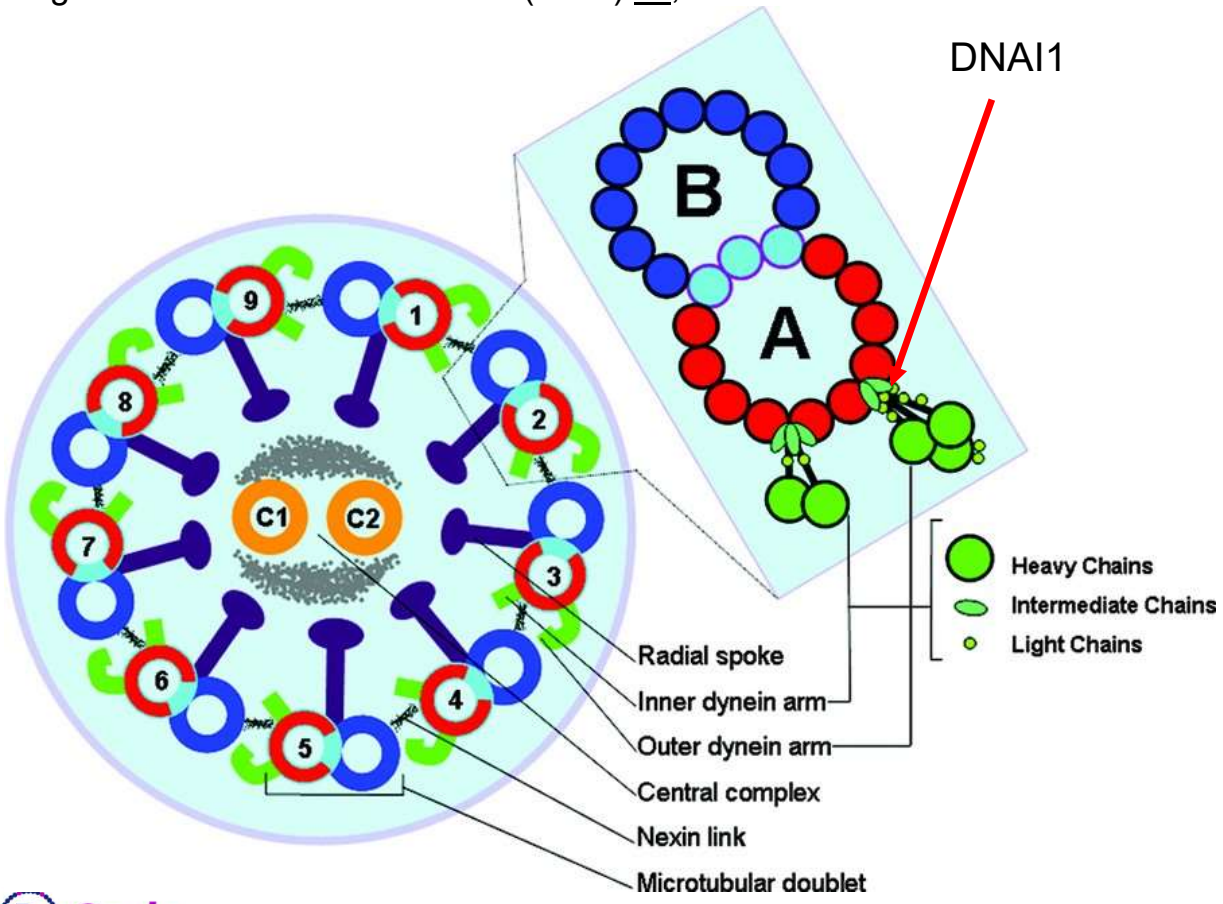
ReCode THERAPEUTICS

Rescue of Ciliary Function in Primary Ciliary Dyskinesia using Nebulized LNP-formulated DNAI1 mRNA

October 4, 2022

Mutations in DNAI1 impair ciliary movement

Leigh MW et al. *Genetics in Med* (**2009**) 11, 473



- 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 A/B 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.

