Rescue of CFTR function in primary bronchial epithelial cells from patients with cystic fibrosis using lipid nanoparticle delivery of RNA-based therapies

WS09 Workshop 9 -Future therapeutic approaches and insights into our current practice

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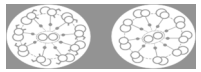
FOCUSED ON DELIVERING GENETIC MEDICINES TO PATIENTS

Primary Ciliary Dyskinesia



- mRNA
- Multi-gene/drug pipeline

Cystic Fibrosis

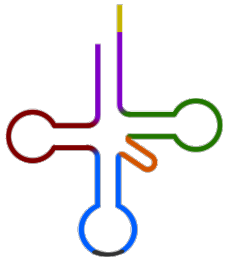
- **tRNA for nonsense mutations**
- **mRNA (mutation-independent)**
- **Gene editing**

- ✓ **Delivery to CF patient-derived hBE* cells**
- ✓ **Rescue of CFTR function in hBE cells**
- ✓ **Aerosol delivery onto hBEs and into the lungs of mice**
- ✓ **Generally well-tolerated in human cells, mice, NHPs**

NOVEL LNP PLATFORM SUPPORTS ALL PROGRAMS

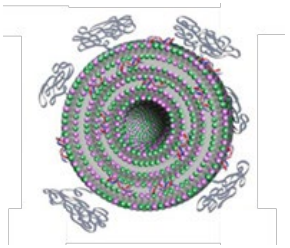
hBE: human bronchial epithelial

RCT223 (tRNA) for Arginine Opal Nonsense Mutations



tRNA Payload

- Chemically synthesized
- Specific for nonsense mutations
- Reads premature stop (nonsense) codon as signal for arginine to make a full-length functional CFTR protein



Formulation

- Proprietary lipid nanoparticle (LNP) optimized for tRNA
- Formulation well tolerated in rats and patient-derived human bronchial epithelial cells

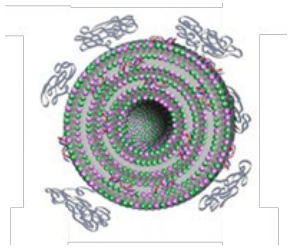
Delivery

- Delivered as an aerosol using a commercially available mesh nebulizer

Mutation-Independent CFTR mRNA

mRNA Payload

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



Formulations

- Proprietary lipid nanoparticle (LNP) optimized for mRNA
- Compatible with nebulization
- Formulation well tolerated in mice and patient-derived human bronchial epithelial cells

