Repeat mRNA LNP administrations to normal and tumor-bearing mice to advance safe and efficacious mRNA therapeutics

Leanna Yee, Martin Rabel, Sams Sadat, Zhengyu Chen, Kalyan Golla, Tony Wu, Vicente Lacap, Richard Jiang, Jay Paquette, Nikita Jain, Anitha Thomas

Cytiva, Vancouver, Canada

Introduction

Lipid nanoparticles (LNPs) have transformed mRNA delivery, offering a promising approach for advancing cancer immunotherapy. Effective and safe delivery of tumor antigen-encoding mRNA can stimulate robust anti-tumor immune responses. Here, we developed LNP formulations for delivering ovalbumin (OVA)-encoded mRNA and evaluated their safety, efficacy, and therapeutic potential in the B16F10-OVA syngeneic melanoma mouse model.

Methods

Novel ionizable lipids were selected for formulating LNPs. OVA-encoded mRNA was encapsulated in LNPs using NxGen™ mixing technology on the NanoAssemblr™ platform. The LNPs were characterized for hydrodynamic size, polydispersity index (PDI), mRNA-loading, and encapsulation efficiency. To assess long-term safety and stability, healthy mice received multiple intramuscular (IM) injections of the LNP formulations at 21-day intervals. Physicochemical stability and toxicity were evaluated through the standard assays and clinical observations. Following this series, B16F10-OVA tumor-bearing mice were vaccinated intramuscularly with OVA mRNA-loaded LNPs. Clinical symptoms and survival outcomes were monitored and compared to untreated controls.

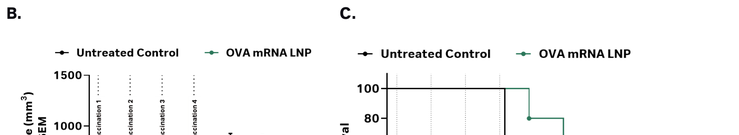
Results

1.Critical quality attribute (CQA)

		Dosing information		CQA (ranges)		
#	Group	Payload (mRNA)	IM dose (µg)	Size (nm)	PDI	EE (%)
1	PBS	–	–	–	–	–
2	Control 1	OVA	5	77–90	0.01–0.09	95–99
3	Control 2	OVA	5	80–92	0.01–0.06	91–98
4	LNP 1	OVA	5	71–82	0.03–0.10	96–99
5	LNP 2	OVA	5	70–80	0.01–0.09	96–99
6	LNP 3	OVA	5	70–80	0.02–0.09	96–100
7	LNP 4	OVA	5	70–79	0.04–0.09	97–99
8	Control 1 empty	N/A	N/A	77–90	0.01–0.05	N/A
9	Control 2 empty	N/A	N/A	87–108	0.01–0.07	N/A
10	LNP1 empty	N/A	N/A	43–56	0.08–0.20	N/A
11	LNP2 empty	N/A	N/A	63–78	0.01–0.10	N/A
12	LNP3 empty	N/A	N/A	66–76	0.01–0.08	N/A

*Abbreviations: OVA = ovalbumin; PDI = polydispersity index; EE = encapsulation efficiency; N/A = not applicable.
CQA ranges of LNPs were measured over the time course over 6 months.
Both mRNA-containing control LNPs and control empty LNPs were made using benchmark ionizable lipids.

2. Study design and clinical observations for repeated intramuscular (IM) administration of Cytiva LNPs



B.

Clinical score:	0 – normal	1 – mild	2 – mild to moderate	3 – moderate	4 – severe

Treatment group	Before 7th injection	3 days after 7th injection	1 day after 9th injection	5 days after 9th injection	12 days after 9th injection	19 days after 9th injection
PBS	Normal	Normal	Normal	Normal	Normal	Normal
Control 1	Normal	Normal	Ruffled fur	Ruffled fur	1 animal found dead	Ruffled fur
Control 2	Normal	Fur slightly scruffy	Ruffled fur	Ruffled fur (1 animal)	Ruffled fur (1 animal)	Ruffled fur (1 animal)
LNP 1	Normal	Normal	Normal	Normal	Normal	Normal
LNP 2	Normal	Normal	Normal	Normal	Normal	Normal
LNP 3	Normal	Normal	Normal	Normal	Normal	Normal
LNP 4	Normal	Normal	Normal	Normal	Normal	Normal
Control 1 empty	Normal	Normal	Normal	Normal	Normal	Normal
Control 2 empty	Normal	Normal	Normal	Normal	Normal	Normal
LNP 1 empty	Normal	Normal	Normal	Normal	Normal	Normal
LNP 2 empty	Normal	Normal	Normal	Normal	Normal	Normal
LNP 3 empty	Normal	Normal	Normal	Normal	Normal	Normal

