A novel delivery system to enhance the immune response and shelf-life stability of mRNA vaccines

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BACKGROUND

Since the COVID-19 pandemic, mRNA vaccines have become the preferred vaccine platform for developing new vaccines due to its rapid and adaptable manufacturing processes. However, the platform has several limitations, including ultra-cold chain storage, waning immune responses, high manufacturing costs, and systemic distribution / side effects. While mRNA vaccines encapsulated in lipid nanoparticles (LNP) have been described as a self-adjuvanting platform, the addition of an adjuvant could significantly improve the vaccine's efficacy, stability, and increase duration of protection.

VaxForm is investigating using aluminum adjuvants as a new delivery vehice and adjuvant for mRNA LNP vaccines. In addition to increasing the vaccine's efficacy and length of protection, adding aluminum adjuvant has the potential to reduce side effects, enhance stability, and to our knowledge, has not been investigated.

Aluminum adjuvants enhance immune responses through several mechanisms. One key mechanism is known as the "depot effect," in which antigens accumulate on the aluminum particles, enabling extended interactions between the antigens and immune cells, leading to stronger immune responses. Aluminum adjuvants increase the uptake of antigens by facilitating phagocytosis. They induce the production of cytokines, promoting the differentiation of naive CD4+ T cells into Th2 cells and enhancing antibody production. In addition to improving vaccines' immune responses to antigens, aluminum adjuvants reduce the prevalence and severity of systemic adverse reactions by binding and slowly releasing molecules thereby reducing toxicity.

In this study, VaxForm used mRNA LNP vaccine expressing Green Fluorescent Protein (GFP) and commercially available aluminum adjuvants to demonstrate proof-of-concept. Formulation experiments showed better adsorption of LNP to aluminum phosphate (AdjuPhos) compared with aluminum hydroxide (Alhydrogel). When the formulations were evaluated for potency in HepG2 cell transfection assay, aluminum adjuvanted mRNA LNP elicited up to two-fold increase in GFP expression compared with mRNA LNP alone and suggested depot effect mechanisms. The addition of aluminum adjuvant to mRNA LNP broadened and magnified the T cell response by inducing secretion of Th1 and Th2 cytokines in human PBMCs. Formulations with aluminum adjuvants also showed better stability: 100% transfection efficacy was retained after 4 months of storage at 4°C while baseline formulations without aluminum adjuvant only retained 40%.

The enhanced potency has the potential to reduce the dose needed per immunization (dose-sparing). Dose sparing will allow filling of more final drug product vials for per mRNA batch produced and therefore improve global access during epidemic or pandemic. The reduced dose and specific targeting of immune cells with the aluminum adjuvant will reduce systemic adverse reactions and could help improve patient compliance. These preliminary data show great potential to improve mRNA vaccine platform and will further be evaluated *in vivo*.

