# Lipid Nanoparticle Composition Shapes Immune Response to mRNA Vaccine and Potency of Anticancer Immunity

Yining Zhu[a,b] , Jingyao Ma[b,c], Ruochen Shen[a,b], Ivan Vuong[a,b] and Hai-Quan Mao[a,b,d,e,*]
[a]Department of Biomedical Engineering, [b]Institute for NanoBioTechnology,
[c]Department of Chemical and Biomolecular Engineering, [d]Department of Materials Science and Engineering,
[e]Translational Tissue Engineering Center, Johns Hopkins University and Medicine, Baltimore, MD

**Statement of Purpose:** Lipid nanoparticles (LNPs) have been successfully designed as immunostimulatory delivery platforms for antigen-encoding mRNA, as exemplified by the LNP-based COVID-19 vaccines.[1-2] However, there is a lack of systematic investigation into the effect of LNP composition on properties and efficacy of the immune responses elicited by mRNA LNP vaccines. Here, we developed a multi-step screening method to optimize the type of helper lipid and component ratios in an LNP formulation to efficiently deliver antigen-encoding mRNA to antigen presenting cells for vaccine-based cancer immunotherapy. By screening *in vitro* transfection activity, dendritic cell (DC) maturation and antigen presentation, and *in vivo* for immune activation and suppression of tumor growth, LNP formulations with potent antitumor efficacy are investigated as delivery systems for anti-cancer mRNA vaccines.

**Methods:** To generate a 1,080 formulation LNP library, we used DLin-MC3-DMA as the ionizable lipid, cholesterol, DMG-PEG2000, and one of six helper phospholipids (DOTAP, DDAB, DOPE, DSPC, 14PA, and 18PG) that were previously used in FDA-approved or experimental LNP formulations. By varying the following parameters: (1) combined molar percentage of DLin-MC3-DMA and helper lipid ranging from 20% to 80%; (2) weight ratio of cholesterol to DMG-PEG2000 ranging from 10 to 500; (3) weight ratio of DLin-MC3-DMA to helper lipid ranging from 1 to 200; and (4) the molar ratio of chargeable groups in ionizable lipid to phosphate groups in pDNA (N/P ratio) ranging from 4 to 12, a series of LNP formulations were generated using a luciferase reporter mRNA to screen *in vitro* for transfection efficiency in DCs. Selected LNP formulations were then loaded with ovalbumin (OVA) mRNA to assess the ability of antigen presentation, and induction of co-stimulatory molecule expression by DCs. LNPs with the highest DC transfection, antigen presentation, and maturation were further tested *in vivo* for transport to the draining lymph nodes (dLNs) via subcutaneous (s.c.) injection. LNPs showing effective transfection in dLN cells were administered s.c. into C57BL/6J mice to evaluate the immune responses and anticancer efficacy in prophylactic and therapeutic treatment models of melanoma.

**Results:** Based on the initial library screening, top-performing LNP formulations exhibited higher *in vitro* transfection efficiency in DC2.4 cells were tested in freshly isolated bone marrow-derived DCs (BMDCs) for antigen presentation and maturation analyses (Fig. 1A). The antigen presentation and maturation levels of APCs in dLNs were examined after s.c. injection of selected LNPs (C10, D6, and F5) (Fig. 1B). These three LNPs showed higher SIINFEKL-H-2Kb+ DC levels in dLNs and were tested for their vaccination efficiency. A significantly higher numbers of Th1 cells (CD4+IFN-γ+) were observed for all three formulations (Fig. 1C), though C10-treated group also showed the highest Th2 response along with a high OVA-specific IgG titer (Figure 1D-E). All three formulations are then tested on B16-OVA model and showed strong tumor growth inhibition efficacy with a prolonged overall survival time (Figure 1F). Furthermore, compared to D6 and F5, which generated strong Th1 response only, C10 LNPs, triggering both Th1 and Th2 responses, yielded a markedly improved protection effect.

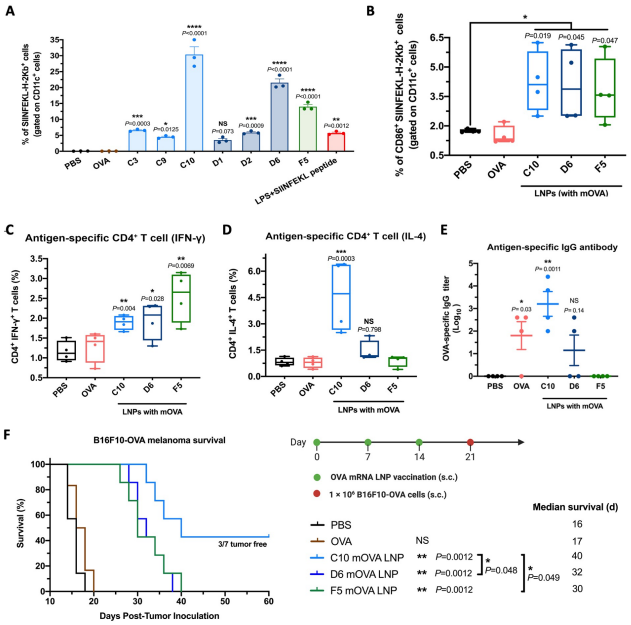


**Figure 1.** (A-B) SIINFEKL-MHC-I presentation by DCs treated with OVA mRNA LNPs *in vitro* (A) or *in vivo* (B). (C-E) Intracellular staining for IFN-γ (C) and IL-4 (D) in CD4+ T cells, and OVA antibody (E) after vaccinated with OVA mRNA LNPs. (F) Mouse survival following a prophylactic vaccination model for OVA-B16-F10 melanoma in C57BL/6 mice (n = 7; 10 μg mOVA per injection).

**Conclusion:**
This LNP screening platform allows for identification of the best-performing mRNA LNPs for APC-specific gene expression, and tuning Th1/Th2 skewed immune response. Among the top candidates, C10 elicits potent Th1 and Th2 responses and mediates most potent antitumor efficacy compared to formulations with only Th1-skewed response.

**References**

1. Oberli, MA, *et al.* **Nano Lett.,** 2017; 17(3): 1326-35.
2. Miao, L, *et al.* **Nat. Biotechnol.,** 2019; 37(10):1185.