Identification and Characterization of a Novel AAV Capsid & Product for the Treatment of Cystic Fibrosis Lung Disease

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Disclosure to Learners

- **Financial relationships with relevant companies within the past 24 months:**
  - 4D Molecular Therapeutics, Inc., *Full-time Employee*

Unmet Need & Limitations of Conventional AAV Vectors for CF Lung Disease

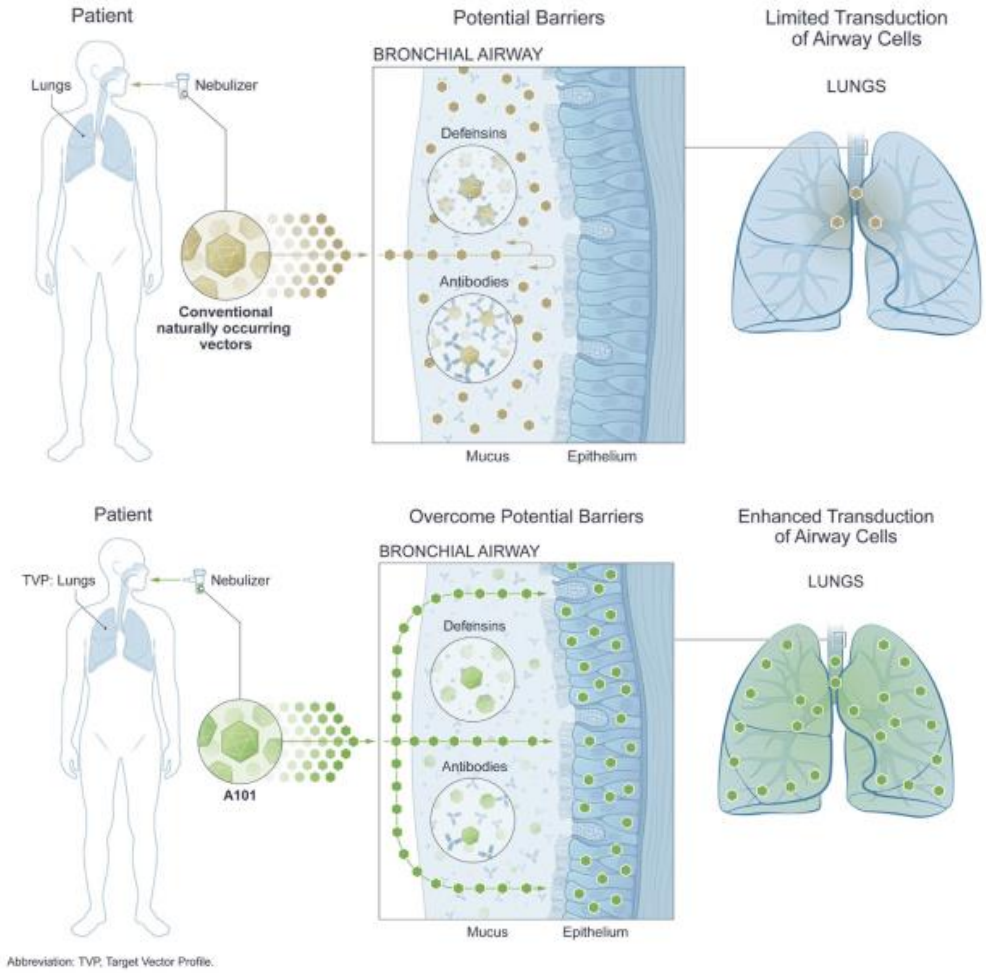
DURABLE REPLACEMENT OF CFTR BY AAV GENE THERAPY HOLDS PROMISE

Background:

- Cystic Fibrosis (CF): monogenic disorder caused by mutation in *CFTR*
- High unmet medical need in CF lung disease remains despite modulator therapy
- Aerosolized AAV-CFTR gene therapy failed to show efficacy in clinical trials
- AAV delivery through mucus barrier limited

Program Objectives:

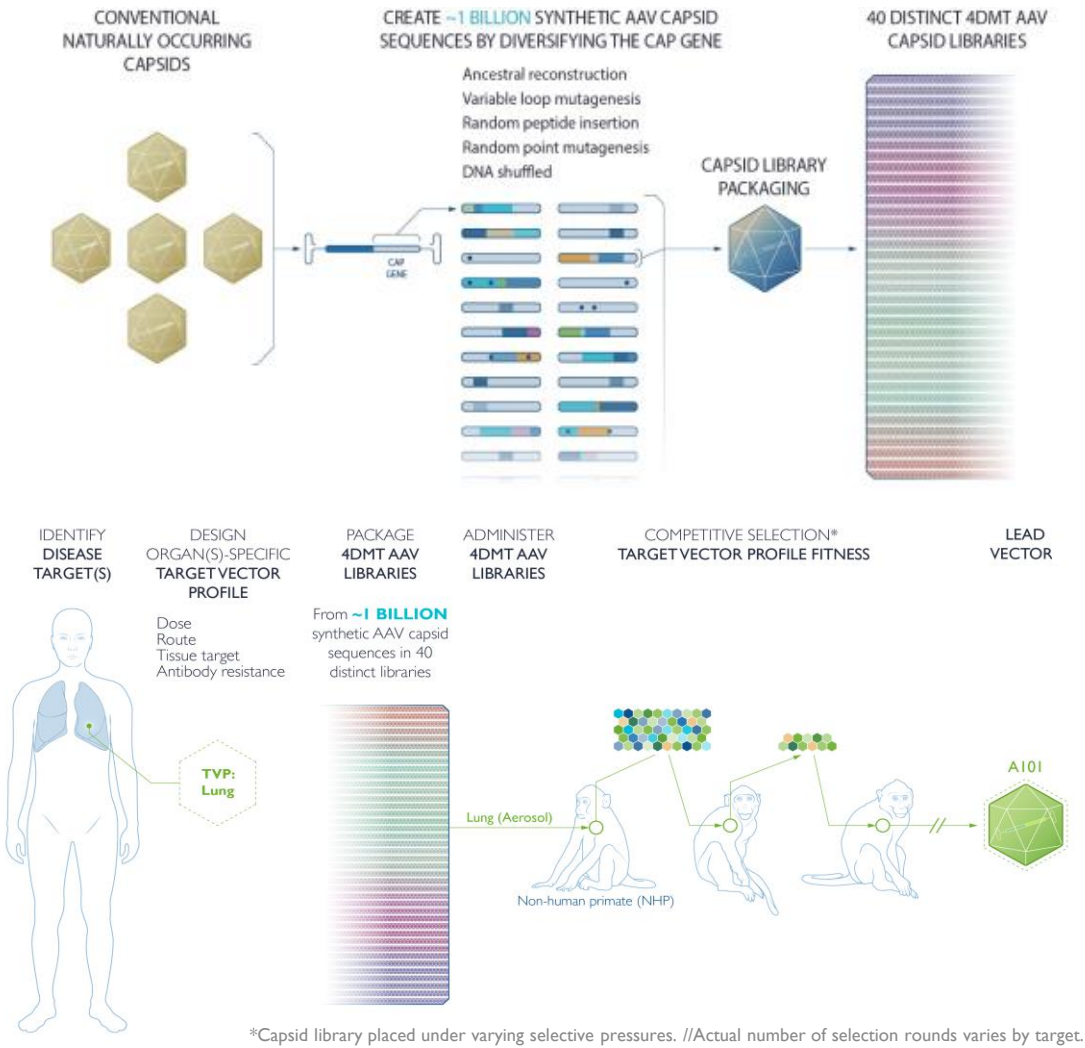
- Invent vector with optimized Target Profile
- Design, engineer & package promoter/*CFTR* payload in vector
- Preclinical pharmacology, toxicology & biodistribution studies to support IND & clinical trial initiation



Therapeutic Vector Evolution in Primate Lung: A101 Invention

4DMT AAV DISCOVERY PROGRAM FOR AEROSOL DELIVERY TO LUNG AIRWAYS

- Industrialized Therapeutic Vector Evolution platform
- ~ 1 BILLION synthetic AAV capsid sequences
- Primate (NHP) model most relevant
- Aerosol delivery with clinically approved nebulizer
- A101 Target Vector Profile designed for:
  - Widespread efficient aerosol delivery to lung airway and alveolar cells
  - Penetration through mucus barrier
  - Resistance to pre-existing neutralizing antibodies in human population
  - Efficient lung airway cell transduction and transgene expression



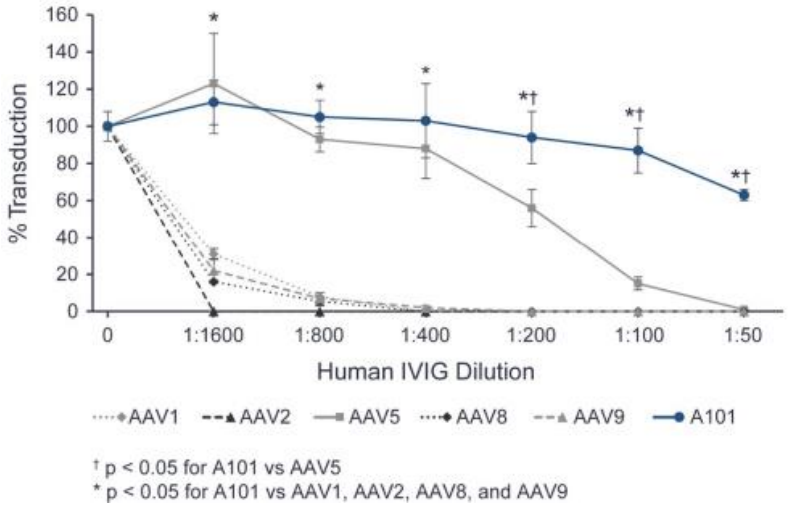
*Capsid library placed under varying selective pressures. //Actual number of selection rounds varies by target.

