Design and Development of Novel Ionizable Lipid Which Exhibits Extrahepatic Gene Delivery Aspiring to ex/in vivo CAR-T Therapy

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Abstract

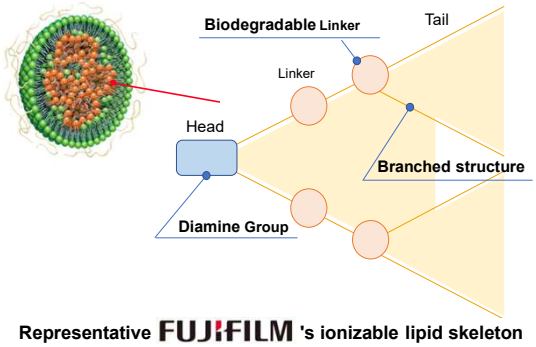
[Introduction & Objectives] Delivering nucleic acids to immune cells, such as T cells, holds significant potential for cancer therapy. In general, it is well known that most lipid nanoparticles (LNPs) tend to bind to apolipoprotein E (ApoE) in the bloodstream, leading to predominant uptake by the liver in vivo. We have developed proprietary GMP-compliant ionizable lipids (e.g. FL-0445) for use in mRNA vaccines and liver-targeting ionizable lipids. However, the extrahepatic delivery of LNPs remains a challenge. In this study, we designed a novel ionizable lipid that exhibits extrahepatic gene delivery aiming for ex vivo and in vivo CAR-T therapy.

[Methods] ①We prepared various LNPs using our ionizable lipids library and evaluated their mRNA delivery efficiency to human primary T cells in the presence or absence of ApoE ②assessed the in vivo delivery efficiency of expression of human erythropoietin(hEPO) and luciferase(Luc) ③evaluated the utility of CAR-knock-in ex vivo ④prepared antibody-conjugated LNPs and administered them to PBMCs, followed by assessment of mRNA delivery efficiency in each cell type.

[Results] **FL-1779T- LNP** exhibited ①Small particle size and high mRNA delivery efficiency to primary human T cells independent of ApoE presence ②Significantly lower hEPO and Luc expression ③Efficient CAR knock-in in T cells ex vivo ④Cell-specific mRNA delivery by antibody-conjugation.

[Conclusions] These findings suggest that FL-1779T-LNP is a promising platform for nucleic acid delivery in both ex vivo and in vivo CAR-T cell therapies.

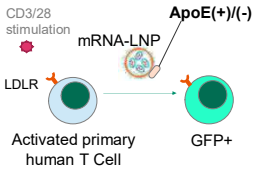
Design concept of novel ionizable lipid



Representative FUJIFILM 's ionizable lipid skeleton

✓ **Head :** Introduction of substituents to adjust the apparent pKa
✓ **Tail :** Control of hydrophobicity to make particle size smaller

Result 1: mRNA delivery to primary human T cell

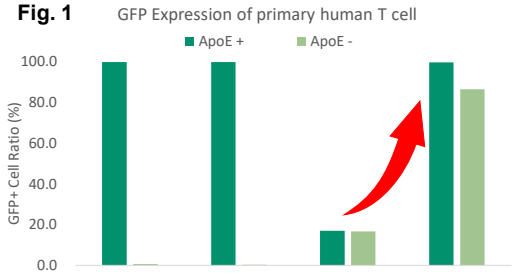


(**Fig 1.**) Primary human T cells were stimulated for 3 days before adding mRNA-GFP-loaded LNPs, and GFP-positive cells were measured by flow cytometry after 24 hours.

Table 1. Characterization of LNPs

Lipid	Size (nm)	PDI	EE (%)
Lipid 5	81	0.11	95
FL-1245T	115	0.08	90
FL-1010T	**231**	0.06	84
FL-1779T	**79**	0.13	98

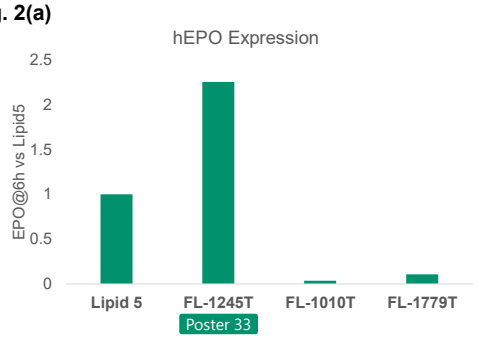
Fig. 1



✓ **FL-1779T-LNP** has small particle size compared with FL-1010T.
✓ **FL-1779T-LNP** exhibited high mRNA delivery efficiency to primary human T cell even in the absence of ApoE.

