# Two Novel ionizable Lipids for mRNA Delivery: Improved Safety in Rats and transfection in Non-human Primates

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**FUJIFILM corporation**

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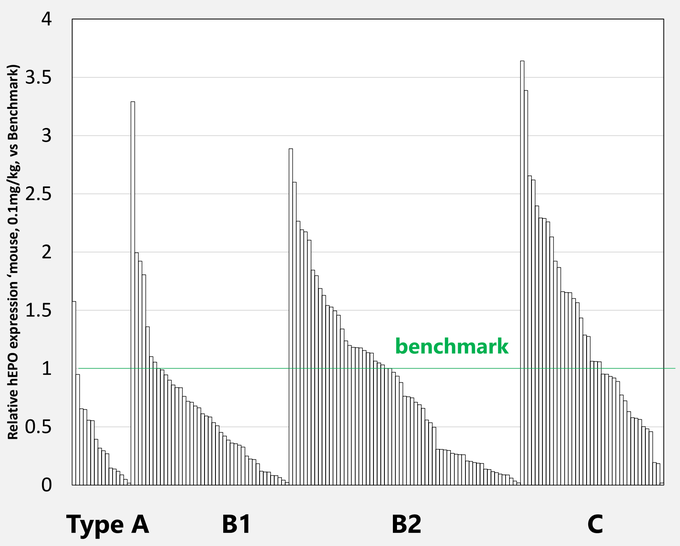
## Abstract

Lipid nanoparticle (LNP) technology has realized mRNA pharmaceuticals and provides vast opportunities for applying mRNA to various therapeutics in addition to COVID-19 vaccines, such as protein replacement and gene editing. Although many academics and companies are working on LNPs, it is still difficult to achieve both high efficacy and low toxicity. In this conference we will introduce our novel, low toxic ionizable lipids.

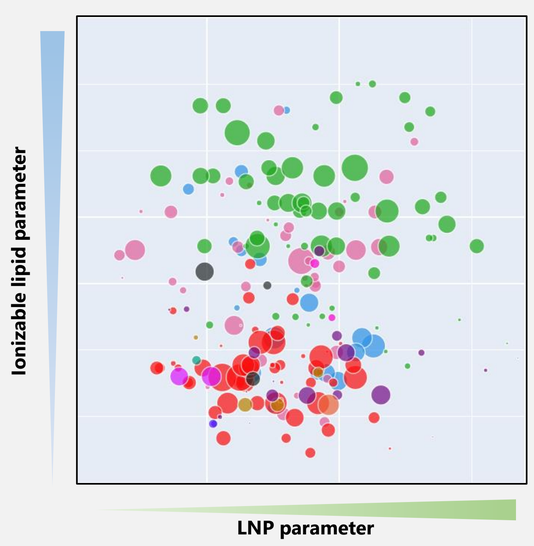
We developed two next-generation ionizable lipids, FL-0207T and FL-0179T, for efficient delivery of mRNA with low toxicity. The intravenous administration of mRNA encapsulated LNPs showed comparable protein expression in mice (Figure 1), and higher protein expression than benchmark LNPs in rats (Figure 2). In rats, intravenous administration of 1 mg/kg of LNP formulated mRNA did not cause significant toxicity in blood cell counts, blood biochemistry, and complements. Furthermore, these LNPs showed the protein expression in cynomolgus monkeys that exceeded the benchmark LNPs in both Cmax and AUC, which is attributed to their long blood circulation. In conclusion, FL-0207T and FL-0179T are promising materials for mRNA containing LNP formulations, which will contribute the expanding mRNA therapeutics in the future.

## FUJIFILM ionizable lipid library

hEPO expression screening
(mouse, 0.1 mg/kg, i.v. injection)




Data analysis




- We developed next-generation ionizable lipids, FL-0207T and FL-0179T.
- We are still working to expand the Lipid Library on an ongoing basis.