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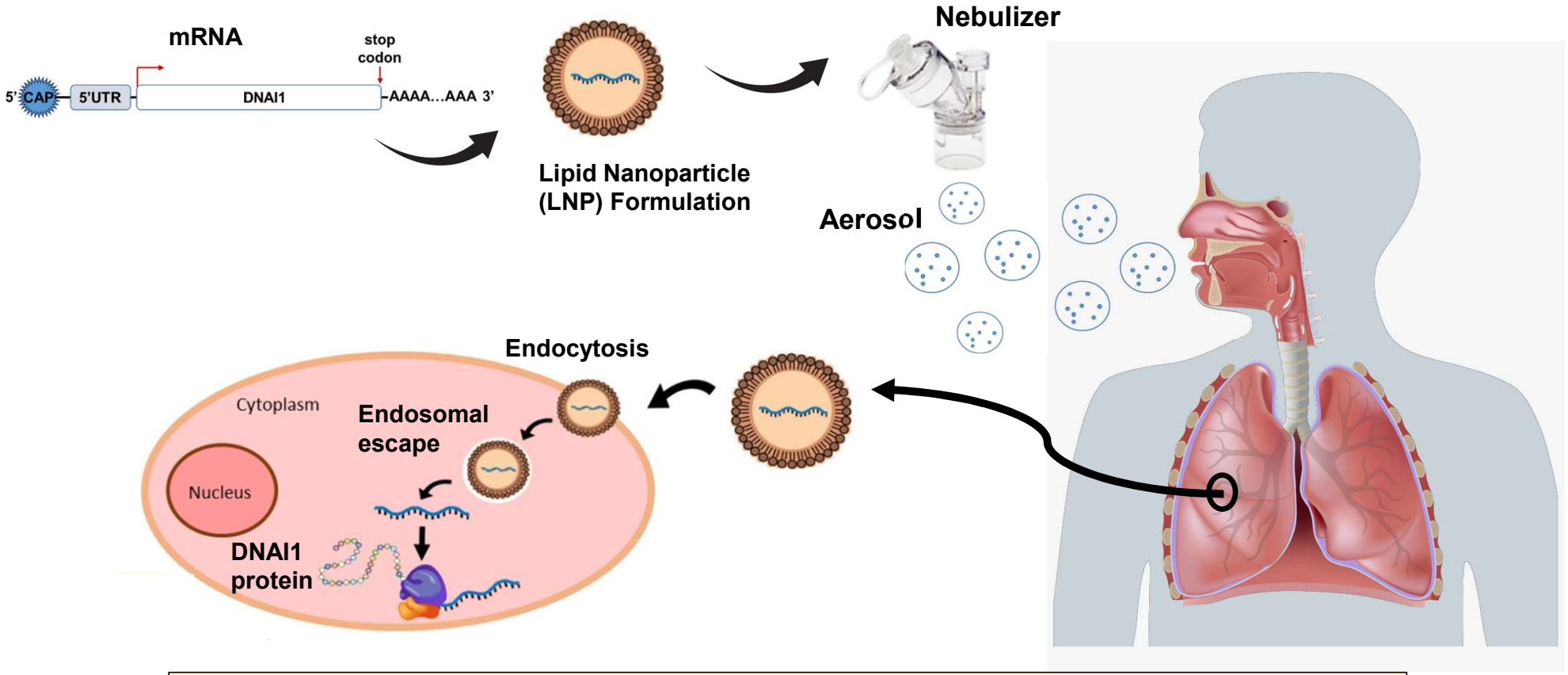
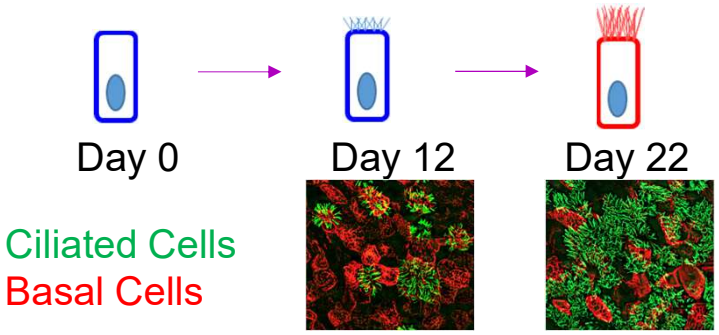
Inhaled mRNA therapeutics to treat PCD



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.

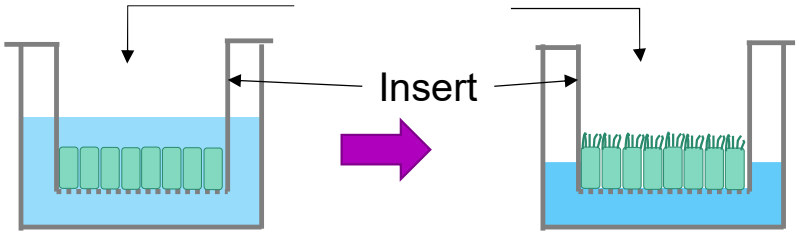
Primary human bronchial epithelial cell (hBEC) cultures at the air-liquid-interface (ALI) – optional shRNA-mediated DNAI1 knock-down

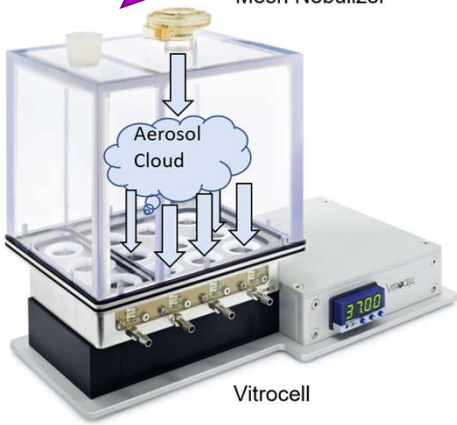


LNP/DNAI1

Human bronchial epithelial cells are obtained from harvested tissue

Respiratory epithelial cells





shRNA-mediate Knock-down of DNAI1

DNAI1-deficient, Immotile cilia

- Treatment time: 9 min (2-3 min nebulization + time for “cloud” to settle)

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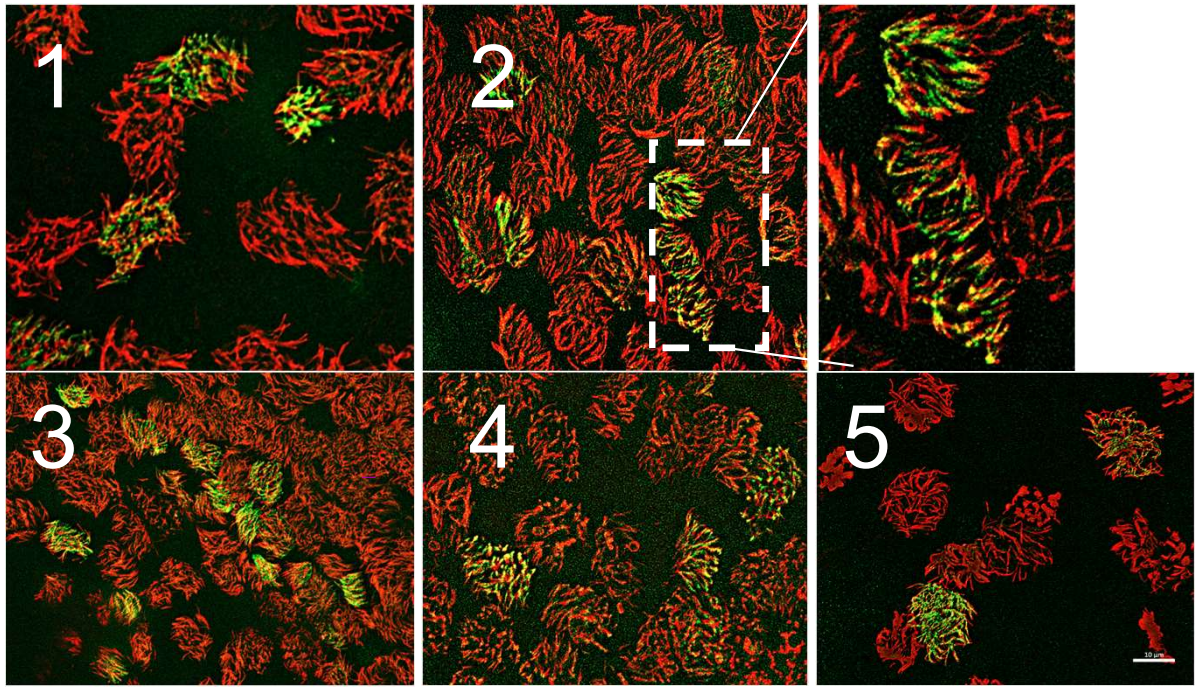
A single administration is sufficient for newly-made DNAI1 protein incorporation into cilia of hBECs