Delivery

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

RCT223 (tRNA) for Arginine Opal Nonsense Mutations

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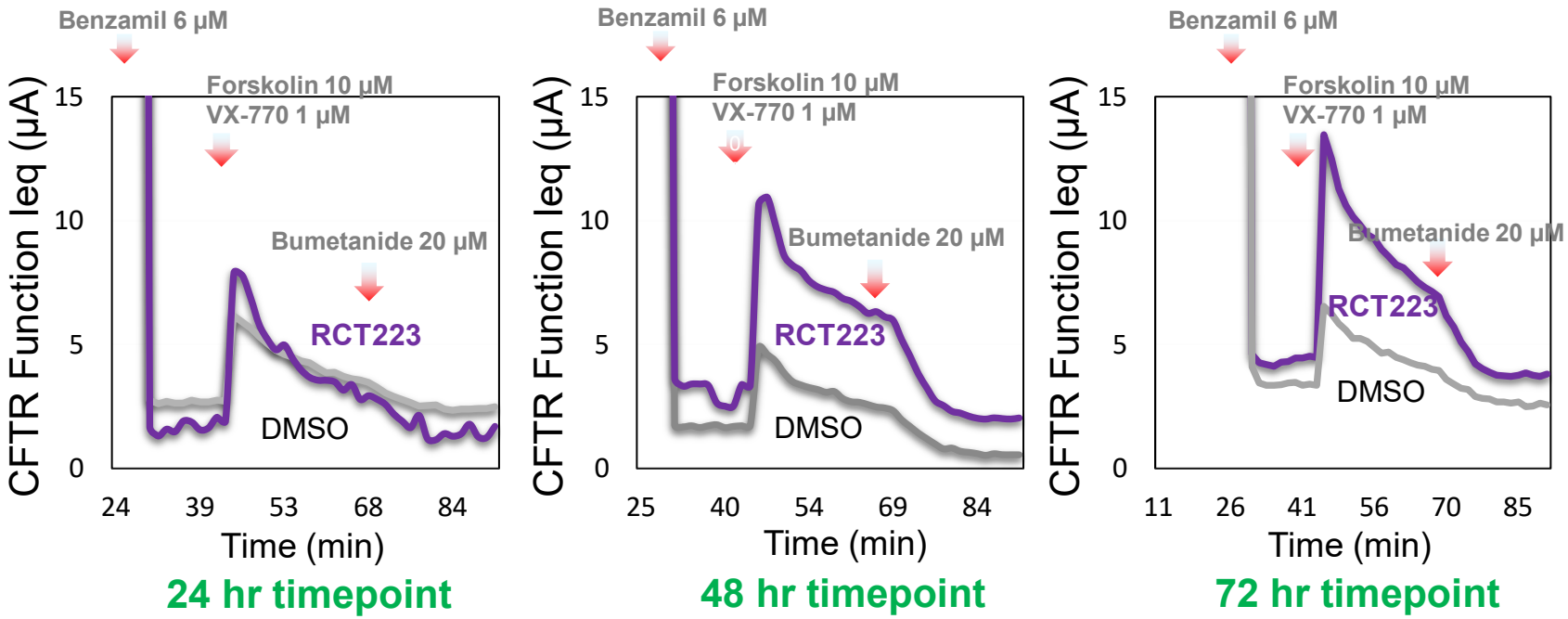
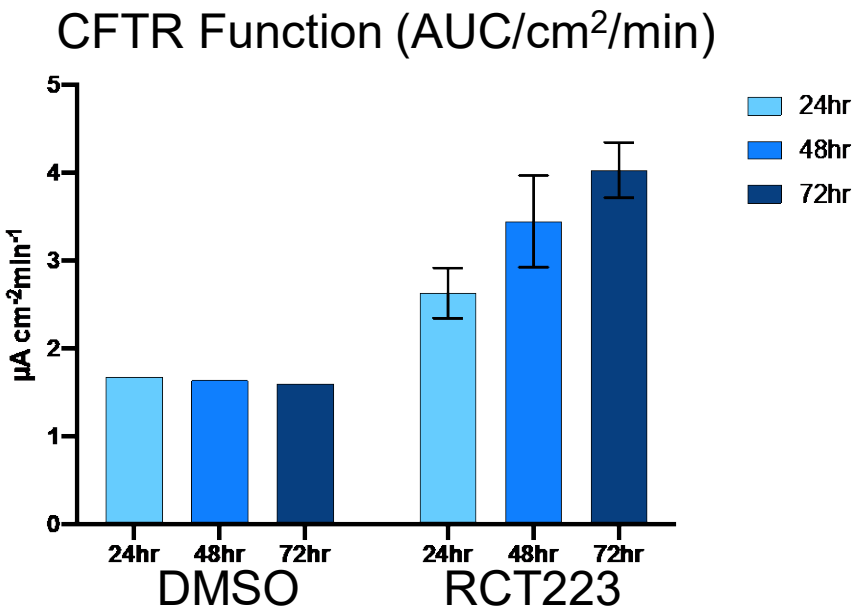
RCT223 (tRNA) Restores CFTR Function in Patient-Derived CF hBE Cells with a Single Administration