## fLuc mRNA delivery in mice

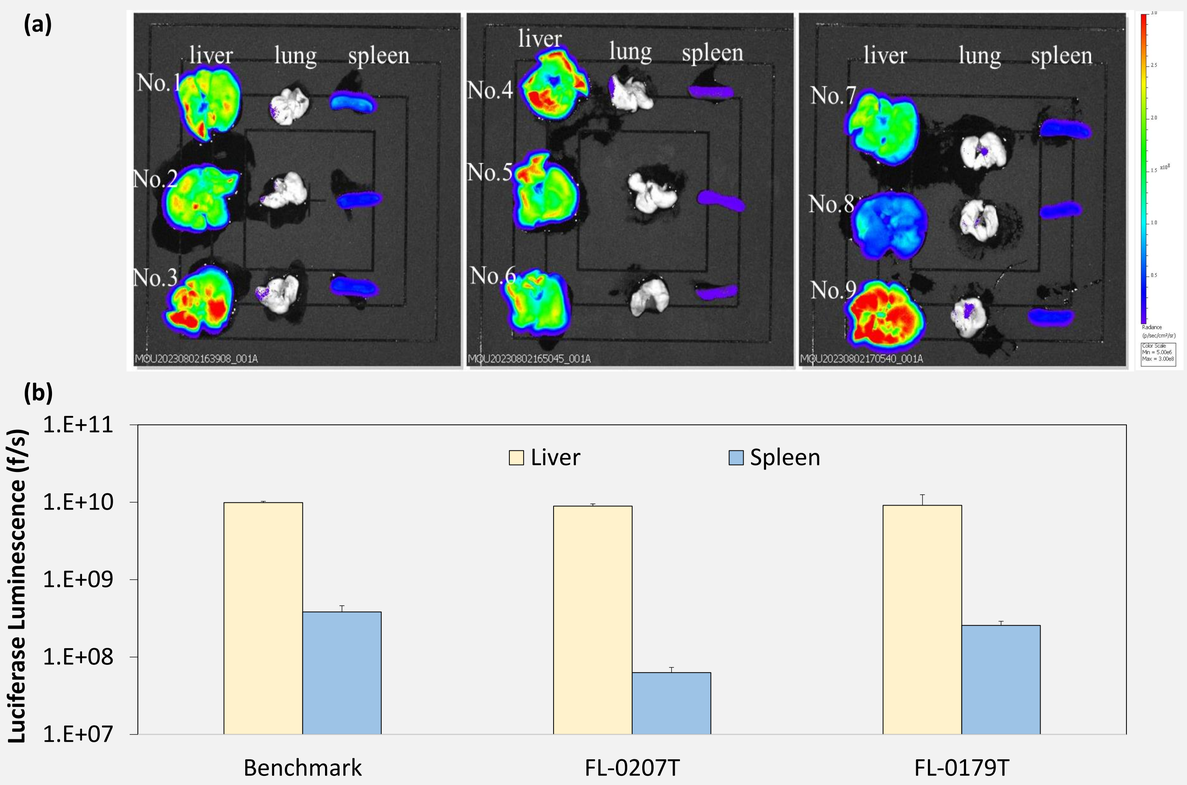



**Figure 1. Luminescence imaging in mice**

(a) Bioluminescence imaging and (b) quantitative results of firefly Luciferase (fLuc) expression 6hr after dosing of 0.2 mg/kg fLuc mRNA (Trilink, CleanCap®) in LNPs, i.v., single dose, n=3, mean + SD.

- Our LNPs performed same expression level as the benchmark LNP.
- In both groups, luciferase expressions were seen almost exclusively in the liver.

