ARCT-032 (LUNAR®-CFTR) Improves Mucociliary Clearance in CF Ferrets

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Arcturus Therapeutics and University of Iowa
Arcturus Therapeutics has developed mutation-agnostic mRNA treatment for CF lung disease
ARCT-032 is currently being evaluated in a Phase 1 clinical trial
Epithelial Cell Types in Airways

Major Proximal Airway Cell Types (Cartilaginous Airways)

Terminal Bronchiole Cell Types

Respiratory Bronchiole Cell Types

CFTR Expression (~15 to >90%)

<table>
<thead>
<tr>
<th>Goblet</th>
<th>Ionocyte</th>
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<tr>
<td>Trans</td>
<td>basal</td>
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<tr>
<td>Club</td>
<td>AT2</td>
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CF Ferret Models at University of Iowa

Airways

CFTR$^{WT}$

CFTR$^{KO}$

CFTR$^{G551D}$

CFTR$^{G551D}$ + VX-770

Airway Glands

Science Translational Medicine 2019

Human Gene Therapy 2022
LUNAR® Mediated Delivery of mRNA Payloads to Ferret ALI Cultures

**Rosa-Tg Cre Reporter**

<table>
<thead>
<tr>
<th>Exon 1</th>
<th>EGFP</th>
<th>Stop</th>
<th>tdTomato</th>
<th>Intron</th>
<th>CAG</th>
<th>Exon 2</th>
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<td>LoxP2</td>
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</table>

**Rosa-Tg Wild Type**

- Airway basal cells
- ALI
- LUNAR®-hCFTR
- 2-days

**Short Circuit Current**

**CFTR KO**
- LUNAR® Vehicle
- LUNAR®-CFTR mRNA

**CFTR ΔF508**
- LUNAR® Vehicle
- LUNAR®-CFTR mRNA

**Vehicle (V)**
- KO
- ΔF
- WT

**CFTR (C)**
- V
- C
LUNAR® Mediated Delivery of mRNA Payloads to Ferret Airways

Cre mRNA + LUNAR®

ROSAT-TG Intratracheal

En Face Trachea

Vehicle
EGFP Tomato
LUNAR®-Cre

Intralobar Airways

EGFP Tomato DAPI

EGFP Tomato DAPI

% EGFP epithelial cells

100
80
60
40
20
0
Trachea ILB
LUNAR®-CFTR and LUNAR®-tdTomato Delivery to Ferret Airways

LUNAR®-Tomato mRNA

LUNAR®-CFTR and LUNAR® hCFTR

Act-Tubulin
LUNAR® Platform Targets Ciliated Cells
Pulmonary Ionocytes in the Proximal Airway

Major Proximal Airway Cell Types (Cartilaginous Airways)

- Basal
- Goblet
- Ionocyte
- Transitional

Yuan et al. *Nature* 2023

Ferret Trachea Whole Mount

ATP6V1G3 (Ionocyte) Act-Tubulin (Ciliated)

- WT
- FOXI-KO

CFTR-KO FOXI1-KO

- pH
- ASL Volume
- ASL Viscosity
- Clearance

CFTR Expression

~1%

~30%

~30%

Human Ferret

Glands

CFTR Expression

Trachea

Bronchi

Bronchioles

Yuan et al. *Nature* 2023
LUNAR® Platform Predominantly Targets Non-Ionocytes in the Ferret Proximal Airways
LUNAR®-Cre Transfection in ROSA-TG Ferrets

LUNAR® platform efficiently delivered mRNA payloads to multiple epithelial cell types including ciliated cells and some ionocytes in ferret airways.
Mucociliary Clearance (MCC) Endpoints in CF Ferrets

Gallium-68-albumin SPECT/CT (Trachea)

PET-CT

Proximal

Distal

WT  CF On-VX  CF Off-VX
Airway clearance in the CF ferret model is CFTR-dependent.
ARCT-032 (LUNAR®-hCFTR) Improved MCC in CF Ferrets

- G551D ferrets were removed from VX-770 to achieve a MCC defect (20-40% of WT)
- MCC evaluated 24 hr post-dosing with LUNAR®-hCFTR or LUNAR®-TdTomato

![Graph showing MCC clearance over time](image)
ARCT-032 (LUNAR®-hCFTR) Improved MCC in CF Ferrets

Rate of Clearance (First 6 min)

One-way ANOVA

P = 0.0001

P = 0.0001

P = 0.9151

P = 0.9979

Pre Post Diff

LUNAR®-hCFTR (N=8)

Paired Analysis

LUNAR®-tdTomato (N=6)
Conclusions

- LUNAR® platform demonstrates efficient delivery of mRNA payloads and transfection in various cell types in ferret airways.
- ARCT-032 (LUNAR®-CFTR) improved CFTR activity in CF ferret model as demonstrated by significantly improved MCC.
- These in vivo results complement findings from in vitro studies in human bronchial epithelial cells (HBEs) derived from CF patients where ARCT-032 restored CFTR-mediated chloride conductance.
- ARCT-032 is currently being studied in a Phase 1 trial in healthy volunteers and adults with CF.