Fig 1. A) Animal study design and **B)** clinical observation for repeated IM administration study of Cytiva LNPs in female CD-1 mice.

3. Anti-cancer efficacy of lead Cytiva OVA mRNA-LNP in B16F10-OVA syngeneic mouse model

Fig 3. A) Experimental schedule for *in vivo* test of lead Cytiva OVA mRNA-LNP and its anti-cancer efficacy against B16F10-OVA subcutaneous (SC) allograft syngeneic model in female C57BL/6 mice. A comparative average of **B)** tumor growth and **C)** % survival of the mice groups that received four repeated IM doses of untreated control and OVA mRNA-LNP, respectively, with 7 days apart. Two days before the first IM administration, 3×10^5 B16F10-OVA cells were inoculated subcutaneously. Tumor volumes were calculated by 0.5236×length×width×high.

2.1. Clinical biochemistry: Cytiva LNPs-injected mice maintain similar liver enzyme profiles as PBS control

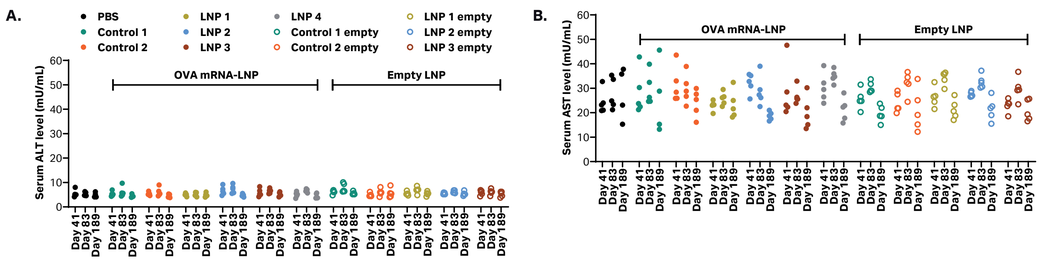


Fig 2.1 Serum **A)** alanine aminotransferase (ALT) and **B)** aspartate aminotransferase (AST) level measured on days 41, 83 and 189 for repeated IM administration study in female CD-1 mice (n = 5).

