AAV-Mediated Gene Therapy for Cystic Fibrosis (4D-710)

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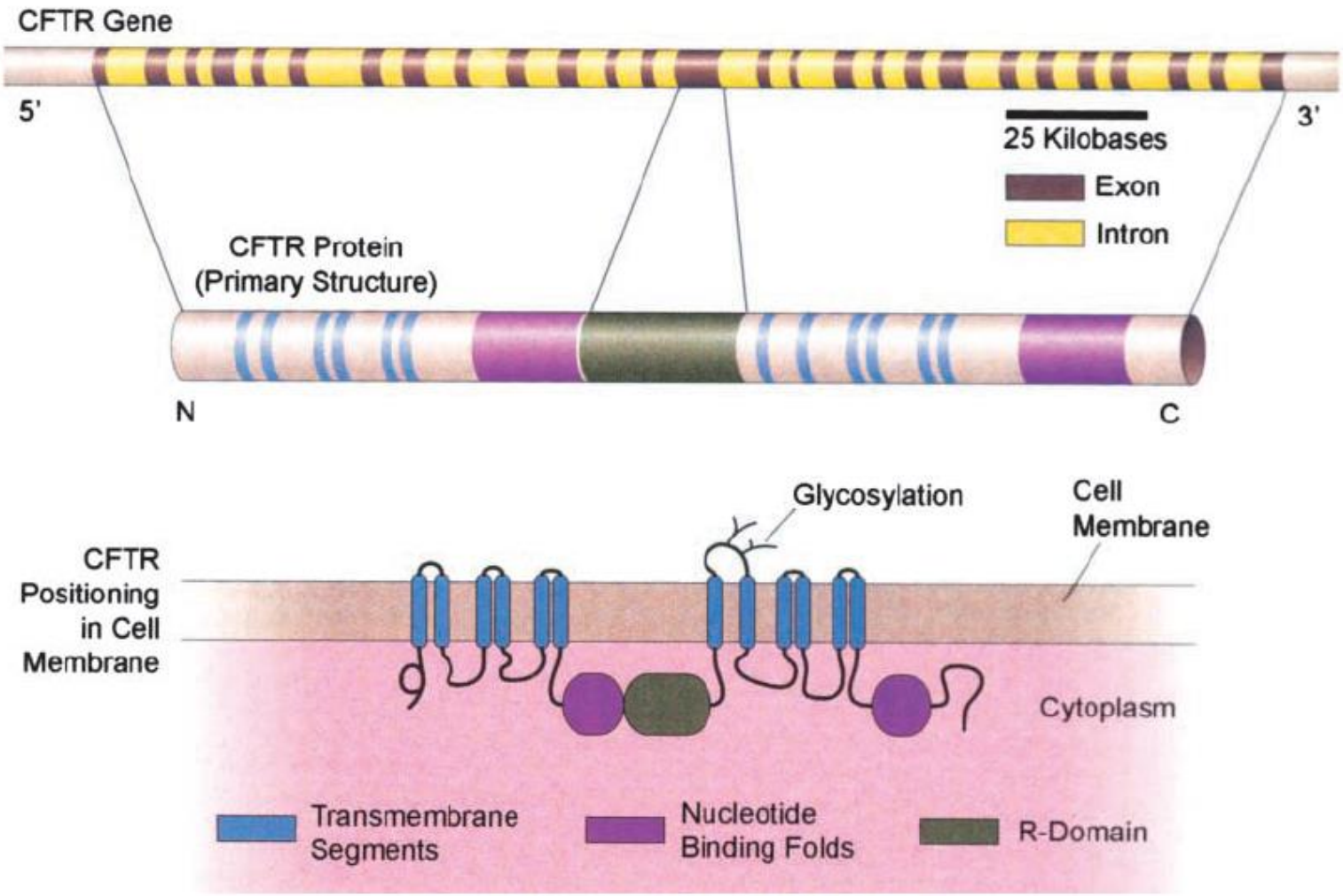
Disclosures

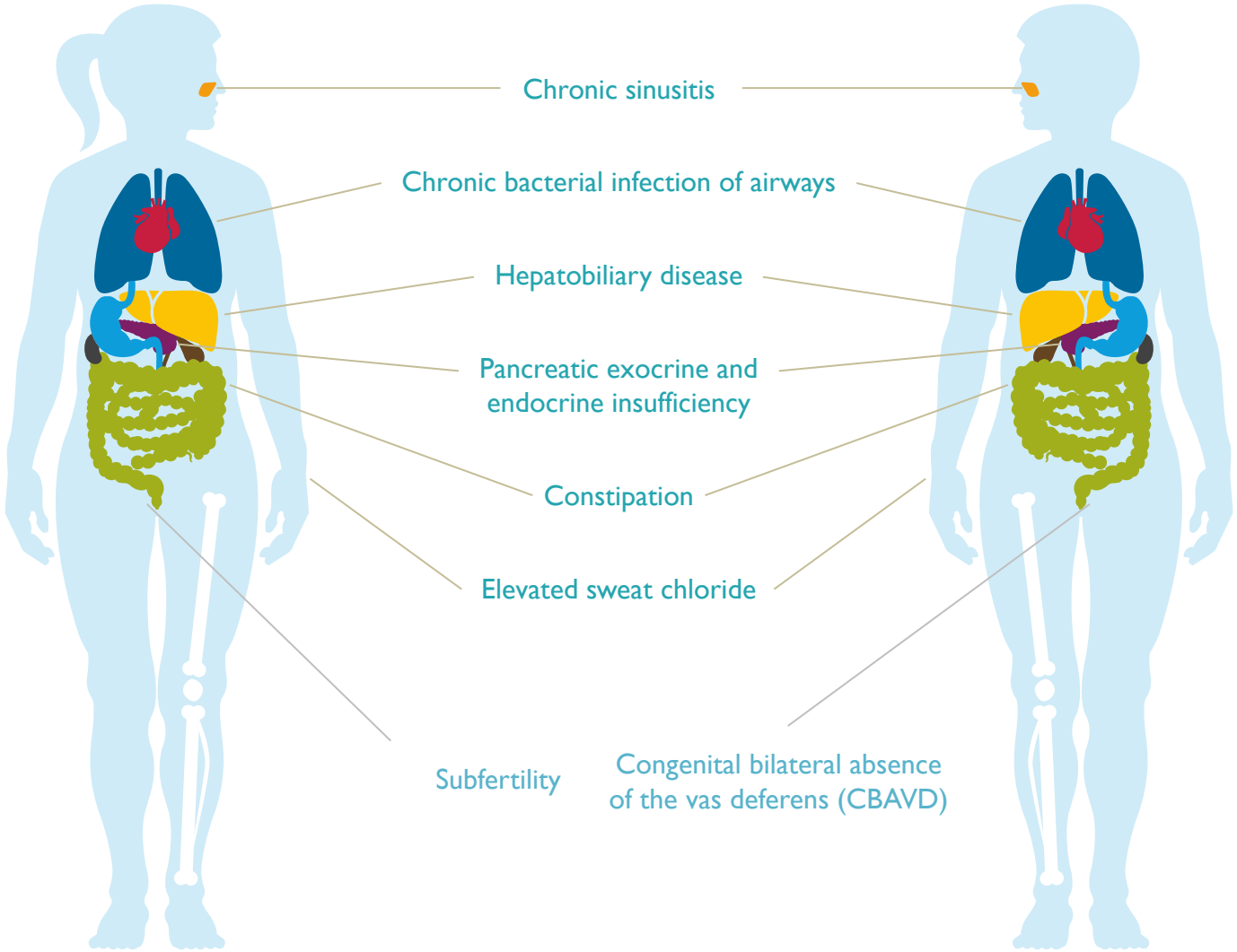
- Personal financial relationships with commercial interests relevant to medicine, within the past year:
  - As faculty at an institution that is part of the CF TDN, I am/have been site/national PI on studies for 4DMT, Vertex, and Eloxx.
  - I have done clinical trial consulting for Vertex.
  - I serve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