- Well-differentiated human DNAI1 knock-down hBECs were treated with a single dose of LNP/DNAI1-HA mRNA
- Integration of newly-made DNAI1-HA into axoneme of cilia peaks between time points 2 and 3 after treatment.

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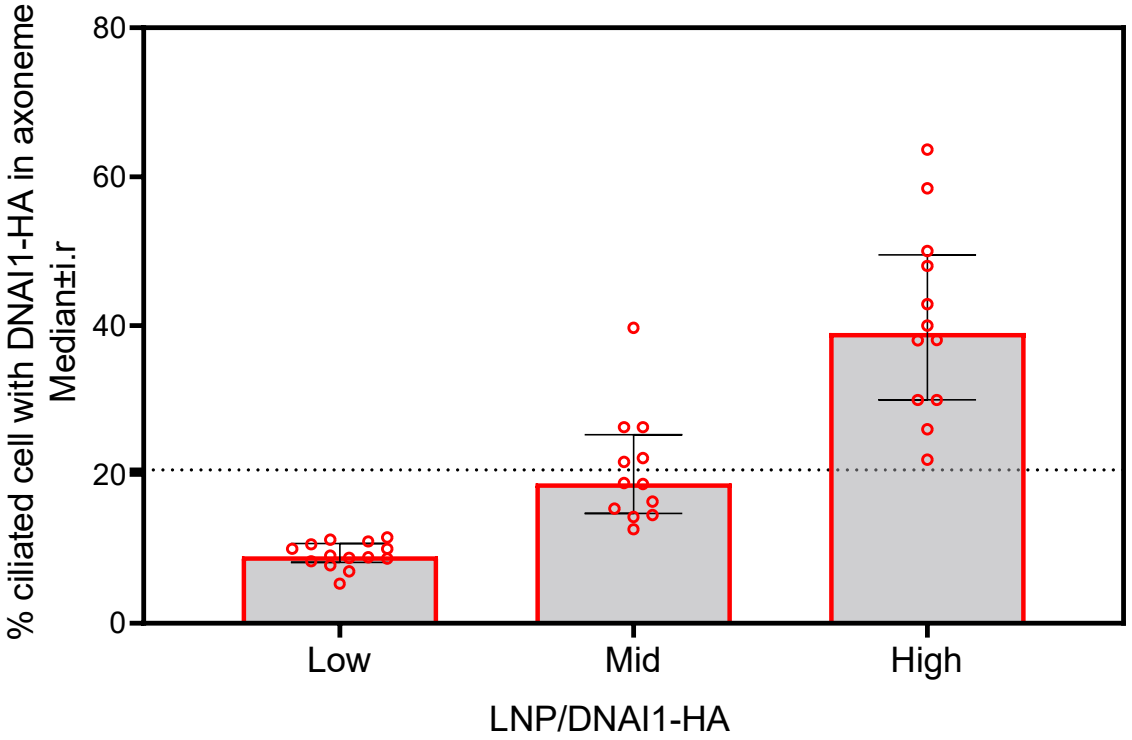
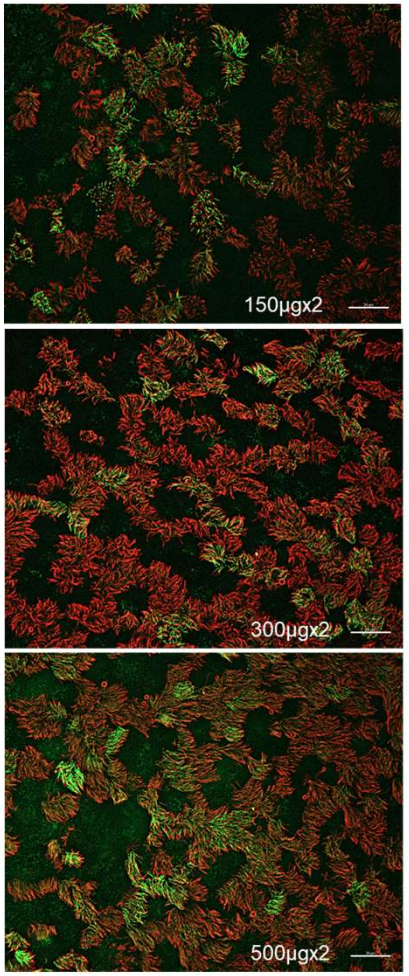
The number of ciliated cells positive for axonemal incorporation increases as you increase the dose, but the intensity of staining for a cell remains constant





- DNAI1-HA positive cells show similar fluorescence intensity: higher doses (or additional treatments) **increase the number of DNAI1 positive cells but DNAI1-incorporation into axoneme plateaus**

