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

LUNAR®-OTC is Arcturus’ human OTC mRNA-mediated enzyme replacement therapy to treat patients suffering from ornithine transcarbamylase deficiency (OTCD). OTCD is a rare metabolic, Urea cycle disease in which the enzyme OTC does not convert Ornithine to Citrulline efficiently.

**Conventional Potency Assay for Protein Biologic May Not Apply to mRNA Therapeutic**

- mRNA drug substance is not the ultimate product in vivo as mRNA has to be converted to protein
- Activity of the enzyme may not linearly correlate with mRNA transfected
- Plateau does not represent maximum levels of activity of enzyme expressed in cells but rather a combination of factors – substrate depletion, enzyme inactivity, product inhibition and other non-physiological mechanisms during the reaction

**Novel ways to Measure Potency of mRNA Therapeutic Drug Substance**

**Different assays for different stages of development**

- Potency for mRNA therapy defined as follows:
  1. Expressivity of the protein (Cell-Free Translation)
  2. Stability of expressed protein (In Cell Translation)
  3. Activity of expressed protein (Cell based activity)

**Cell-free Expressivity**

- Cell-Free Translation system as a Potency test for Expressivity
- Identify Conditions where the protein expression is linearly dependent on mRNA concentration

**Cell-based Expressivity**

- In vitro protein expression determined by Western blotting
- Cellular protein expression was evaluated by transfecting immortalized human cell line with OTC mRNA
- Degree of expression was linearly dependent on amount of mRNA transfected

**Enzyme Activity**

- Potency determined by measuring initial velocity in a steady-state experimental set up
- Cellular protein expression was evaluated by transfecting immortalized human cell line with OTC mRNA
- Degree of expression was linearly dependent on amount of mRNA transfected

**Conclusions**

- mRNA Therapeutics require development of potency assays early during the pre-clinical stage
- Conventional potency methods for protein biologic may not apply directly to mRNA therapeutic drug substance
- Cell-based potency assays have to be carefully evaluated to make sure that the read-outs correspond to the actual potency of the drug substance and not an artifactual value
- mRNAs can be evaluated for potency at level of protein expression (cell-free), cellular protein expression/stability and enzyme activity in vitro