Selected traces

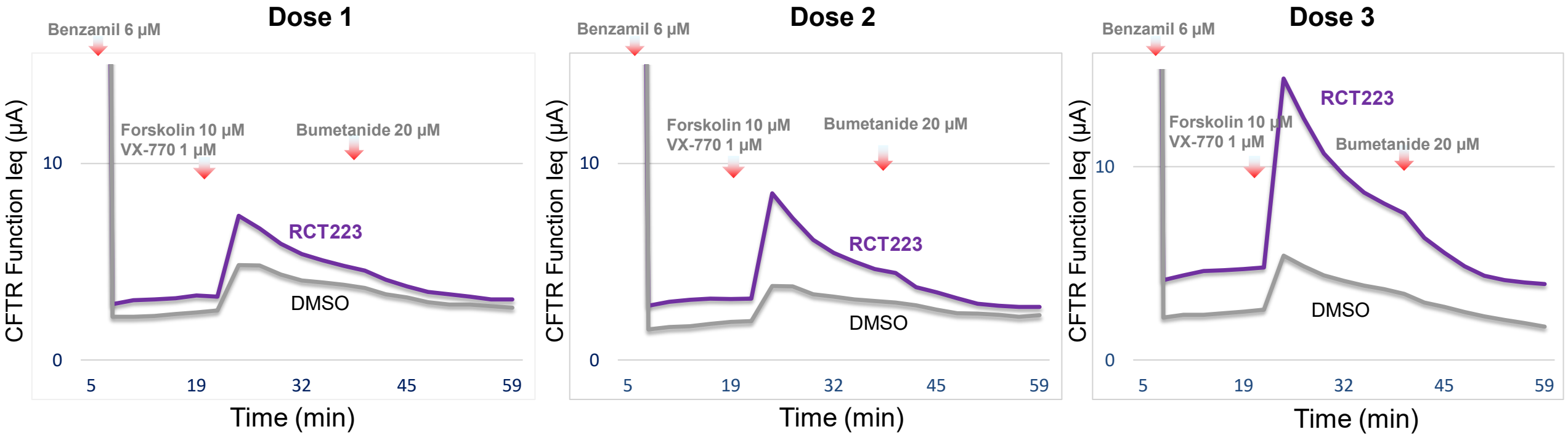




Effect persists for at Least 72hrs

Genotype: R553X/F508del
Treatment: single apical dose, 12 µg/well tRNA

CFTR Function Increases Over Time with 2x/week Dosing of RCT223 (tRNA) in Patient-Derived CF hBE Cells



- **Increasing activity with repeated administrations**
- **Longer term studies are ongoing**

Genotype: R553X/F508del
Treatment: 12 µg/well tRNA every 3 days (apical)
Measure of CFTR function: 24 hrs post treatment

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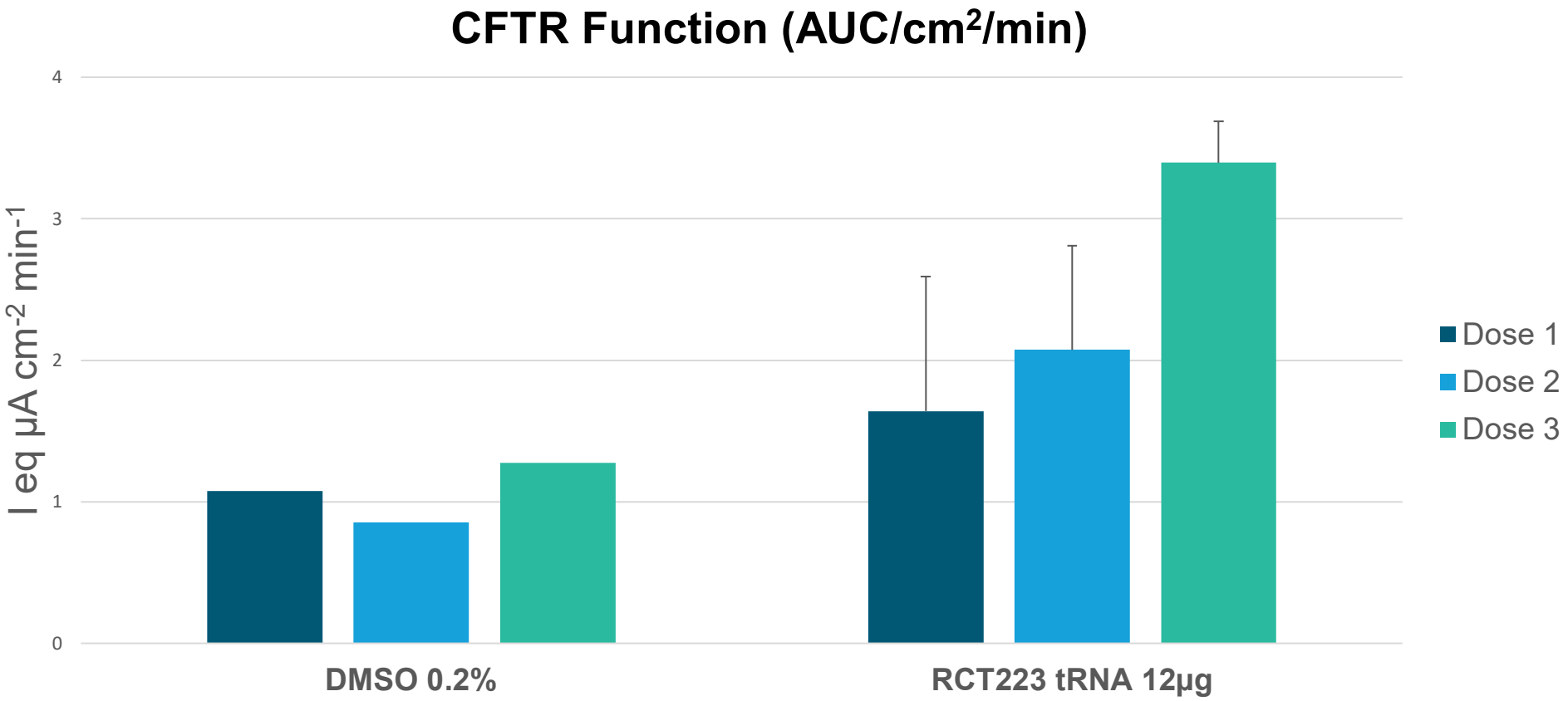
CFTR Function Increases Over Time with 2x/week Dosing of RCT223 (tRNA) in Patient-Derived CF hBE Cells



