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VIENNA, AUSTRIA

46th EUROPEAN CYSTIC FIBROSIS CONFERENCE

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

AEROW

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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

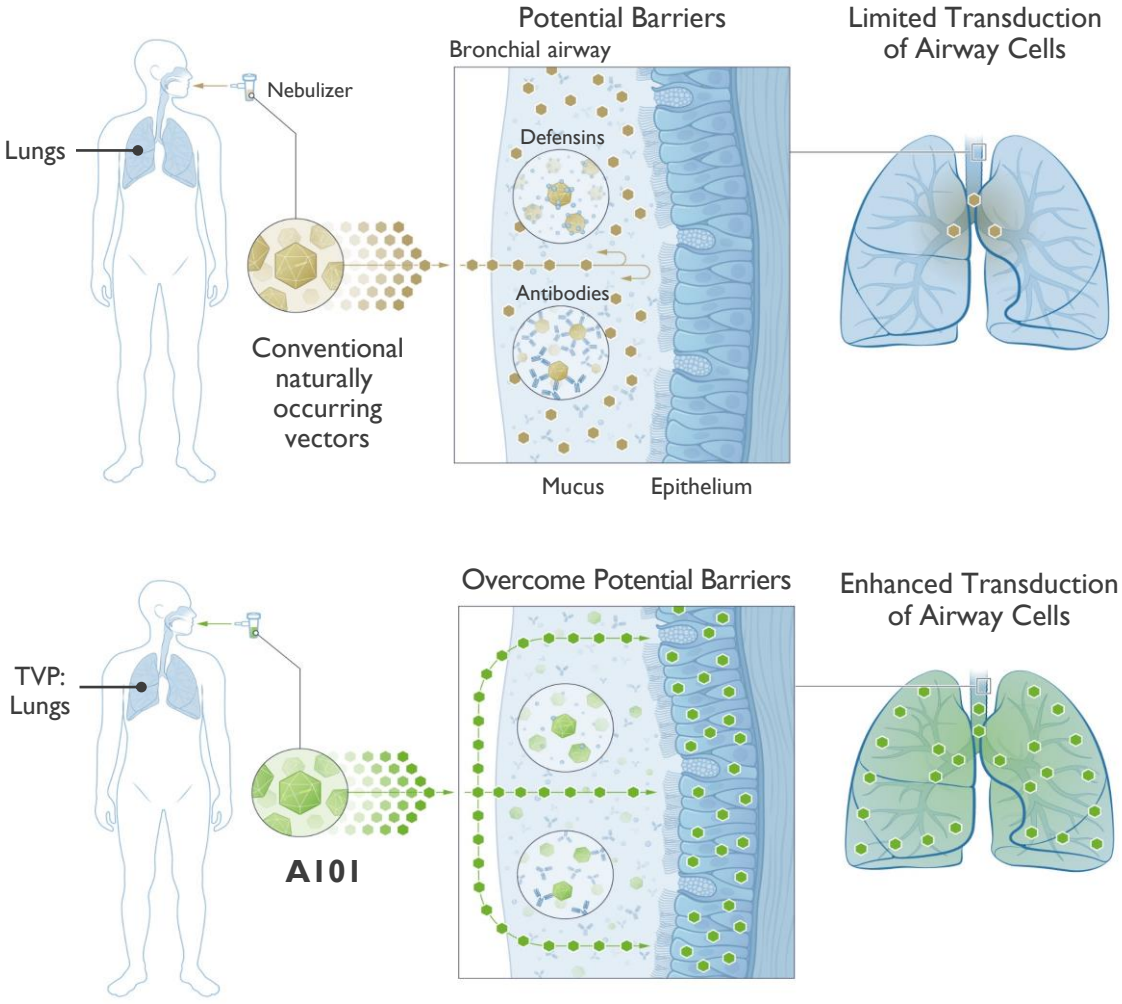
PREVIOUS CLINICAL TRIAL EXPERIENCE WITH TGAAVCF

- 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**

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.

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

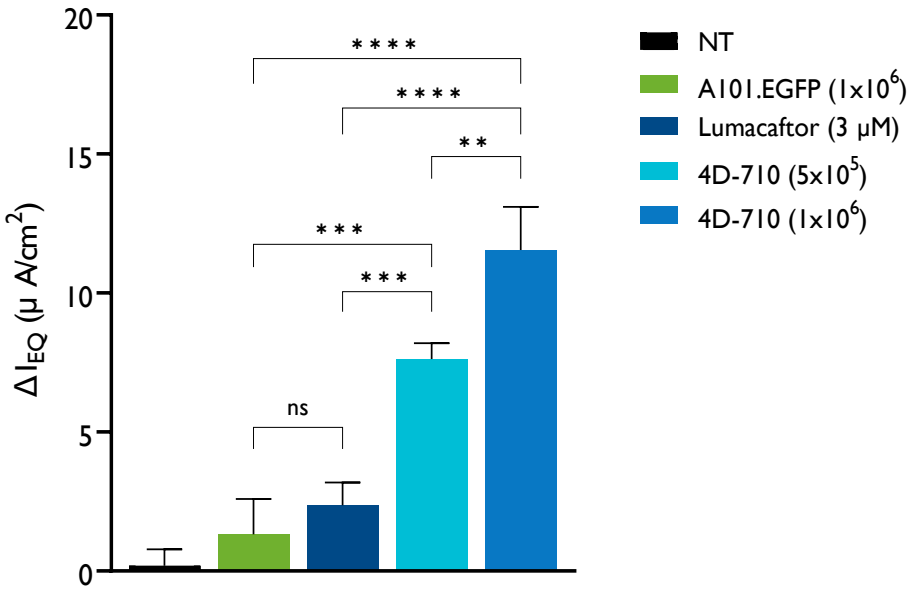
A101 TARGET VECTOR PROFILE AND 4D-710 PRODUCT DESIGN AND KEY ATTRIBUTES



CFTR Function Assays: 4D-710 Function Comparable to Trikafta

DOSE-RELATED 4D-710–MEDIATED CFTR FUNCTION

Dose-dependent CFTR Activity > Lumacaftor*

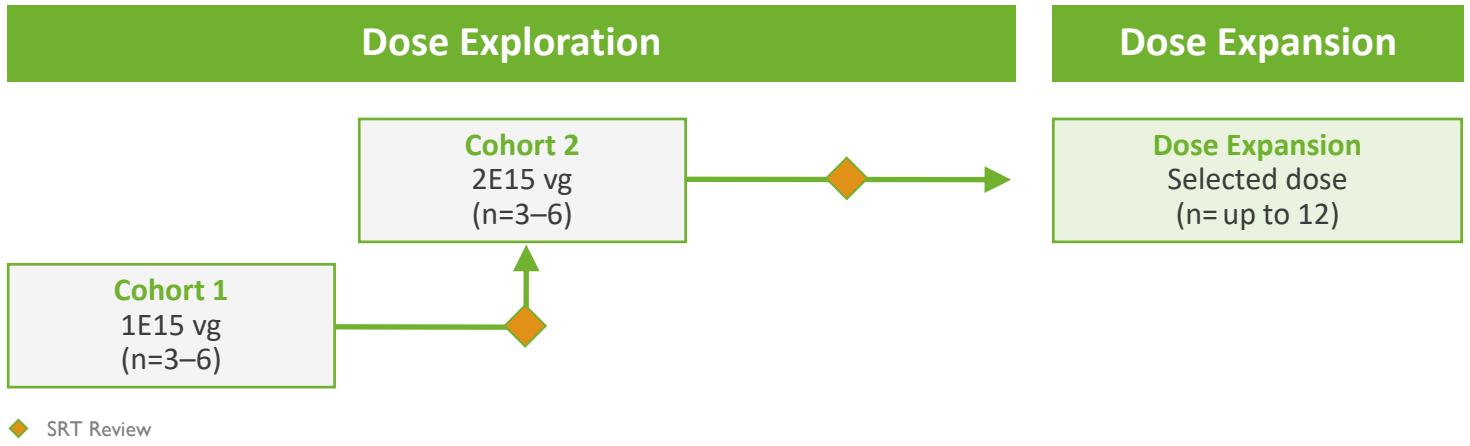


**p<0.01; ***p<0.001; ****p<0.0001.

*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.

