**Evaluating lipid-based delivery of dsDNA agonists for activation of cGAS-STING pathway**

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**Introduction:** In eukaryotes, DNA is primarily found in the nucleus and mitochondria. The presence of cytosolic DNA is typically a sign of internal dysregulation or the presence of pathogens.[1] The cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway is responsible for detecting cytosolic DNA and eliciting an innate immune response to protect the cell.[2] Activation of this pathway plays an important role in cancer immunotherapies.[3] To activate this pathway, exogenous double-stranded DNA (dsDNA) must be delivered into the cytosol of a cell to stimulate cGAS. When cGAS detects dsDNA, cGAMP is produced, which activates STING. In response, type I interferons are produced, eliciting an immune response, such as dendritic cell maturation, which can lead to more adaptive immune cell activity, and overcoming an immunosuppressive tumor environment.[4] This study looks to use lipid-based nanoparticles (LBNPs) encapsulated with a dsDNA agonist to safely and effectively stimulate the cGAS-STING pathway.

**Figure 1.** Encapsulation efficiency of G3YSD in each LBNP determined via Quant-iT RiboGreen RNA assay.

**Methods:** FDA-approved lipid formulations (Dlin-MC3-DMA, SM-102, and ALC-0315) were combined with 18:0 DSPC, plant cholesterol, and DMG-PEG-2000 via pipette mixing. G3-YSD was prepared in a sodium acetate solution and encapsulated within each LNP. Encapsulation efficiency was determined via RiboGreen RNA assay. G3-YSD-LNPs were incubated in THP-1 dual reporter cell line for 16 hours before evaluating interferon (IFN) and nuclear factor-κB (NF-κB) induction using Quanti-Luc and Quanti-Blue reagents. Cell viability will also be assessed using Calcein-AM.

**Results:** Each LNP formed monodisperse nanoparticles that successfully encapsulated G3-YSD. After 16 hours of incubation, there were no signs of cytotoxicity. The only experimental group that produced interferons was the commercially available LyoVec transfection reagent. LNPs can reliably be synthesized both on a small-scale using hand-mixing and on a large scale using a microfluidics system.

**Conclusions:** LyoVec proved to be the best at activating the cGAS-STING pathway in vitro. LNP formulations can successfully encapsulate nucleic acid cargo, however, more research needs to be done to investigate the internalization mechanisms of these nanoparticles to better design methods to successfully deliver the cargo.

**References:**

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