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Lipid nanoparticle formulations optimized for delivery and *in vivo* gene editing using novel synthetic RNA guided nucleases (sRGN)

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Lipid nanoparticles (LNPs) are a robust and effective technology for delivering nucleic acids to the liver, including multi-component systems incorporating both mRNA and gRNA for gene editing. However, typical mRNAs used for Cas9 expression are large and complex RNA payloads. A separate abstract details the engineering of a set of novel synthetic RNA Guided Nucleases (sRGN). When formulated as mRNA into LNPs, we report here the improved performance of sRGN compared to the widely used SpCas9 genome editing endonuclease. We believe that the small size (~3.5 kb) of sRGN mRNA is responsible for the observed packaging advantage into LNPs compared to SpCas9 mRNA (~4.4 kb). When evaluated *in vivo* in rodent, LNPs harboring alternative sRGNs each showed comparable or higher editing efficiency to SpCas9-LNPs—even at equimolar doses. Toward understanding the mechanism for these LNP delivery improvements, cryoTEM analysis of sRGN-LNPs gives evidence for improved LNP morphology and physicochemical characteristics, which suggest a better quality LNP formulation with these smaller payloads. Interestingly, sRGN-LNPs also showed enhanced particle stability compared to SpCas9-LNPs; sRGN-LNPs were less prone to aggregation over time and retained *in vivo* potency significantly longer than SpCas9-LNPs. Over the same storage conditions, a ~70% drop in editing efficiency was observed with SpCas9-LNPs whereas a potency drop as small as ~20% was observed with sRGN-LNPs. Overall, these novel sRGN-LNPs represent a promising alternative to known CRISPR/Cas delivery systems showing initial favorable *in vivo* gene editing performance and enhanced particle stability.