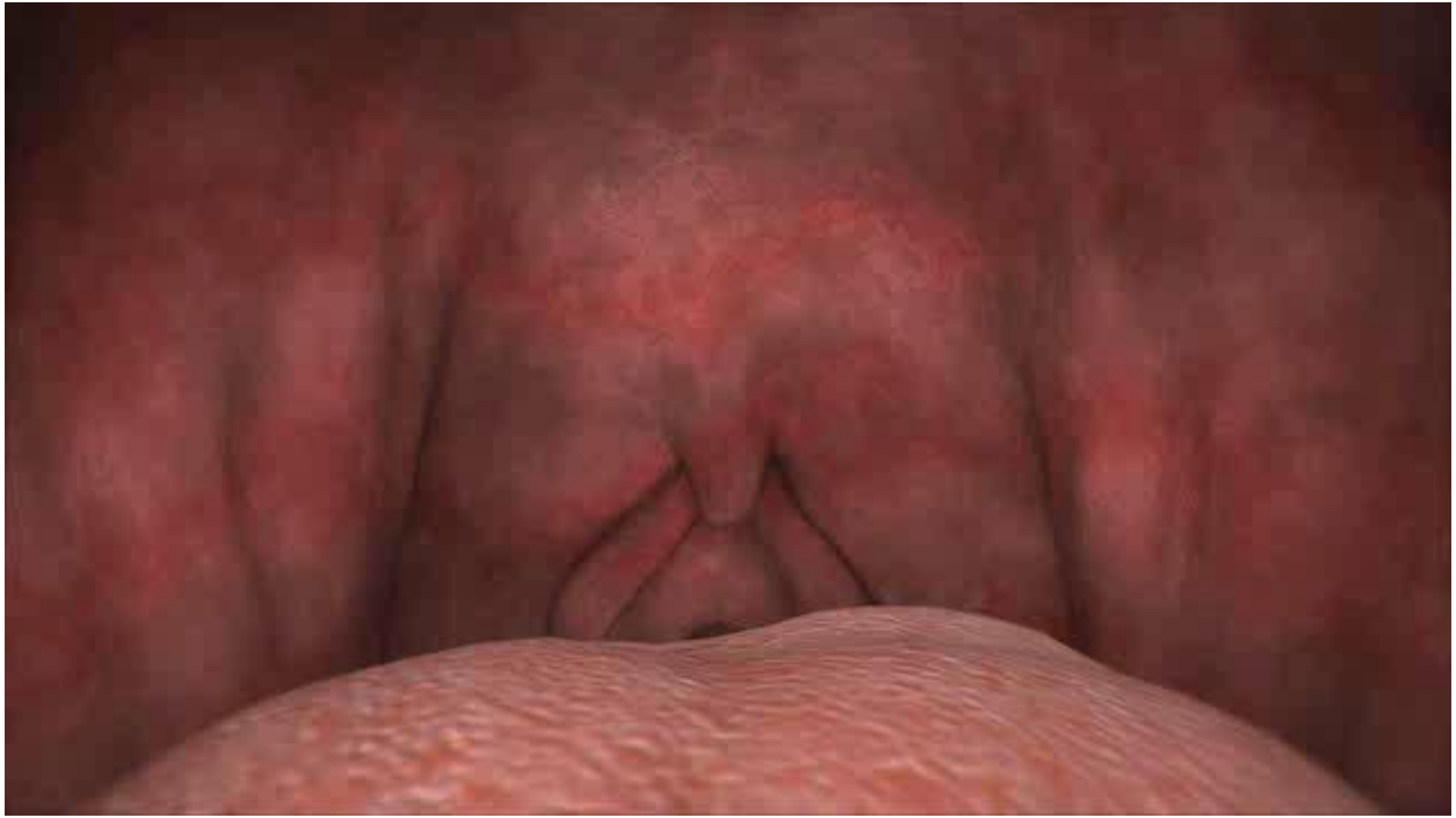
Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)



Multisystem Autosomal Recessive Disorder

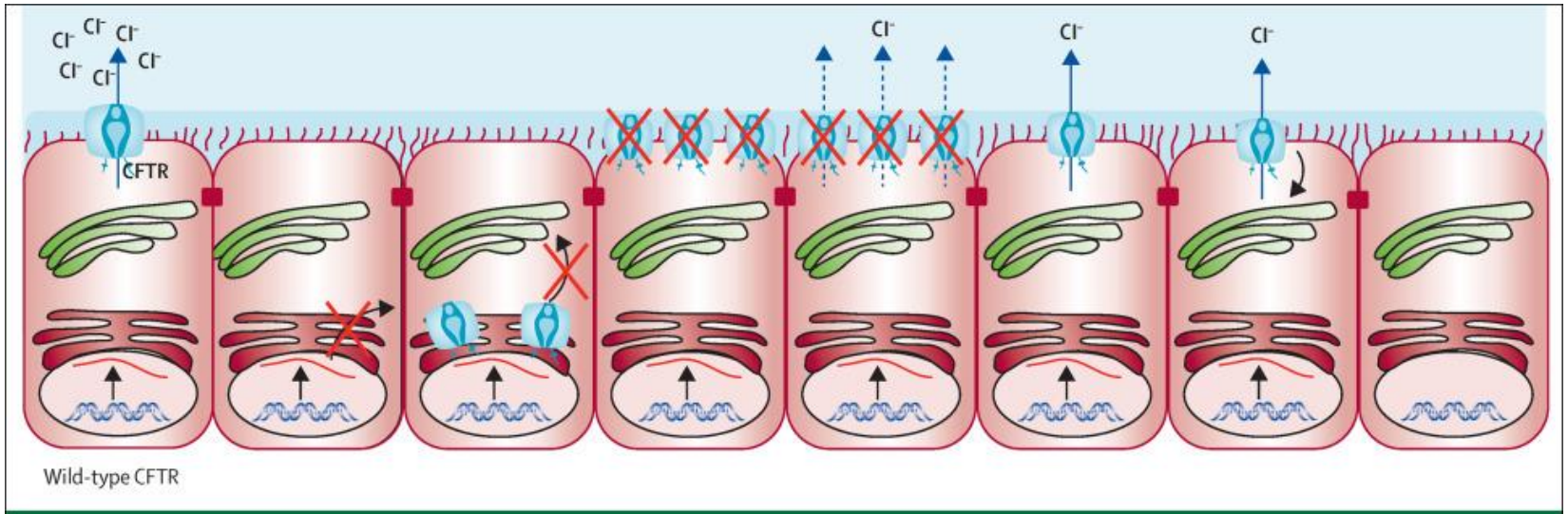


Slide courtesy of CFF



Video courtesy of CFF

Classifying CFTR Mutations



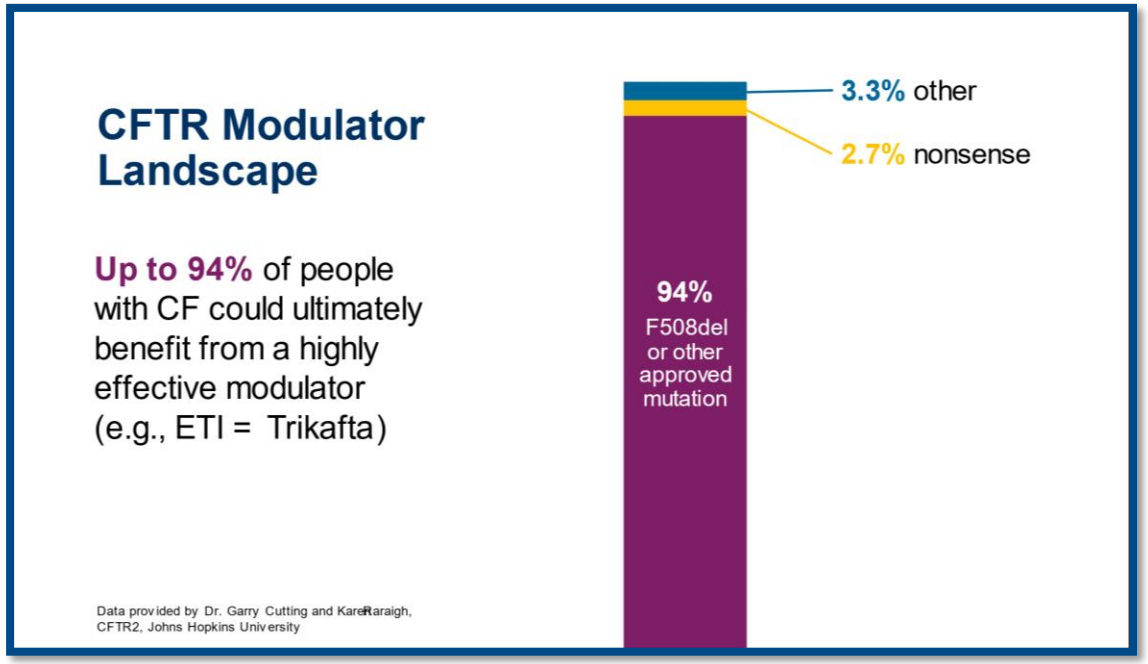
	Class I	Class II	Class III	Class IV	Class V	Class VI	Class VII
CFTR defect	No protein	No traffic	Impaired gating	Decreased conductance	Less protein	Less stable	No mRNA
Mutation examples	GLy542X, Trp1282X	Phe508del, Asn1303Lys, Ala561Glu	Gly551Asp, Ser549Arg, Gly1349Asp	Arg117His, Arg334Trp, Ala455Glu	Ala455Glu, 3272-26A→G, 3849+10 kg C→T	c. 120del23, rPhe508del	dele2,3(21 kb), 1717-1G→A
Corrective therapy	Rescue synthesis	Rescue traffic	Restore channel activity	Restore channel activity	Correct splicing	Promote stability	Unrescuable
Drug (approved)	Read-through compounds (no)	Correctors (yes)	Potentiators (yes)	Potentiators (no)	Antisense oligonucleotides, correctors, potentiators? (no)	Stabilisers (no)	Bypass therapies (no)

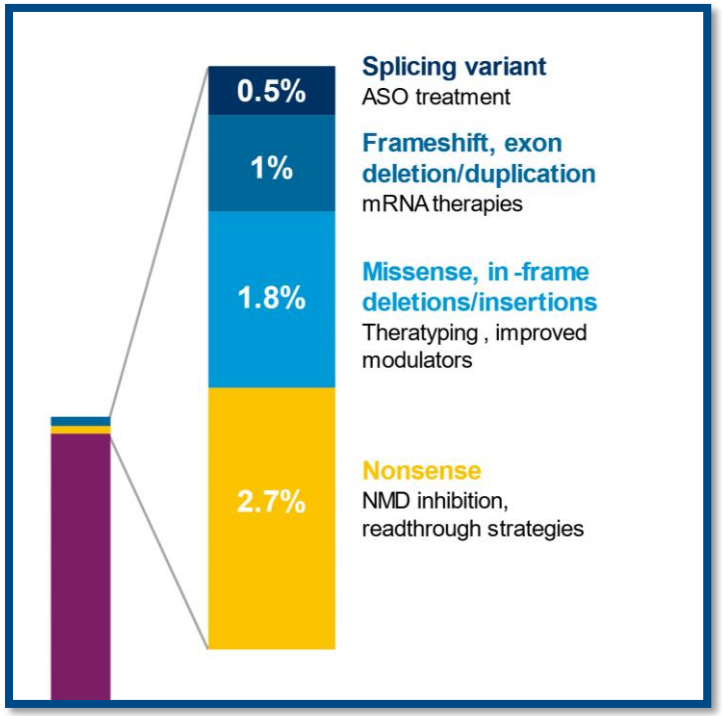
De Boeck, Amaral. *Lancet Resp Med* 2016