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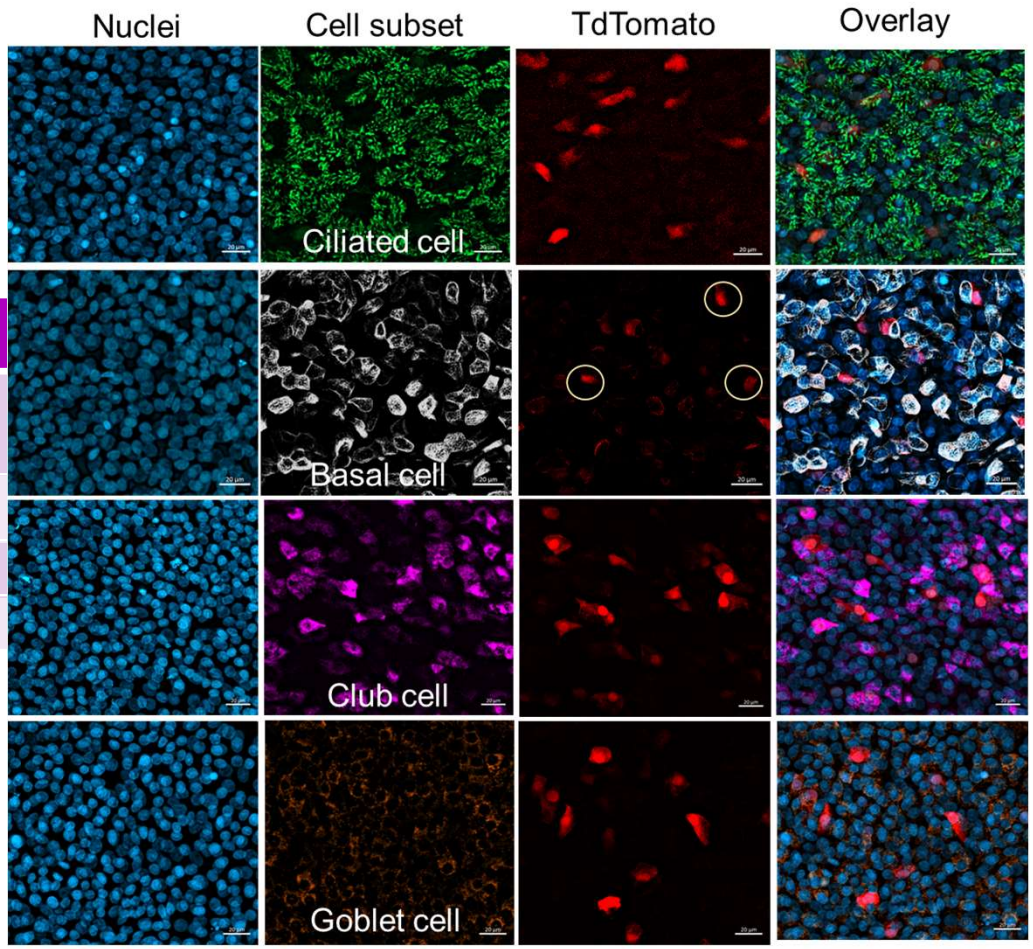
Transfection of hBECs with tdTomato mRNA reveals cell tropism signatures

Cell Subset	Antibody
Club	Secretoglobin Family 1A Member 1 (SCGB1A1)/CC10
Goblet	Mucin 5AC (MUC5AC)
Basal	Cytokeratin 5 (CK5)
Ciliated	acetylated-tubulin (TUBA)



- Well-differentiated hBECs were treated once with LNP-formulated tdTomato mRNA (200 μg) using Vitrocell nebulization)
- For staining ciliated and club cell, the entire membrane was used; one membrane was cut in half and used for basal and goblet cells.
- Z stack images (21) were taken at 40x. Images on this slide were deconvoluted and compressed.

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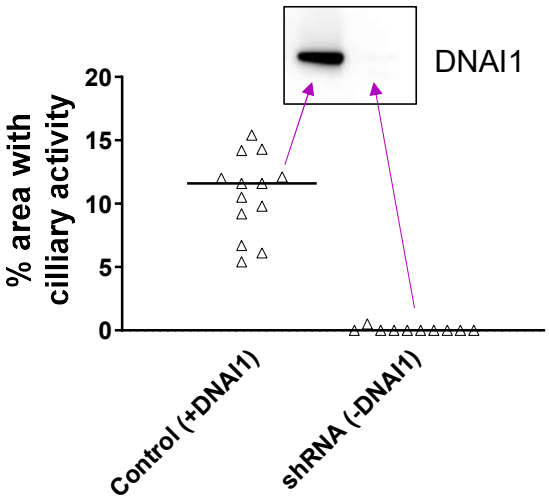
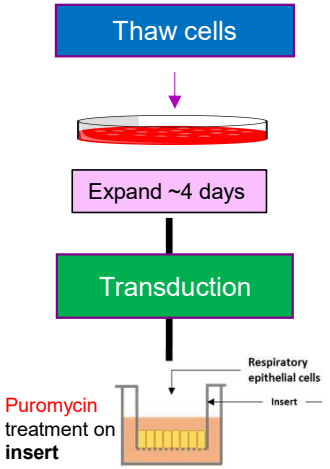
Human DNAI1 shRNA knock-down ALI cell model

DNAI1 protein expression and ciliary activity in control and DNAI1 shRNA KD cultures



- ALI cultures 21 days post lifting.
- +DNAI1 (non-transduced)
- -DNAI1 (transduced with DNAI1 shRNA)



- Human bronchial epithelial cells transduced with shRNA lentivirus targeting DNAI1 prior to plating on inserts
- Transduced cells are kept under puromycin selection during differentiation (21 days)