- Increasing activity with repeated administrations
- Longer term studies are ongoing

Genotype: R553X/F508del
Treatment: 12 µg/well tRNA every 3 days (apical)

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Mutation-Independent CFTR mRNA

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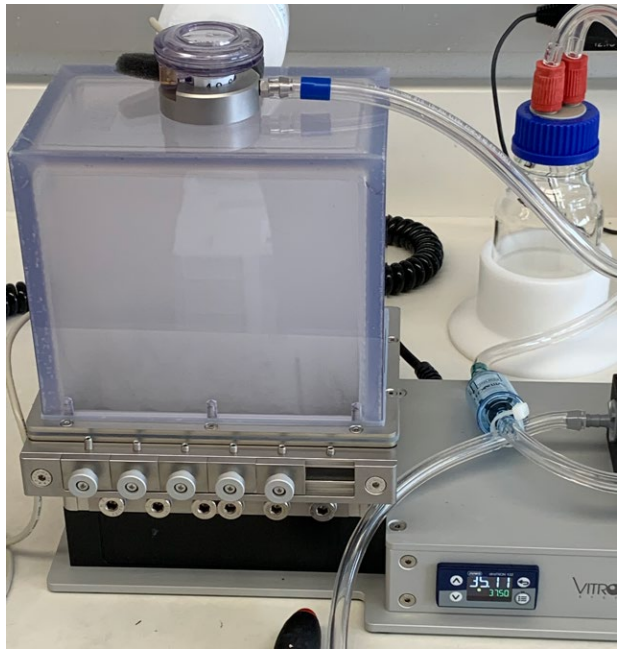
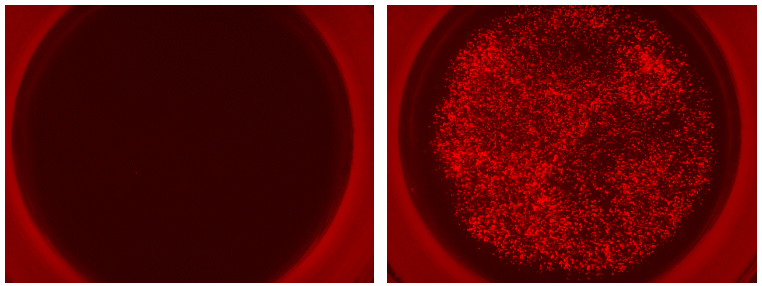
Aerosol Delivery of LNP-formulated mRNA to CF hBEs Using a VitroCell Exposure System



VitroCell Exposure System and human bronchial epithelial ALI model used for nebulization feasibility/device compatibility, *in vitro* potency & cell tropism studies



- Fully-differentiated CF hBE cells
- 4 µg/well dose Tomato Red mRNA

- **LNP formulated mRNA penetrates CF mucus**
- **High levels of expression distributed throughout the well after a single delivery**
- **VitroCell Exposure system used for delivery of CFTR mRNA to CF hBEs**