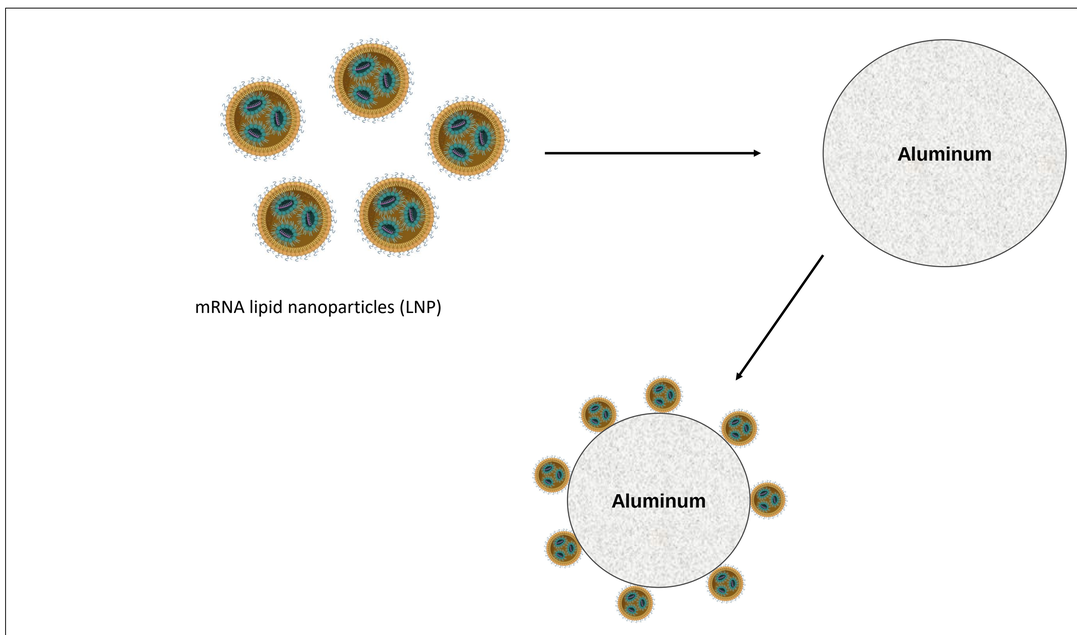


Figure 1. Concept of formulating mRNA LNP with aluminum adjuvant. LNPs (top left, about 100 nm in size) are mixed with aluminum adjuvant (top right, particles 1-3 µm in size). LNPs adsorb to the aluminum adjuvant (bottom image).

RESULTS

	Zeta potential (mV)		Adsorption	
	AlOOH	AlPO4	AlOOH	AlPO4
Adjuvant alone	25.2	-26.6	N/A	N/A
LNP alone	22.6		N/A	N/A
1:20 ratio LNP:Al	14.5	-5.32	89%	99%
1:10 ratio LNP: Al	11.4	8.63	89%	95%
1:5 ratio LNP:Al	4.8	13.2	73%	80%

Table 1. Evaluating adsorption capacity of LNPs with aluminum adjuvants. Zeta potential and adsorption was measured after mixing LNPs with either Alhydrogel (AlOOH) or AdjuPhos (AlP04) at increasing ratios. AlOOH is positively charged while AlPO4 is negatively charged. LNPs adsorbed to AdjuPhos better than Alhydrogel due to their positive charge. Adsorption % reduces as increasing amount of LNPs are added.

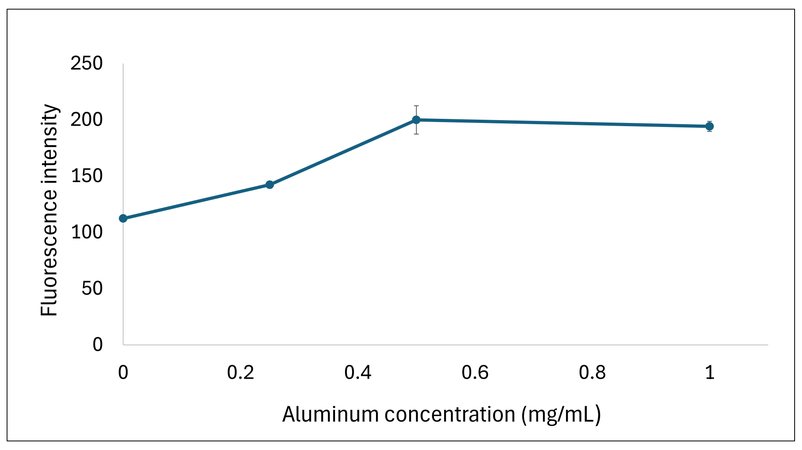


Figure 2. Dose-response of aluminum adjuvant on mRNA LNP transfection in HepG2 cells. 100 µg/ml mRNA was mixed with increasing amount of AdjuPhos (0.25, 0.5, and 1 mg/mL). Cells were dosed with appropriate dilutions of the vaccines (200-fold) for 24 hours. Enhanced transfection effect of aluminum adjuvant seems to plateau at 0.5 mg/mL where it results in 2-fold increase in GFP expression compared with mRNA LNP formulation with no adjuvant.