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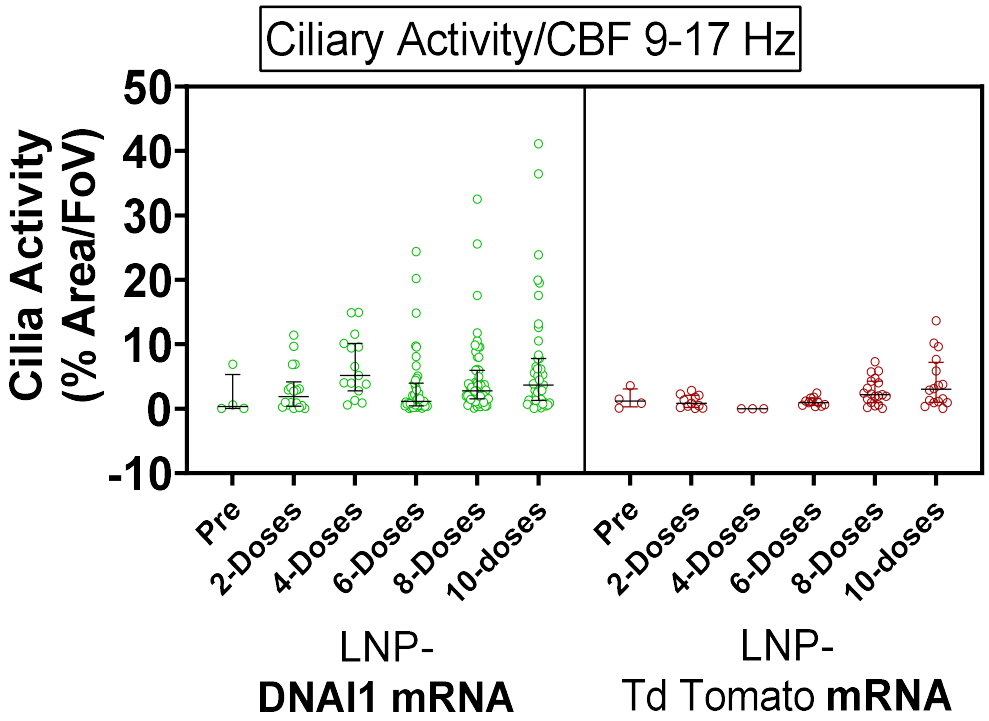
Aerosol administration of LNP/DNAI1 mRNA rescues ciliary activity in knock-down primary hBEC ALI cultures



- Well-differentiated human DNAI1 knock-down cells (KD-hBECs) were treated 2x/week with LNP-formulated DNAI1 (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 can be detected with normal beat frequency (9-17 Hz) that appeared synchronized.

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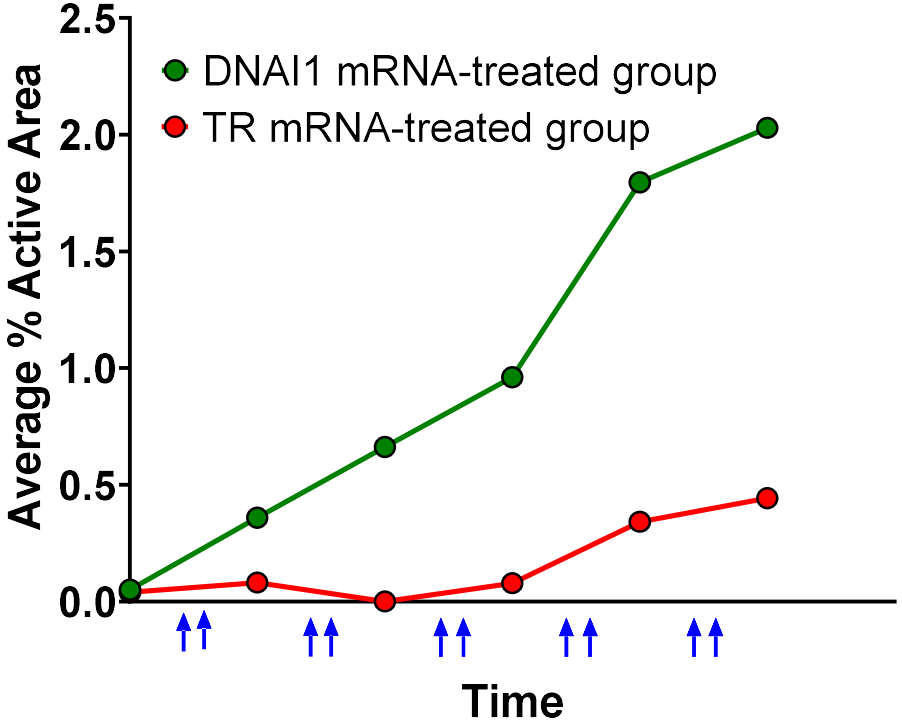
Aerosol administration of LNP/DNAI1 mRNA rescues ciliary activity in knock-down primary hBEC ALI cultures (cont'd)



- 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.

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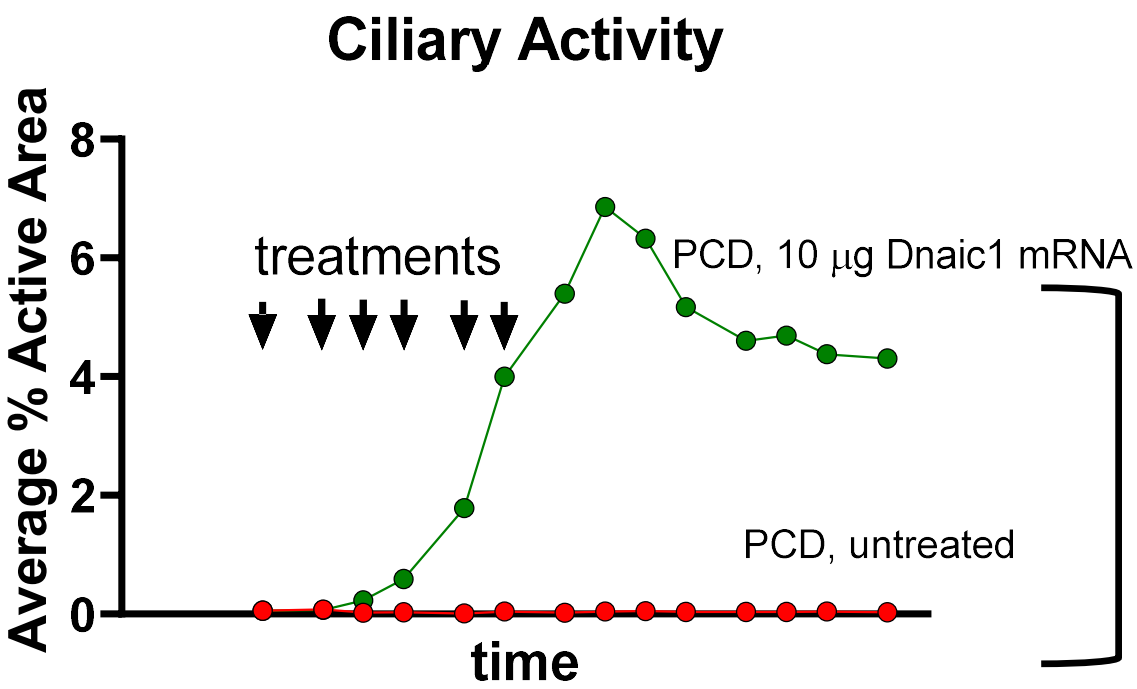
Rescue of ciliary activity in KO mouse ALI cultures persists for weeks after last treatment