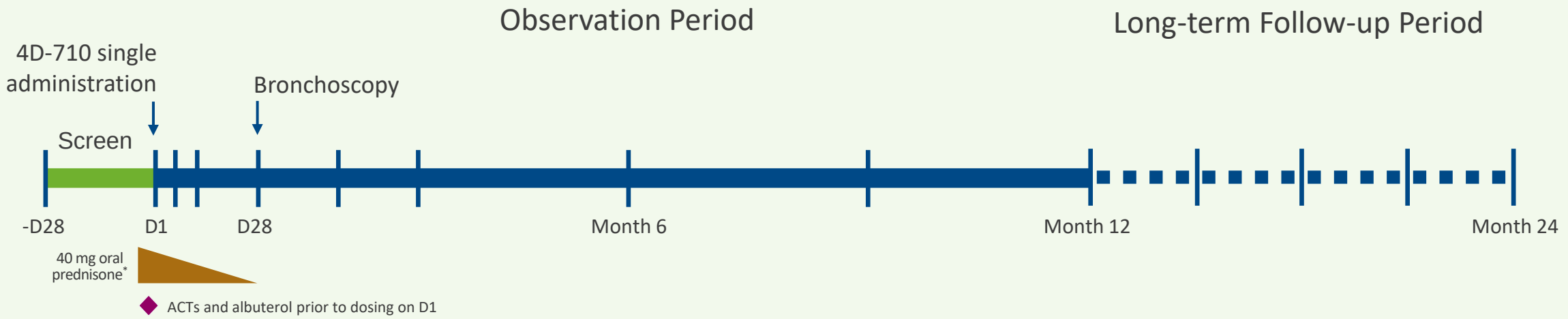
4D-710 Phase 1/2 Clinical Trial Study Design (4D-710-C001)

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



4D-710 Administration

- Pre-dose procedures (Day 1):
  - Airway clearance technique
  - Aerosol bronchodilator (albuterol)
  - Pre- & post-bronchodilator spirometry
- 4D-710 administered via AeroEclipse® II breath-actuated nebulizer



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 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

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	Ineligible variant	Ineligible variant
CFTR variant (class)	II/V	I/I*	I/II
Historical sweat chloride, mmol/L	74	103	110
Percent predicted FEV_1	83	69	94
Pre-dose NAb to A101 capsid†	Low	Negative	Moderate
Pre-dose anti-drug antibody titer†	Low	Negative	Moderate

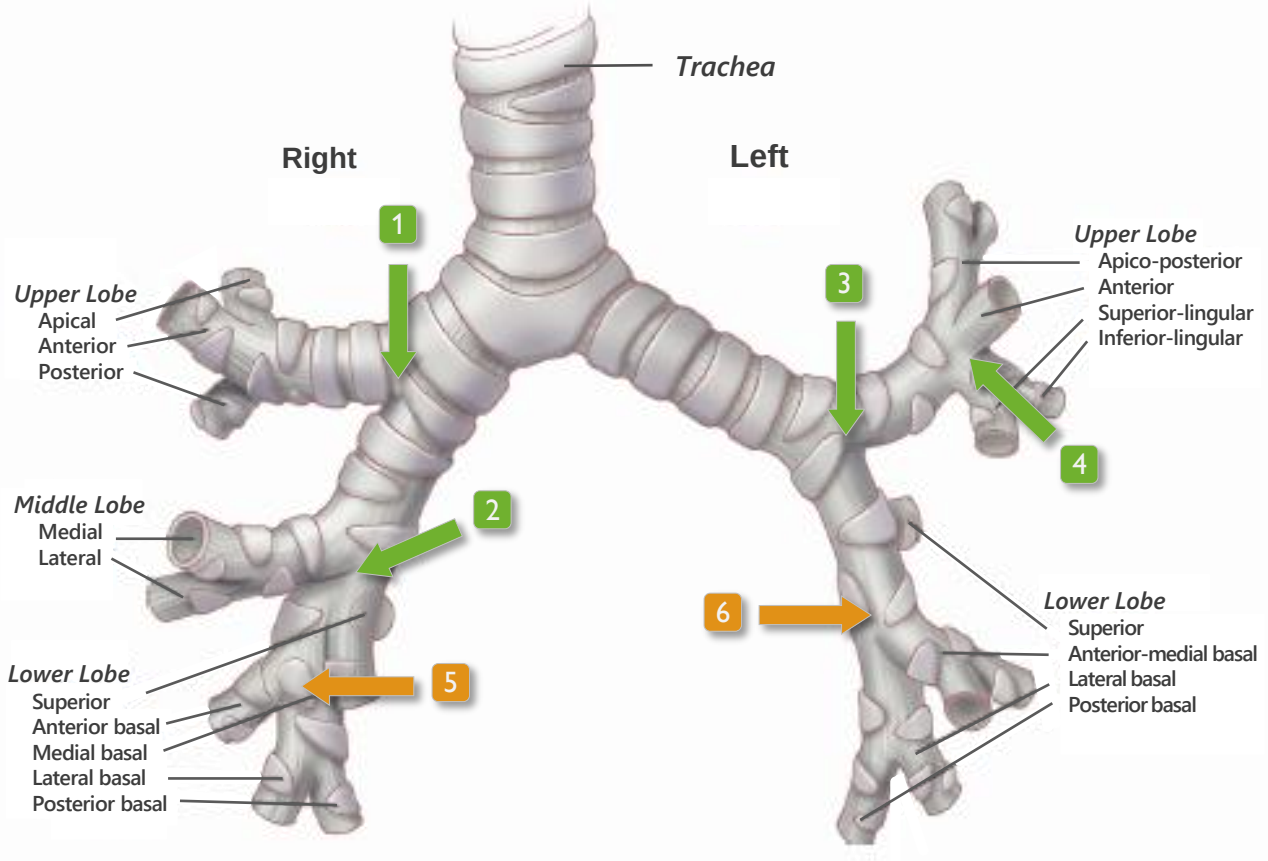
*Large gene deletion projected to result in a null variant profile. †Nab and antibody titer categories defined as negative (0), low (1:1–1:999), moderate (1:1000–1:14,999,) and high (≥1:15,000).
Sweat chloride normal range ≤29 mmol/L, *Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation* (2017). CFTR, cystic fibrosis transmembrane conductance regulator; NAb, neutralizing antibodies.

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	

*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.



