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
Human Antibody Resistance & Efficient Transduction of Human Cells

A101 DRIVING EXPRESSION OF REPORTER OR microCFTR (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

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

<table>
<thead>
<tr>
<th>4D-710</th>
<th>Lung</th>
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<tr>
<td>Genome (qPCR)</td>
<td>46/48 (95.8%)</td>
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<tr>
<td>mRNA (RT-qPCR)</td>
<td>44/48 (91.7%)</td>
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Number of positive tissue samples across three NHPs are indicated.

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

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

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Thank You
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