Rescue of Ciliary Function in Cell-based Primary Ciliary Dyskinesia Models using Nebulized, LNP-formulated mRNA

Rumpa B. Bhattacharjee[1], Mirko Hennig[1], Daniella Ishimaru[1], David Liston[1], Jackson K. Eby[2], Christian C. Corona[1], Ishita Agarwal[1] Jade E. Casillas[1], Touhidul Molla[1], Maninder S. Sidhu[1], Julia Poniatowski[1], Sierra R. Comini[1], Ally Ashworth[1], Xueliang Yu[2], Peiyang Gao[2], Harriet E. Lister[1], Omid Mousa[1], Hannah L. Golliher[3], Patrick R. Sears[3], Weining Yin[3], Michael J. Torres[2], Daniel J. Siegwart[4], Vladimir G. Kharitonov[1], Lawrence E. Ostrowski[3], David J. Lockhart[1], Brandon A. Wustman[1]

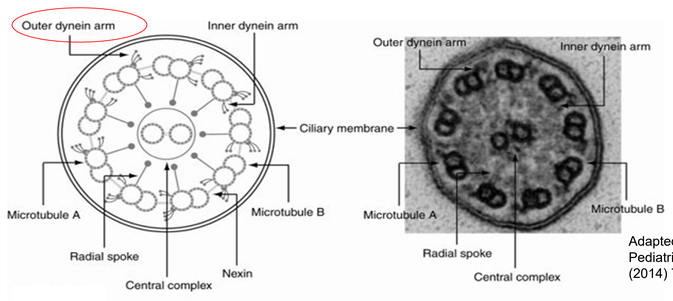
[1]ReCode Therapeutics, Inc., Menlo Park, CA, [2]ReCode Therapeutics, Inc., Dallas, TX,

[3] Dept of Cell Biology and Physiology, UNC-Chapel Hill, NC, [4]UT Southwestern Medical Center, Dallas, TX

INTRODUCTION

Primary ciliary dyskinesia (PCD) is a rare 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.

DNAI1, dynein axonemal intermediate-chain1, is the first-identified PCD causative gene (1). The DNAI1 protein (699 amino acids) 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 (axoneme) and hydrolyze ATP to generate ciliary movement. DNAI1 is expressed in ciliated cells and mutations in DNAI1 impair ciliary motility.



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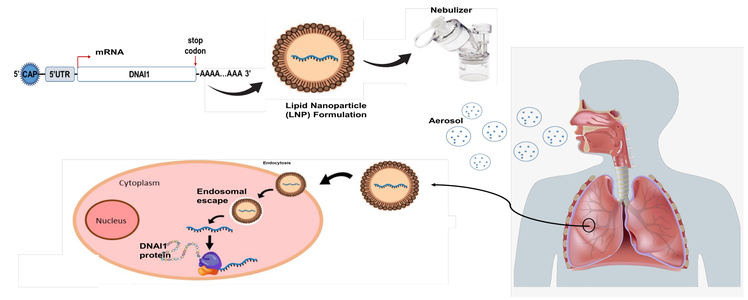
Currently, there are no disease-modifying therapies available, and treatments are limited to palliative care for the management of symptoms. Thus, there is a clear unmet medical need for therapeutic approaches to treat the underlying causes of PCD.

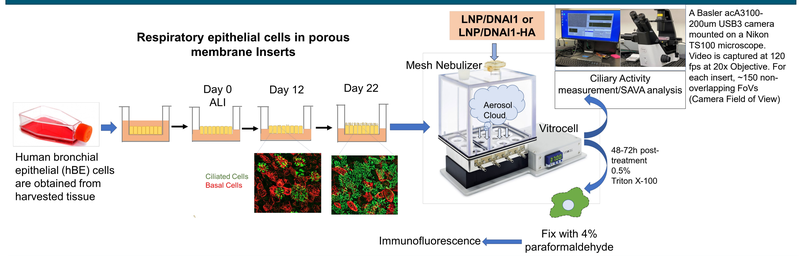
Inhaled mRNA therapeutics for PCD



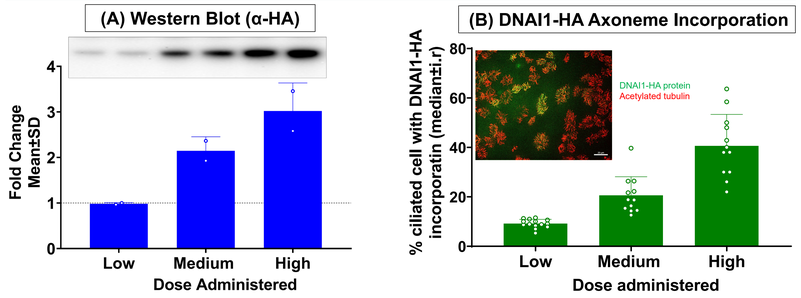
ReCode Therapeutics is developing an mRNA-based therapy for the treatment of PCD caused by mutations in *DNAI1*. The DNAI1 mRNA is sequence optimized and formulated in a proprietary SORT lipid nanoparticle (LNP). The LNP formulated mRNA is nebulized and delivered as an aerosol directly into the airway. Using knockdown human bronchial epithelial (hBE) cultures and knockout mouse tracheal cultures we show that ReCode's LNP can restore ciliary activity.