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

Widespread Transgene Delivery and Expression: Cohort 1

CONSISTENT TRANSDUCTION ACROSS PARTICIPANTS AND LUNG REGIONS

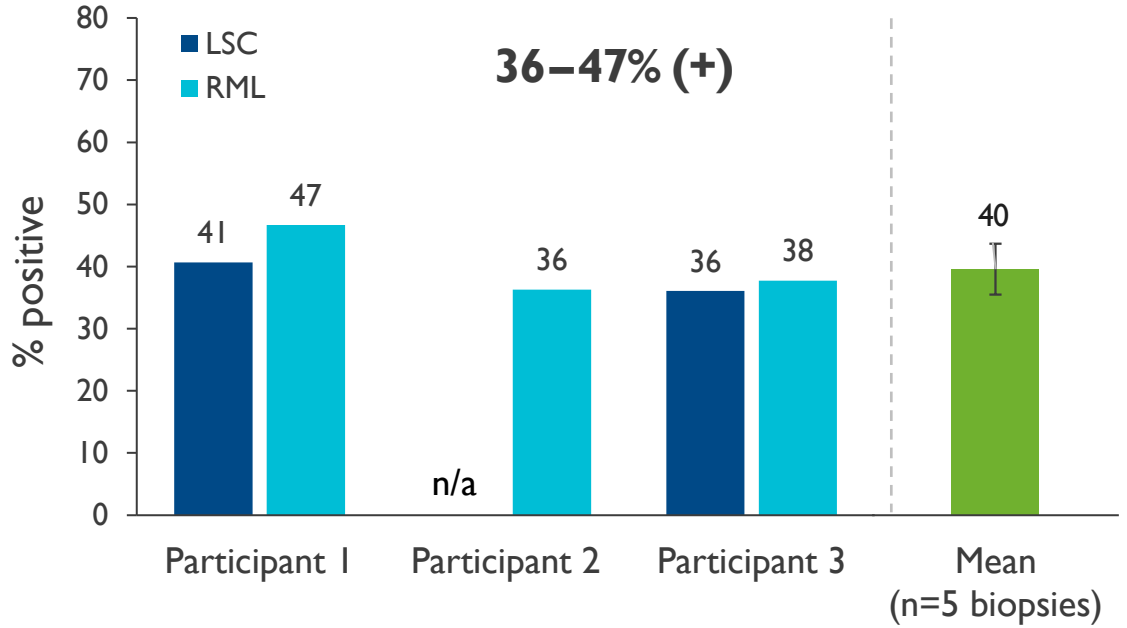
4D-710 DNA (+) Lung Biopsies

CFTRΔR DNA qPCR Results*
5 of 5 biopsies (+)†

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

4D-710 RNA Expression (+) Lung Biopsies

CFTRΔR RNA ISH
% Positive Epithelial Cells†

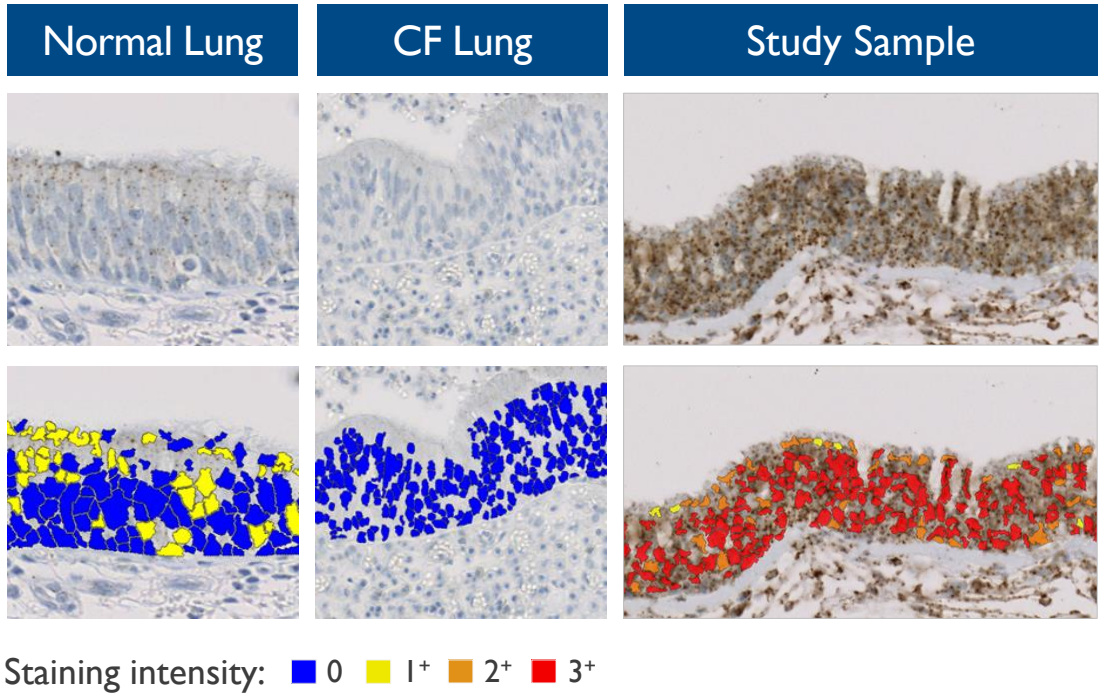


*qPCR assay range: 25 – 25,000,000 copies.
†Participant 2 LSC not sampled. Quantification by Visiopharm AI Machine Learning Analysis. ISH, *in situ* hybridization; LSC, left secondary carina endobronchial biopsy; RML, right middle lobe endobronchial biopsy.

CFTR Protein Expression

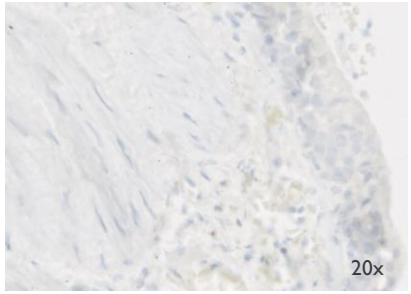
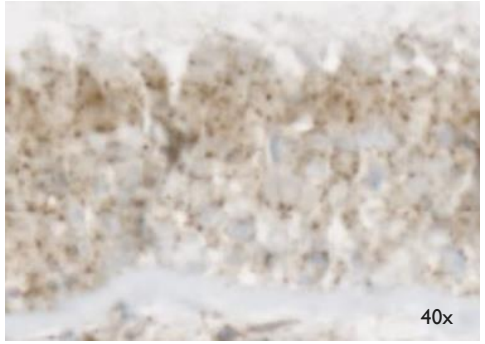
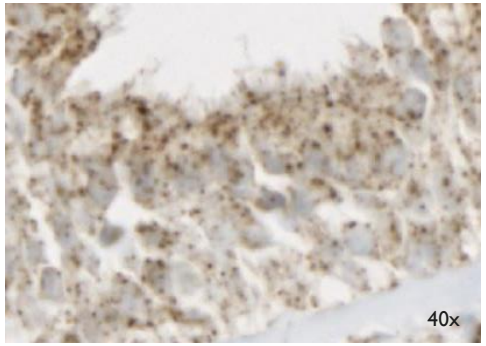
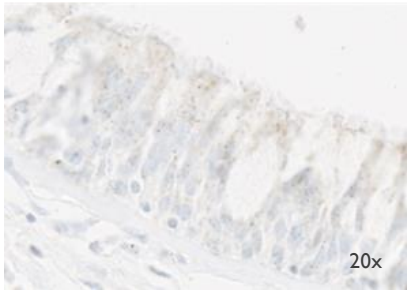
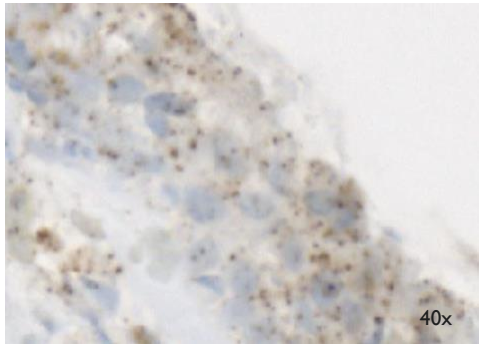
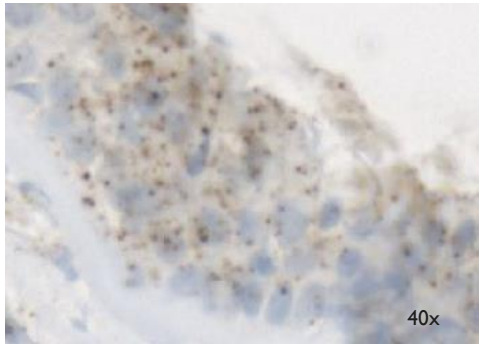
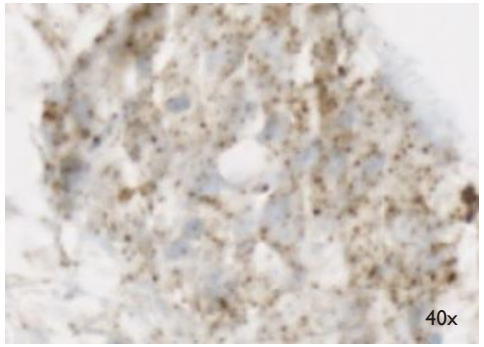
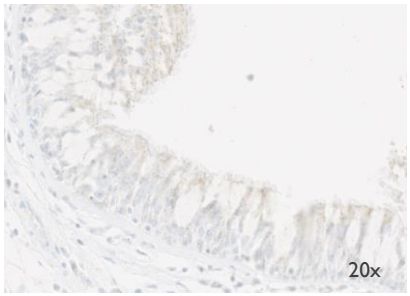
QUALITATIVE AND QUANTITATIVE ANALYSES

- 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



Widespread CFTR Protein Expression

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

	Controls	Participant 1	Participant 2	Participant 3
Mouse IgG Isotype				
Left Secondary Carina			*Not obtained*	
Normal Lung				
Right Middle Lobe				
CF Lung				

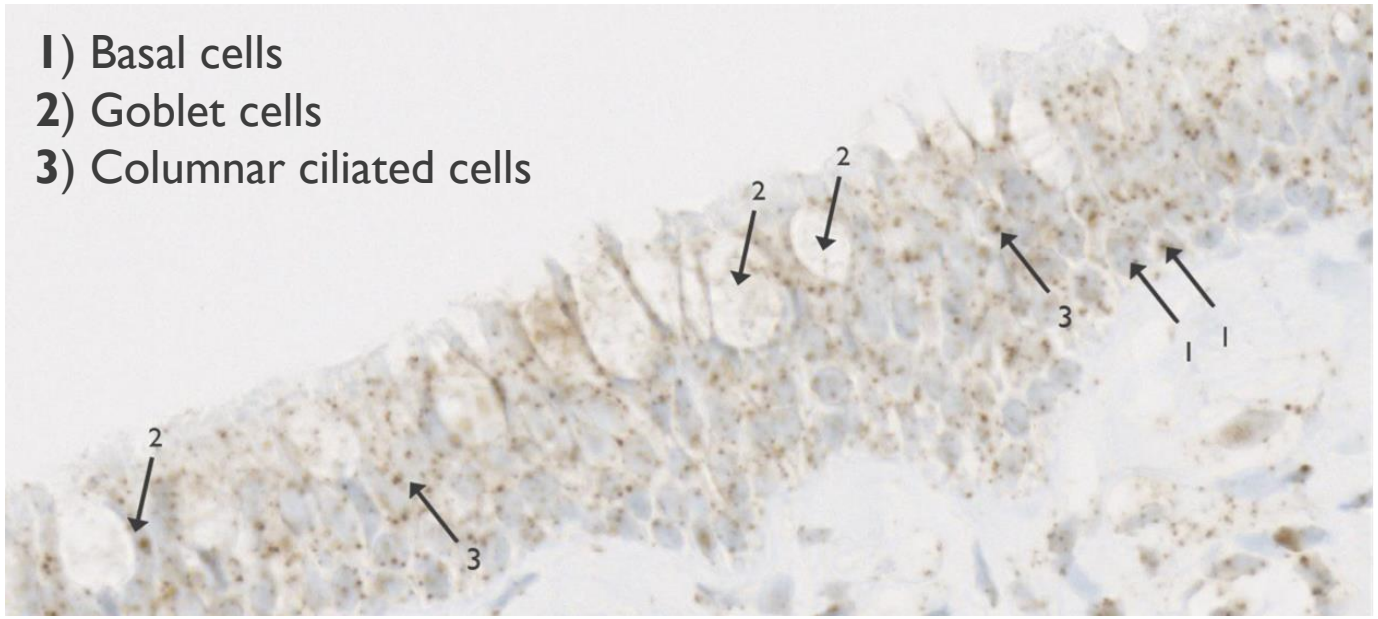
- 100% (3/3) of participants positive for CFTR protein in the lung
- 100% (11/11) of lung samples positive for CFTR protein

