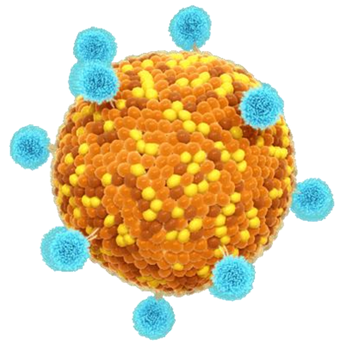
LUNAR-CF, a mRNA Replacement Therapy for Cystic Fibrosis

Carlos G. Perez-Garcia, Jerel Vega, Samantha Murphy, Thanhchau Dam, Mike Cuddy, Carina Wimer, Mike Matsumoto, Jose A. Gonzalez, Marciano Sablad, Daiki Matsuda, Rajesh Mukthavaram, Priya Karmali, Amita P. Thakerar, Ella Meleshkevitch, Robert J. Bridges, Pad Chivukula, Christine Esau

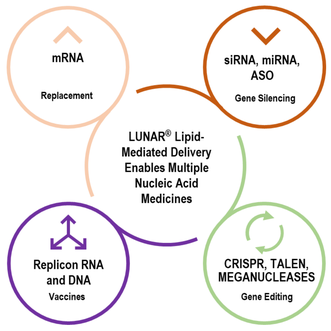
Arcturus Therapeutics, Inc., San Diego, CA, USA

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

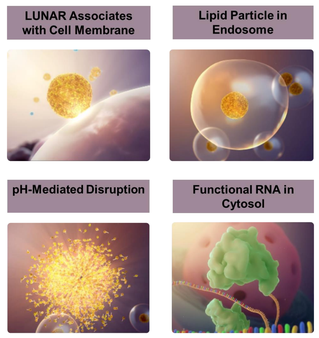


INTRODUCTION

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.



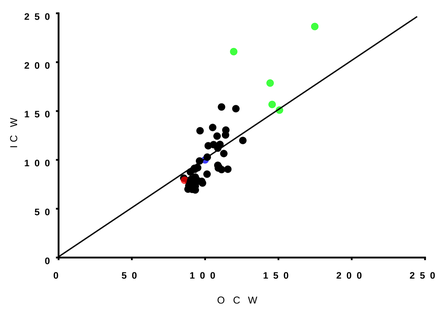
LUNAR® lipid nanoparticle carrying the mRNA payload reaches the target cell, where it fuses with the plasma membrane forming an intracellular endosome. This endosomic particle undergoes a pH-mediated disruption that causes the breakdown of the biodegradable nanoparticle and the delivery of the mRNA into the cytoplasm. Therefore, 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 any 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 were generated for Arcturus' LUNAR-CF program.

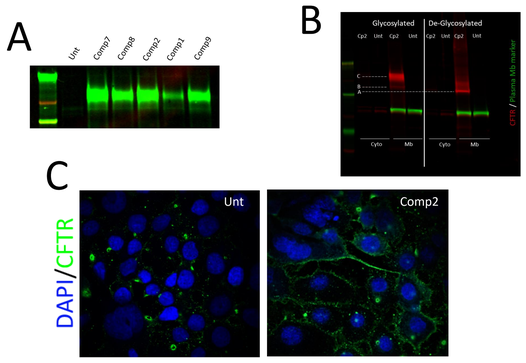
RESULTS

Codon-optimized sequences have an impact on expression levels



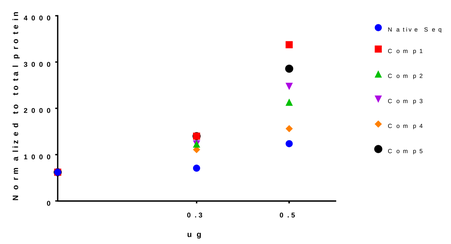
Codon-optimized sequences were designed based on the human natural CFTR sequence. mRNAs were made by IVT and then transfected into CFBE cells. 24h post-transfection, expression levels were determined by ICW and OCW using a CFTR antibody. Correlation between both assays was plotted. Compounds were rank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

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



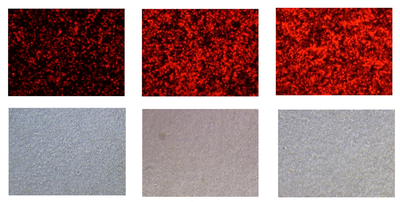
A: WB showing CFTR C-band expression in transfected CFBE cells. Different compounds show different levels of C-band whereas un-transfected was negative. B: CFBE cells were transfected with an optimized CFTR mRNA, followed by fractioning and de-glycosylation. CFTR expression was only observed in the plasma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

Lead compounds can be rank ordered based on C-band expression in FRT transfected cells



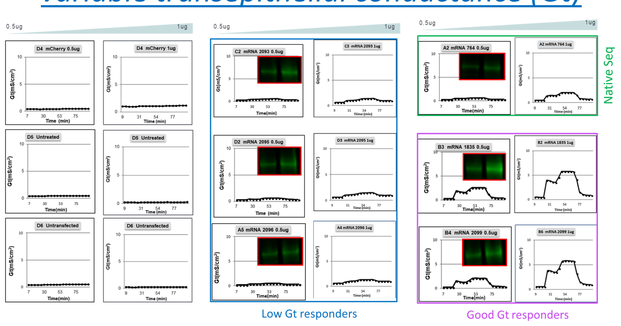
Dose response of selected mRNAs transfected in FRT cells. C-band was measured by WB using a CFTR antibody. At 0.5ug/well, we can rank order compounds based on C-expression levels.

