

Manufacturing and Stability Testing of a GNE-Lipoplex for a Clinical Trial for the Treatment of Hereditary Inclusion Body Myopathy Type (HIBM)

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Hereditary Inclusion Body Myopathy Type (HIBM) is a rare muscle disease related to familial transmission of a mutation of the GNE gene, which encodes the bifunctional enzyme UDP-GlcNAc 2-Epimerase / ManNAc Kinase (GNE/MNK). This is the rate limiting bifunctional enzyme that catalyzes the first 2 steps of sialic acid biosynthesis. Decreased sialic acid production consequently leads to decreased sialylation of a variety of glycoproteins. We have created a GNE-Lipoplex comprised of a DOTAP:Cholesterol BIV (bilamellar invaginated vesicle) liposomal delivery vehicle and a plasmid-based GNE vector payload. The BIV liposomes are manufactured according to the publication from Templeton et al (Nature Biotech, 1997). The GNE-plasmid was validated in our publication (Jay, et al. Gene Regs and System Biol. 2008). The plasmid DNA is spontaneously encapsulated into the BIV liposome upon mixing. The final concentration of DNA is 0.5ug/ul. Argon gas is layered onto the liquid product before the vial is sealed. The final product (GNE-Lipoplex) is stored at 2-8°C and individual vials were analyzed at different time points for to assess the stability of the product. Testing included optical analysis (optical density, particle size, and zeta potential), extraction, quantitation, and quality analysis of the plasmid DNA, and expression analysis of the GNE-Lipoplex (transient transfection followed by RT-qPCR analysis). Third party USP-Sterility and LAL-Endotoxin testing indicate no biological growth and endotoxin levels <0.5 EU/ml. In-house test results indicate that the GNE-Lipoplex is stable up to 1 year, with no indication of changes in the optical characteristics of the complex, or changes in the physical characteristics of the plasmid DNA. Additional tests are under investigation to improve the characterization and stability testing of the GNE-Lipoplex.