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

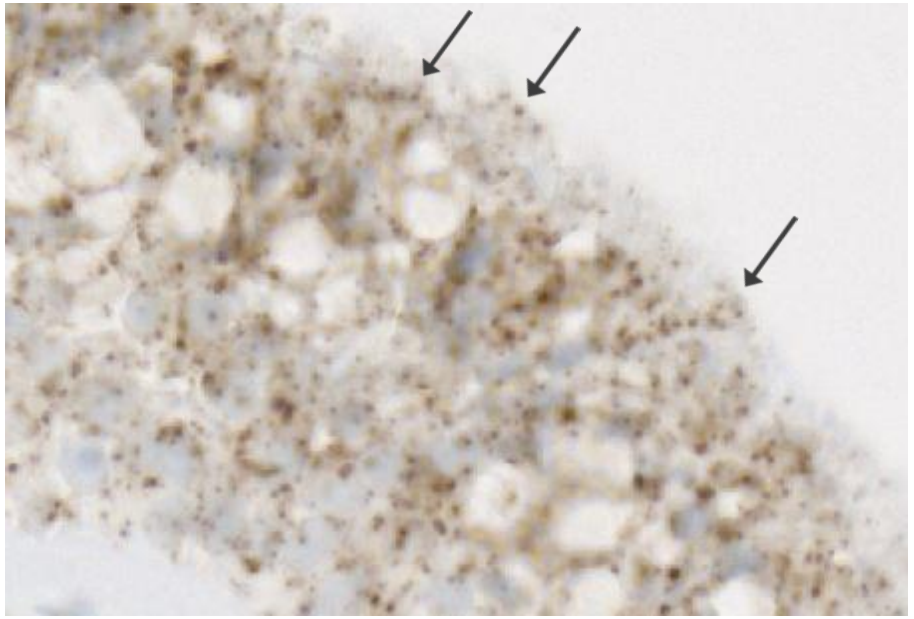
CFTR Protein Localization (IHC)

CFTR PROTEIN EXPRESSED IN MULTIPLE AIRWAY EPITHELIAL CELL TYPES

CFTR Protein Expressed in Multiple Cell Types



Localization to Apical Membrane



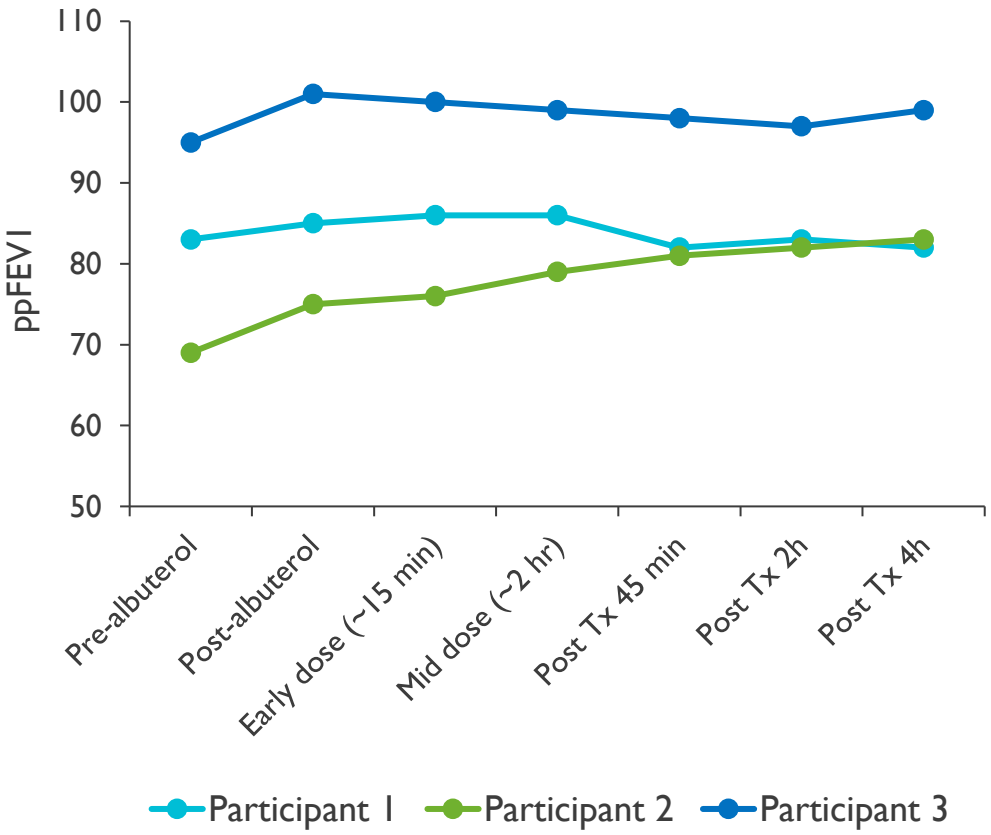
Images from Participants 1 and 3, respectively. IHC, immunohistochemistry.

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

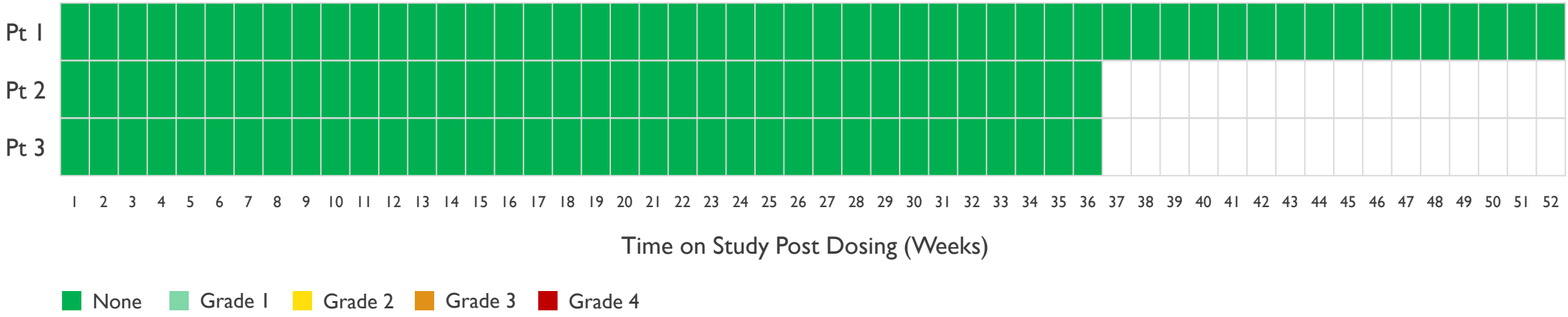


AE, adverse event; $ppFEV_1$, percent predicted forced expiratory volume in 1 second.

Cohort 1 Safety and Tolerability

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

4D-710–Related Adverse Events

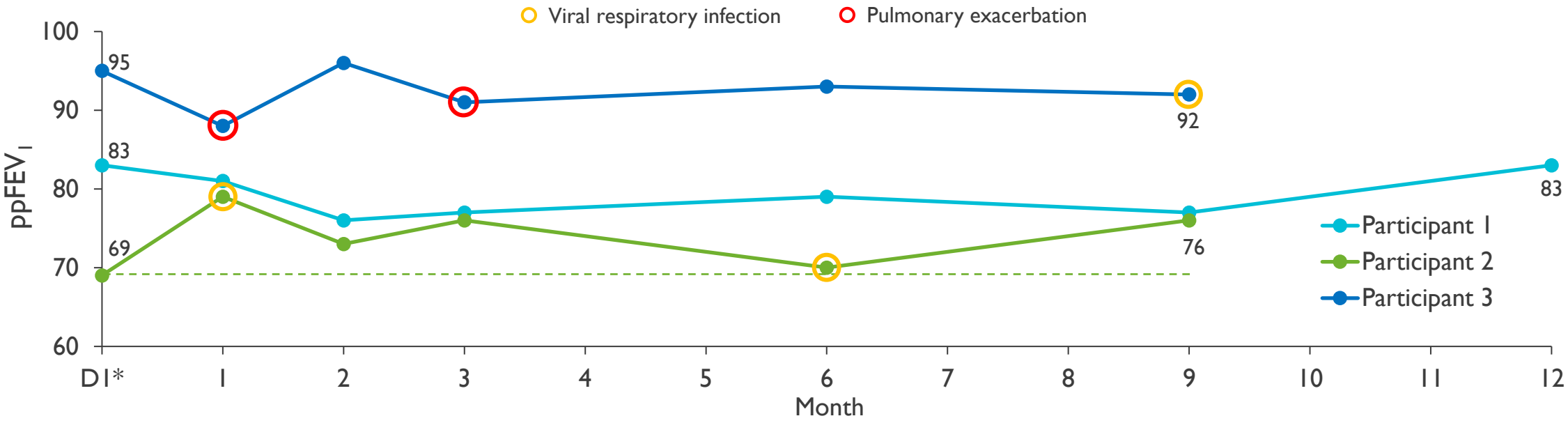


- No 4D-710–related adverse events
- No 4D-710–related serious adverse events
- No dose-limiting toxicities

