Ethan P. Hoasjoe
L2 Medical Student
LSU Health Sciences Center, New Orleans, LA

Yawen Hu Ph.D. & Guoshun Wang DVM, Ph.D.
Department of Immunology, Microbiology and Parasitology, LSU Health Sciences Center

"Lipid Nanoparticles for Gene Editing to Correct Cystic Fibrosis"

Introduction: Lipid nanoparticles (LNPs) have emerged as a novel vehicle to deliver gene editing machinery to correct diseased genome. This development is ushering in a new era in medical treatment. Traditionally, gene editing is achieved through physical or viral delivery methods, but the limitations come in less efficient and quick degradation. LNPs optimize safety and delivery through being composed of natural components which means lower risk in causing unwanted immunogenicity or mutation; additionally, LNPs can be modified substantially to cause a more specific target location. Our group has rationalized to apply the LNP technology to the genetic condition of cystic fibrosis.

Methods: Our formulations for lipid nanoparticles came from utilization of multiple gangliosides, natural lipid components, that can be varied with the other components of an ionizable lipid, cholesterol, amphipathic phospholipid, and a polyethylene-glycol component. We have characterized 4 different compositions that target 2 different tissues: hemopoietic stem cells and epithelium. To test the application, we have bred tdTomato mice, in which the red fluorescent protein expression requires Cre recombinase (Cre) to cut out the stop codon before the gene. For in-vitro experiments, we extracted the bone marrow cells from the mouse tibia and femur. LNPs packed with the Cre mRNA were added to the cells and cultured for 24 and 48 hours. Then fluorescent cell percentages were determined via flow cytometry. For in vivo experiments, the formulated LNPs packed with the Cre mRNA were injected into the tdTomato mice via I.V. administration. Blood leukocytes were examined for fluorescence. Furthermore, we are currently designing a gene editing to correct the *G542X* CFTR mutation in mice, which will prove the feasibility of this approach for CF genetic therapy.

Preliminary Results: There seems to be a correlational benefit to the use of gangliosides in LNP form. The composition including GM3 ganglioside at a concentration of 0.5% of total lipid nanoparticle composition yielded 0.68% expression of tdTomato in live bone marrow cells. When lowering the dosage of GM3 to 0.25% of total lipid nanoparticle composition, the results showed 0.028% of tdTomato expression in the live cells. These formulations with different concentrations of GM3 are now being tested in vivo for mRNA delivery.

Discussion: Ideally, LNPs present a new horizon concerning how we deliver gene-editing therapies. Confirmation of the devised compositions will confirm the experiments to move to the in vivo phase which can present more details about the intracellular effects as a therapy. In a more longitudinal aspect, the group hopes to develop a prime editing mechanism for application to correct *G542X* for CF, a premature termination mutation to which there is no effective modulator therapy. From the preliminary confirmations of our group's LNP compositions, we decided to move ahead with in-vivo experimentation on neonatal and adult mice with the inclusion of GT1b ganglioside with the retained composition We are still awaiting these results.