Collaboration with L. Ostrowski, UNC Chapel Hill



Tamoxifen-treated
Dnaic1 KO ALIs

- Dnaic1 KO mouse cells treated 3x/week (M,W,F)
- Ciliary activity in treated Dnaic1 KO cultures was first detected after administration of the second dose
- Activity in treated Dnaic1 KO cells reached 36% of normal (vs PCD/no TAM controls) after 6 doses
- **Ciliary activity in treated Dnaic1 KO cells remained above 20% of normal (more than 50% of max) for weeks after the last treatment (the last timepoint assessed)**

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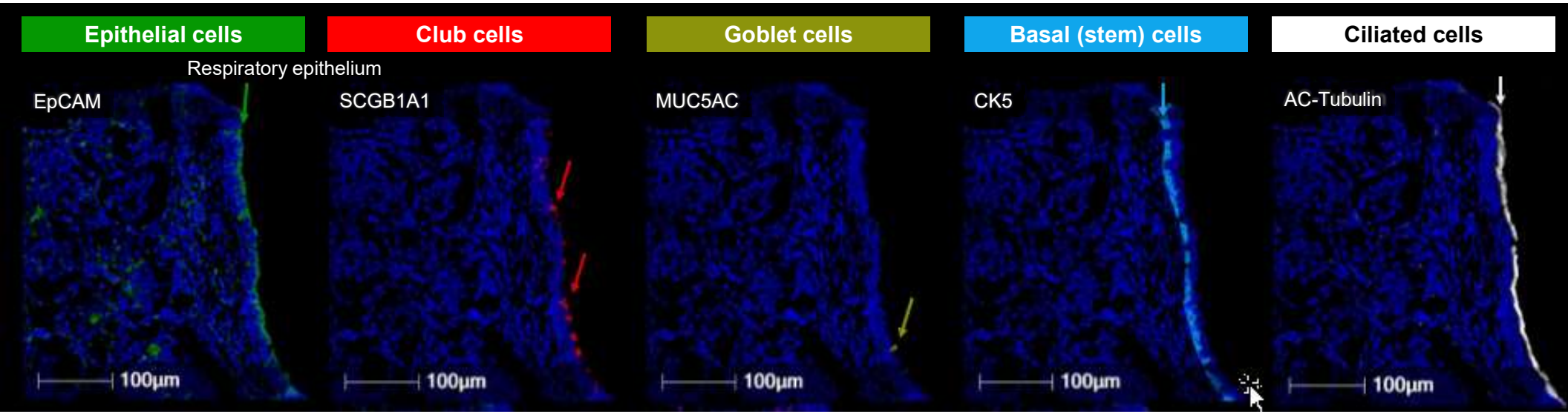
Novel tool developed to understand protein expression at cellular level in Non-human Primates (NHP)

Multiplex immunofluorescence (IF) panel from NHPs with markers for lung cells highlighted after single aerosol delivery



- AC-Tubulin, acetylated-tubulin; CK5, cytokeratin; MUC5AC, mucin 5AC; SCGB1A1, secretoglobin Family 1A Member 1; EpCAM, Epithelial cell adhesion molecule.

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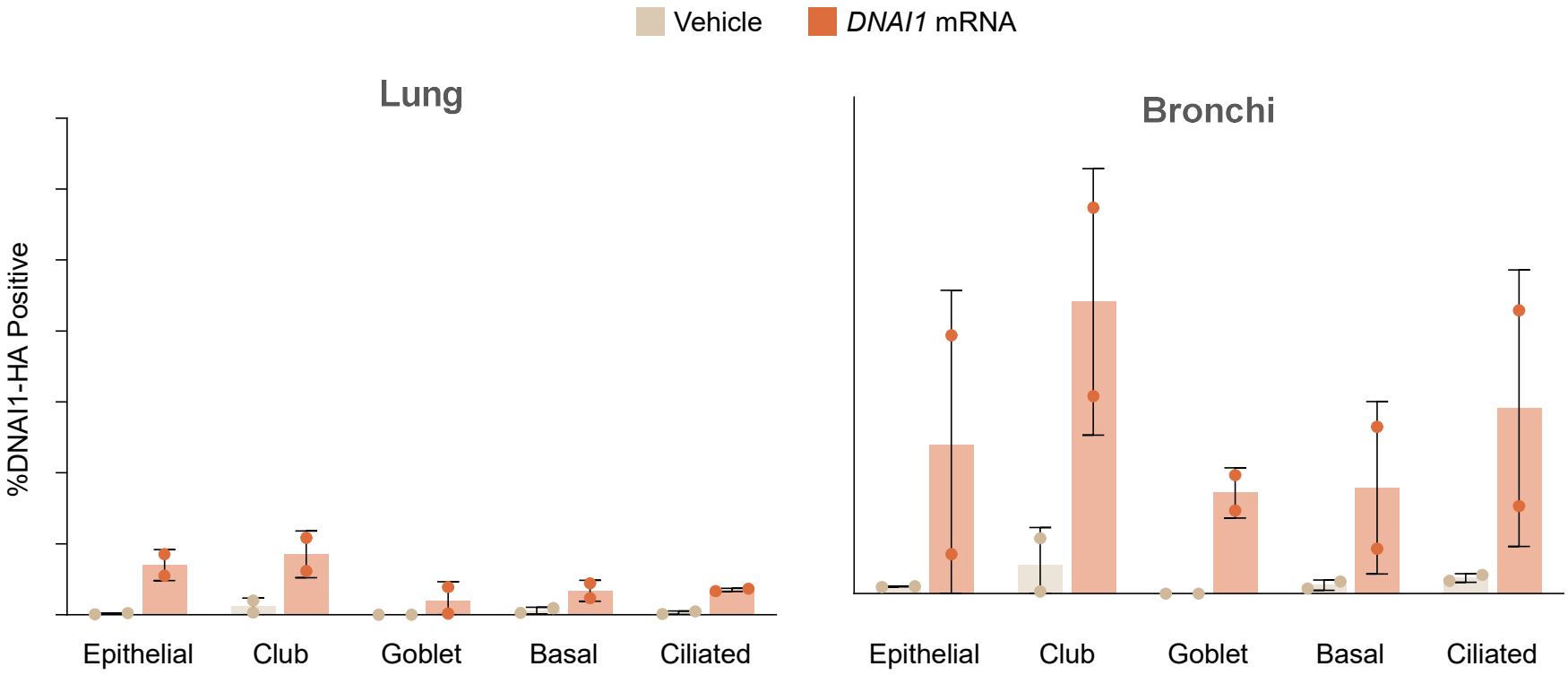
Newly-made DNAI1-HA protein observed in target cells of NHP lungs and bronchi after inhaled (intubated) delivery of LNP/DNAI1-HA mRNA