*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_1: Cohort 1

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



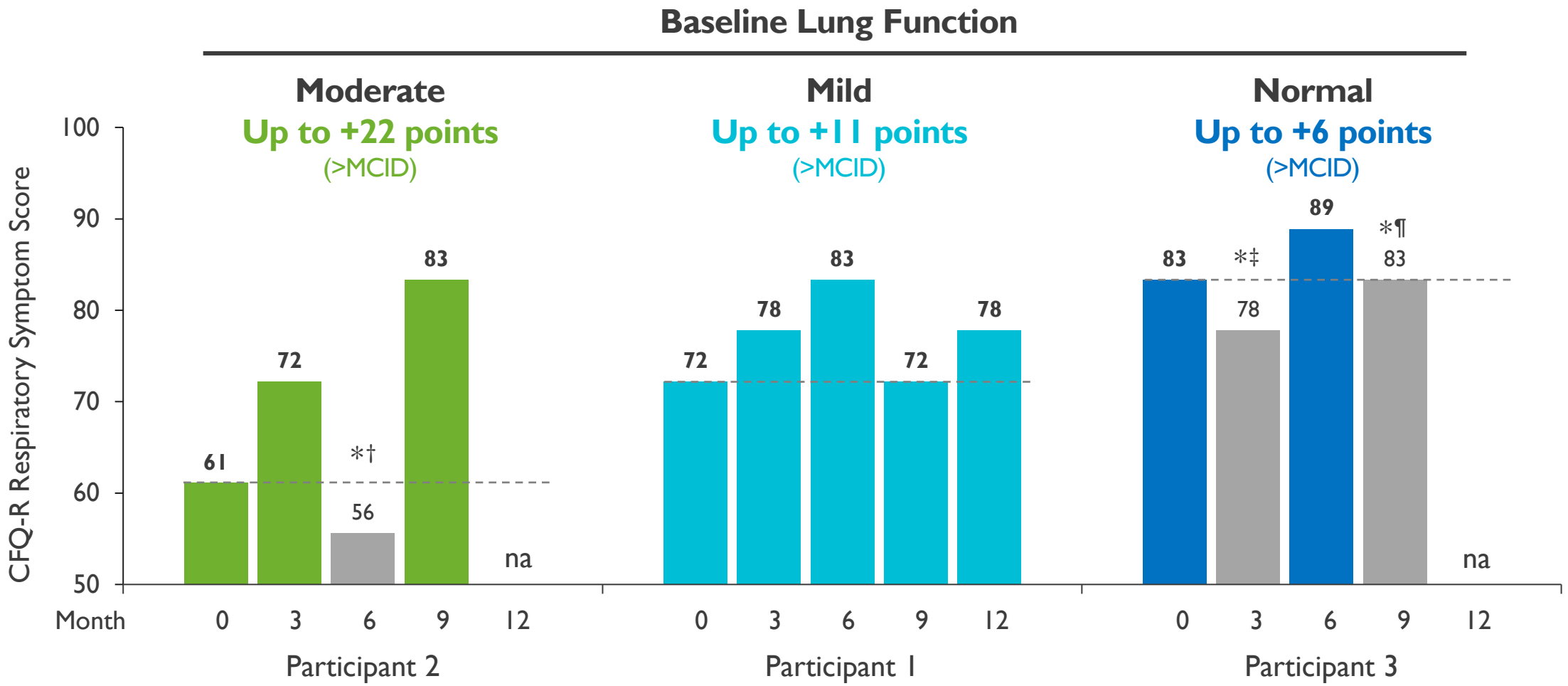
Pulmonary Exacerbation/Viral Respiratory Infection (not related to 4D-710)

Cohort 1	Month 1	Month 3	Month 6	Month 9	Month 12
Participant 1	none	none	none	none	none
Participant 2	D 8: Grade 3 COVID-19, dyspnea	none	D 176: Grade 1 rhinovirus	none	*pending*
Participant 3	Day 28: Grade 2	D 88: Grade 1	none	Day 266: Grade 1 COVID-19	*pending*

*Pre-dose spirometry assessment. $ppFEV_1$, 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)



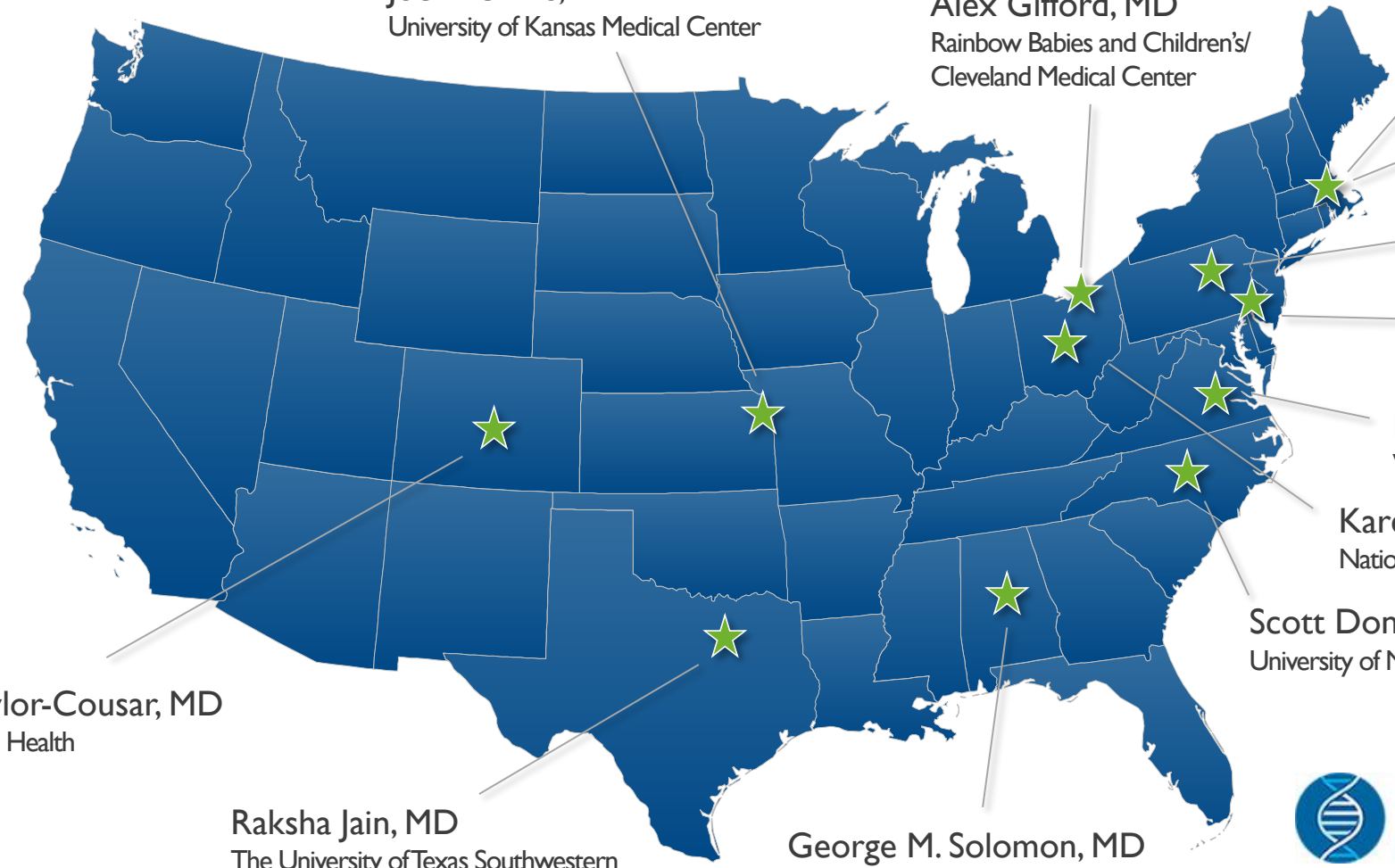
CFQ-R, Cystic Fibrosis Questionnaire-revised. Scores range from 0 to 100, with higher scores indicating better health. MCID=4 points (individuals with CF and stable respiratory disease) [1].
*Respiratory-related adverse event within 21 days of assessment. †Grade 1 rhinovirus (D176). ‡Grade 1 infective pulmonary exacerbation (D88). ¶ Grade 1 COVID-19. 1. Quittner AL et al. *Chest* 2009;135:1610–18.

