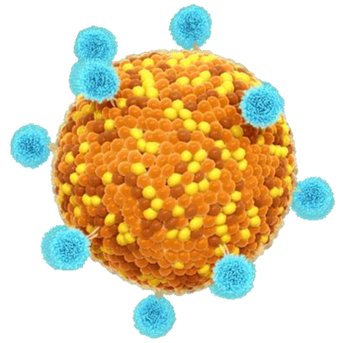
LUNAR® selective delivery of nebulized mRNA into murine lung epithelial cells



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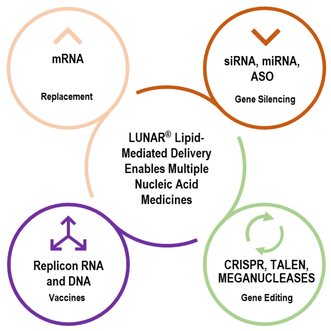
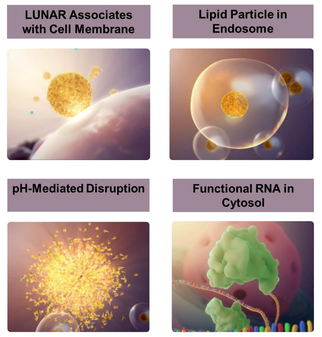
INTRODUCTION

Arcturus Therapeutics is a nucleic acid medicines company focused on developing RNA therapeutics to treat rare diseases. Our proprietary LUNAR® lipid-mediated delivery technology enables the efficient delivery of any mRNA into a variety of cell types and tissues, and can be optimized for multiple routes of administration.



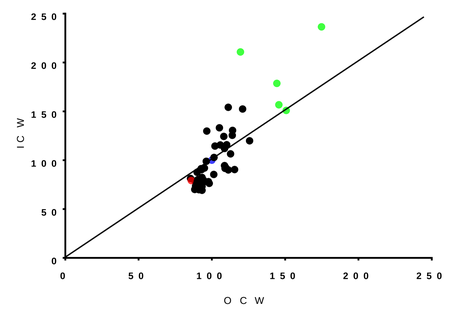
LUNAR® lipid nanoparticles carrying the mRNA payload reaches the target cell, where it fuses with the plasma membrane forming an intracellular endosome. This endosomic particle undergoes a pH-mediated disruption that causes the breakdown of the biodegradable nanoparticle and the delivery of the mRNA into the cytoplasm. The mRNA follows natural translational and post-translational routes to generate the protein of interest.



LUNAR®-CF is a CFTR mRNA replacement therapy to treat patients independent of genotype. Novel codon optimized sequences were generated and different LUNAR® formulations were screened to specifically target lung epithelial cells by a nebulization approach. Proof-of-concept generated for Arcturus' LUNAR®-CF.