Majority of PwCF are Variant-eligible for Highly Effective Modulator Therapy

Modulator Therapy Landscape

- Up to 94% of people with CF could ultimately be eligible for a highly effective modulator
- It's not known (exactly) how many pwCF are not taking modulators (probably >10%)





Slide courtesy of JP Clancy; https://www.cff.org/Research/Developing-New-Treatments/CFTR-Modulator-Types/

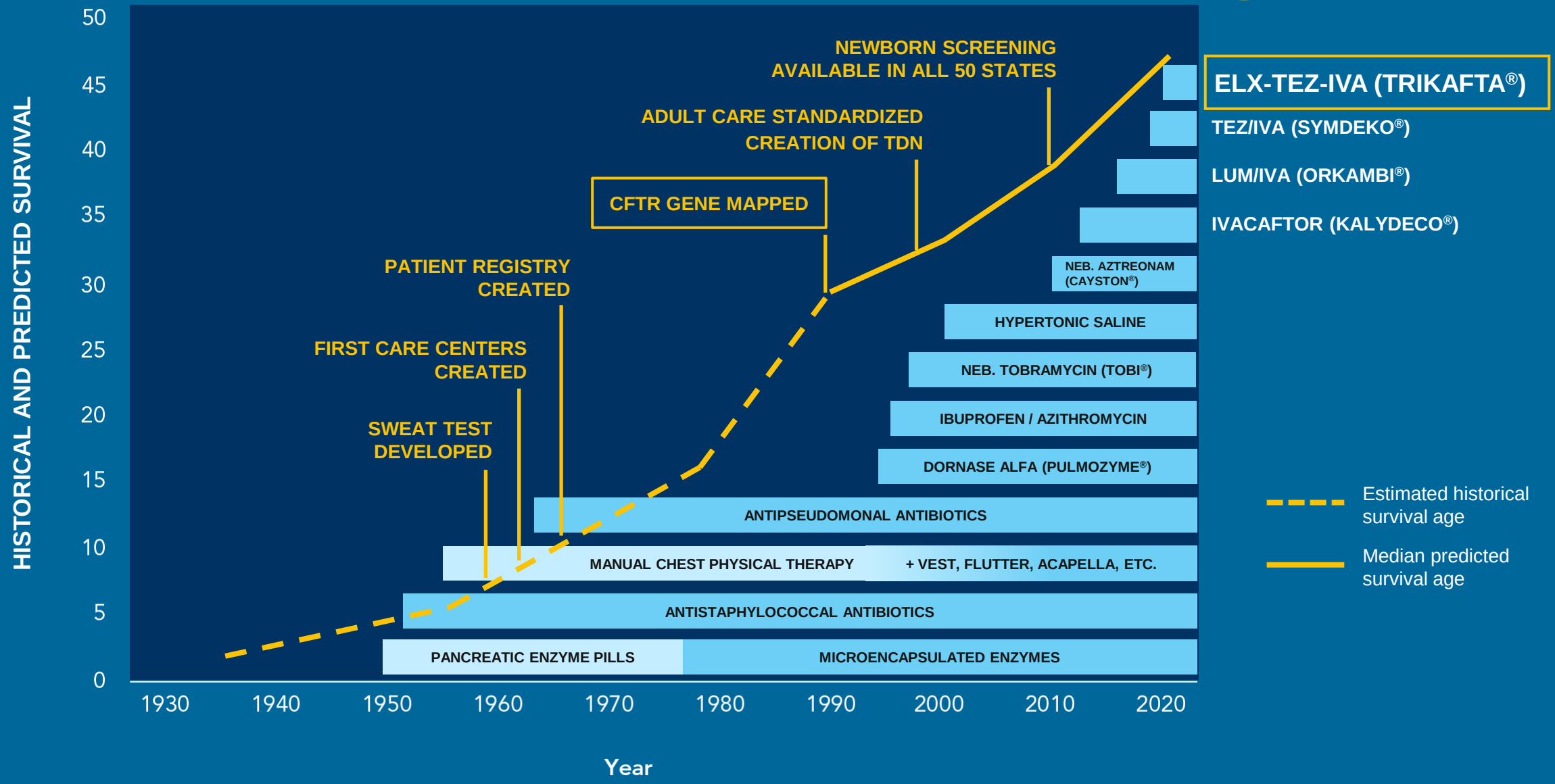
Unequal Eligibility for CFTR Modulator Therapy

Ethnic Ancestry	White	Hispanic	Black	Asian	Native American
# with 0 copies of F508del*	2,298 (10%)	588 (30%)	458 (38%)	63 (40%)	29 (17%)

PwCF from historically marginalized groups are less likely to qualify for modulators

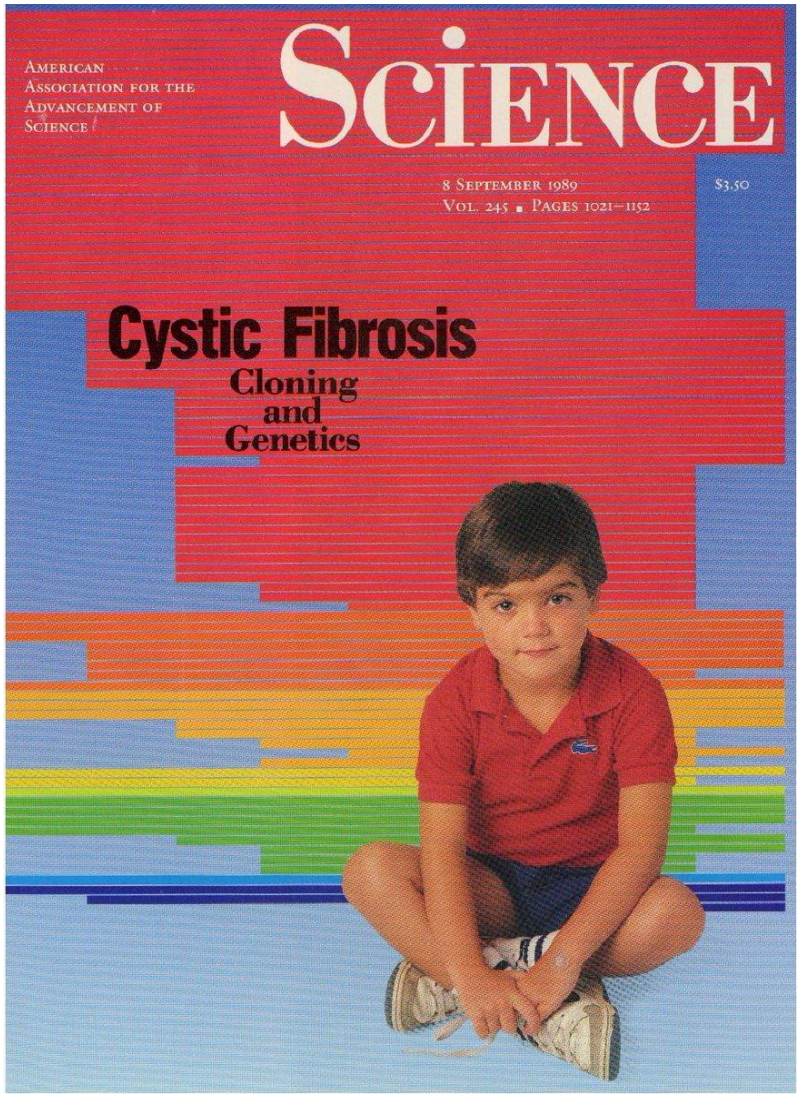
Table adapted from Schrijver et al *J Mol Diagn* 2016*; McGarry and McColley, *Peds Pulm* 2021; Desai et al *Resp Med* 2022

