LUNAR-CF, a mRNA Replacement Therapy for Cystic Fibrosis
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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 nanoparticle carrying the mRNA payload reaches the target cell, where it fuses with the plasma membrane forming an intracellular endosome. This endosome 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

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)

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

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