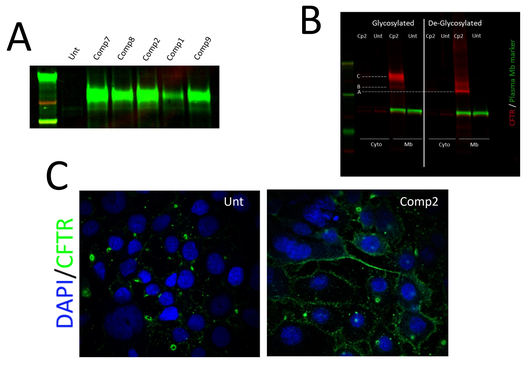
RESULTS

1. Codon-optimized sequences have an impact on expression levels



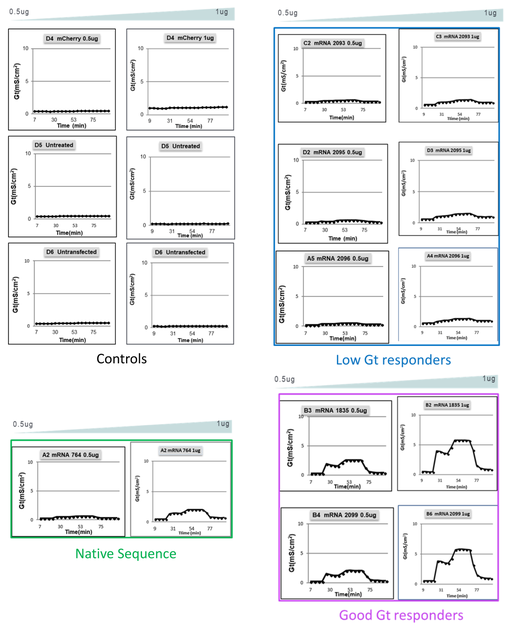
Codon-optimized sequences were designed based on the human natural CFTR sequence. mRNAs were made by IVT and then transfected into CFBE cells. 24h post-transfection, expression levels were determined by In/On-Cell Western (ICW, OCW) using a human CFTR antibody. Correlation between both assays was plotted. Compounds were rank ordered based on their expression profile. Highest expressers, native sequence and baseline are in green, blue and red dots, respectively.

2. Codon-optimized mRNAs generate C-band glycosylated plasma membrane proteins



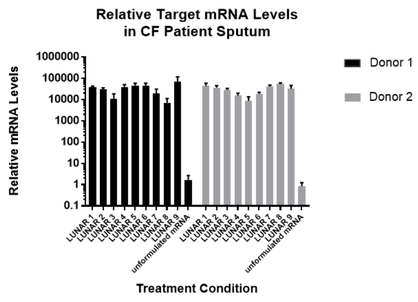
A: WB showing CFTR C-band expression in transfected CFBE cells. B: CFBE cells were transfected with an optimized CFTR mRNA, followed by fractioning and de-glycosylation. CFTR expression was only observed in the plasma membrane fraction of transfected cells. C-band transitioning to A-band was observed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

3. Selected C-band based compounds show variable transepithelial conductance (Gt)



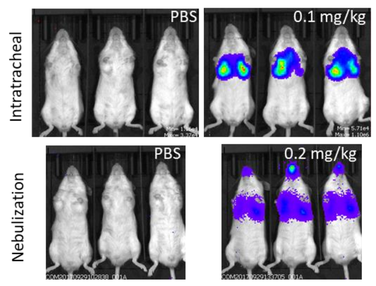
FRT cells were transfected with CFTR codon-optimized mRNAs and the transepithelial conductance (Gt) was measured after activation with Forskolin (Fsk), followed by VX770 and posterior inhibition with Inh-172. Negative controls did not shown any activity (Controls). A low Gt response to Fsk and VX770 was observed in a subset of compounds (Low Gt responders). Native sequence had a good response with a Gt ~2mS/cm^2. A subset of mRNAs showed a Gt response of ~6mS/cm^2, a 3-fold increase over the native sequence, indicating the chloride channel is active and responsive (Good Gt responders).

4. LUNAR® formulations shield and protect the mRNA in CF sputums



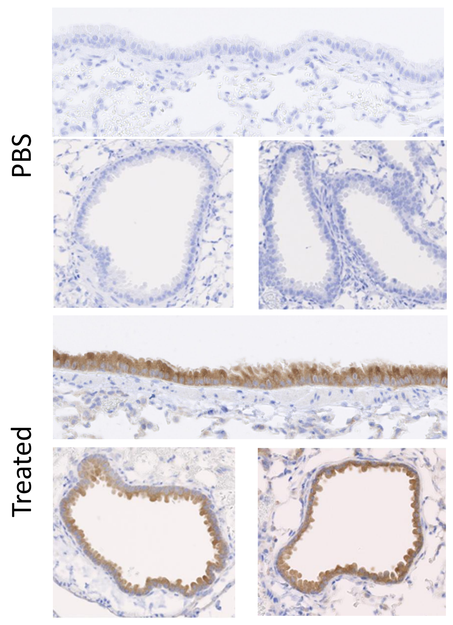
Relative mRNA quantitation of LUNAR® encapsulated mRNA in CF sputums. Unformulated mRNA was used as a control. Samples were incubated for 24h and qPCR was used to assess mRNA levels. All the LUNAR® formulations shield the mRNA and protect it from degradation in both CF sputums. Unformulated mRNA was degraded.