Timeline of Advances in CF

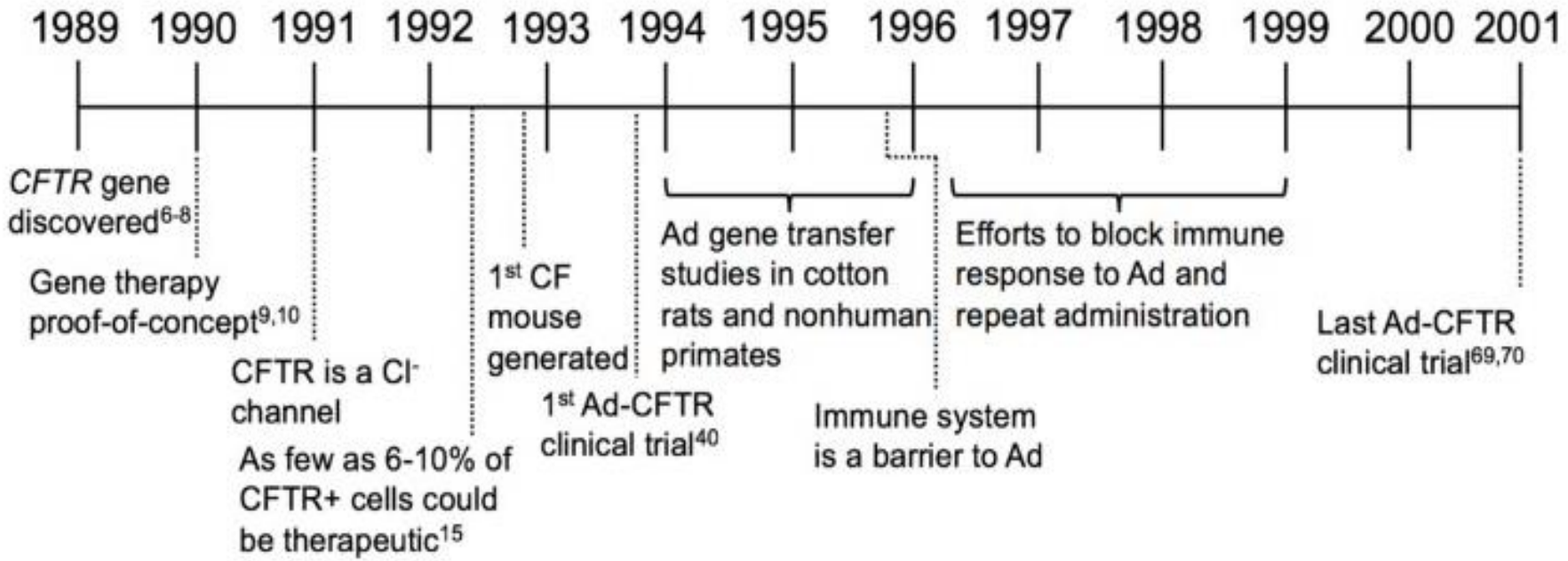


Adapted from Ramsey & Welsh. *Am J Respir Crit Care Med* 2017;**195**(9):1092–9.

Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene Discovered



Timeline of CF Gene Therapy 1989–2001



Timelines of CF gene therapy eras: Important milestones impacting the CF field are represented in timelines at the beginning of each era. The timelines are intended to orient the reader to new developments relative to other events and are not comprehensive of all contributions to the field (1989–2001).

Cooney AL at el. *Genes* 2018; 9(11):538.

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**

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;13:1349-1359. 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.

Advantages and Disadvantages of Conventional AAV as a Vector

Advantages

- Lower risk of pathogenicity
- Duration of expression (in non-proliferating cells)
- Broad range of target organs based on # of serotypes
- Lack of strong immune response to many serotypes
- Low risk of insertional mutagenesis

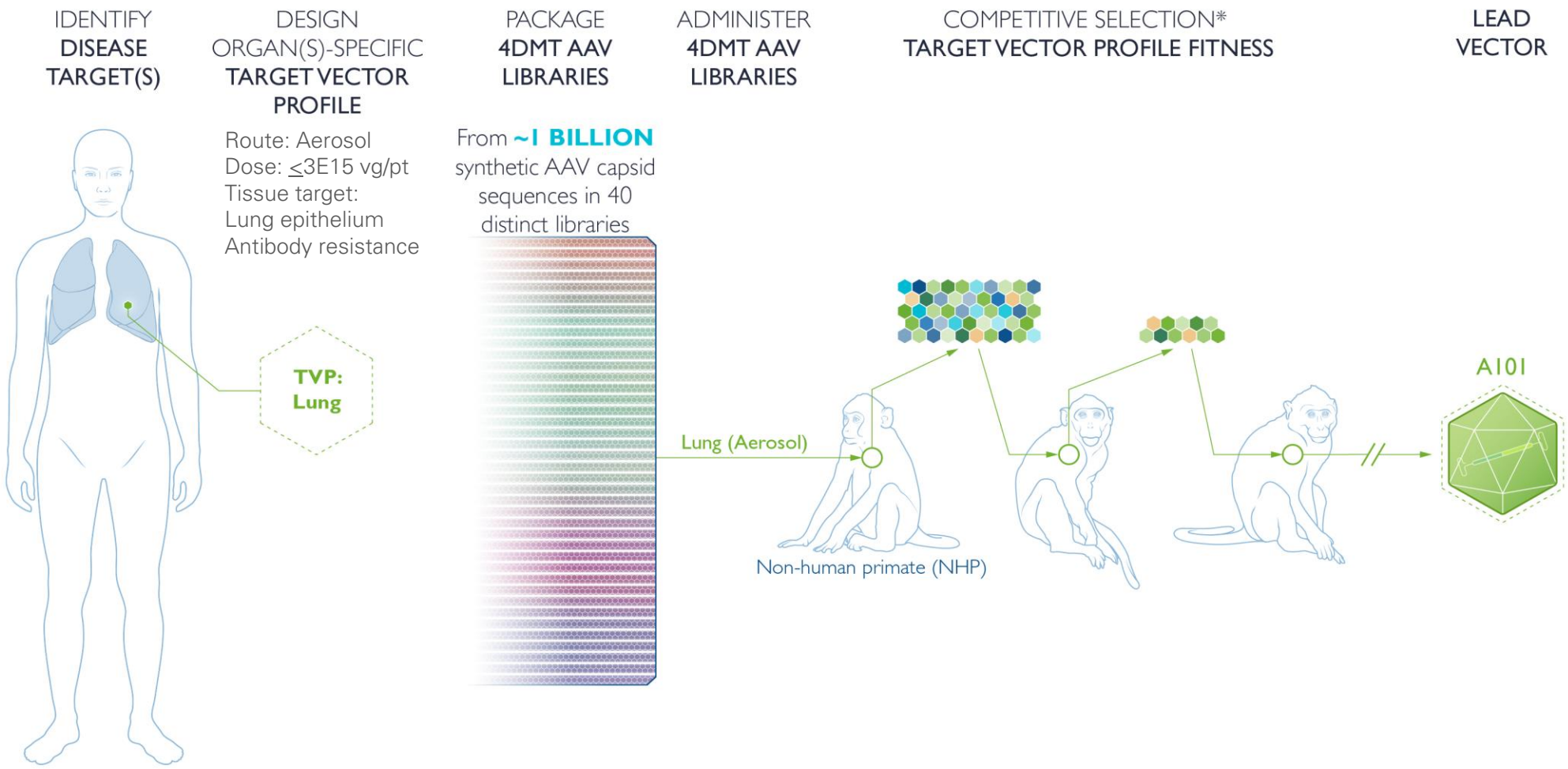
Disadvantages

- AAV receptor on basolateral (rather than apical) surface
- Small genome packaging capacity
- Potential for pre-existing or inducible Ab
- Potential for hepatotoxicity at high dose intravenous delivery
- Unclear if repeat dosing is possible

McLachlan et al. *Hum Genet Ther* 2022

Therapeutic Vector Evolution: A101 Aerosol Delivered Synthetic AAV

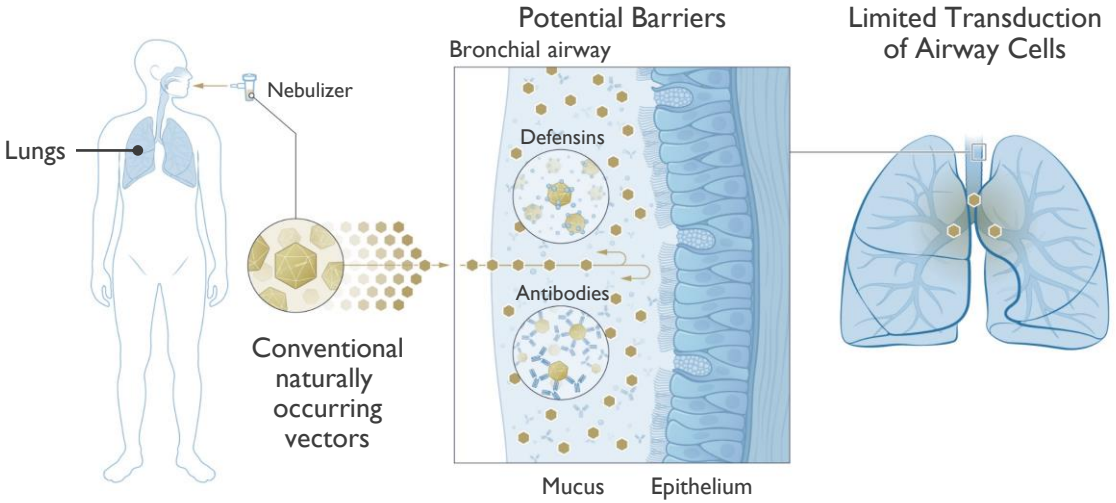
PROPRIETARY SYNTHETIC VECTOR DISCOVERY PLATFORM