4D-710 Phase 1/2 Clinical Trial Interim Data Summary

COHORT 1 (1E15 vg): DURATION OF FOLLOW-UP, 9–12 MONTHS

- 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
  - $ppFEV_1$ 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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Raksha Jain, MD
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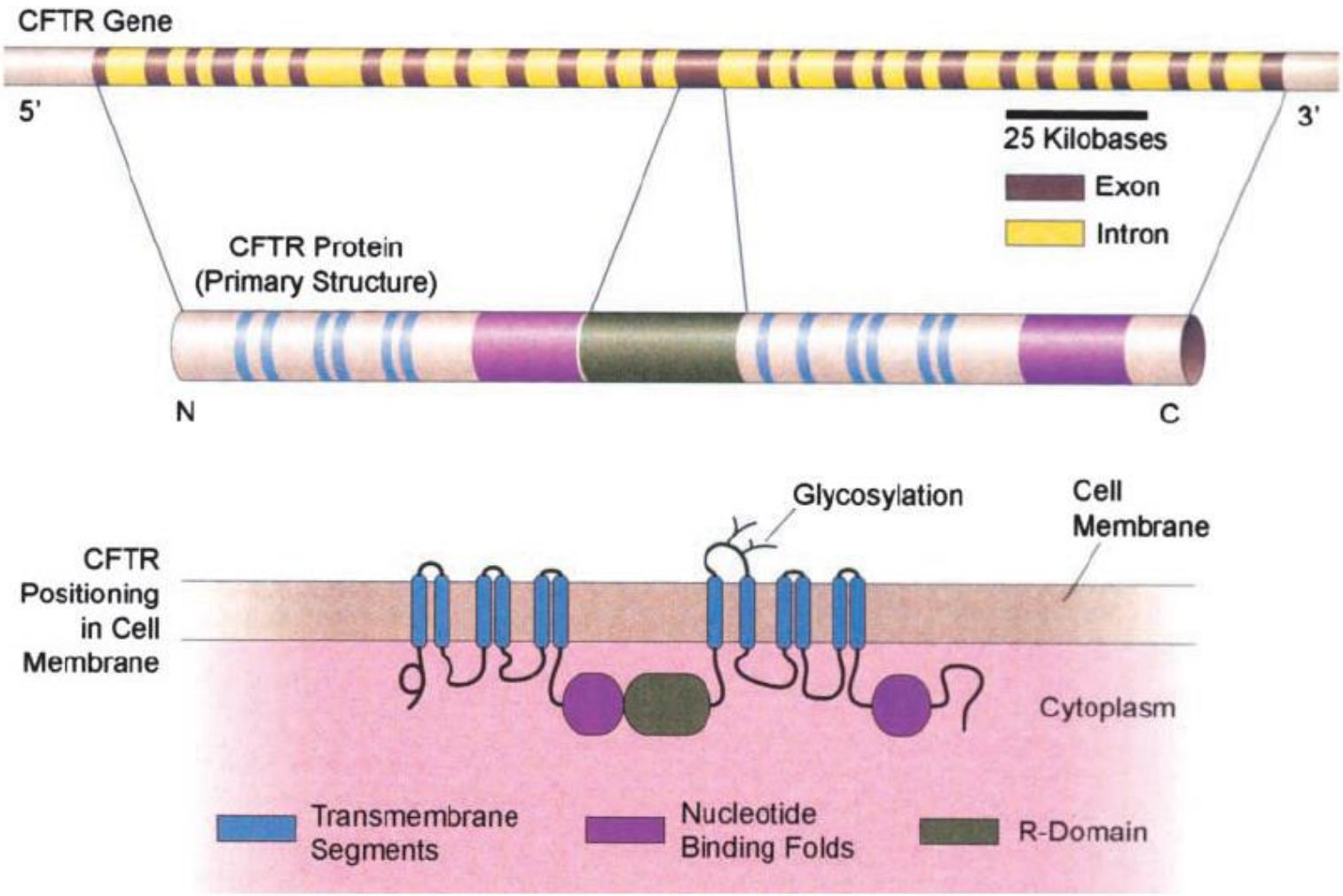
UNTIL IT’S DONE

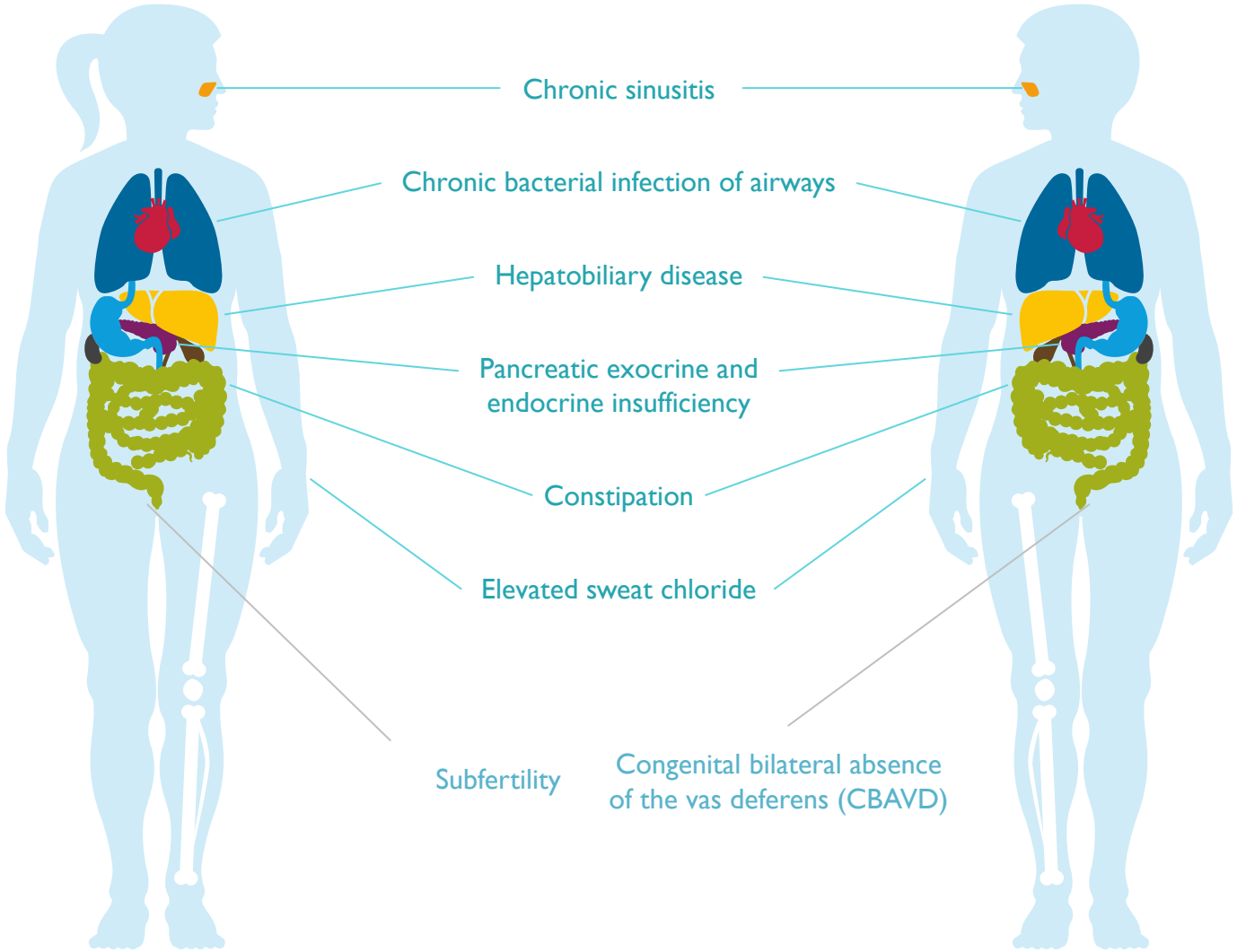
- People with CF and their families
- Participating CF clinical and research centers
- JP Clancy and 4DMT for slides

Back-up

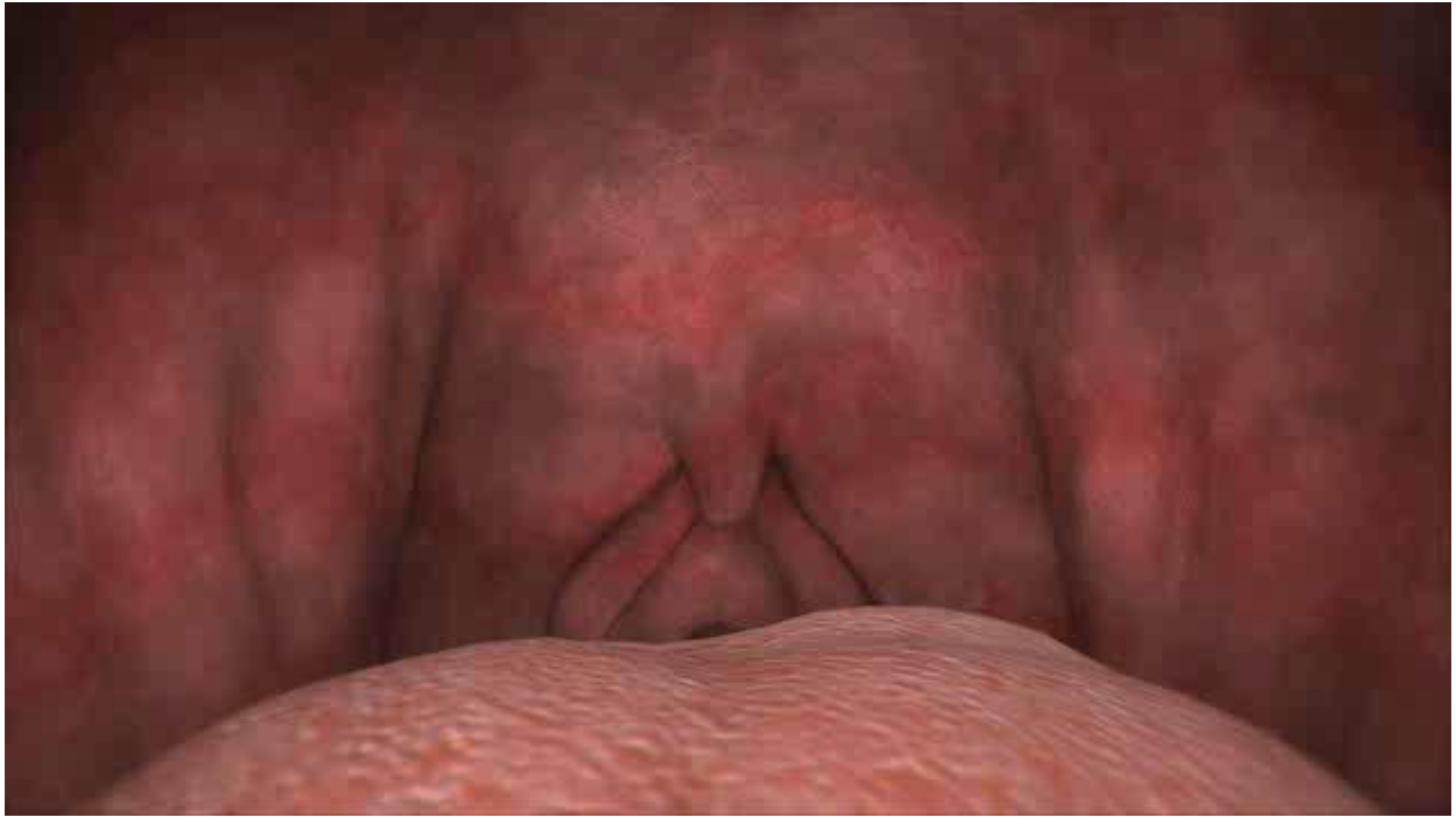
Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)