Human Antibody Resistance & Efficient Transduction of Human Cells

A101 DRIVING EXPRESSION OF REPORTER OR *micro*CFTR (4D-710) TRANSGENE

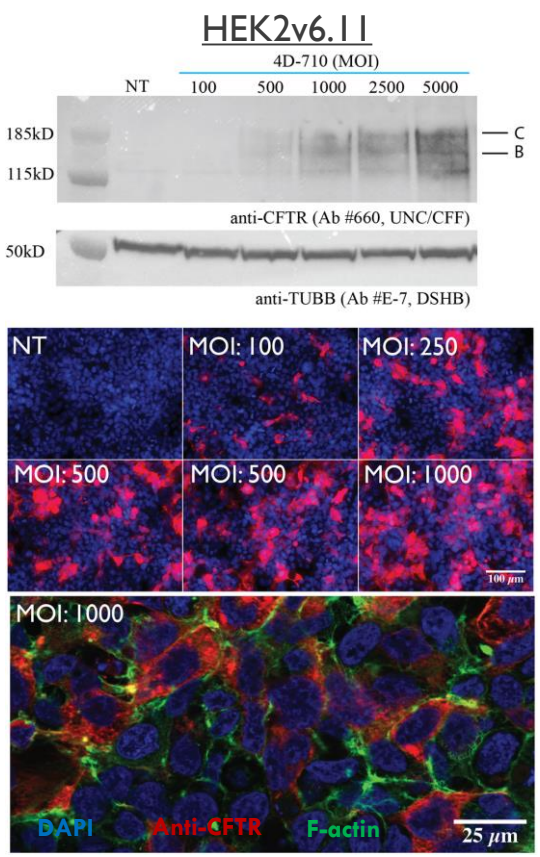
A101 HUMAN ANTIBODY RESISTANCE

- Compared to conventional AAV vectors *in vitro*
- All vectors driving expression of a luciferase reporter transgene



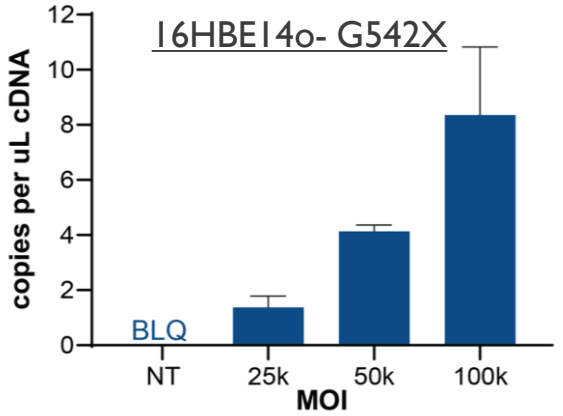
4D-710 CFTR PROTEIN EXPRESSION

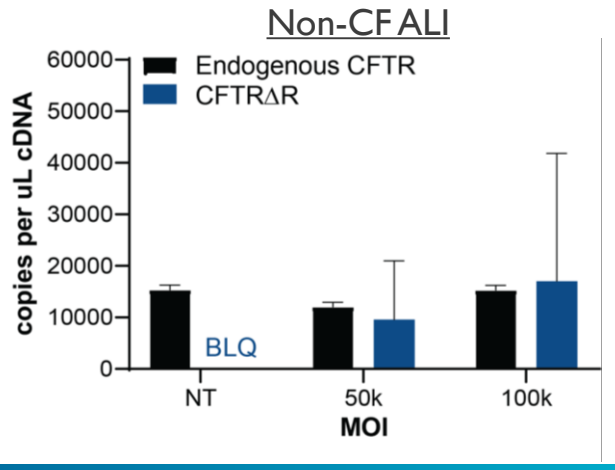
- Dose-dependent & cell membrane localization