## hEPO expression and toxicological parameters in Rats

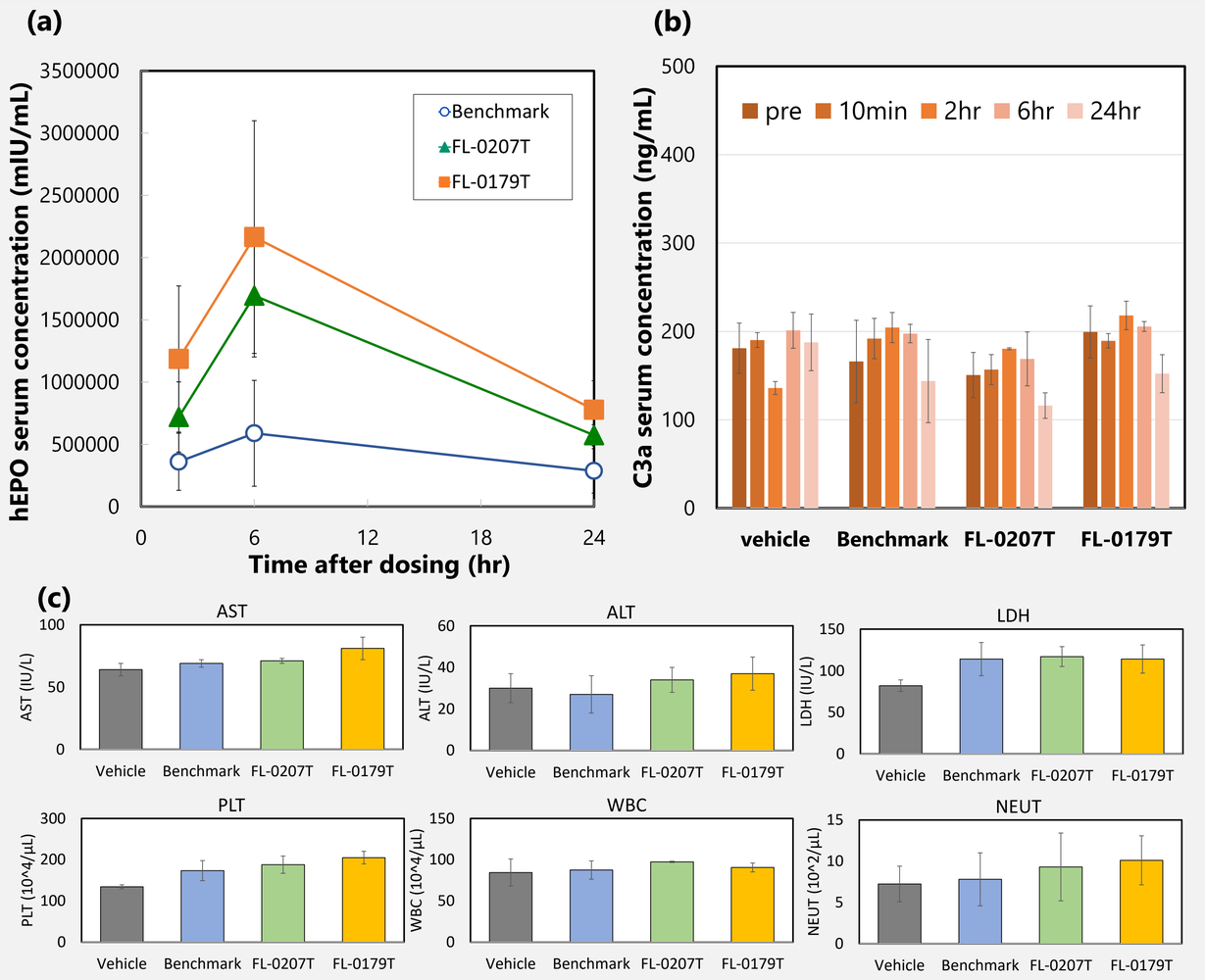



**Figure 2. Expression and Immunogenicity Profile in Rat**

(a) hEPO serum concentrations and (b) complement 3a fragment (C3a) serum concentration after delivery of 1.0 mg/kg hEPO mRNA (Trilink, CleanCap®) in LNPs, i.v., single dose, n=3, mean ± SD. (c) biochemistry patameter and blood test 1week after the administration, n=3, mean ± SD

- Our two lipids showed 3 times higher hEPO expression than benchmark LNP.
- Our LNPs showed no complemental activation (C3a), biochemical parameter, and blood test did not significantly change 1 week after the administration.

## hEPO expression study in NHPs

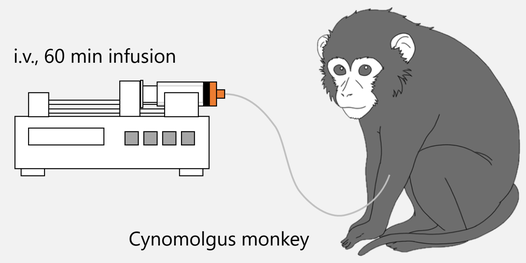



**Table. 2 Physiological Parameters of LNPs**

| Ionizable lipid | diameter (nm) | Zeta potential (mV) | | EE* (%) |
|---|---|---|---|---|
| | | pH7.4 | pH5.5 | |
| Benchmark | 86 | -8.5 | 19.3 | 87.5 |
| FL-0207T | 81 | -19.6 | 4.6 | 90.2 |
| FL-0179T | 87 | -24.8 | 13.2 | 90.3 |