Result 2: in vivo Study – hepatic, extrahepatic mRNA delivery

Fig. 2(a)

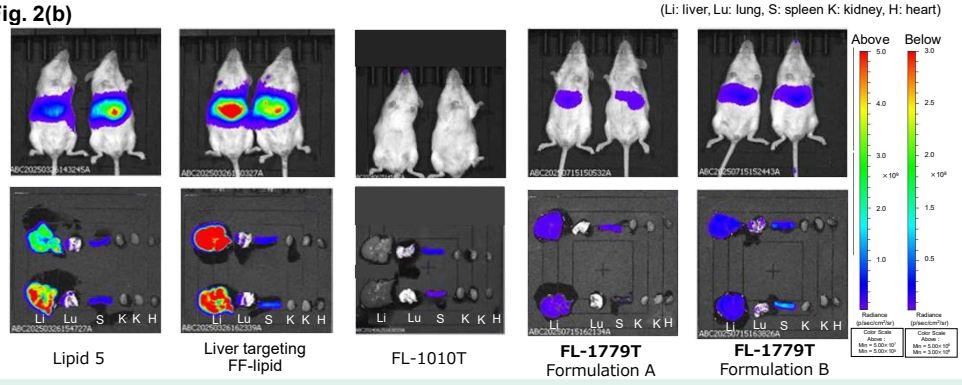


(**Fig 2(a).**) hEPO protein expression levels of mRNA-LNP in plasma compared with benchmark LNP, 6 hours after i.v. administration (0.1 mg/kg as hEPO mRNA),

(**Fig 2(b).**) fLuc-mRNA encapsulated LNP was administered to ICR mice at a dose of 0.2mg/kg (i.v.) and fLuc luminescence was imaged and quantified by IVIS. (Top: 5hr, Bottom: 6hr)

Fig. 2(b)

(Li: liver, Lu: lung, S: spleen K: kidney, H: heart)



✓ **FL-1779T** exhibited little protein expression in the liver.
✓ Spleen delivery might be controlled by formulation. We are analyzing which specific cell populations are targeted.

Result 3: ex vivo CAR-Knock-In Study

Fig. 3(a)

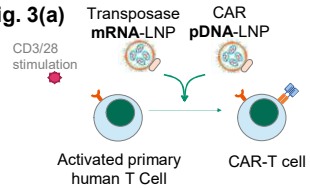


Fig. 3(b)

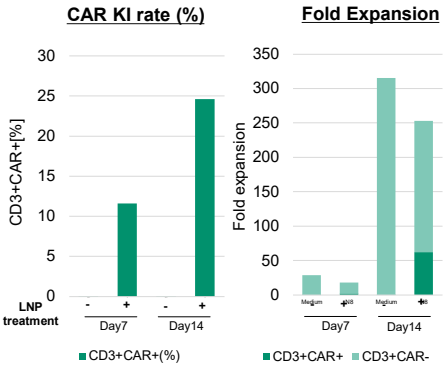
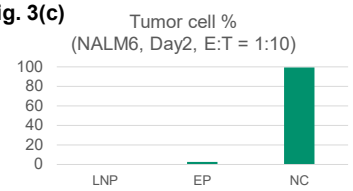


Fig. 3(c)

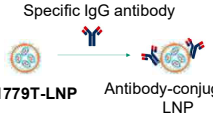


(**Fig 3.**) (a) Protocol : Day 0, primary human T cells were activated with anti-CD3 and anti-CD28 antibodies. Day 3, Transposase mRNA LNP and CAR pDNA LNP were added to the cultures. (b) Percentage of CD3+ CAR+ cells 1 day after adding LNPs followed by CAR knock-in with LNP, then cultured until Day 7 and Day 14. (c) Percentage of Tumor cells in NALM6 cells after 2 days.

✓ **FL-1779T-LNP** exhibited CAR knock-in efficiency of 24.6% in 14 days.
✓ **FL-1779T-LNP** exhibited low cell growth suppression.
⇒ **FL-1779T** has potential for use in ex vivo CAR-T production.

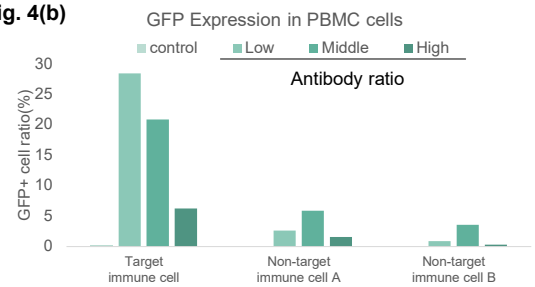
Result 4: Potential of active-targeting

Fig. 4(a)



(Fig4.)(a) Specific antibody was conjugated to **FL-1779T-LNP** by maleimide chemistry.
(b) Antibody-conjugated-LNPs including GFP-mRNA were added to non-activated PBMC cells. Percentage of GFP+ cells 1 day after adding LNPs in each cells.

Fig. 4(b)



✓ **FL-1779T-LNP conjugated with antibody** exhibited selective payload delivery to target immune cells.
⇒ **FL-1779T** is potential lipid for active targeting.

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

【Disclosure Statement of COI】
The authors have no financial conflicts of interest disclose concerning the study.

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