4D-710 CFTR mRNA EXPRESSION

- Dose-dependent mRNA expression



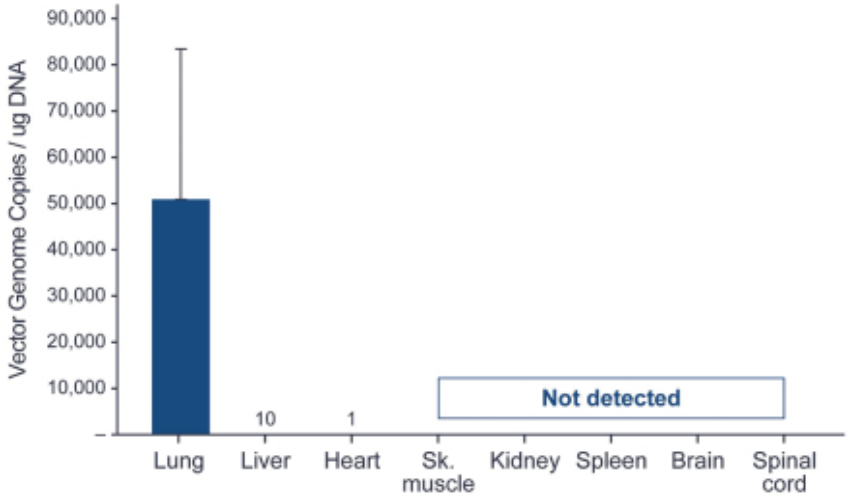


A101 Aerosol Delivery & Transgene Expression in Primates

VECTOR CHARACTERIZATION & 4D-710 PILOT SAFETY/DOSE FINDING NHP STUDIES

A101-EGFP: BIODISTRIBUTION

- Single aerosol delivery with clinical nebulizer
- High levels of genome localization in lungs
- Minimal systemic distribution



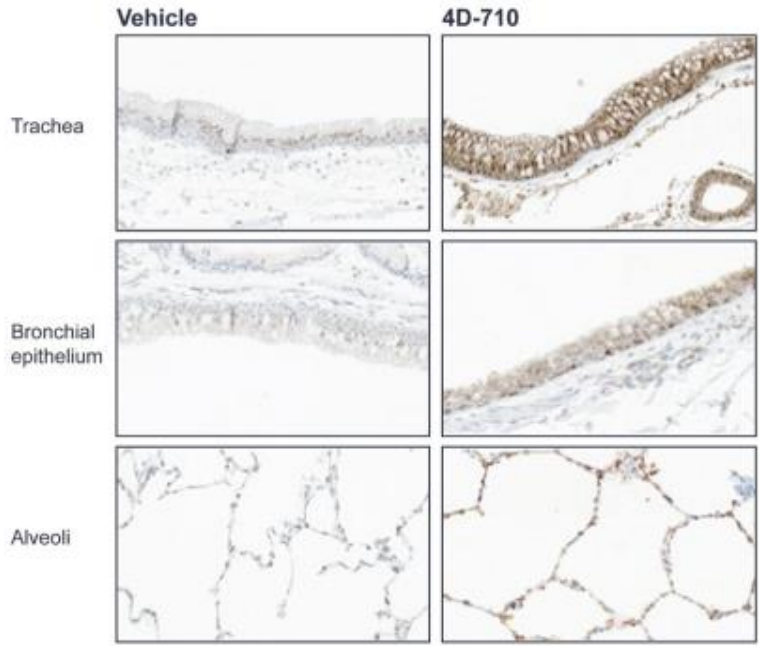
A101 genome localization was limited in liver and heart, and not present in other tissues outside the lung.

4D-710: TOLERABILITY & CFTR TRANSGENE EXPRESSION

- Single aerosol dose of product candidate 4D-710 (3E13 vg per NHP)
- No adverse findings or inflammation reported
- CFTR transgene expression detected throughout all lung segments

4D-710	Lung
Genome (qPCR)	46/48 (95.8%)
mRNA (RT-qPCR)	44/48 (91.7%)

Number of positive tissue samples across three NHPs are indicated.



Illustrative images highlight transduction of the NHP lung at the 3E13 vg dose.

Conclusions

4D-710 FOR THE TREATMENT OF CYSTIC FIBROSIS LUNG DISEASE

- Therapeutic Vector Evolution used in primates to invent A101 vector
- A101 matches Target Vector Profile:
  - Aerosol delivery throughout NHP airways
  - Mucus barrier penetration in NHP
  - Resistance to pre-existing human antibodies
  - Efficient lung airway cell transduction & transgene expression in NHP
- 4D-710: product candidate for patients with CF lung disease
  - Designed, engineered, & packaged the promoter/CFTR payload in A101
  - Performed preclinical pharm, tox & biodistribution studies to support IND filing
- Next Steps: Advance 4D-710 to IND filing & clinical development

Acknowledgments

- Our special thanks to the Cystic Fibrosis Foundation

- 4DMT Process & Analytical Development

- 4DMT Project Management

Thank You

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