*EE; Encapsulation Efficiency

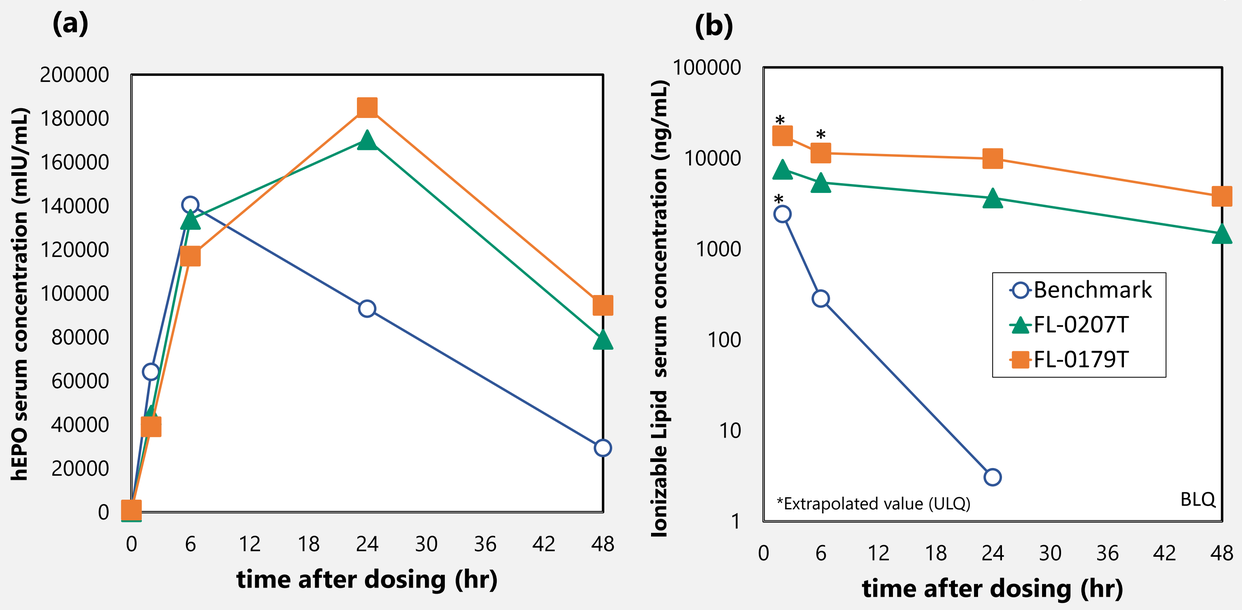



**Figure 3. Expression and Lipid Concentration Profile in Cynomolgus monkey**

(a)hEPO serum concentrations and (b) ionizable lipid serum concentration after delivery of 0.2 mg/kg hEPO in LNPs, i.v., 60 min infusion, n=2. U/BLQ: Upper/Below the Limit of Quantification.

- Our LNPs performed higher expression Cmax than benchmark LNP.
- Our LNPs showed different pharmacokinetics and more sustained protein expression than benchmark LNP.

## Fujifilm CDMO service

**For customers at research phase**

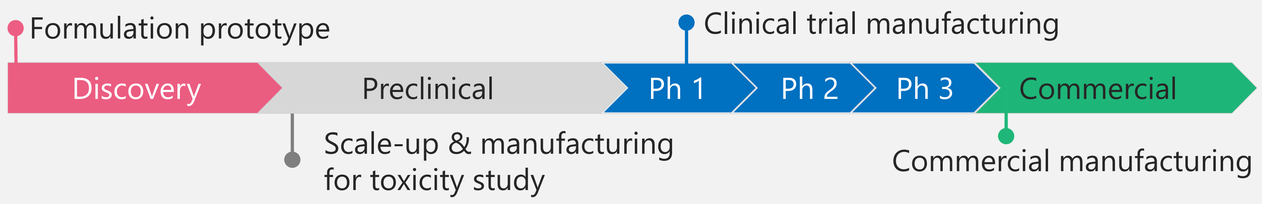
✓Original lipids including FL-2266 and FL-0445 (Patent granted in JP)
- high expression, rapidly metabolized, and applicable to mRNA

✓Formulation development and optimization from discovery to clinical

**For customers at development phase**

✓Microfluidic mixing system (NanoAssemblr® system)
- Scalable & reproducible (upto GMP manufacturing)
- Supported by Precision Nanosystems through strategic alliance
- Analytical services including the development of test methods

- The contribution of formulation technology throughout development phases




- Seamless scale-up manufacture using NanoAssemblr® system

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