An mRNA-Based Therapy to Treat Primary Ciliary Dyskinesia: Aerosol Delivery, Biodistribution and Tolerability

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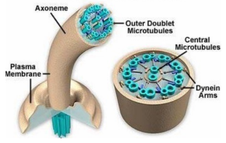
Rationale

Primary ciliary dyskinesia (PCD) is a respiratory disease caused by inherited mutations in more than 40 different genes. Bi-allelic mutations in any of the PCD genes result in a loss of ciliary activity and mucociliary clearance. People with PCD suffer from recurrent respiratory tract infections and inflammation leading to bronchiectasis. Currently, there are no disease-modifying therapies available, and treatments are limited to palliative care.

ReCode Therapeutics is developing an mRNA-based therapy for the treatment of PCD caused by mutations in DNAI1. The DNAI1 mRNA is formulated in proprietary lipid nanoparticles (LNPs), nebulized, and delivered as an aerosol directly into the airway. Here we present the nebulization methods, aerosol characteristics, biodistribution, and tolerability in a non-human primate (NHP) model.

Mutations in DNAI1 Impair Ciliary Movement

- 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.
- DNAI1 mutations impair ciliary activity with loss of mucociliary clearance (MCC).



Inhaled mRNA Therapy to Treat PCD

mRNA Payload

- Optimized sequence for improved stability, quality and translation efficiency
- Modified nucleotides for reduced immunoreactivity

Formulations

- Proprietary 5-component SORT lipid nanoparticle (LNP) optimized for mRNA[1]
- Delivers to ciliated cells

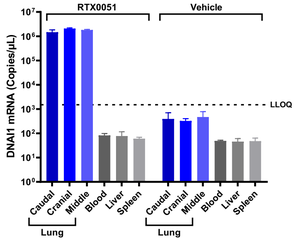
Delivery

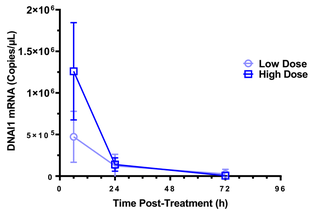
- Delivered as an aerosol to the respiratory epithelium using a commercially available mesh nebulizer

Using a Next Generation Impactor (NGI), we measured the mass median aerodynamic diameter (MMAD) of nebulized RTX0051-DNAI1. Aerosol droplet sizes were appropriate for deposition in the conducting airways of NHPs. Losses in encapsulation efficiency post-nebulization were modest and mRNA encapsulation efficiency remained high.

[1]Cheng Q. et al. (2020). *Nat. Nanotechnol* 15:313.

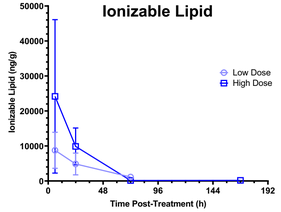
Delivery of DNAI1 mRNA to the Lung Without Systemic Exposure in NHPs

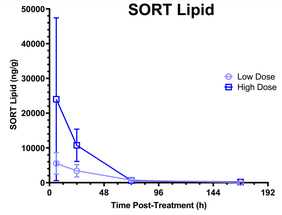




ReCode's DNAI1 mRNA-LNPs were administered to cynomolgus macaques by aerosol administration. Following treatment, total RNA was extracted from the indicated tissues and delivered human DNAI1 mRNA was detected by digital RT-PCR using primers specific for the codon and sequence-optimized mRNA. On the left panel, animals were treated with a single dose of proprietary LNP formulation using an intubated exposure system. Blood was collected at 1 h and tissues were harvested at 6 h post-treatment. High levels of hDNAI1 mRNA were present in samples taken from three lung locations (caudal, cranial, middle lobes) of treated animals. No hDNAI1 mRNA was detected above background in blood, liver, or spleen tissue from treated animals. Assay LLOQ is indicated by the dotted line. Shown on the right panel are results from animals treated with a single high or low dose of another proprietary LNP formulation using a facemask exposure system. Lung tissue was collected at 6 h, 24 h, and 72 h post-exposure. Detected levels of DNAI1 at 6 h dropped rapidly at 24 h and 72 h.

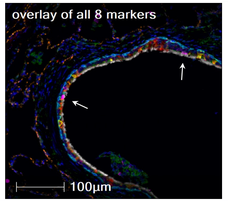
LNP Lipids Are Rapidly Cleared From the Lung Following Delivery



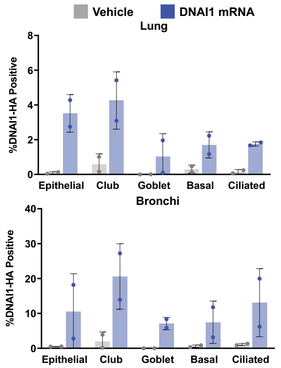


A single high or low dose of proprietary LNP ormulation was administered to cynomolgus macaques using a facemask exposure system. Blood and tissues (Lung, Liver, Spleen) were collected. LNP component lipids were detected by LC-MS. Shown on the left panel are results for ReCode proprietary ionizable lipid in lung tissue. Levels of the ionizable lipid rapidly dropped following administration. On the right panel are levels of SORT lipid in lung tissue. Following treatment, levels also declined rapidly with 0.7 to 0.8% of starting amounts remaining at 7 d. Limited exposure of either lipid was observed outside of the lung.

