mRNA-based Therapies for Primary Ciliary Dyskinesia

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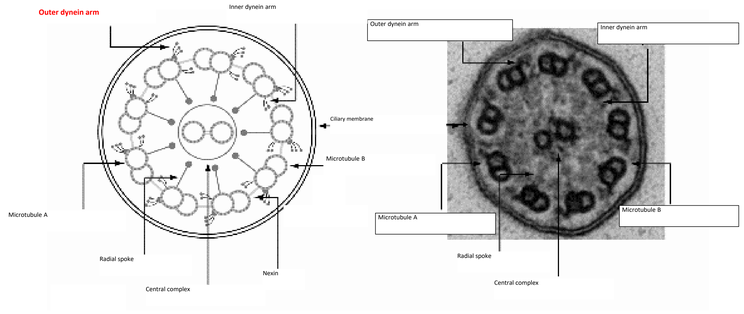
Introduction:

Primary ciliary dyskinesia (PCD) is a genetic respiratory disease caused by inherited mutations in more than 40 different genes. Bi-allelic mutations in any of the PCD genes result in loss of ciliary activity and mucociliary clearance. People with PCD suffer from recurrent respiratory tract infections and inflammation leading to bronchiectasis with varying severity. Currently, there are no disease-modifying therapies available, and treatments are limited to palliative care for the management of symptoms and airway infections. Thus, there is a clear unmet medical need for therapeutic approaches to treat the underlying causes of PCD.

Mutations in DNAI1 impair ciliary movement



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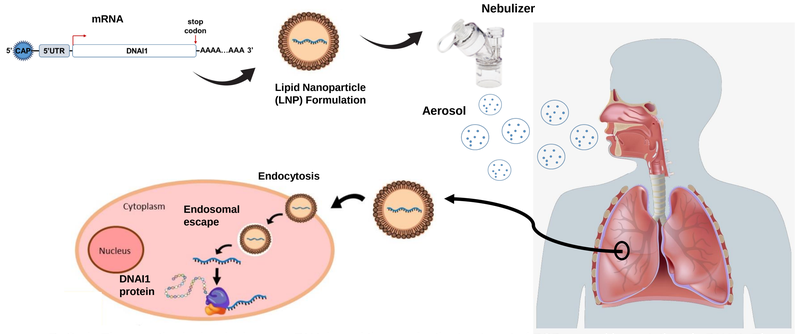
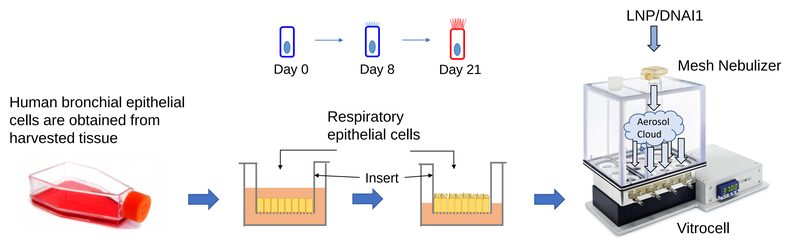
- DNAI1 (699 amino acids), a dynein axonemal intermediate-chain 1 protein, is an integral component of the outer dynein arm complex that is essential for ciliary movement.
- Dynein arms are located along the length of central microtubule doublets and hydrolyze ATP to generate ciliary movement.
- DNAI1 is expressed in ciliated cells lining the airways of nasal cavity, middle ear, paranasal sinuses, lower respiratory tract, fallopian tubes, and ventricles in the brain.

Inhaled mRNA therapeutics to treat PCD

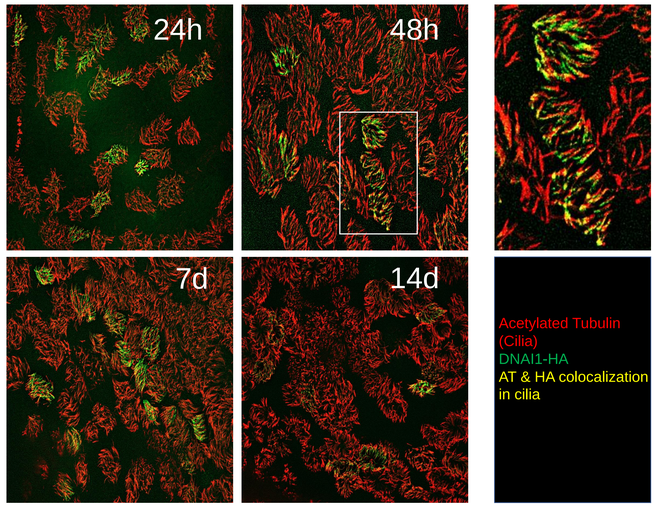


ReCode Therapeutics is developing an mRNA-based therapy for the treatment of PCD caused by mutations in DNAI1. The DNAI1 mRNA is formulated in a proprietary lipid nanoparticle (LNP), nebulized and delivered as an aerosol directly into the airway.

