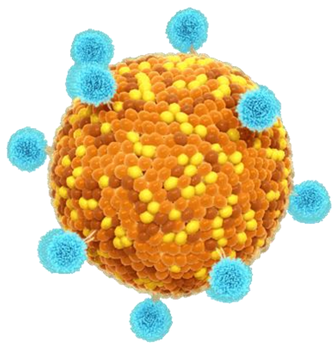


LUNAR®-CF, an aerosolized mRNA replacement Therapy for Cystic Fibrosis Lung Disease

Carlos G. Perez-Garcia, Yihua Pei, Yanjie Bao, Priya Karmali, John Engelhardt, Xiaoming Liu, Craig Hodges, Thanhchau Dam, Jose A. Gonzalez, Marciano Sablad, Suezanne Parker, Tamar R. Grossman, Pad Chivukula

BUILDING INNOVATIVE RNA MEDICINES

Arcturus Therapeutics, Inc., San Diego, CA, USA

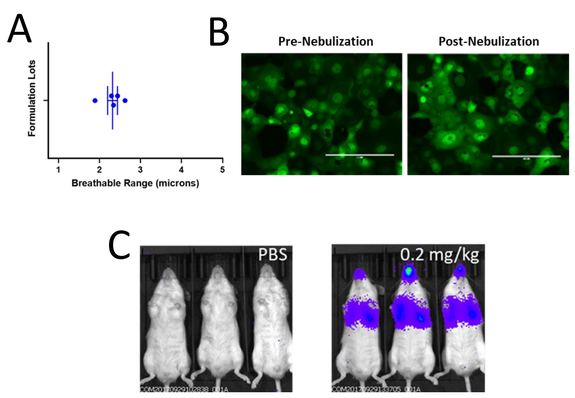
Contact: Carlos G. Perez-Garcia, carlos@arcturusrx.com

LUNAR®-CF OVERVIEW

LUNAR®-CF is an aerosolized mRNA replacement therapy to treat Cystic Fibrosis (CF) Lung Disease, a therapeutic approach agnostic to a patient's genotype. A healthy copy of the human CFTR mRNA is encapsulated into lipid nanoparticles (LUNAR®-hCFTR), aerosolized to patient's airways using a vibrating mesh nebulizer to directly deliver a de novo human CFTR mRNA into epithelial cells. This human CFTR mRNA encodes a fully functional human CFTR protein that will be beneficial to facilitate mucociliary clearance and improve CF lung disease.

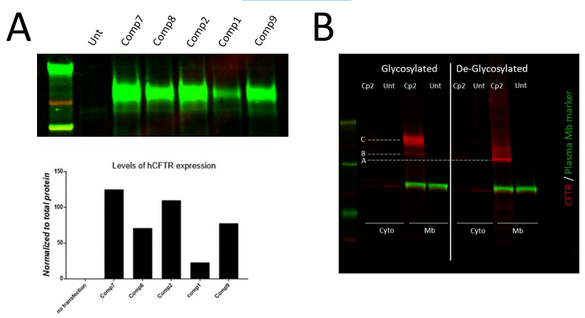
RESULTS

1. Aerosolized LUNAR®, A Delivery Platform for Lung



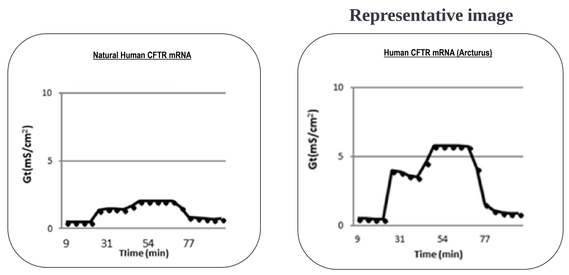
A: Aerosolized LUNAR® droplets (2-3 microns) are highly breathable and optimal for lung delivery. B: To determine if LUNAR® formulations are biologically active as an aerosol, LUNAR®-EGFP mRNA formulations were nebulized, pre- and post-nebulized fractions were collected and used to transduce HBE cells. After a 6h incubation, EGFP expression was visualized under the microscope with similar levels of EGFP expression in both fractions, indicating that the functional properties of LUNAR®-EGFP mRNA were maintained as an aerosol. C: LUNAR®-Luciferase mRNA was nebulized in WT mice and IVIS system was used for imaging. Luminescence was observed in lower and upper airways. PBS controls were negative.