Expression of DNAI1-HA in PCD Target Cells of NHP Lung and Bronchi After a Single Administration

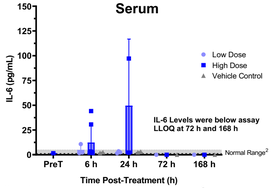


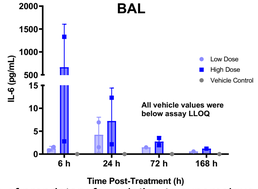
Cell Type	Marker	Color
Epithelial	EpCam	Green
Club	SCGB1A1	Red
Goblet	MUC5AC	Yellow
Basal	Cytokeratin 5	Light Blue
Ciliated	Acetylated Tubulin	White
Endothelial	CD31	Orange
hDNAI1-HA	HA tag	Pink



A single dose of proprietary LNP formulation was administered to cynomolgus macaques using an intubated exposure system. At 6 h post-exposure, lung and bronchi tissue was collected from two NHPs (1M, 1F) and stained with a multiplex immunofluorescence panel containing antibodies for each cell type (shown in table above). mIF stained slides were then scanned with a Vectra Polaris microscopy system, the images were unmixed with inForm® software and analyzed with HALO® software. For the bar graphs, the percent DNAI1-HA positive value for each cell type was calculated by combining the total cell counts from four lung sections or one bronchi section per animal. Total number of cells scored per animal ranged from ~500,000 to 1,400,000 (lung) to ~16,000 to 65,000 (bronchi). Shown are the individual data points for each treated animal and the mean ± std. dev. for each group (N=2).

Transient Increase in IL-6 Following Treatment





Following a single administration of a high or low dose of proprietary formulation to cynomolgus macaques (1M,1F/dose/time point) using a facemask system, a panel of 10 cytokines (IFN-α2a, IFN-γ, IL-1β, IL-4, IL-6, IL-10, IL-17A, IP-10, MCP-1, TNFα) and two complement factors (C3a, sC5b-9) were measured in serum and bronchial alveolar lavage fluid (BAL) at 6 h, 24 h, 72 h, and 168 h. Of the 10 cytokines, only IL-6 in serum and IL-6 and IP-10 in BAL showed transient increases following treatment. Shown above are results for IL-6, which peaked at 6 to 24 h post-treatment and returned to near baseline by 72 h to 168 h. A similar pattern was observed for IP-10 in BAL. No significant changes in C3a or sC5b-9 were seen in serum or BAL. [2]Normal NHP serum IL-6 levels from: Hocum Stone, L., et al. (2021). Sci Rep 11(1): 2340.

Formulation was Well-Tolerated in Non-Human Primates

A comprehensive assessment of histopathology, clinical chemistry, hematology, and clinical observations was performed on cynomolgus macaques treated with a high or low dose of RTX0051-DNAI1 using a facemask exposure system. Animals (1M,1F/dose/time point) were assessed at 6 h, 24 h, 72 h, and 7 d following treatment.

Key Findings:

- No adverse clinical signs observed
- No significant changes in body or organ weight
- No treatment-related changes in clinical chemistry parameters
- No changes in hematology or coagulation parameters
- Slight increase in BAL neutrophils was observed with no changes in other immune cells (lymphocytes, monocytes, macrophages, eosinophils)
- Outside of the lung, no macro- or microscopic histopathology findings were observed in other organs.

Lung Histopathology Scores

6 HRS	Vehicle		Low Dose		High Dose	
	M	F	M	F	M	F
Number of Animals Scored	0	0	1	1	1	1
No Abnormalities			0	1	0	0
Alveolar macrophages, increased			1	0	1	0
Infiltrate; inflammatory cell, Alveolus			0	0	1	1

24 HRS	Vehicle		Low Dose		High Dose	
	M	F	M	F	M	F
Number of Animals Scored	1	1	1	1	1	1
No Abnormalities	0	0	1	1	0	0
Alveolar macrophages, increased	1	1	0	0	1	1
Infiltrate; inflammatory cell, Alveolus			0	0	1	1

72 HRS	Vehicle		Low Dose		High Dose	
	M	F	M	F	M	F
Number of Animals Scored	0	0	1	1	1	1
No Abnormalities			0	1	0	0
Alveolar macrophages, increased			1	0	1	0
Cellularity, increased; lymphocyte, Balt			1	0	0	0
Infiltrate; inflammatory cell, Alveolus			0	0	0	1

168 HRS	Vehicle		Low Dose		High Dose	
	M	F	M	F	M	F
Number of Animals Scored	0	0	1	1	1	1
No Abnormalities			1	1	1	0
Alveolar macrophages, increased			0	0	0	1

Scoring Legend	
Minimal	1
Mild	2
Moderate	3

Conclusions

These data demonstrate the ability of our LNP-formulated mRNA to be nebulized and delivered directly to the lungs as an inhaled aerosol without significant exposure to other tissues. We observed robust delivery of DNAI1 mRNA to the lung and expression of human DNAI1 in relevant target cell types (ciliated, club, basal cells). The DNAI1 protein has a long half-life once incorporated into the cilia axoneme, thus we anticipate accumulation of DNAI1 with repeated administrations. These results support further development of inhaled mRNA as a promising disease-modifying therapy for PCD and IND-enabling toxicology studies have been initiated.

Disclosures

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NO PICTURES OR RECORDING ARE ALLOWED