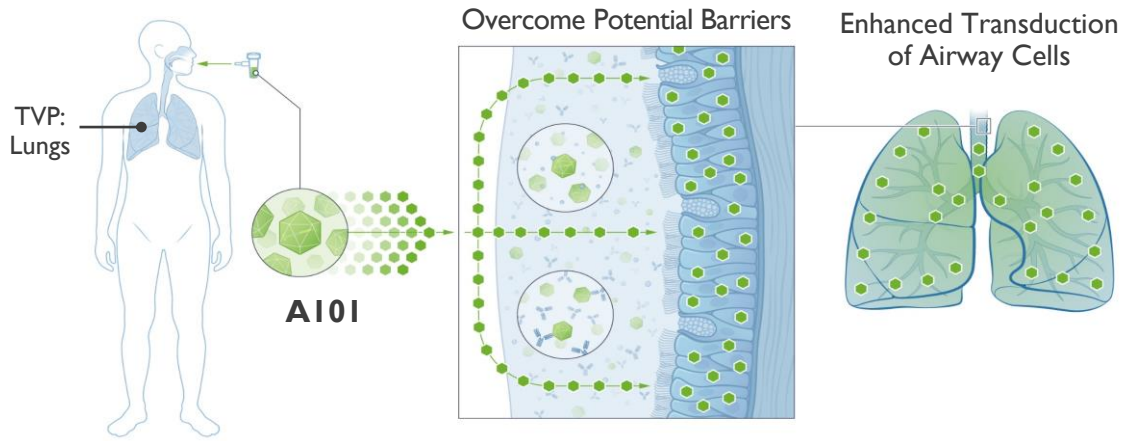


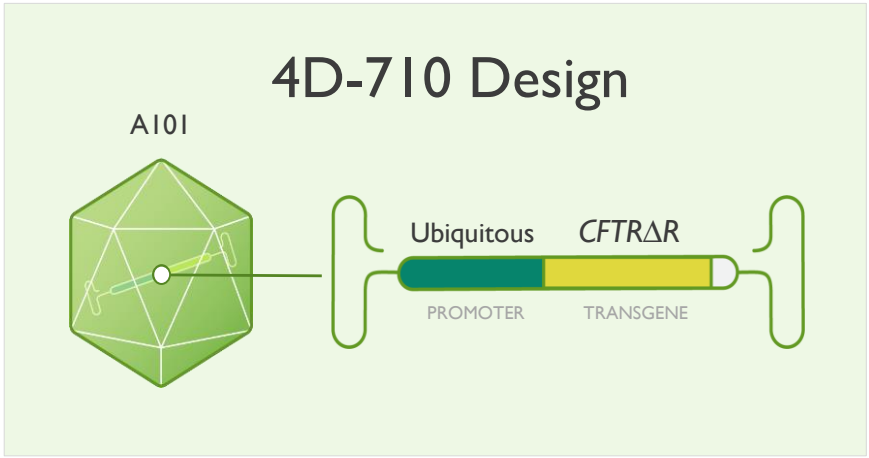
TVP, Therapeutic Vector Evolution; vg, vector genome

4D-710: Next-Gen Aerosolized Genetic Medicine for Cystic Fibrosis Lung

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







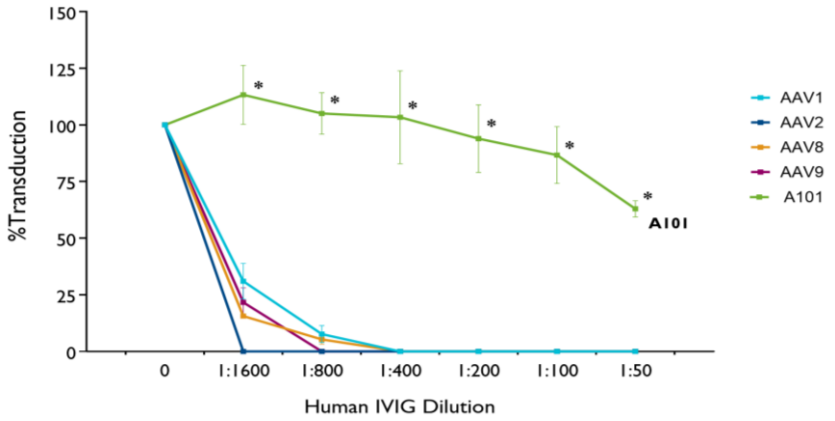
A101 KEY ATTRIBUTES

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

4D-710 Preclinical Characterization

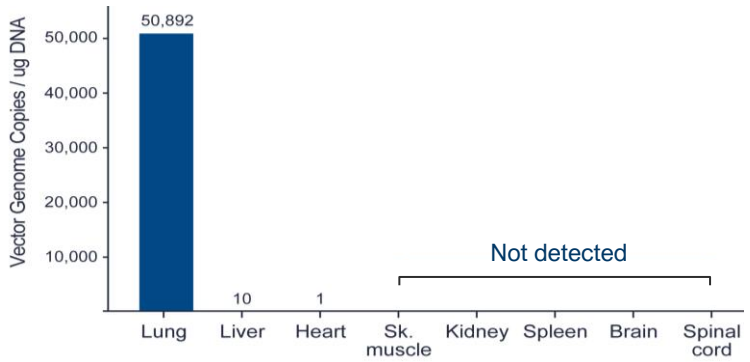
A101 VECTOR RESISTANCE TO HUMAN IVIG, 4D-710 BIODISTRIBUTION, AND CFTR EXPRESSION IN PRIMATES

Human Antibody Resistance: IVIG



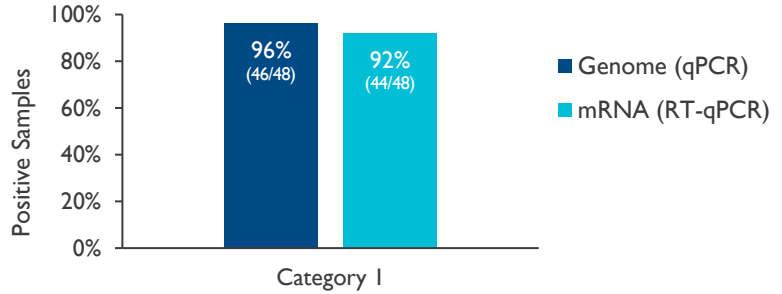
Human Hek2v6.11 cells. *p<0.05.

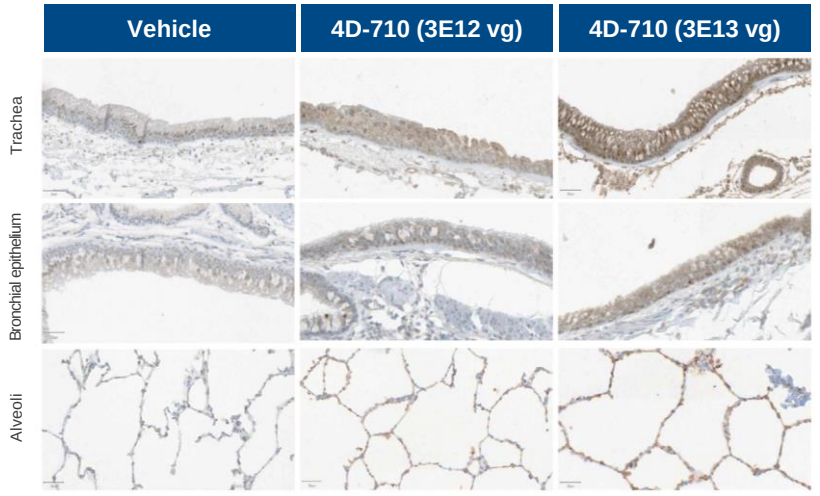
Lung-Specific Delivery: Aerosol in NHP



Delivery and Transduction: Aerosol NHP

4D-710 Biodistribution in NHP Lung (n=3 NHP; 48 samples)





CFTR immunohistochemistry staining of lung tissue samples from nonhuman primates, representative images (10x).

Calton M. American Thoracic Society International Conference, May 14-19, 2021. Abbreviations: NHP, nonhuman primate.

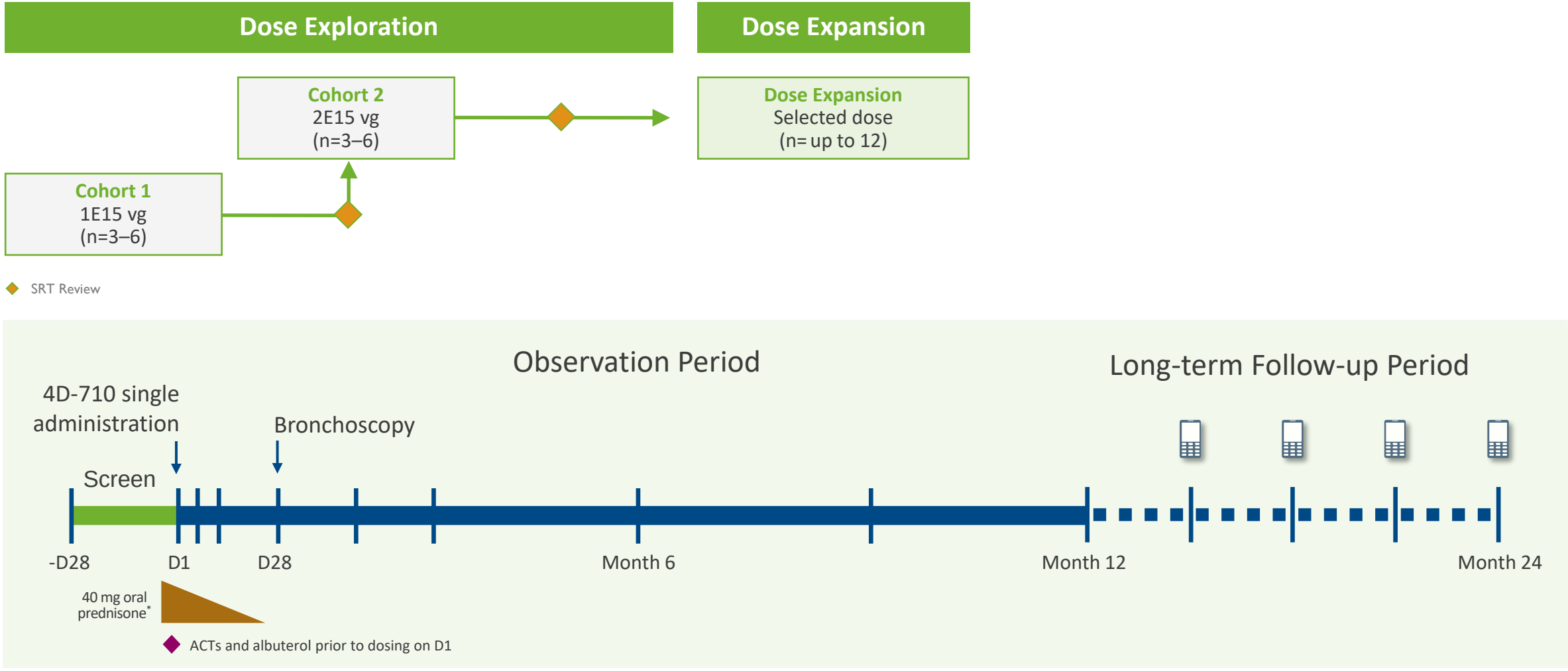
Next-Generation 4D-710 AAV Gene Therapy in Cystic Fibrosis:

4DMT APPROACH TO OVERCOMING HURDLES WITH TGAAVCF

- A101 novel synthetic vector:
  - Directed Evolution in primates for aerosol delivery
  - Efficient mucus penetration & transgene expression
  - High resistance to pre-existing human AAV antibodies
  - Lung retention >99.9%
- 4D-710 genetic medicine product:
  - Targeted and evolved vector (A101): tgAAVCF used AAV2
  - Strong promoter: tgAAVCF had no exogenous promoter

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



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 and 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 FEV_1 ≥50% and <100%
- Resting O_2 sat ≥92% on room air

CFTR, cystic fibrosis transmembrane conductance regulator; $ppFEV_1$, 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 $ppFEV_1$ from baseline (through Month 12)
  - Change in Cystic Fibrosis Questionnaire-revised (CFQ-R) scores (through Month 12)

$ppFEV_1$, percent predicted expiratory volume in 1 second.

4D-710 Phase 1/2 Clinical Trial: Cohort 1 Participants

DEMOGRAPHICS AND BASELINE CHARACTERISTICS

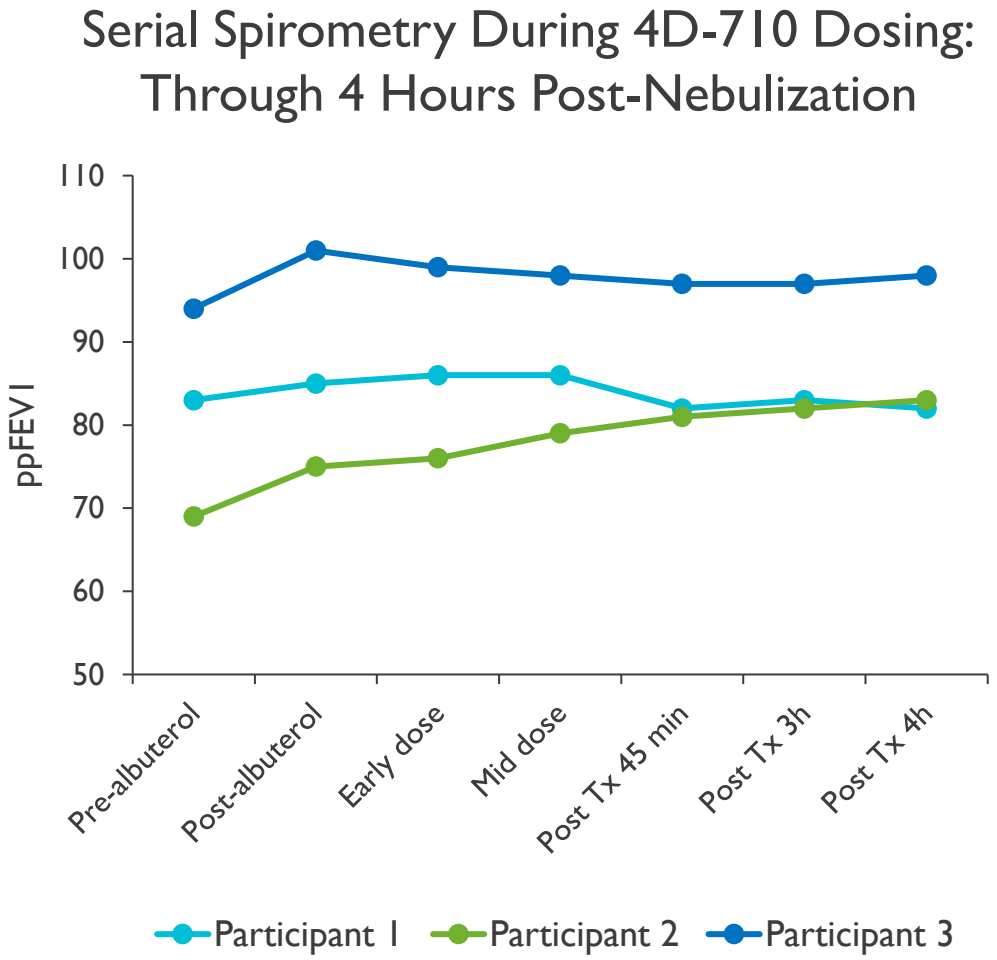
Characteristic	Cohort 1 (1E15 vg dose)		
	Participant 1	Participant 2	Participant 3
Age, y	36	24	20
Sex	Male	Male	Female
Race/ethnicity	Non-Hispanic white	Non-Hispanic white	Non-Hispanic white
CFTR modulator eligibility	Tolerability issues	Ineligible variant	Ineligible variant
CFTR mutation class	II/V	I/unknown	I/II
Historical sweat chloride, mmol/L	74	103	110
Percent predicted FEV_1 ($ppFEV_1$)	83	69	94
Pre-dose NAb to A101 capsid	Positive	Negative	Positive

Sweat chloride normal range ≤29 mmol/L, *Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation* (2017).
CFTR, cystic fibrosis transmembrane conductance regulator

4D-710 Phase 1/2 Clinical Trial: Cohort 1 Acute Safety

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

- Full volume administered (1E15 vg)
- Participant 1: a mild, self-limited AE
  - Grade 1 dry throat, fatigue during nebulization
- No bronchospasm



AE, adverse event; $ppFEV_1$, percent predicted forced expiratory volume in 1 second.

4D-710 Phase 1/2 Clinical Trial: Cohort 1 Safety Summary

NO 4D-710–RELATED ADVERSE EVENTS UP TO 12 MONTHS AFTER COMPLETION OF DOSING

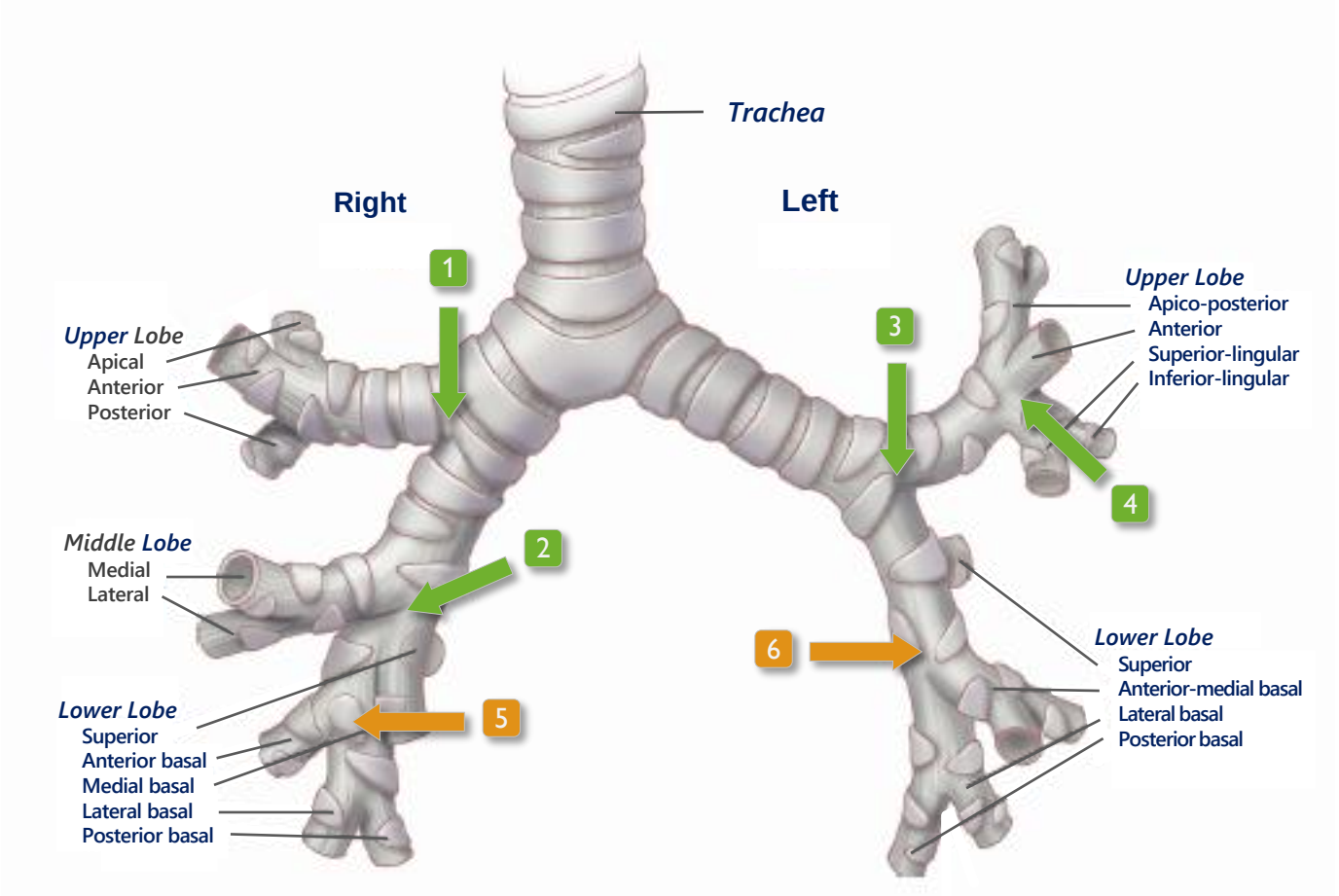
- No 4D-710–related adverse events
- No 4D-710–related serious adverse events
- No dose-limiting toxicities

Duration of Cohort 1 safety follow-up as of 12-APR-2023: 12 months for Participant 1, 9 months for Participants 2 and 3

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

Bronchoscopy: Week 4*

Bronchoscopic Sampling Sites			Biomarker	
			ISH	PCR
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	



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

*Participant 3 bronchoscopy conducted at Week 8 due to pulmonary exacerbation (unrelated to study drug).