2. Codon-Optimized mRNAs Generate C-Band Glycosylated Plasma Membrane Proteins



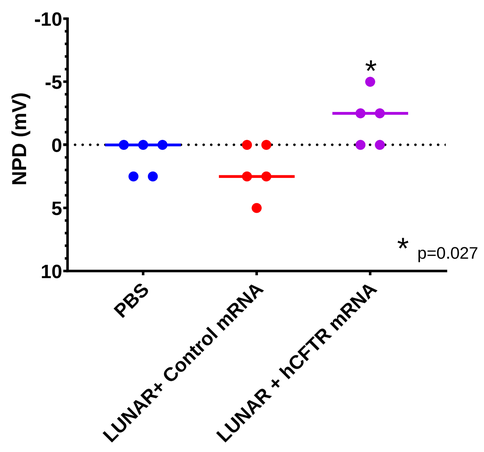
A: WB and quantitation of CFTR C-band expression levels in transfected CFBE cells. B: CFBE cells were transfected with an optimized hCFTR mRNA, followed by fractioning and de-glycosylation. hCFTR expression was only observed in the plasma membrane fraction of transfected cells. C-band transitioning to A-band was observed in the de-glycosylated samples.

3. Codon-Optimized hCFTR mRNAs Show Increased Transepithelial Conductance



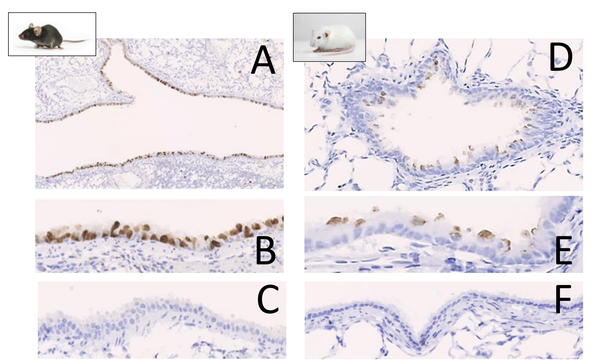
FRT cells were transfected with hCFTR codon-optimized (CO) mRNAs and the transepithelial conductance (Gt) was measured after activation with Forskolin, followed by VX770 and posterior inhibition with Inh-172. Negative controls did not show any activity (not shown) . Natural hCFTR mRNA was active with a Gt ~2mS/cm². CO-hCFTR mRNAs (a representative image is shown) showed a Gt response of ~6mS/cm², a 3-fold increase over the natural sequence, indicating the chloride channel is active and responsive.

4. LUNAR®-hCFTR mRNAs Restore Chloride Channel Activity in a Class I CFTR KO mice



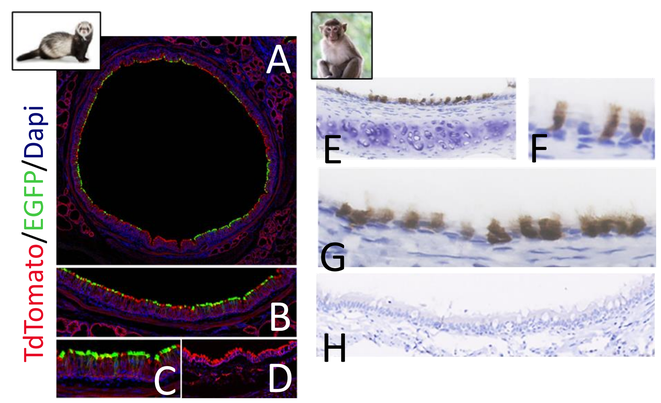
LUNAR®-hCFTR mRNAs were delivered intranasally in a Class I CFTR KO mice at a 1mg/kg dose for two consecutive days. Thirty-six hours post-last dose, Nasal Potential Difference (NPD) was measured by using a Hepes Buffered Ringers (HBR) solution with Amiloride to inhibit ENAC followed by a Chloride-free HBR-Amiloride-Forskolin solution to establish a CFTR gradient and facilitate its secretion into the nasal lumen where hCFTR activity could be registered. The results show that hCFTR mRNA was consistently active considering the variability typical of this assay. Statistical significance was also observed. PBS and a LUNAR®-TdTomato mRNAs were used as negative controls.