5. LUNAR® is distributed in upper/lower airways



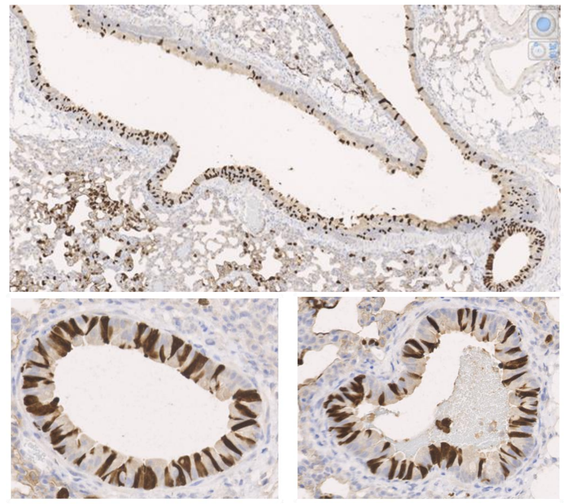
Functional delivery of luciferase mRNA in both lower and upper airways. LUNAR® formulations were prepared with luciferase mRNA and delivered intratracheally or nebulized using the Aerogen Solo device in WT mice. IVIS system was used for imaging.

6. LUNAR®-eGFP delivered in epithelial airways



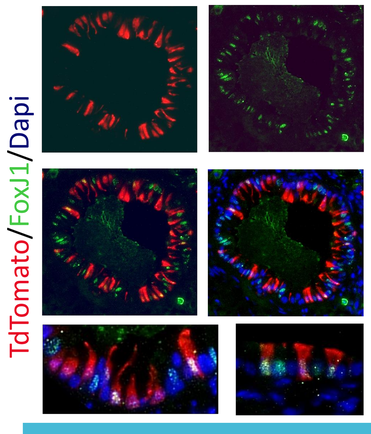
LUNAR® delivery of a reporter mRNA into murine lung epithelial cells. Animals were dosed intratracheally at 0.4 mg/kg with optimized LUNAR® formulations carrying an eGFP mRNA. Control animals were treated with PBS. Animals were sacrificed 24h later and lungs were processed for histology. Paraffin sections were prepared and stained for eGFP and counterstained with Hematoxylin. Top panel, PBS treated mice lacking eGFP immunostaining. Bottom panel, LUNAR®-eGFP treated animals immunostained for eGFP, with moderate-to-high eGFP staining in the epithelium from upper and lower airways. No other lung structure was positive for eGFP.

7. TdTomato is selectively detected in epithelial airways after LUNAR®-Cre delivery



Molecular proof-of-concept showing efficient delivery of LUNAR® formulations into epithelial airways using a Flox-TdTomato transgenic mouse model. Animals were dosed intratracheally at 0.4 mg/kg with LUNAR® formulations carrying Cre-mRNA. At 24h post-dose, animals were sacrificed and lungs processed for histology with a TdTomato antibody. Immunostaining indicates a selectivity in the cellular population expressing TdTomato in the epithelial airways.

8. LUNAR® efficiently targets airway epithelial cells



TdTomato expression after intratracheal delivery of LUNAR®-Cre in a Flox-TdTomato mouse model. FoxJ1 is used to co-localize with ciliated lung epithelial cells in the airways. Dapi is used as counterstaining. Detailed co-localization between TdTomato and FoxJ1 is observed in the high power views.

CONCLUSIONS

- Codon-optimization is a feasible approach to develop improved CFTR sequences with higher protein levels and active chloride channels.
- C-band expression does not directly correlate with an active chloride channel.
- LUNAR® is compatible with nebulization and shields the mRNA from degradation in CF sputums.
- Efficient LUNAR®-mediated delivery of mRNA into ciliated lung epithelial cells.

ACKNOWLEDGEMENTS

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