Lung sections collected from two NHPs 6 hrs after dosing.

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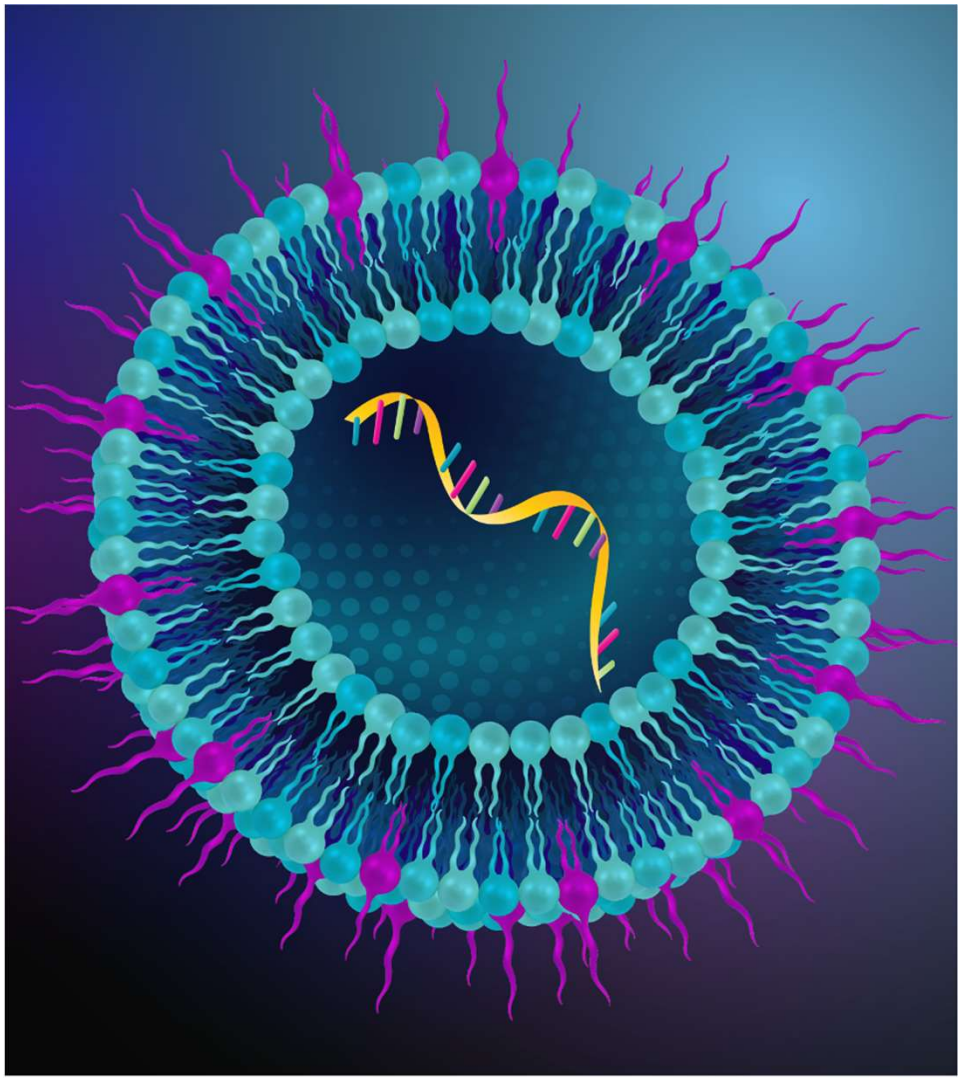
Conclusions

- 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 Non-Human Primates
- LNP-formulated DNAI1 mRNA delivered as an aerosol rescues ciliary function in cell-based PCD models in the presence of mucus.
- Ciliary function in cell-based knock-out mouse PCD models rescued by LNP-formulated Dnaic1 persists for weeks after the last treatment.

