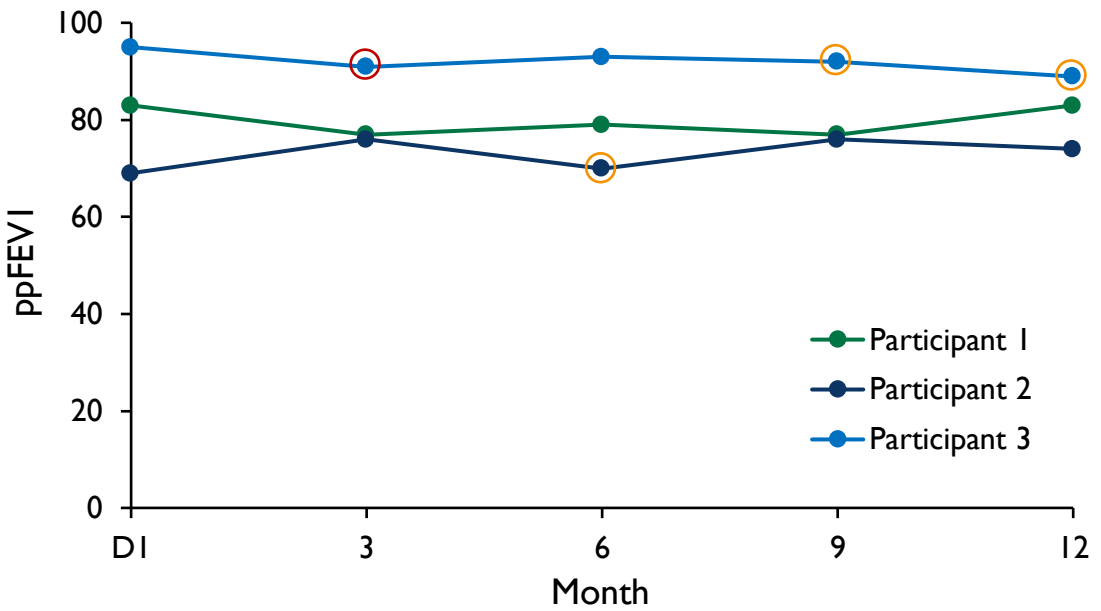
4D-710 (2×10^{15} vg)



Prednisone

Expected transgene expression

4D-710 (1×10^{15} vg)