Widespread Transgene Delivery & Expression: Biopsies

CONSISTENT TRANSDUCTION ACROSS PATIENTS, LUNG REGIONS

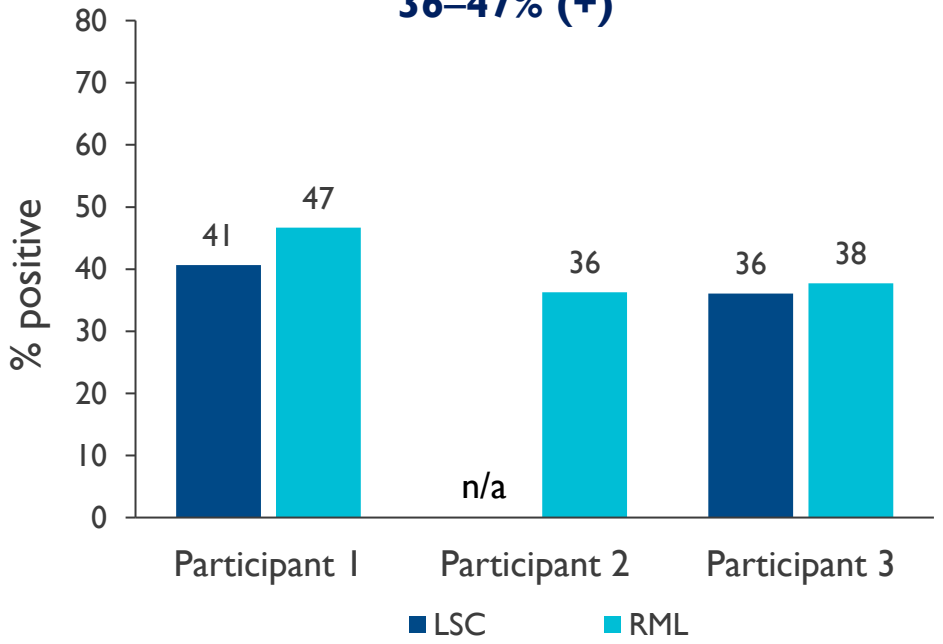
4D-710 DNA (+) Lung Biopsies

CFTRΔR DNA qPCR[1] Results
5 of 5 biopsies (+) (All 3 pts)

Participant	Left Upper Lobe/ Lingula Carina DNA	Right Secondary Carina DNA
1	**Positive**	**Positive**
2	n/a	**Positive**
3	**Positive**	**Positive**

4D-710 RNA Expression (+) Lung Biopsies

CFTRΔR RNA ISH
% Positive Epithelial Cells[2]
36–47% (+)



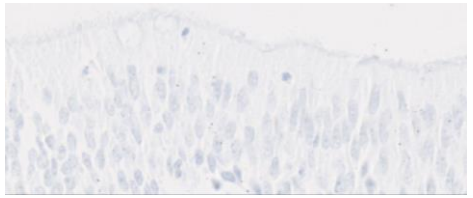
[1] qPCR assay range: 25 – 25,000,000 copies.
[2] Participant 2 LSC not sampled. Quantification by Visiopharm AI Machine Learning Analysis. ISH, in situ hybridization; LSC, left secondary carina endobronchial biopsy; RML, right middle lobe endobronchial biopsy.

Widespread CFTR Expression in Lung: All 5 Biopsies (+)

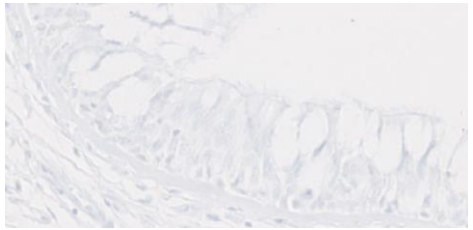
CFTRΔR RNA EXPRESSION BY ISH

Controls

DAPB ISH
(negative control probe)



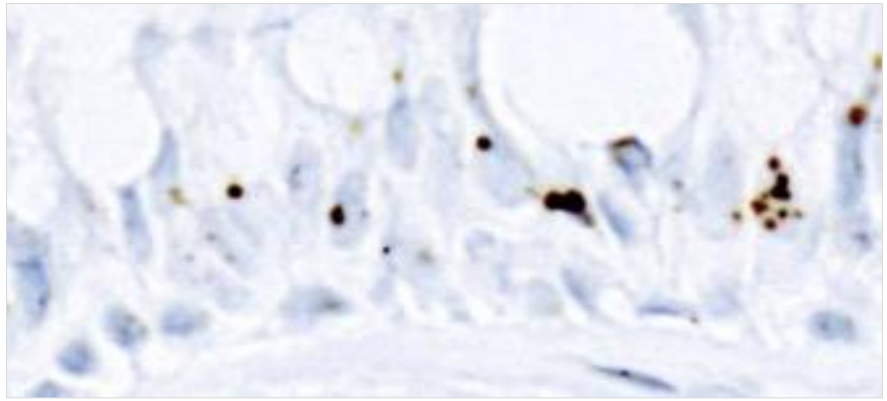
CFTRΔR ISH
Untreated CF lung tissue



4D-710–Treated *CFTRΔR* RNA probe*

Left Secondary Carina
Endobronchial Biopsy (40X)

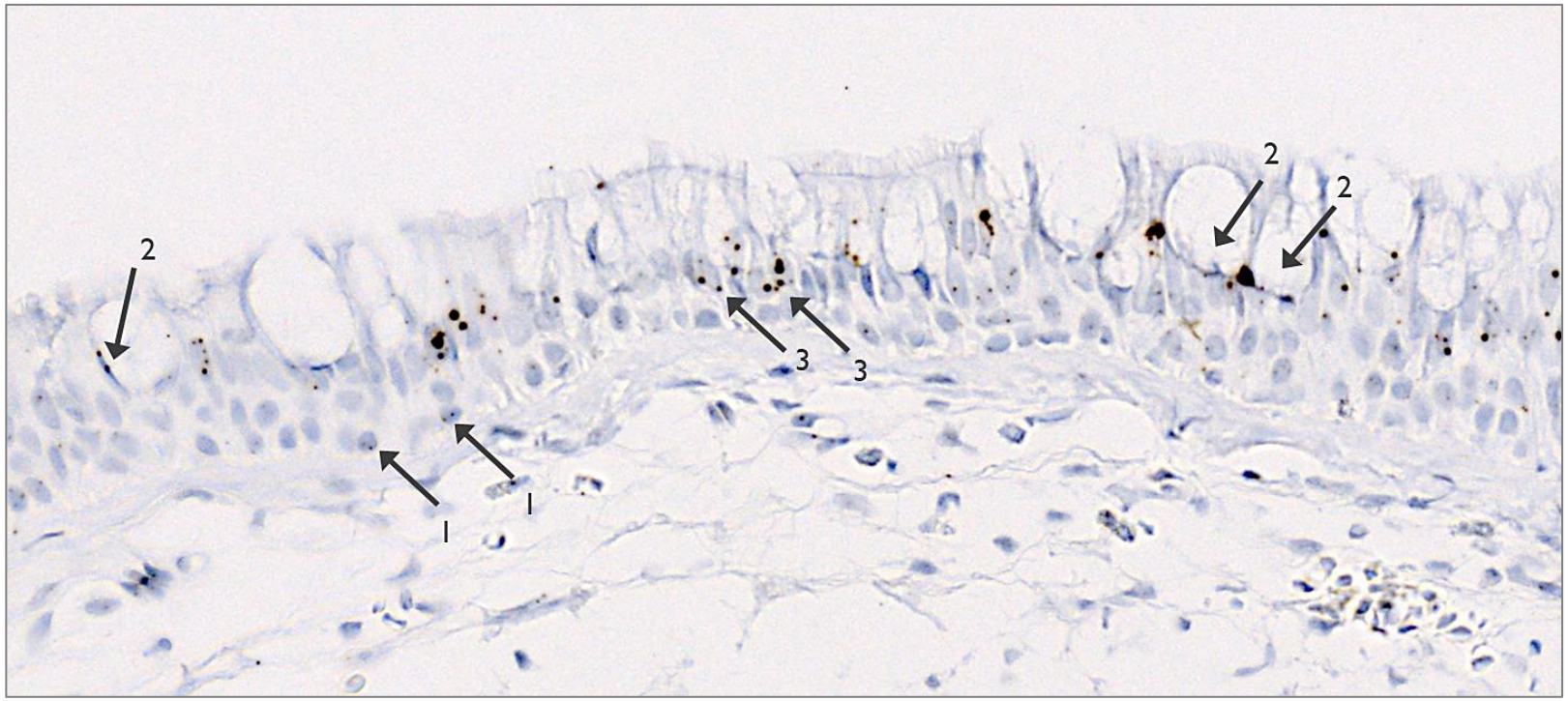
Left Secondary Carina
Endobronchial Biopsy (80X)



*Representative images from Participant1. *CFTRΔR* ISH signal observed in all evaluable biopsies from all 3 participants (Participant 2 LSC not sampled). ISH, in situ hybridization; LSC, left secondary carina.