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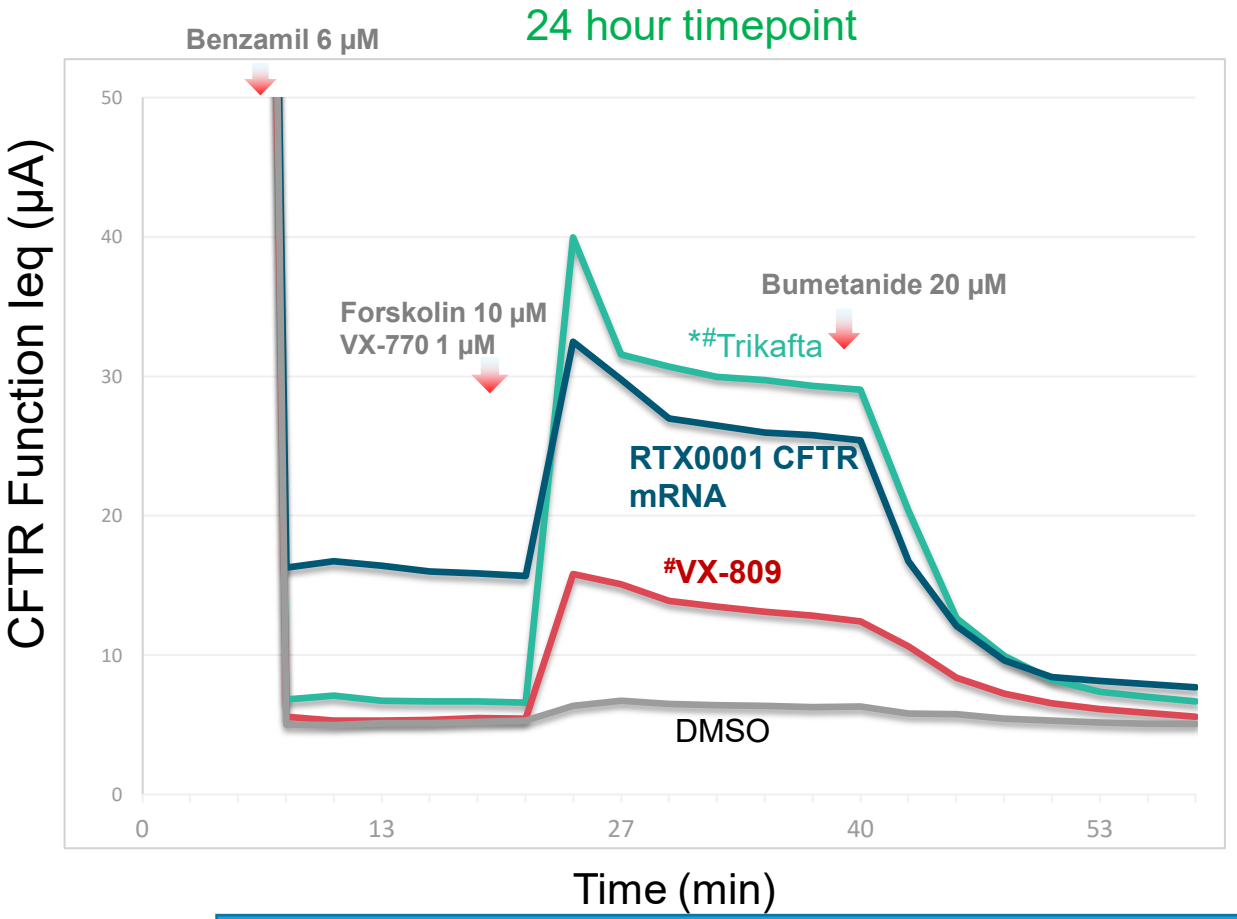
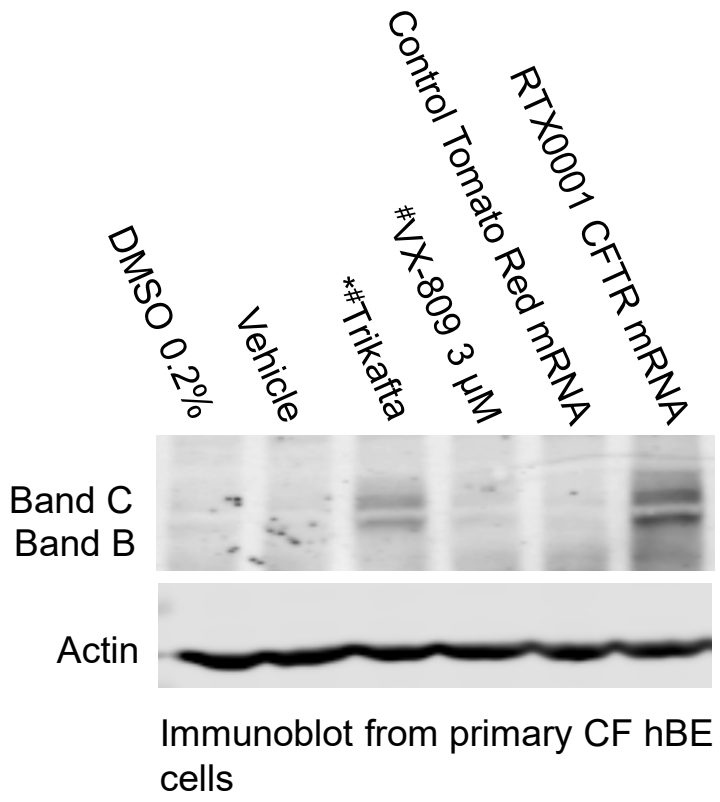
LNP Formulated CFTR mRNA Produced CFTR Protein and Restores Function in Patient-Derived CF hBE Cells