Prednisone

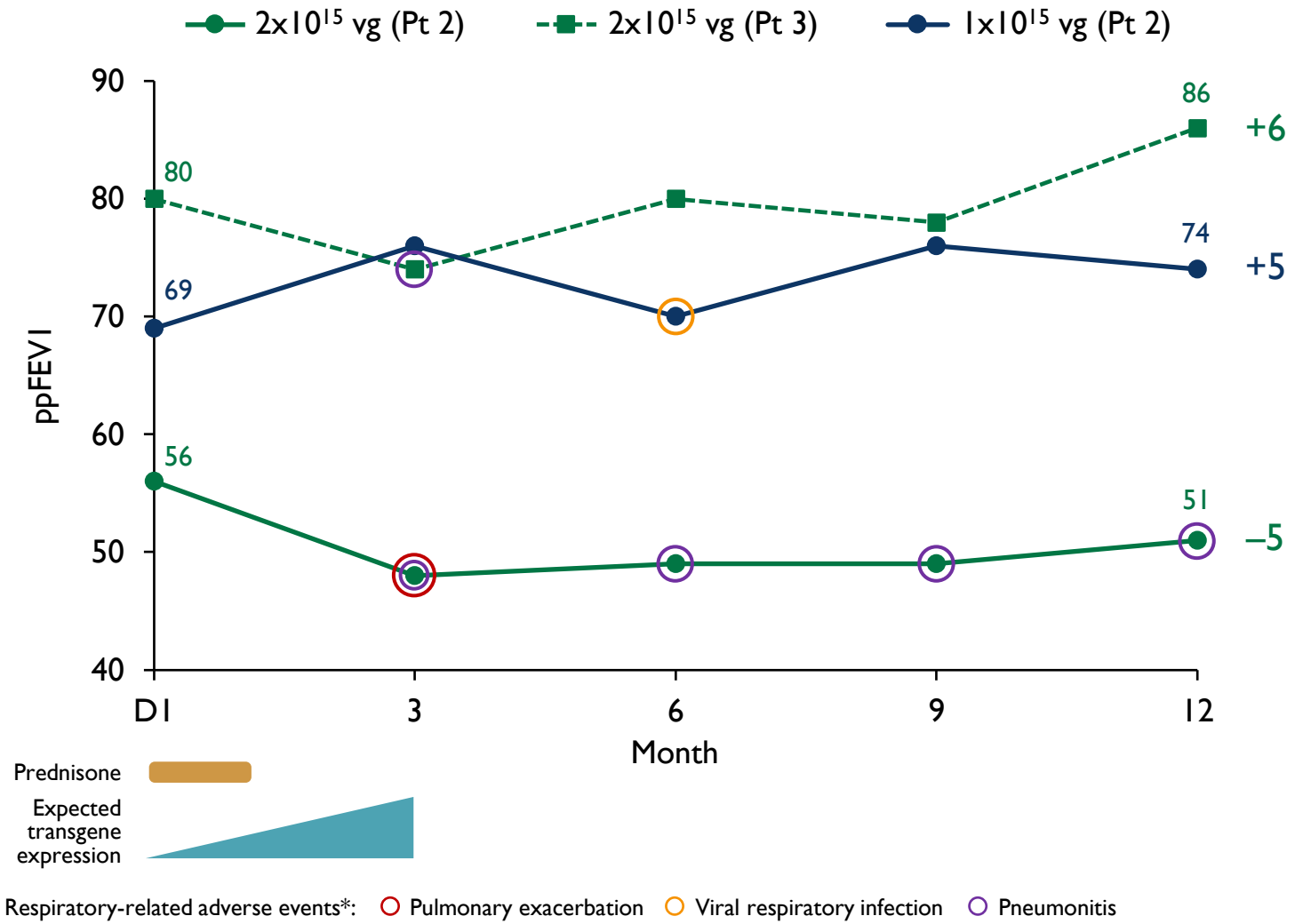
Expected transgene expression

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 FEV_1 in Participants with a Baseline Value ≤80% (N=3)



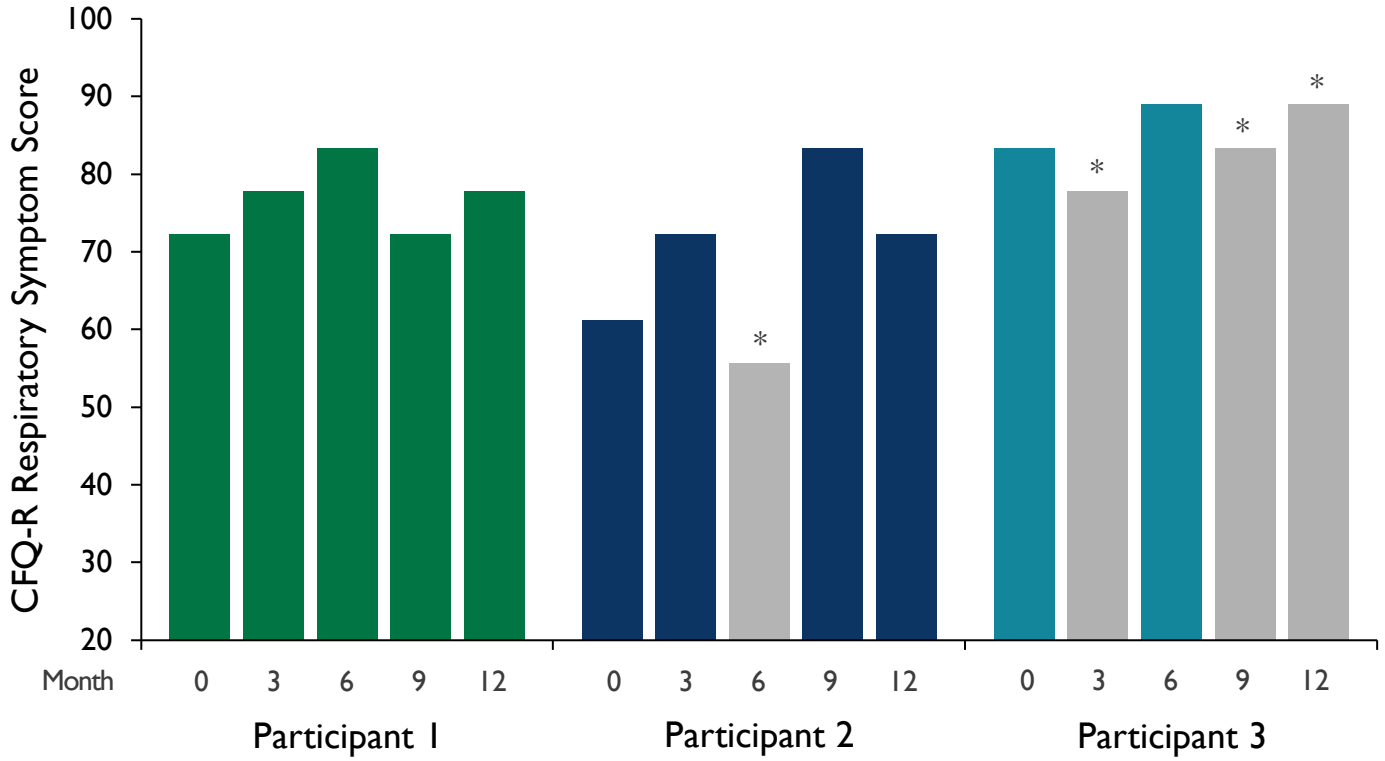
- Three participants had a baseline ppFEV$_1$ ≤80% and ≥6 months of follow up
- Two showed improvement in ppFEV$_1$ at 12 months
  - 2x10^{15} vg (n=1): +6%
  - 1x10^{15} vg (n=1): +5%