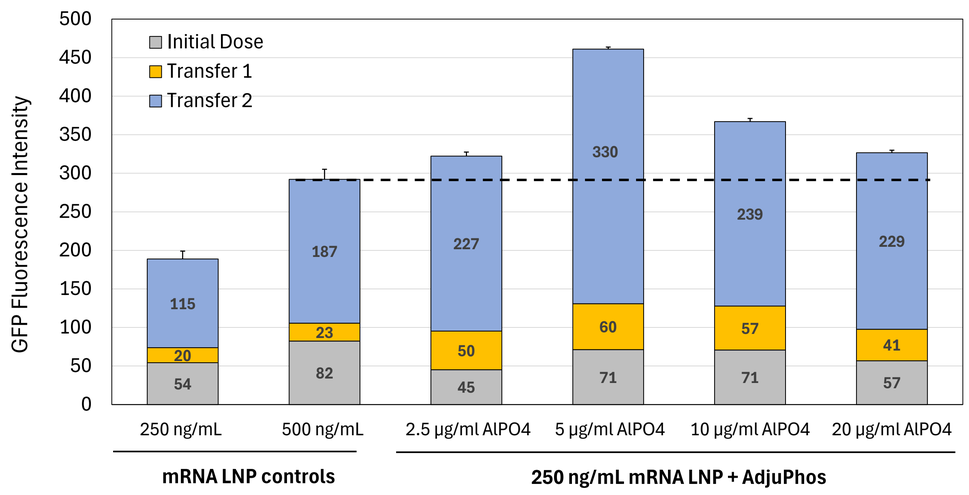


Figure 3. Aluminum adjuvant enhances mRNA LNP transfection potential with depot effect. Formulations were added to HepG2 cells and transferred twice into a new set of cells each time before measuring GFP fluorescence after 24 hours. The GFP expression of the initial dose for 250 ng/mL doses with or without aluminum were comparable. However, the first and second transfer of doses resulted in double GFP expression in formulations with aluminum (orange and blue bars) compared with mRNA LNP control. This suggests that AdjuPhos facilitates a depot effect of mRNA LNP into the cells by slowly releasing LNPs. This also demonstrates the potential for dose sparing where half a dose (250 ng/ml) with the adjuvant results in an equivalent or better response as 500 ng/mL non-adjuvanted doses (dotted line).

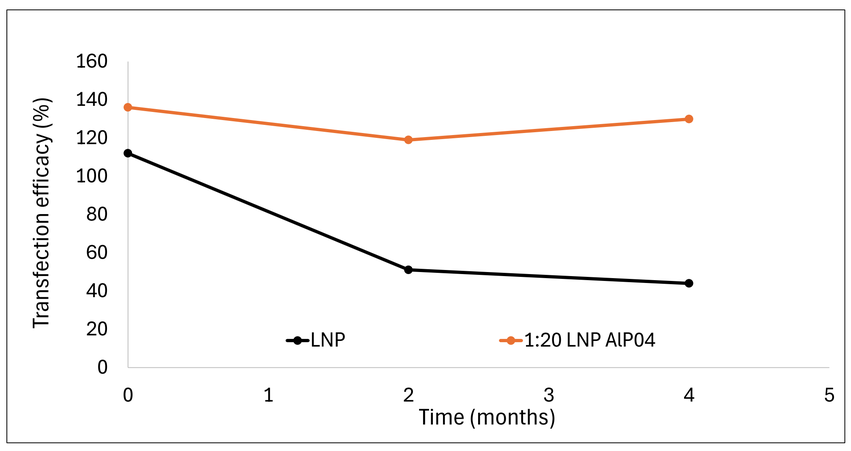
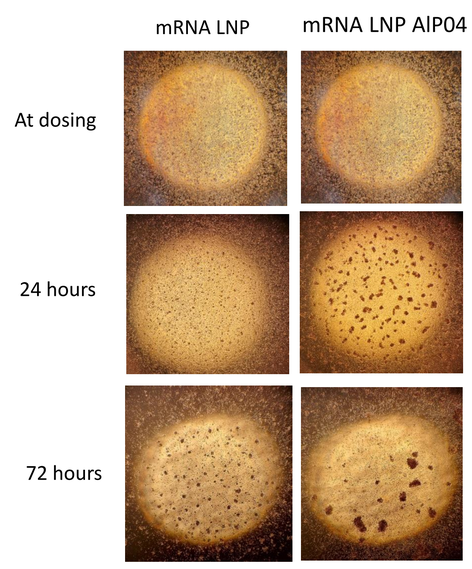


Figure 4. Stability of LNP vs aluminum-adjuvanted LNP formulations over 4 months at 4°C. 1:20 ratio of LNP to aluminum adjuvant (50 ug/ml mRNA LNP with 1 mg/mL aluminum) was evaluated for stability by cell transfection assay in HepG2. Over 100% transfection efficacy was retained after 4 months of storage at 4°C while baseline formulations without aluminum adjuvant only retained 40%.