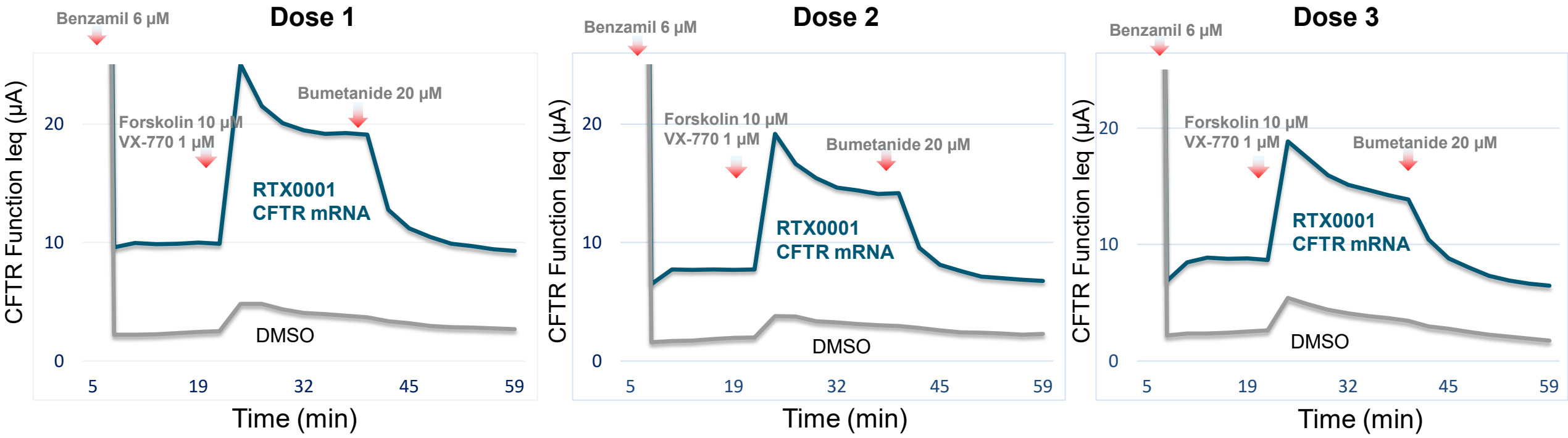
Immunoblot from primary CF hBE cells

A single, low dose significantly restores CFTR function

Genotype: G542X/F508del
Treatment: single administration of 4 µg/well CFTR mRNA delivered as an aerosol