*Occurring within 21 days of pulmonary function assessment.

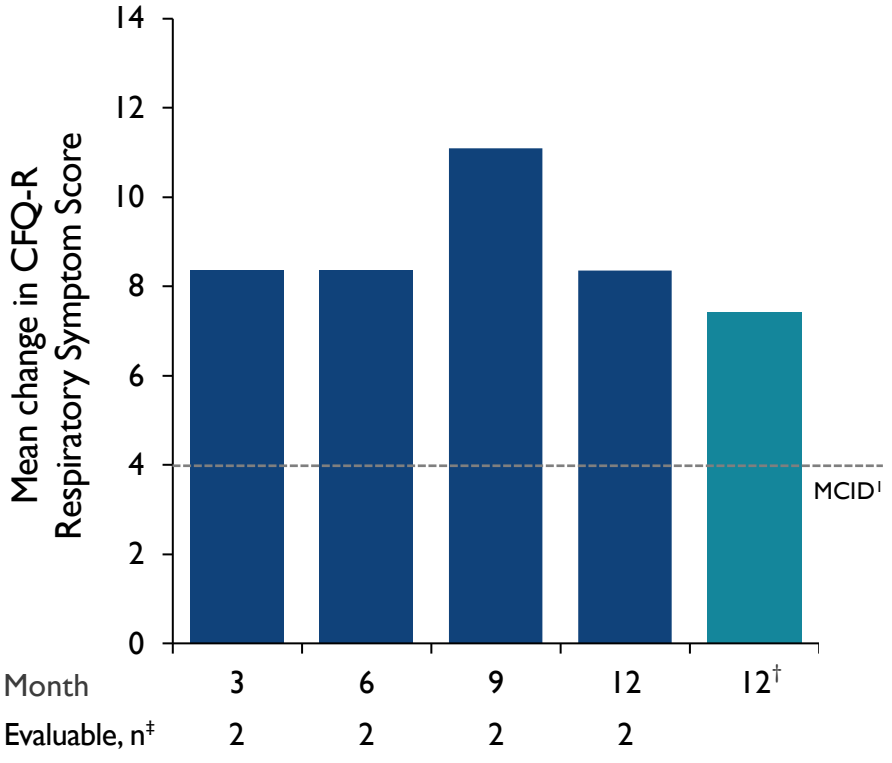
4D-710 (1×10^{15} vg): Durable Improvement in CFQ-R-R Score