Multisystem Autosomal Recessive Disorder

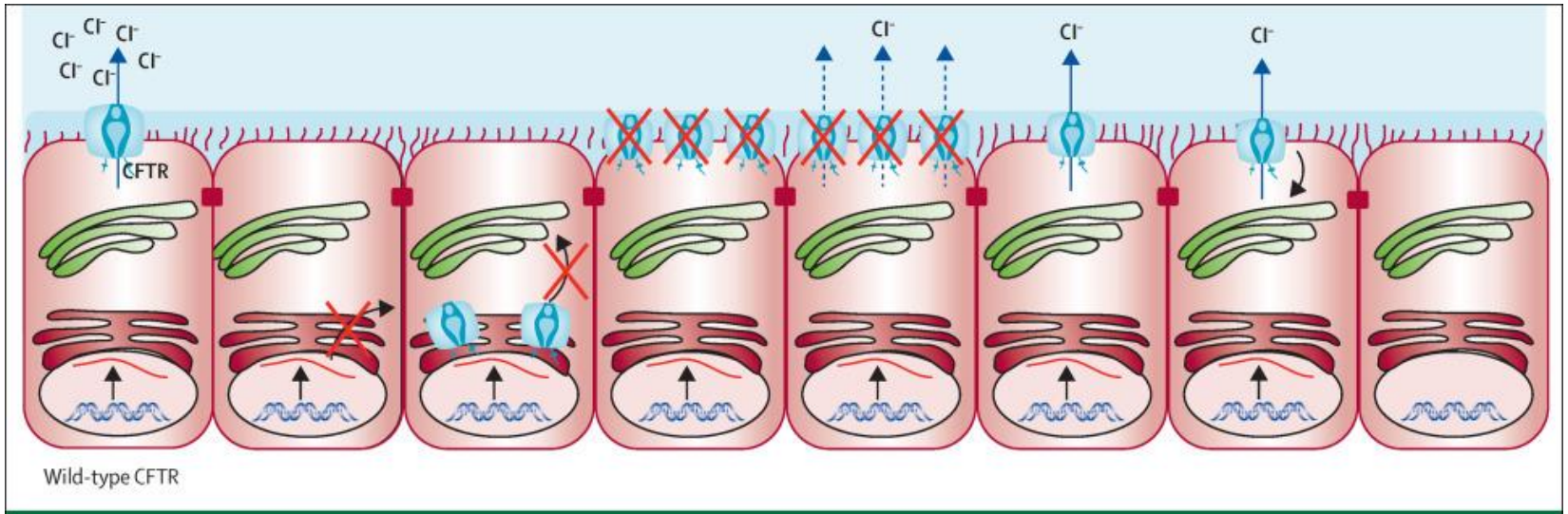


Slide courtesy of CFF



Video courtesy of CFF

Classifying CFTR Mutations



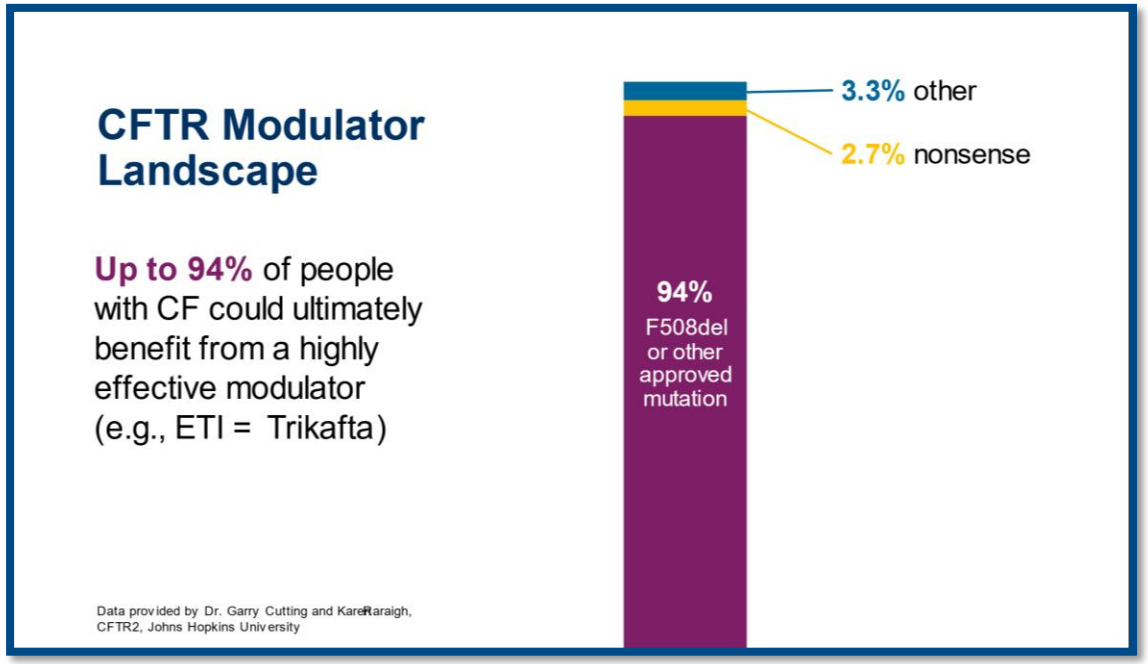
	Class I	Class II	Class III	Class IV	Class V	Class VI	Class VII
CFTR defect	No protein	No traffic	Impaired gating	Decreased conductance	Less protein	Less stable	No mRNA
Mutation examples	GLy542X, Trp1282X	Phe508del, Asn1303Lys, Ala561Glu	Gly551Asp, Ser549Arg, Gly1349Asp	Arg117His, Arg334Trp, Ala455Glu	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)

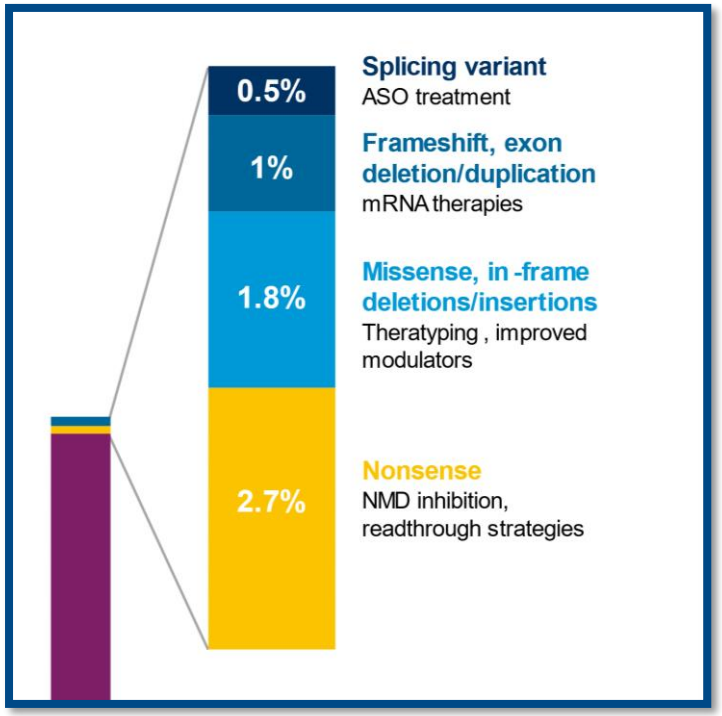
De Boeck, Amaral. *Lancet Resp Med* 2016

