Novel ionizable lipid FL-0445T for prophylactic cancer vaccine

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Abstract

Lipid nanoparticles (LNPs) have been regarded as a leading option for mRNA delivery in clinical use including COVID-19 vaccines. As reported in the previous conference, we presented our novel patented two ionizable lipids performed high protein expression, FL-2266 LNPs via an i.v. administration and FL-0445T LNPs via a local administration[1]. Here, we demonstrate the prophylactic cancer vaccine applicability of FL-0445T LNPs. First, the biodistribution profile of FL-0445T LNPs after an intramuscular administration in mice was measured (Figure 1). FL-0445T LNPs encapsulating luciferase mRNA showed the comparable luciferase expression to the benchmark LNPs in the muscle. The luciferase expression and the mRNA biodistribution of FL-0445T LNPs in the liver were lower than those of benchmark LNPs. Subsequently, we evaluated the prophylactic anti-tumor effects of FL-0445T LNPs. Intramuscular immunization with FL-0445T LNPs encapsulating ovalbumin (OVA) mRNA inhibited the tumor growth and improved the survival rates in mice inoculated with E.G7-OVA cells (Figure 2). The observed anti-tumor effects were comparable to those induced with OVA protein and poly (I:C). These results suggest that the FL-0445T LNPs are applicable for prophylactic cancer vaccines.

Our patented ionizable lipids FL-0445T and FL-2266T are available for customers in Fujifilm's DDS CDMO services. Regarding the manufacturing, we have successfully synthesized patented FL-0445T under GMP.

[1] Makita-Suzuki K, Novel Ionizable Lipids FL-0445T and FL-2266T for mRNA lipid nanoparticles, 10th International mRNA Health Conference (2021)

Biodistribution - intramuscular

Table 1. Characterization of LNPs

Lipid	Size (nm)	PDI	ζ potential (mV) pH 7.4	ζ potential (mV) pH 5.5	EE (%)
Benchmark	93	0.09	-18.4	17.1	90
FL-0445T	95	0.13	-17.0	11.1	94

- FL-0445T LNPs showed comparable protein expression to the benchmark LNPs.
- Intramuscularly administered FL-0445T LNPs were less expressed in the liver than the benchmark LNPs, which was consistent with mRNA biodistribution.

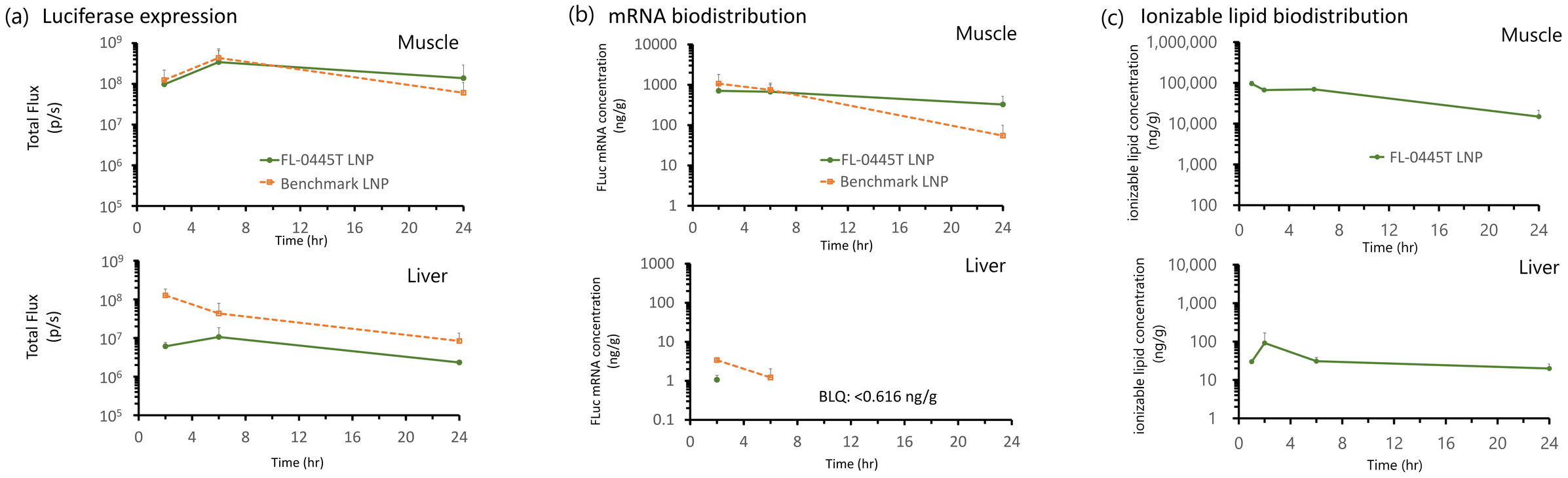


Figure 1. Luciferase expression and biodistribution of intramuscularly administered FL-0445T LNPs.

A single dose of FL-0445T-LNPs or the benchmark LNPs encapsulating FLuc mRNA (Trilink, CleanCap®) was intramuscularly administered to ICR mouse (2 μg mRNA/head, N=3/group). The luciferase expression (a), the mRNA biodistribution (b), and the ionizable lipid biodistribution (c) were quantified at the injected muscle and liver by IVIS (a), by branched DNA assay (b), and by LC-MSMS (c), respectively (mean + SD). The evaluated time points were 2, 6, 24 hr (a, b) or 1, 2, 6, 24 hr (c) after administration.