Mean Increase Over 12 Months Consistently Above MCID

CFQ-R Respiratory Symptom Score



Mean Change in CFQ-R Score



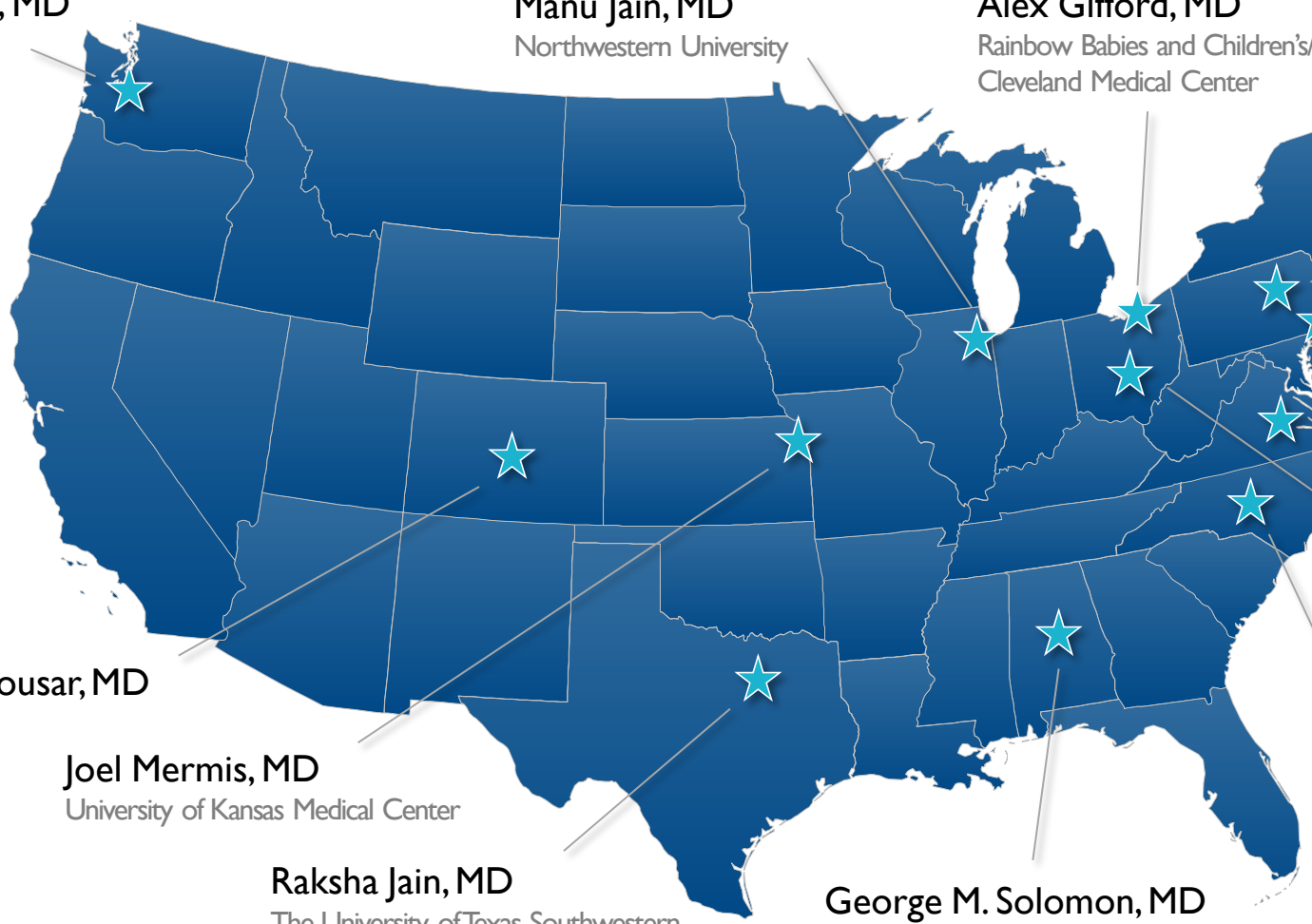
*Respiratory-related adverse event within 21 days of assessment. †All enrolled participants (n=3). ‡Excludes participants with a respiratory-related event within 21 days of assessment. CFQ-R-RD, Cystic Fibrosis Questionnaire-Revised (respiratory symptoms scale). Scores range from 0 to 100, with higher scores indicating better health. MCID=4 points [1]. 1. Quittner AL et al. *Chest* 2009;135:1610–18.

4D-710 Phase 1/2 Clinical Trial Interim Analysis
Summary and Conclusions

- Administration of a single aerosolized dose of 4D-710 to adults with CF lung disease was generally well tolerated at doses up to 1×10^{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 <u>not</u> prevent** transgene expression/biological activity
- Enrollment in 2.5×10^{14} and 5×10^{14} vg cohorts ongoing
  - Biological activity (CFQR-R QOL and $ppFEV_1$) to be reported at 12 months

Acknowledgments

Participants and Their Families, Principal Investigators and Study Staff, CFF



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4DMT CFTR IHC Assay Development

Validated by Extensive Control Testing to Ensure Specificity to CFTR Epitope

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