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/

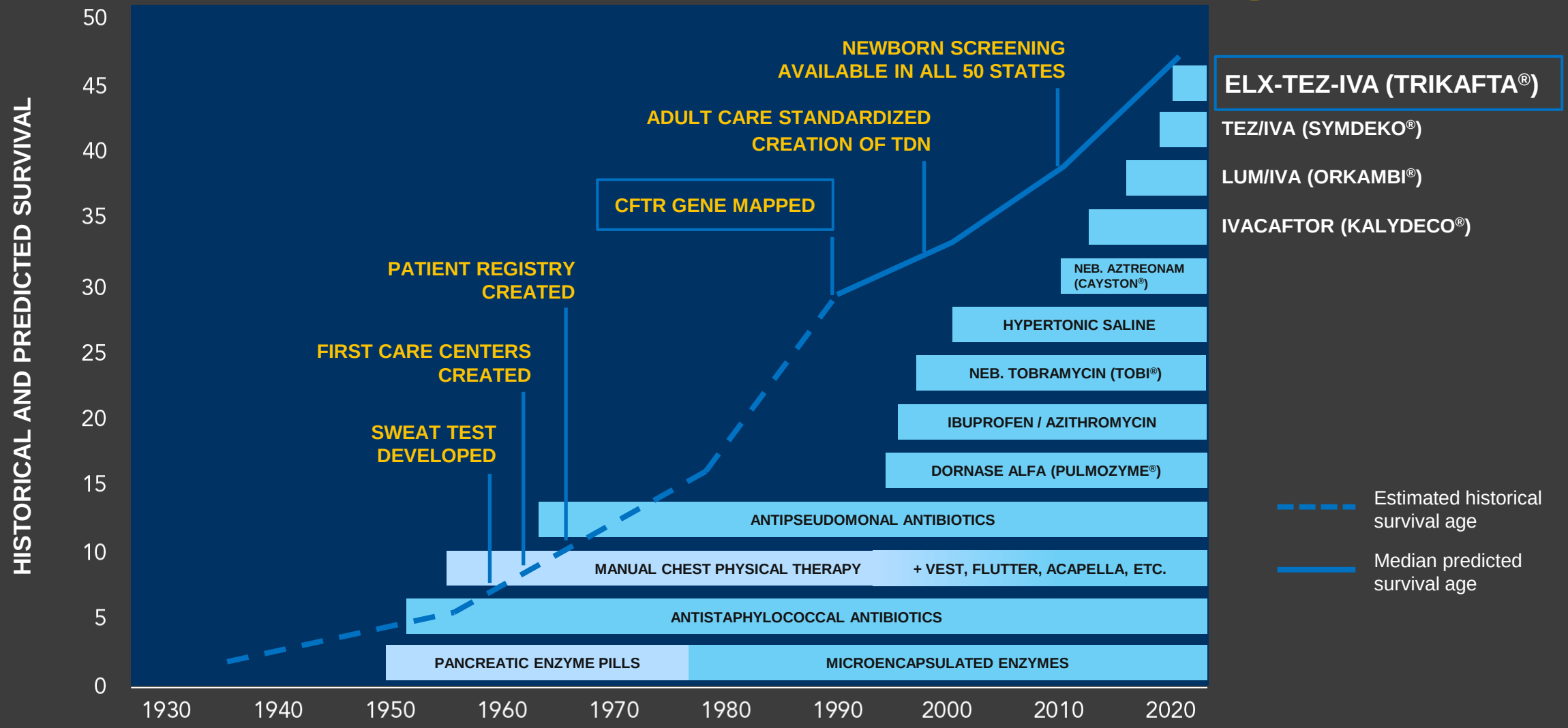
Unequal Eligibility for CFTR Modulator Therapy

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

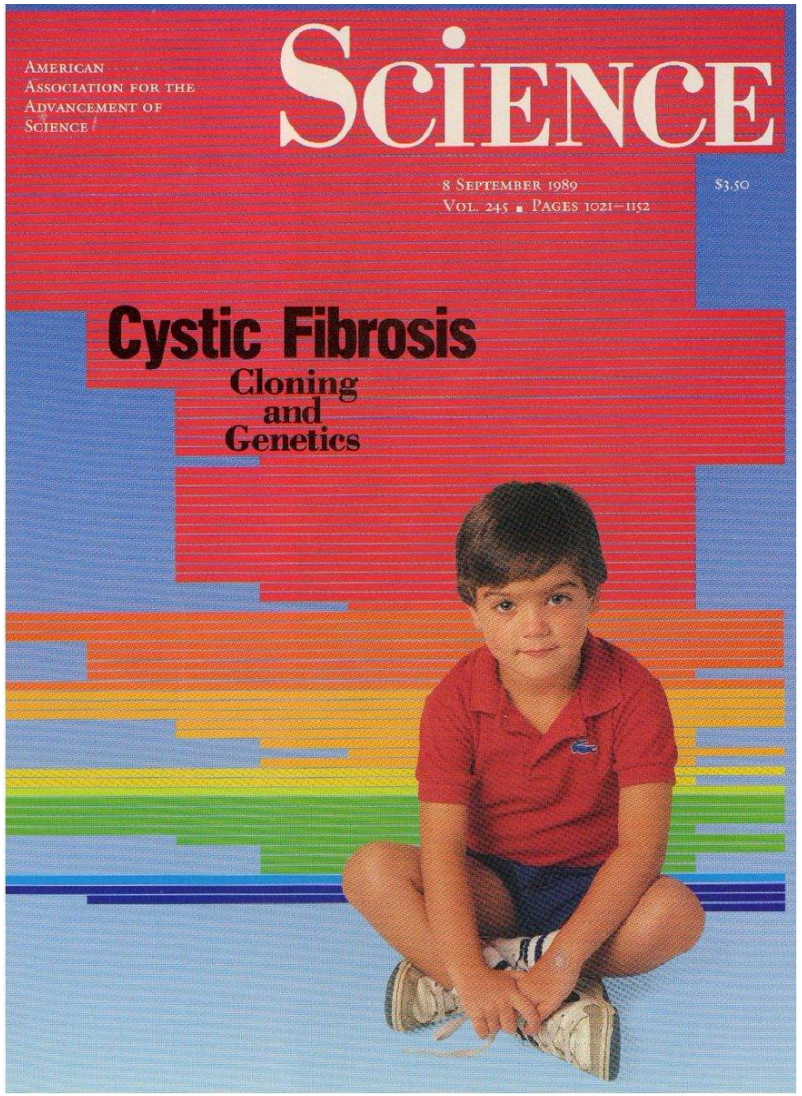
Timeline of Advances in CF



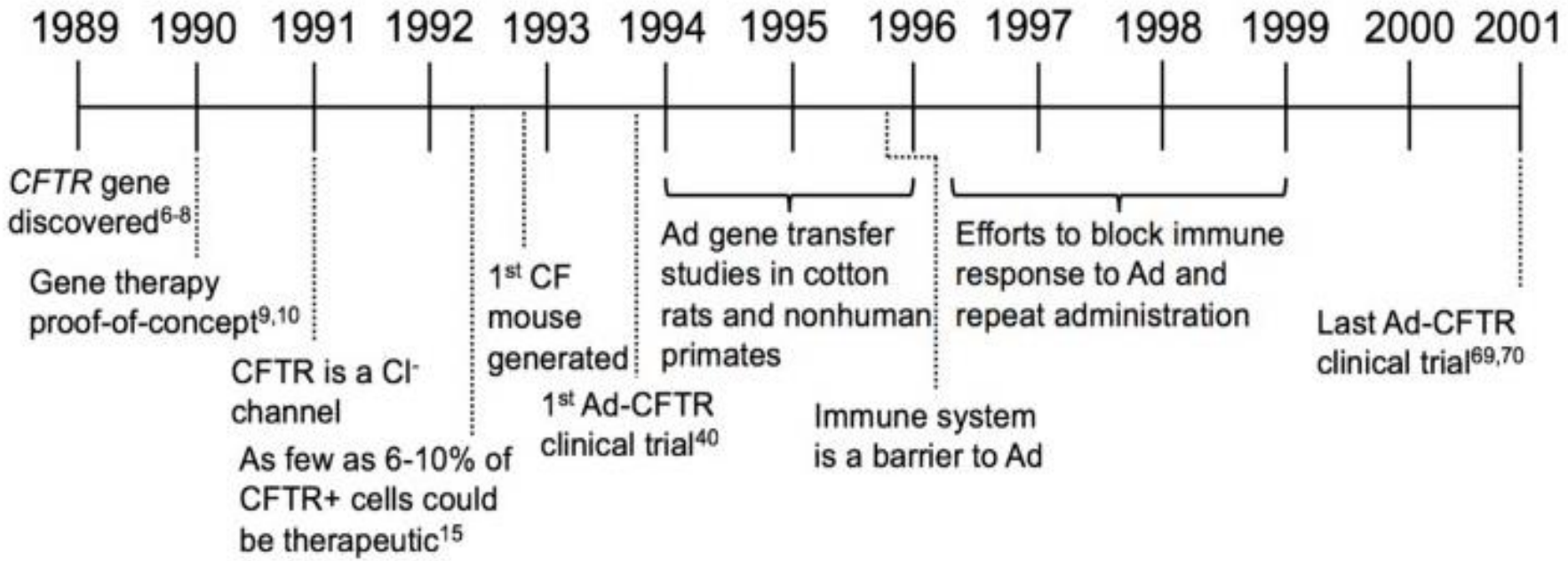
Slide courtesy of JP Clancy; 2021 CFFPR

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.