5. Efficient Delivery of LUNAR®-TdTomato mRNA to Rodent Epithelial Airways



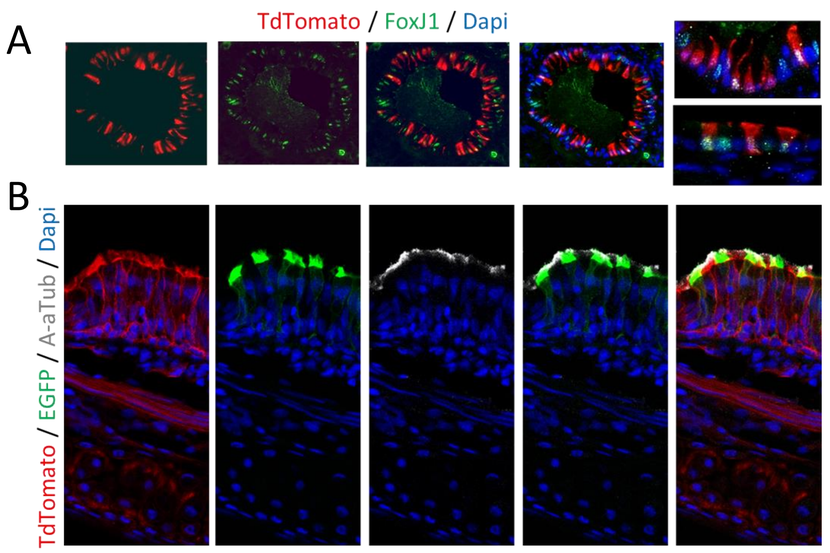
Delivery of LUNAR®-TdTomato mRNA to rodent airways by intratracheal administration of a 1mg/kg dose in mice (A-B) and a nose-only exposure of an aerosolized dose of 0.25 mg/ml in rats (D-E). Animals were sacrificed 24h (A-C) and 48h (D-F) post-dose and lungs were prepared for immunohistochemistry analysis using a TdTomato antibody. Treated animals show expression of TdTomato protein in the epithelial cells in the airways (A-B, D-E). PBS controls (C,F) did not show any staining for TdTomato.

6. LUNAR®-mRNA is Efficiently Delivered to Non-Rodent Epithelial Airways



A-D: The ROSA26TG ferret model constitutively expresses TdTomato (D) in the airways, and upon CRE-mediated recombination EGFP expression is activated. A 0.6 mg/ml dose of LUNAR®-CRE mRNA was delivered using a microsprayer. A robust conversion of the TdTomato into EGFP was observed by immunohistochemistry analysis of lungs (A-C). DAPI was used as counterstaining. E-H: A dose of 1 mg/ml of LUNAR®-TdTomato mRNA was aerosolized and administered to NHP using a face mask exposure system. A robust expression of TdTomato protein was observed by immunohistochemistry analysis of lungs (E-G). Control (H) was negative. Cresyl violet was used as counterstaining.

7. LUNAR® Efficiently Delivers to Ciliated Epithelial Cells in Rodents and Non-Rodents



Delivery of LUNAR®-CRE mRNA to ciliated epithelial cells in rodents (A) and non-rodents (ferrets, B). A ROSA/LoxP transgenic mice (A) and a ROSA26 ferret model were used to administer LUNAR®-CRE mRNA intratracheally (A) or using a microsprayer (B). Efficient recombination is observed by the presence of TdTomato (A) or EGFP (B), which co-express with specific ciliated cell markers: FoxJ1 (A) and Acetylated Alpha-Tubulin (B). Co-localization indicates efficient delivery to ciliated epithelial cells. DAPI was used as counterstaining.

CONCLUSIONS

- Codon-optimization is a feasible approach to develop improved hCFTR sequences with higher protein levels and active chloride channels that might be beneficial for mucociliary clearance in CF-patients' lungs.
- Efficient LUNAR®-mediated delivery of mRNA into ciliated lung epithelial cells has been demonstrated in rodents (mice, rats) and non-rodents (ferrets, NHPs).
- LUNAR® delivery platform is optimal for lung therapeutics such as CF Lung Disease.

ACKNOWLEDGEMENTS

CYSTIC FIBROSIS FOUNDATION ADDING TOMORROWS

We would like to thank CFF for their guidance and funding in support of this program.