Multiple Bronchial Epithelial Cell Types Express CFTR Transgene

INDEPENDENT PATHOLOGISTS' REVIEW: *CFTRΔR* RNA ISH LOCALIZATION



Transduced cell types*

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

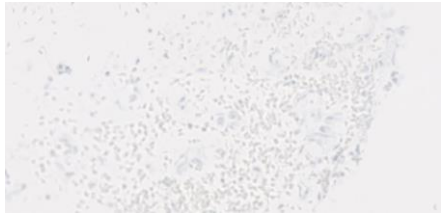
Image from Participant 1. *Assessed by 2 independent pathologists. ISH, in situ hybridization.

Widespread CFTR Expression in Lung: All 6 Brushings (+)

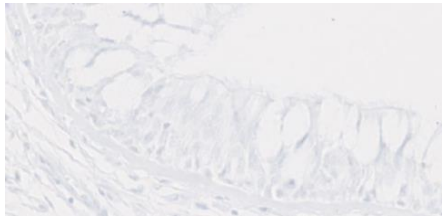
CFTRΔR RNA EXPRESSION BY IN SITU HYBRIDIZATION (ISH)

Controls

DAPB ISH BRUSHING
(negative control probe)

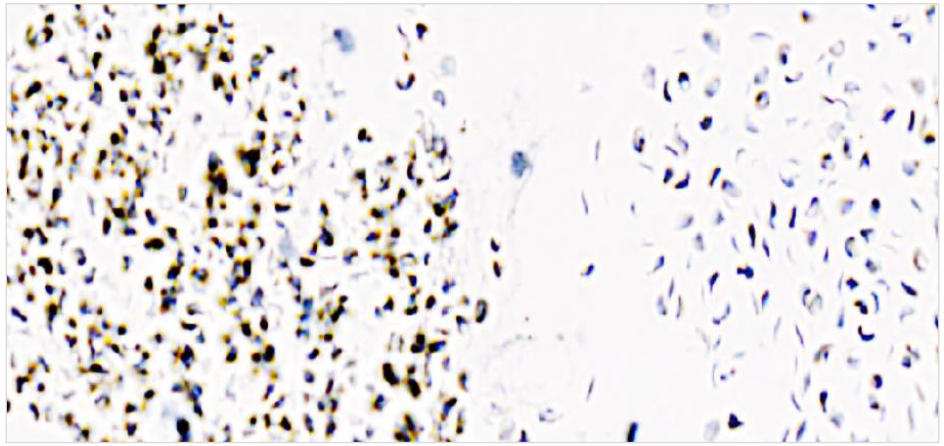


***CFTRΔR* ISH**
Untreated CF lung tissue



4D-710 Treated *CFTRΔR* RNA probe*

Right Lower Lobe



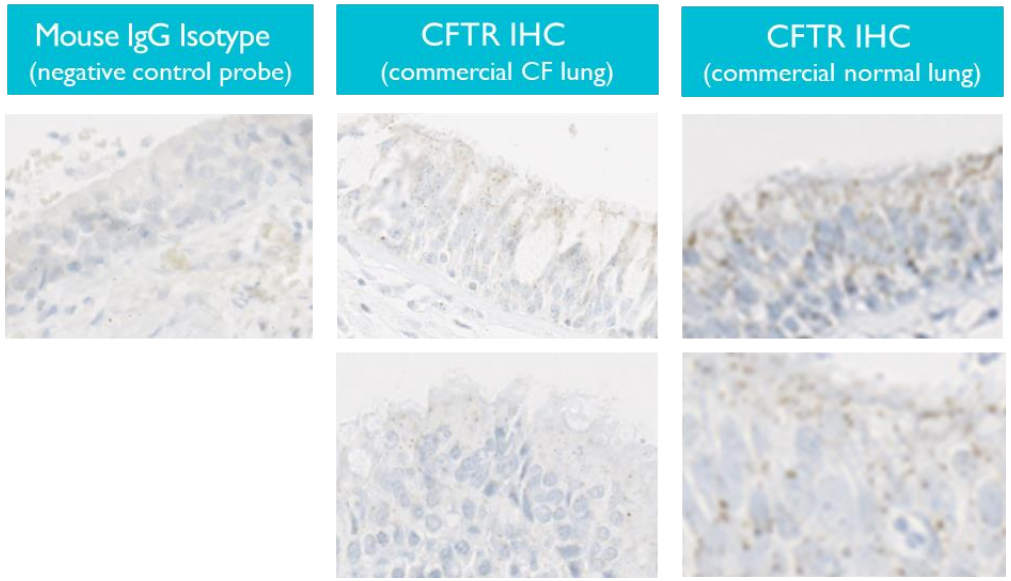
Left Lower Lobe

*Representative images from Participant 1. *CFTRΔR* ISH signal observed in brushings from 2/3 patients (Participant 2 brushings unevaluable).

Widespread CFTR Protein Expression in Lung

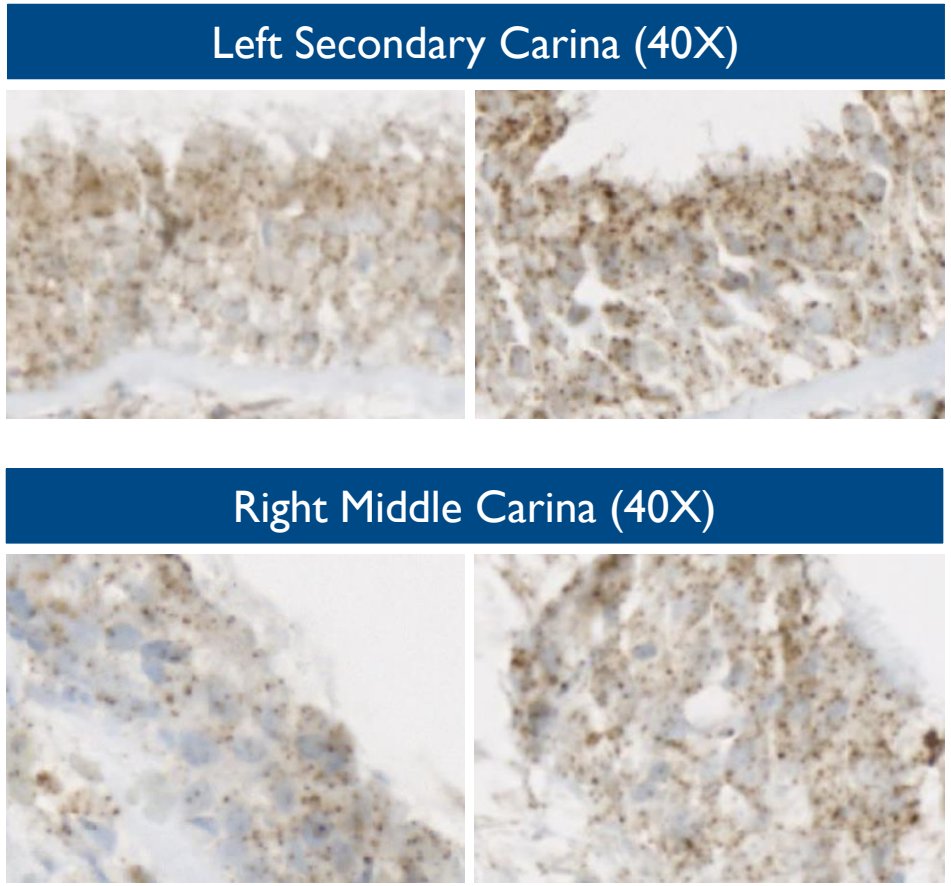
CFTR PROTEIN EXPRESSION BY IHC

Controls



- 3/3 participants positive for CFTR protein expression
- CFTR IHC signal higher than that observed in commercially acquired CF (N=20) and normal lung samples (N=20)

4D-710–Treated CFTR IHC*



*Representative images from Participant 1,3. IHC, immunohistochemistry.

4D-710 Clinical Data Summary, Implications, and Next Steps

CLINICAL PROOF OF CONCEPT FOR SAFETY AND WIDESPREAD TRANSGENE EXPRESSION

- **Cohort 1 Summary:**
  - No 4D-710-related AEs post-dosing
  - Widespread CFTR transgene and protein expression (all 11 lung samples)
  - ~40% of cells expressed CFTR by ISH (including multiple bronchial cell types)
- **Implications:**
  - A101 lung vector validation
  - Clinical proof-of-concept: 4D-710 transgene delivery and protein expression
- **Next Steps:**
  - Cohort 2 enrollment underway (2E15 vg dose); assessment of clinical activity (e.g., ppFEV$_1$; QoL)
  - Assess potential 4D-710 combination therapy in individuals with CF on CFTR modulators

UNTIL IT’S DONE

- People with CF and their families
- Participating CF clinical and research centers
- JP Clancy and 4DMT for slides