Prophylactic anti-tumor effect

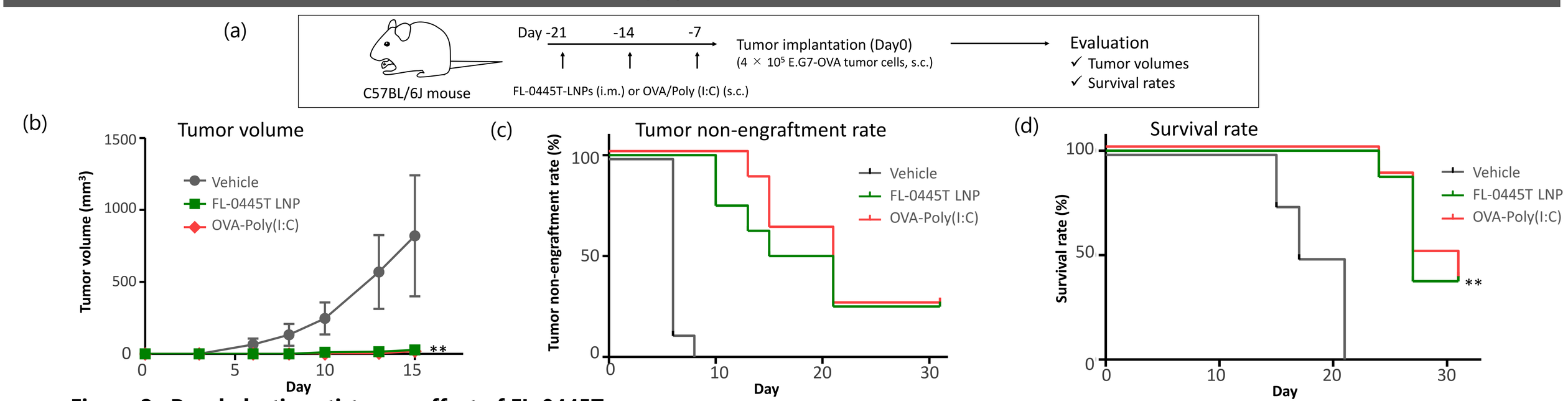


Figure 2. Prophylactic anti-tumor effect of FL-0445T

FL-0445T LNPs encapsulating OVA mRNA (intramuscularly, 10 μg/head) or OVA protein/Poly (I:C) adjuvant mixture (subcutaneously, 100 μg/head) were administered to C57BL/6J mice, weekly 3 times (Day -21, -14 and -7). After vaccination, 4×10^5 E.G7-OVA tumor cells were subcutaneously implanted (Day 0). Each animal was euthanized when the tumor reached 15 mm in longer diameter or Day 31. The tumor volumes of each group until the first individual reached to the endpoint (b), the tumor non-engraftment rate (c) and survival rates (d) were measured. Statistical analysis were performed using Steel's test (b) or Log-rank test (d) (N=8, ** $p<0.01$ vs vehicle).

- FL-0445T LNPs showed comparable tumor growth inhibition and survival rate to protein/Poly (I:C) adjuvant mixture which is known to induce CD8+ T cell response[2].

[2] Takeda, Y. *et al. Cell Rep* **19**, 1874–1887 (2017).

- Preliminary data suggested FL-0445T LNPs can induce CD8+ T cell response (data not shown).

Fujifilm CDMO service

For customers at research phase

✓Original patented lipids ※ including FL-2266T and FL-0445T
- high expression, rapidly metabolized, and applicable to mRNA

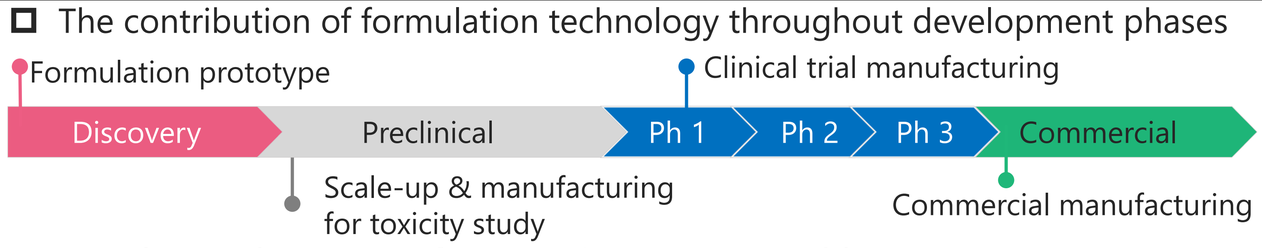
✓Formulation development and optimization from discovery to clinical

※patent: WO2019/235635, WO2021/095876

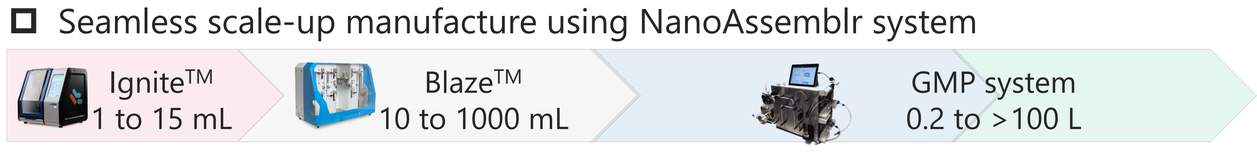
For customers at development phase

✓Microfluidic mixing system (NanoAssemblr® system)
- Scalable & reproducible (upto GMP manufacturing)
- Supported by Precision Nanosystems through strategic alliance

- The contribution of formulation technology throughout development phases



- Seamless scale-up manufacture using NanoAssemblr system



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