1. Kobayashi & Takeda (2012) Differentiation, S23-S29

METHODS

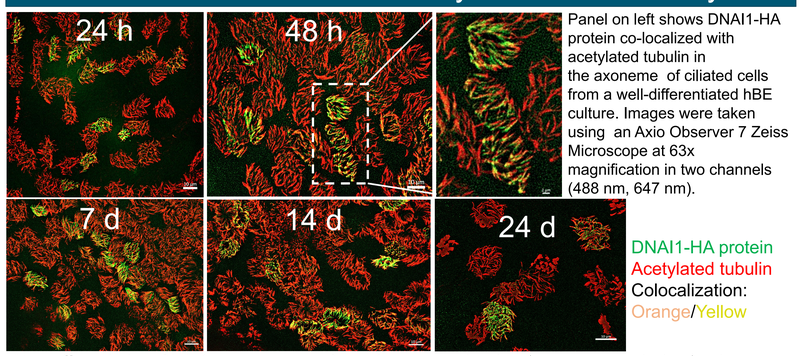


Dose-dependent translation and HA-tagged DNAI1 protein incorporation into ciliary axonemes in hBE cultures



Dose-dependent increase in (A) DNAI1-HA protein expression in cells and (B) DNAI1-HA protein incorporation into ciliary axoneme. Well-differentiated DNAI1 knockdown hBE cultures were nebulized with a single dose of proprietary LNP/DNAI1-HA mRNA using the Vitrocell. 24 h after the treatment, total protein was extracted for WB analysis with HA antibody. Incorporation of DNAI1-HA into the ciliary axoneme was detected by IF microscopy. 72 h after treatment, cultures were washed with TritonX-100 prior to fixing with 4% paraformaldehyde. TritonX-100 wash removes cytoplasmic protein but conserves the axonemal protein. Cells were stained with anti-HA antibody for DNAI1-HA protein and anti-TUBA antibody for acetylated tubulin protein (cilia marker). %DNAI1-HA incorporation = {Total colocalized cells (DNAI1-HA)/total ciliated cells (TUBA)}*100

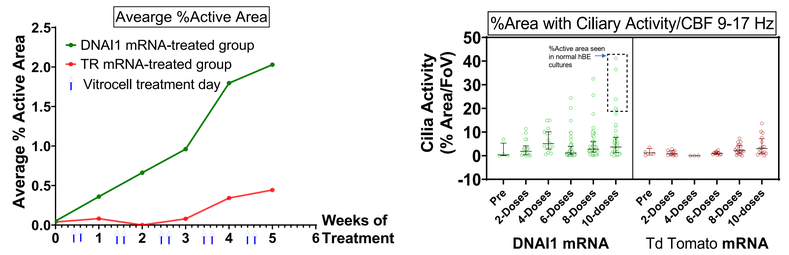
Incorporated exogenous HA-tagged DNAI1 protein can be detected in axonemes 24 days after one delivery



Panel on left shows DNAI1-HA protein co-localized with acetylated tubulin in the axoneme of ciliated cells from a well-differentiated hBE culture. Images were taken using an Axio Observer 7 Zeiss Microscope at 63x magnification in two channels (488 nm, 647 nm).

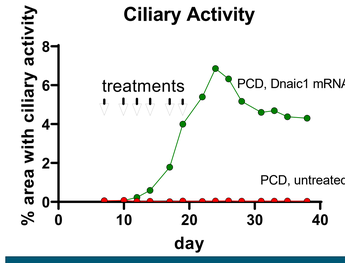
Well-differentiated DNAI1 knock-down hBE cultures were treated (basal administration) with a single dose of proprietary LNP/DNAI1-HA mRNA. Incorporation of DNAI1-HA protein into the axoneme was detected by immunofluorescence microscopy as described above. Z stack images were taken, deconvoluted and compressed to accommodate cells in different planes. Multiple inserts were dosed, cultures were taken out and fixed at each time points. DNAI1-HA protein incorporated along the length of cilia could be observed as early as 24 h and persisted to the last time point (24 d).

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



Well-differentiated human DNAI1 knock-down cells were treated 2x/week with proprietary LNP/DNAI1 mRNA using a Vitrocell exposure chamber. % active area and cilia beat frequency (CBF) was analyzed using SAVA software. Increased ciliary activity in treated DNAI1 knock-down cultures was first detected 7 days after dosing was initiated. Some FoVs in the treated cultures had % active areas in the range as observed in normal hBE cultures. With repeat administration, there was dose-dependent increase in activity. Repeat nebulization treatment was well-tolerated by the cells. There was no leakiness or cell-morphology change during the treatment.

Prolonged rescue of ciliary activity in Dnaic1 knock-out primary tracheal mouse ALI cultures



Differentiating Dnaic1 knock-out (KO) mouse cells were treated 3x/week with proprietary LNP/Dnaic 1 mRNA 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 for 21 days after the last treatment (the last timepoint assessed).

CONCLUSIONS

- Our proprietary LNP-formulated mRNA can be nebulized and is able to penetrate differentiated hBE cell culture mucus. Newly-translated DNAI1 protein incorporates thoroughly the ciliary axoneme of human ciliated cells and can be detected for three weeks after one treatment.
- LNP-formulated DNAI1 mRNA delivered as an aerosol rescues ciliary function in cell-based PCD model. Ciliary function is rescued in LNP-formulated Dnaic1 mRNA and persists for weeks after last treatment.

These results support ReCode's efforts to develop inhaled mRNA as a disease-modifying therapy for PCD. The proprietary LNP/DNAI1 mRNA has been successfully nebulized in mice and GLP tox studies has been initiated in non-human primates.

DISCLOSURES

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