*VX-661 3.3 µM + VX-445 3 µM. #Added to BL media

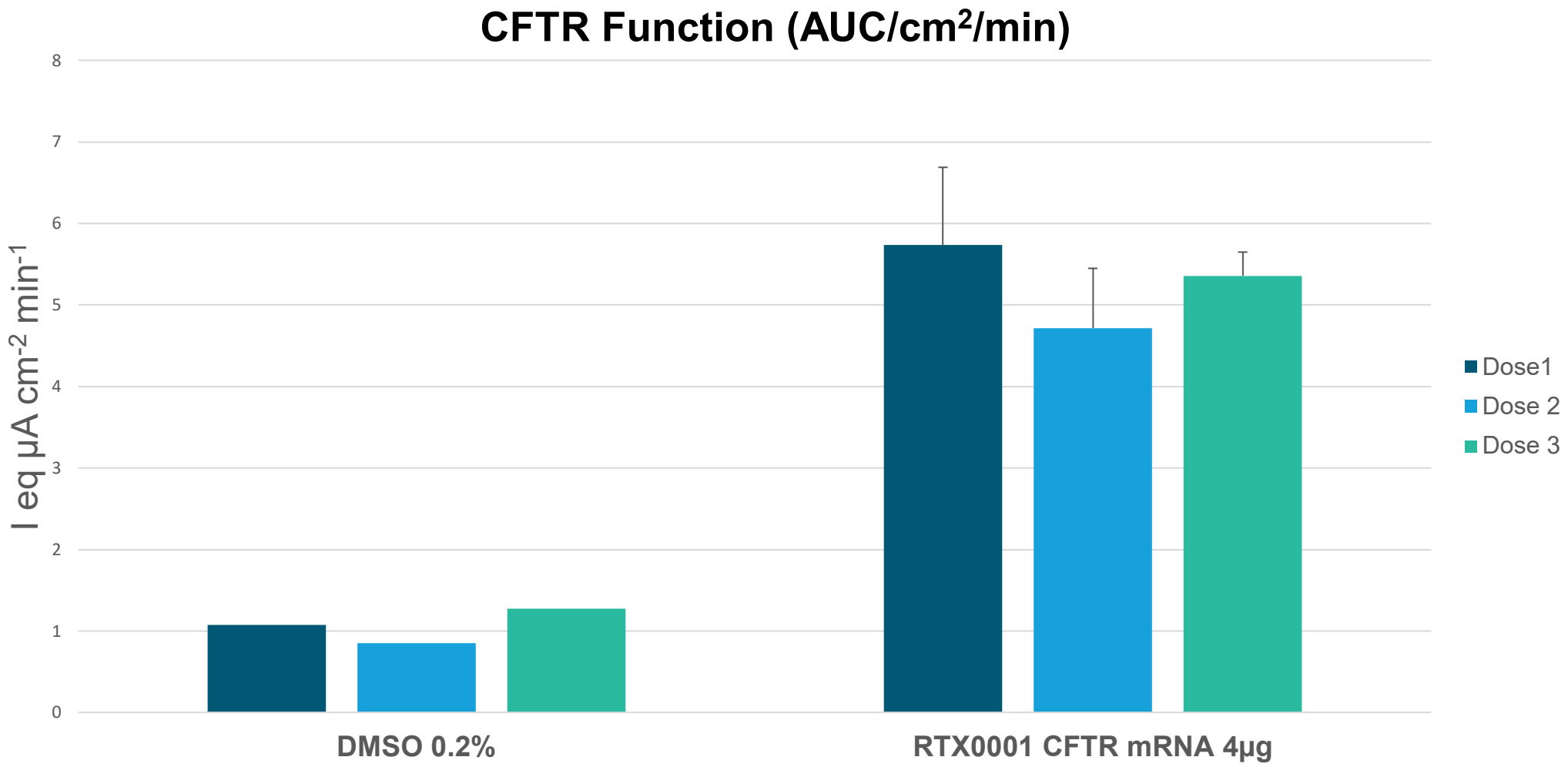
Rescued CFTR Function in CF-Derived hBEs is Maintained with 2x/week Dosing of LNP-formulated CFTR mRNA



CFTR activity is maintained with repeated administrations

Genotype: G542X/F508del
Treatment: single administration of 4 µg/well CFTR mRNA delivered as an aerosol

The RTX0001 mRNA Response is Durable in Patient-Derived CF hBE Cells and Supports 2x/week Dosing