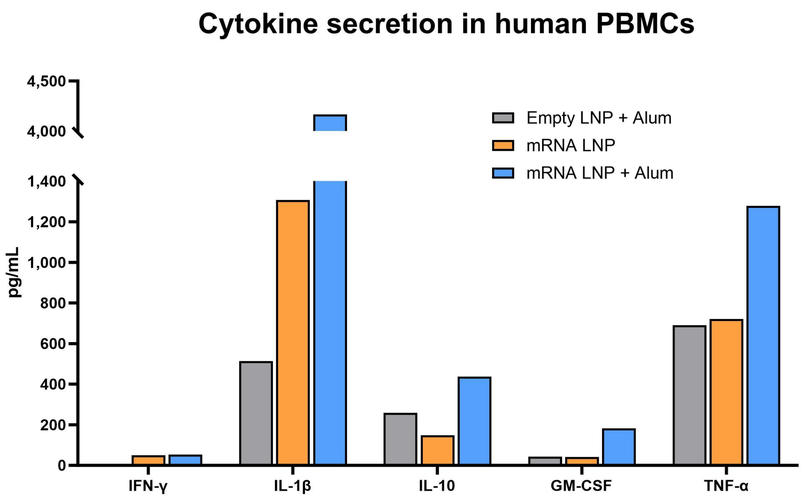


Figure 5. T cell activation is magnified in human PBMCs dosed with mRNA LNP adjuvanted with aluminum as shown with rosette formations (left) and cytokine secretions (right).

METHODS

Formulation

mRNA LNPs expressing green fluorescent protein (GFP) is manufactured by OZ Biosciences. The composition is very similar to approved COVID-19 mRNA vaccines. The lipid nanoparticle encapsulating the mRNA is composed of a 50:10:38.5:1.5 molecular % ratio of ionizable lipid to DSPC to cholesterol to DMG-PEG. GFP mRNA-LNPs were stored at -80°C as supplied at concentrated bulk of 0.5 mg/mL RNA. Alhydrogel (AlOOH) and AdjuPhos were purchased from Invivogen. LNP and aluminum adjuvants are mixed for 30 minutes at room temperature

Transfection Assays with HepG2 cells

HepG2 cells (ATCC, catalog # HB8065) were plated at 40,000 cells per mL density in black 96-well plate. The next day, cells were dosed with mRNA GFP. Fluorescence was measured after 24 hours with SpectraMax M2e Molecular Device plate reader after removing the supernatant. Known concentrations of freshly thawed mRNA GFP-LNP were used to index fluorescence values to determine formulations stability over time (Figures 2 and 4). In the depot and transfer experiment (Figure 3), cells were dosed for 3 hours (initial dose), then the supernatants were transferred to a new set of cells and dosed for another 3 hours (1st transfer), and then again transferred to a new set of cells (2nd transfer). GFP fluorescence raw values were measured after 24 hours from each dose or transfer and added up.

Human PBMC and cytokine secretion

PBMC cells which include a mix of macrophages, dendritic cells, and monocytes purchased from ATCC were used. Cells were seeded in 96-well plates at 200,000 per well density. The next day cells were dosed with 250 ng/mL mRNA GFP-LNPs only or with 1 mg/mL AdjuPhos. Media was collected after 24 hours. Cytokines were measured by ELISA (Acro Biosystems).

mRNA LNP characterization

Zeta potential was measured with Zetasizer Nano ZS from Malvern.

Adsorption: To determine adsorption, the formulations were spun down at 5,000 rcf for 10 minutes (a speed that can pellet the aluminum particles, but not mRNA LNPs, unless they are adsorbed to aluminum surface). Supernatants were collected and tested for mRNA presence by the RiboGreen assay. Sample is exposed to Tris EDTA Triton X-100 to lyse the LNPs and release mRNA. RNA detection by RiboGreen performed following manufacturer instruction (ThermoFisher, catalog # R11490).

CONCLUSION

Using aluminum adjuvant with mRNA LNP vaccine enhances cell transfection by two-fold, elicits a broader T cell response, and improves the vaccine stability. Improving immune-cell targeting of mRNA vaccines with aluminum particles will also lower side effects by reducing off-target biodistribution. Future studies plan to evaluate the improved formulation *in vivo*.

This novel vaccine has the potential to improve global access to vaccines and responses to future pandemic and outbreaks by increasing number of doses available, removing the ultra-cold chain, and improving coverage and patient compliance by reducing side effects caused by systemic distribution.

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