Ex vivo model: Primary human respiratory epithelial cell cultures (hBEs) at the air-liquid-interface (ALI)



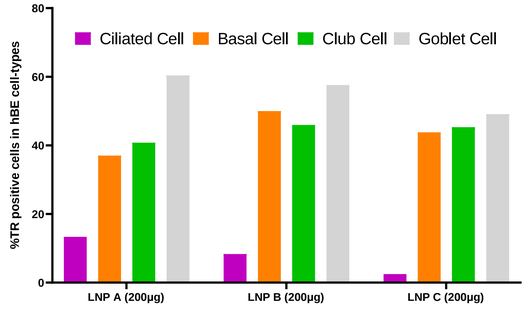
Newly-made HA-tagged DNAI1 protein incorporates into ciliated cells



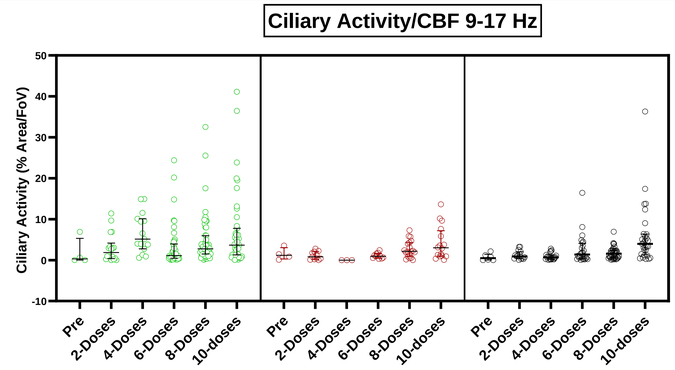
- Well-differentiated human DNAI1 knock-down cells were treated (basal administration) with a single dose of LNP-formulated DNAI1-HA mRNA (10 µg/ml of media).
- Cells were immunostained with anti-acetylated tubulin and anti-HA, 24 h, 48 h, 7d and 14d after dosing. Immunofluorescence imaging show DNAI1-HA can be detected along the length of cilia from 48h to 14d after the treatment.
- Integration of DNAI1-HA into axoneme of cilia peaks between 48 and 72h after treatment.

Transfection of hBEs with Td tomato mRNA reveals formulation-specific cell tropism signatures

- Well-differentiated human hBE cells were treated once with LNP A,B, and C-formulated TR (200µg) using Vitrocell nebulization).
- For immunofluorescence imaging, following cell markers were used:
  - Ciliated cell (acetylated-tubulin; Ac-Tubulin);
  - Basal (Cytokeratin 5; CK5);
  - Club cell (Secretoglobin Family 1A Member 1; SCGB1A1);
  - Goblet cells (Mucin 5AC; MUC5AC).



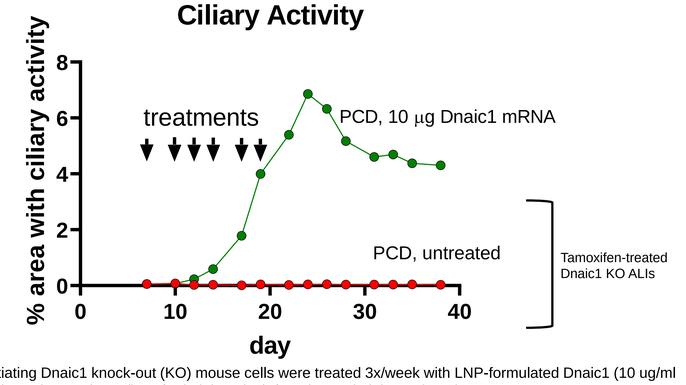
Aerosol administration of formulated DNAI1 mRNA rescues ciliary activity in knock-down primary hBE ALI cultures



LNP/**untagged DNAI1** 25-56 Days post ALI | LNP/**Td Tomato** 25-56 Days post ALI | LNP/**NTC** 25-56 Days post ALI

- Well-differentiated human DNAI1 knock-down cells (hBEs) were treated 2x/week with LNP-formulated DNAI1 (300 µg per Vitrocell nebulization) starting on day 25 post ALI (culture age); last dose administered on day 50 post ALI.
- Increased ciliary activity in treated DNAI1 knock-down cultures was first detected 7 days after dosing was initiated. Rescued ciliary activity had normal beat frequency (9-17 Hz) and appeared synchronized.

Rescue of ciliary activity in KO mouse ALI cultures persists for weeks after last treatment



- Differentiating Dnaic1 knock-out (KO) mouse cells were treated 3x/week with LNP-formulated Dnaic1 (10 ug/ml of media) starting on day 7 (basal administration); last dose administered on day 19
- Ciliary activity in treated Dnaic1 KO cultures was first detected 5 days after dosing was initiated
- Activity in treated Dnaic1 KO cells reached 36% of normal (vs PCD/no TAM controls) by day 24
- Ciliary activity in treated Dnaic1 KO cells remained above 20% of normal (more than 50% of max) 21 days after the last treatment (the last timepoint assessed)

Conclusions:

- Newly-translated DNAI1 protein incorporates throughout the ciliary axoneme of human ciliated cells and can be detected for two weeks after treatment.
- Newly-made DNAI1 protein is detected in ciliated cells, as well as in club and basal cells (precursors of ciliated cells) in hBEs and NHPs (see oral presentation).
- LNP-formulated DNAI1 mRNA delivered as an aerosol rescues ciliary function in cell-based PCD models in the presence of mucus.
- Ciliary function in cells derived from knock-out mice is rescued by LNP-formulated Dnaic1 mRNA and persists for weeks after the last treatment.

Together, these results support ReCode's efforts to develop inhaled mRNA as a disease-modifying therapy for PCD.