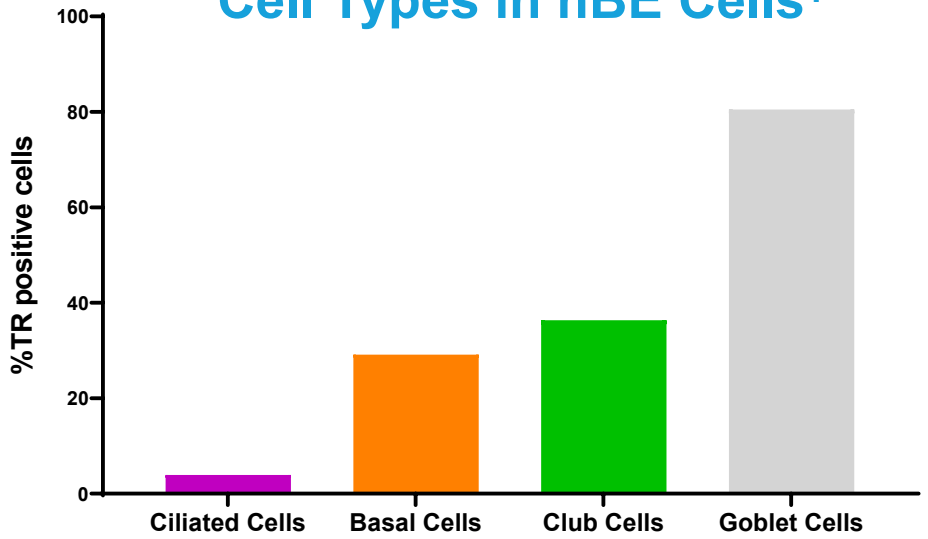


Rescue is maintained with repeated administrations

RTX0001 delivered as an *aerosol*. Dosing frequency every 3 days.

ReCode LNP RTX0001 Delivers mRNA to the Target Cells in Primary hBE Cells and Lung Tissue in Mice

Tomato Red Protein Expressed in Target Cell Types in hBE Cells[1]

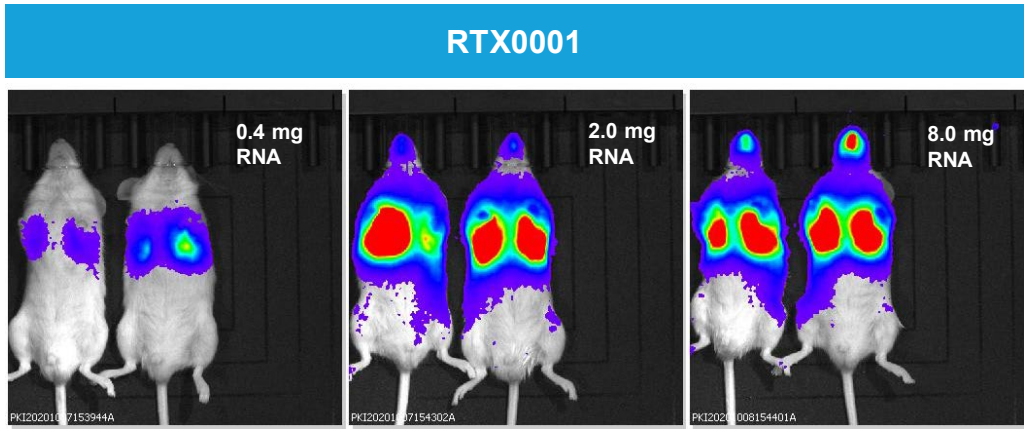


Cell Subsets in HBE cultures

Cell type	Marker
Club	Secretoglobin Family 1A Member 1 (SCGB1A1)
Goblet	Mucin 5AC (MUC5AC)
Basal	Cytokeratin 5 (CK5)
Ciliated	Acetylated-Tubulin (AC-Tubulin)

[1]Study of SORT-LNP delivered as an *aerosol* encoding Tomato Red Protein in a primary ALI hBE model.

Aerosol Delivery of Luciferase mRNA and Protein Expression in the Lungs of Mice



- LNP formulation compatible with Aerogen solo mesh nebulizer
- Good distribution and expression in mouse lungs after a single administration
- Treatments were well tolerated in all mice
- Using the same LNP formulation, protein expression has been detected in the target cells of NHP lungs with a single administration of nebulized mRNA

Doses represent the amount of Luc2 mRNA delivered into the exposure chamber by nebulization. Estimated (not measured) per mouse delivered dose is 0.01, 0.06 or 0.22 mg/kg

Conclusions

tRNA Approach for Nonsense Mutations

- ✓ Functional restoration achieved in the hBE model
  - ✓ Both as a single administration and with repeat dosing
- ✓ Formulations well tolerated in CF patient-derived hBE cultures

Next: Rodent studies in 2021

mRNA Replacement Approach

- ✓ Functional restoration achieved in the CF patient-derived hBE model
- ✓ Responses are durable
- ✓ mRNA delivered to CF target cells and protein produced
- ✓ Formulations well tolerated

Next Steps: NHP studies in 2021

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