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



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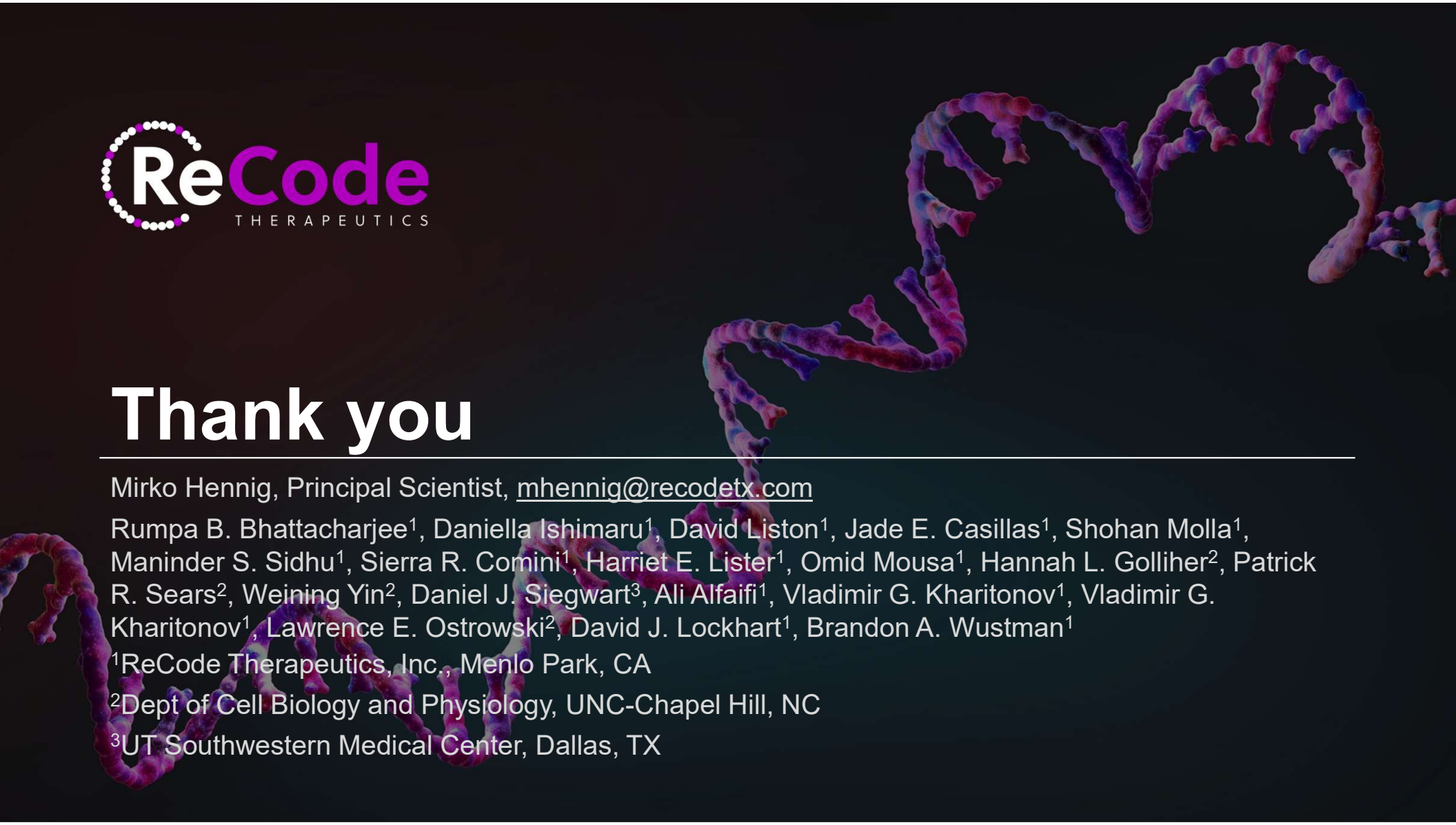

Thank you

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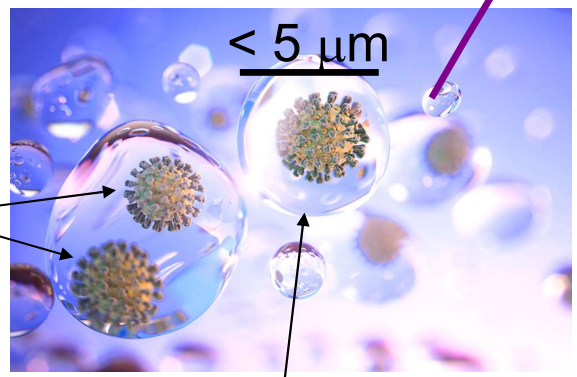
Appendix

Aerosol delivery of LNP-formulated mRNA – importance of droplet sizes



LNPs

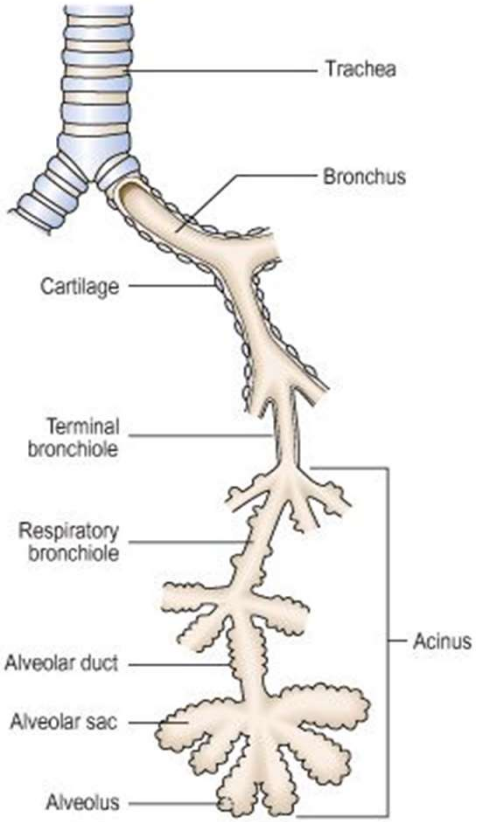
Aerosol droplet

> 5.0µm

(Head)

2.0-5.0µm

0.5-2.0µm



Tracheo-bronchiol region

Peripheral airways

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Epithelial cell type and distribution