A Novel Library of Ionizable Cationic Lipid Nanoparticle-mediated mRNA COVID-19 Vaccines Enables Long-term Storage

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Statement of Purpose: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has led to a worldwide pandemic disastrously impacting public health and the global economy. The two novel and authorized mRNA-based lipid nanoparticle (LNP) vaccines from Pfizer-BioNTech and Moderna were shown to be highly effective against SARS-CoV-2. However, the storage and transportation of these vaccines require ultracold conditions up to -80°C, which drastically reduces viable shipping methods and exponentially increases the cost of transport and storage. Therefore, developing a novel LNP-mediated mRNA vaccine with feasible storage condition and capable of long-term storage is of great interest. Here, we developed a novel library of ionizable cationic lipid nanoparticle, the best ionizable cationic lipid candidate (CLC) was screened from the material libraries and named CLC-003. The CLC-003 was further formulated with helper lipids and tuning the molecular ratios by orthogonal experimental design to create the novel and optimized formulation of lipid nanoparticle (LNP3) for mRNA encapsulation and delivery in vitro and in vivo and carry out the COVID-19 vaccination and long-term storage studies.

Methods: The ionizable cationic lipids were synthesized by enzyme-assist reactions and purified by silica gel column. High-throughput screening was performed in vitro to determine the best ionizable cationic lipid candidate following an orthogonal array experimental design which aimed to screen the optimal molar ratio of the lipids for CLC-003. After identifying the optimized formulation in vitro, the LNP3 were synthesized by a microfluidic chip device with firefly luciferase encoded mRNA for in vivo studies. Meanwhile, the LNP3 were formulated with different concentration of cytoprotectant to evaluate the long-term storage capabilities. After a comprehensive evaluation of LNP3 using a reporter gene in vitro and in vivo, we encapsulated the spike protein encoded mRNA into our newly developed LNP3 via microfluidic device to obtain COVID-19 vaccines which maintained function for at least 3 months storage at -20°C after testing. The mice (n=5) of each group were injected with the LNP3 on a prime-boost manner and the blood and tissue samples were tested accordingly.

Materials: All the materials used for synthesis were purchased from Sigma-Aldrich. The mRNAs were obtained from TriLink Biotechnologies and System Biosciences. All other general reagents and kits were all commercially available.

Results: Through the orthogonal design, the best formulation of LNP3 was determined, which exhibited much higher transfection efficacy than FDA approved MC3 LNPs. Notably, the bioluminescence generated by both fresh and 3-month stored luciferase-mRNA encapsulating LNP3 reached ~$2.4*10^8$ p/s in vivo after intramuscular injection. Immunization with LNP3 mRNA COVID-19 vaccines in BALB/c mice elicited anti-spike antibodies as well as SARS-CoV-2 spike neutralizing antibodies after the priming dose and significantly increased antibody titers after a booster vaccination in a dose-dependent manner with GMTs (geometric mean titers) significantly higher than the placebo vaccinated group. Furthermore, LNP3 COVID-19 vaccines induced CD4+ and CD8+ T cell responses after ex vivo stimulation. The intracellular cytokine staining study detected the secretion of IFN-γ, TNF-α and IL-2, but not IL-4, demonstrating that the LNP3 COVID-19 vaccines successfully induces a Th1-biased SARS-CoV-2 spike-specific immune response in vaccinated mice after 3-month storage at -20°C.

Conclusions: We developed a series of novel ionizable cationic lipids and identified a lipid nanoparticle formulation LNP3, which shows great mRNA transfection efficacy in vitro and in vivo after adjusting the molar ratios of lipids through orthogonal design. The LNP3 was further formulated with spike protein encoded mRNA to develop the COVID-19 vaccines. The LNP3 mRNA COVID-19 vaccines not only generated spike protein expression in vitro but also successfully elicits spike-specific antibodies and Th1-biased T cell immune response in vivo after 3-month storage at -20°C which provide a potential solution to solve current issues of vaccine storage and transportation.

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Conflict of interest disclosure: X.X, and Z.L. are inventors in a patent application that has been filed based on the materials in this abstract. The other authors declare no competing interests.