Transfection efficiency is dose dependent in transfected FRT cells



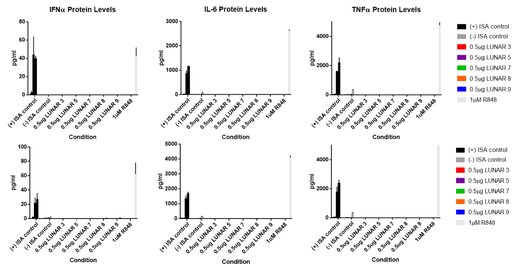
Control mCherry shows a dose dependent increase in expression. mCherry mRNA was used as a transfection control during the functional activity assays done in FRT cells (below).

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



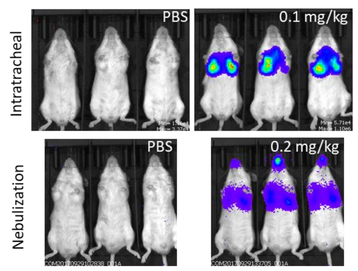
FRT cells were transfected with CFTR codon-optimized mRNAs and the transepithelial conductance (Gt) was measured after activation with Forskolin, followed by VX770 and posterior inhibition with Inh-172. Negative controls did not shown any activity (left panel). Some of the CFTR mRNAs showed low Gt indicating they are not responding properly to the activation (middle panel). Native sequence had a good response with a Gt ~2mS/cm² (right panel, top). Some of the compounds show good Gt response of ~6mS/cm² indicating the chloride channel is active and responsive (right panel, bottom).

Minimal Immunostimulatory activity



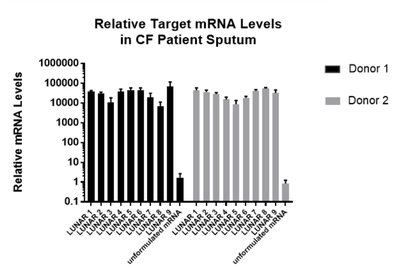
No detectable levels of IFNα, IL-6, or TNFα observed in human PBMCs following LUNAR-mRNA treatment. Fresh PBMCs were isolated from 2 donors, and cells were then treated with 0.5µg LUNAR-mRNA and incubated at 37° C. 24h post-treatment, supernatants were collected and analyzed for cytokine expression levels.

LUNAR® is distributed in upper/lower airways



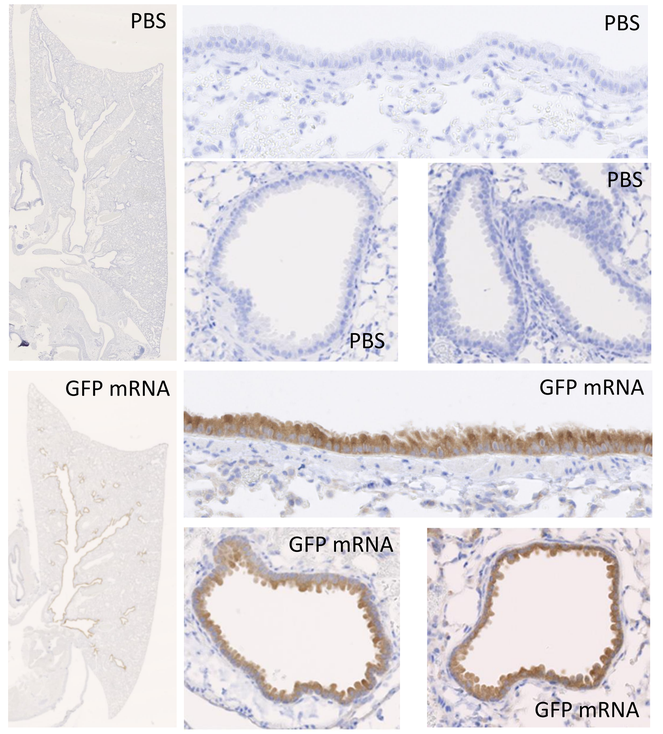
Functional delivery of luciferase mRNA in both lower and upper airways has been demonstrated with LUNAR®. LUNAR® formulations were prepared with luciferase mRNA and delivered intratracheally or nebulized using the Aerogen Solo device in WT mice. IVIS system was used for imaging.

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.

LUNAR®-GFP delivered in epithelial airways



Efficacy studies to determine LUNAR® delivery of a reporter mRNA into murine lung epithelial cells. Animals were dosed intratracheally at 0.1 mg/kg and 0.4 mg/kg with optimized LUNAR® formulations carrying a GFP mRNA. Control animals were treated with PBS. Animals were sacrificed 24h later and lungs were taken down and processed for histology. Paraffin sections were prepared and stained for GFP and counterstained with Hematoxylin. Sections were analyzed by an independent histopathologist. Top panel shows PBS treated mice lacking of any GFP immunostaining. Bottom panel shows a selection of LUNAR-GFP treated animals immunostained for GFP. Histopathological analysis indicated that GFP is present in the upper and lower airways, with moderate-to-high staining in the epithelial cells throughout the trachea, bronchi and bronchioles. No other lung structure was positive for GFP.

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 a reporter mRNA into lung epithelial cells

ACKNOWLEDGEMENTS

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