2.2. Immune modulation: Cytiva LNPs do not induce inflammatory markers long-term

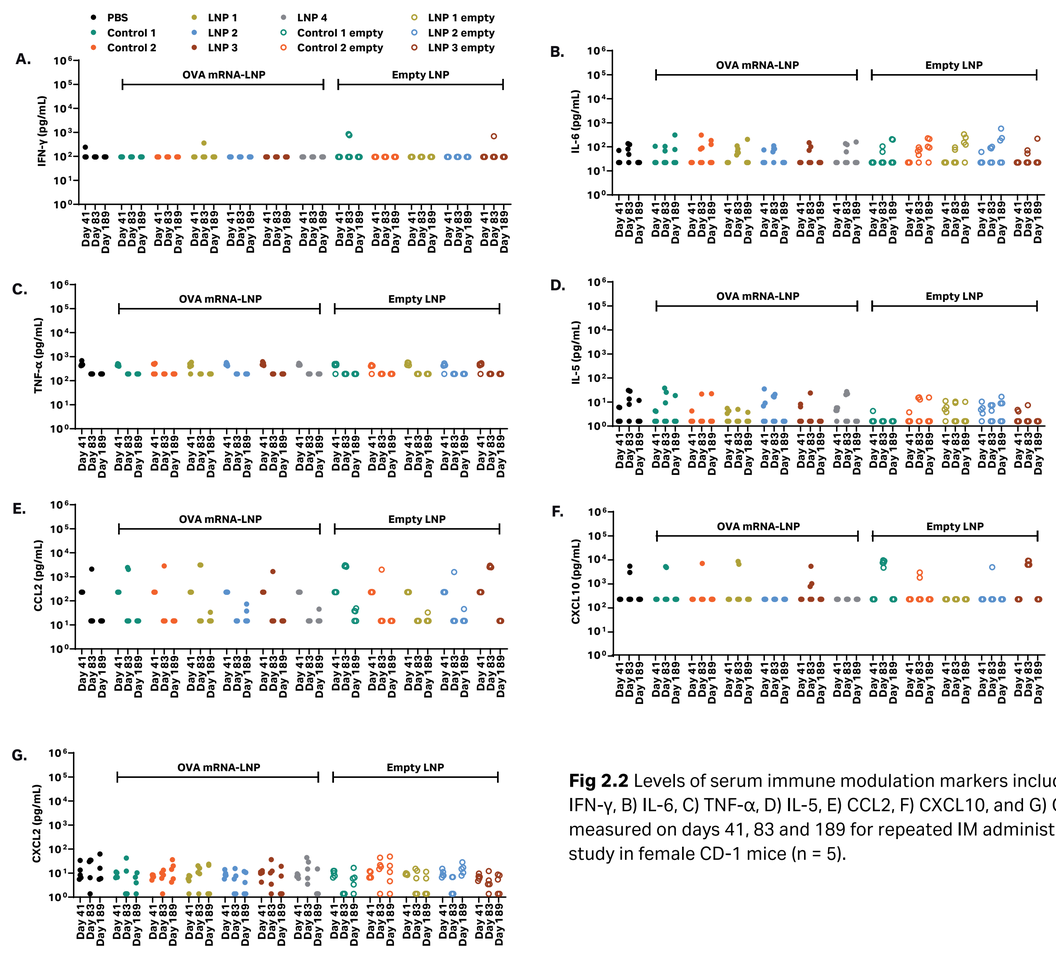


Fig 2.2 Levels of serum immune modulation markers including A) IFN-γ, B) IL-6, C) TNF-α, D) IL-5, E) CCL2, F) CXCL10, and G) CXCL2 measured on days 41, 83 and 189 for repeated IM administration study in female CD-1 mice (n = 5).

2.3. PEG immunogenicity: Cytiva LNPs do not induce any long-term anti-PEG immune response

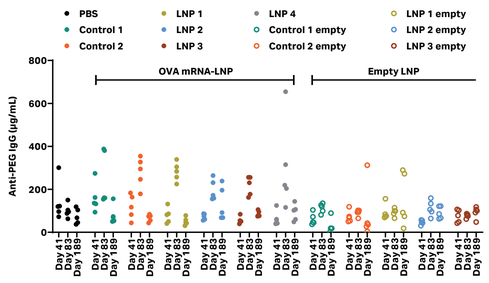


Fig 2.3 Levels of anti-PEG IgG in the serum samples of female CD-1 mice (n = 5) that were collected on days 41, 83 and 189 for the repeated IM administration of Cytiva LNPs.

2.4. Humoral immune response: Cytiva LNPs elicit OVA-specific IgG

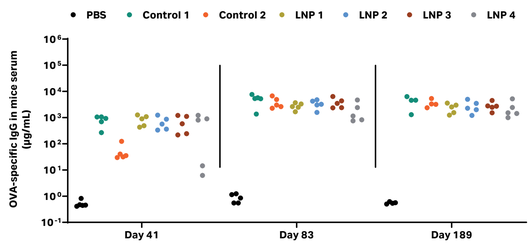


Fig 2.4 Levels of OVA-specific IgG in the serum samples of female CD-1 mice (n = 5) that were collected on days 41, 83 and 189 for the repeated IM administration of Cytiva LNPs.

Conclusions

By retaining the CQAs of the drug products, our novel LNP platform efficiently delivers OVA mRNA as a model for protein replacement applications or cancer vaccines. Additionally, repeated IM administrations of these LNPs over an extended period resulted in minimal adverse events with no indications of systemic toxicity and OVA mRNA-LNPs induced potent anti-tumor effects in the B16F10-OVA model. This favorable safety profile and stability of LNPs upon repeated dosing underscore LNP suitability for long-term immunization strategies.

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