4th Annual LNP Formulation & Process Development Summit @Boston

Novel design of ionizable lipids and LNP formulations for nucleic acid delivery leading to CAR knock-in

Shinichi HASHIMOTO

Head of LNP Formulation Discovery

Bio Science & Engineering Lab.
FUJIFILM Corporation

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FUJIFILM
Value from Innovation

Forward Looking Statements and Regulatory Matters

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Outline

- Our proprietary ionizable lipids
- New Ex Vivo LNP formulations for knock-out and pDNA delivery to T Cell
- New In Vivo Extra-hepatic Lipids discovered from Ex Vivo study

Our mRNA-LNP end-to-end CDMO service

Formulation prototype

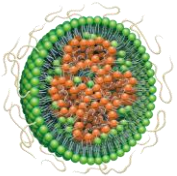
Clinical trial manufacturing

Research | Preclinical | Ph 1 | Ph 2 | Ph 3 | Commercial

Scale-up & manufacturing for toxic study

Commercial manufacturing

CDMO Service



Toyama/JP Factory

Research Phase:

- mRNA design and synthesis
- mRNA-LNP formulation optimization

Development Phase:

- process and analytical methods development
- mRNA-LNP manufacture in GMP

Our mRNA-LNP end-to-end CDMO service

Formulation prototype

Clinical trial manufacturing

Research | **Preclinical** | **Ph 1** | **Ph 2** | **Ph 3** | **Commercial**

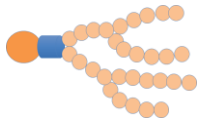
FUJIFILM's ionizable lipids

Scale-up & manufacturing for toxic study

Commercial manufacturing

Today's Topics

Lipid Licensing



Kanagawa/JP Lab

Discovery Phase:

- Provide FUJIFILM's proprietary ionizable lipids depending on client's purposes

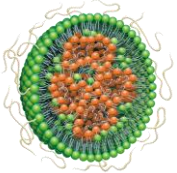
Track Record : **FL-0445**

✓ **Used as client's vaccine in clinical trial Ph3**

✓ GMP manufacture is available

Cell Reports Medicine 2023, *4*, 101134.

CDMO Service



Toyama/JP Factory

Research Phase:

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Development Phase:

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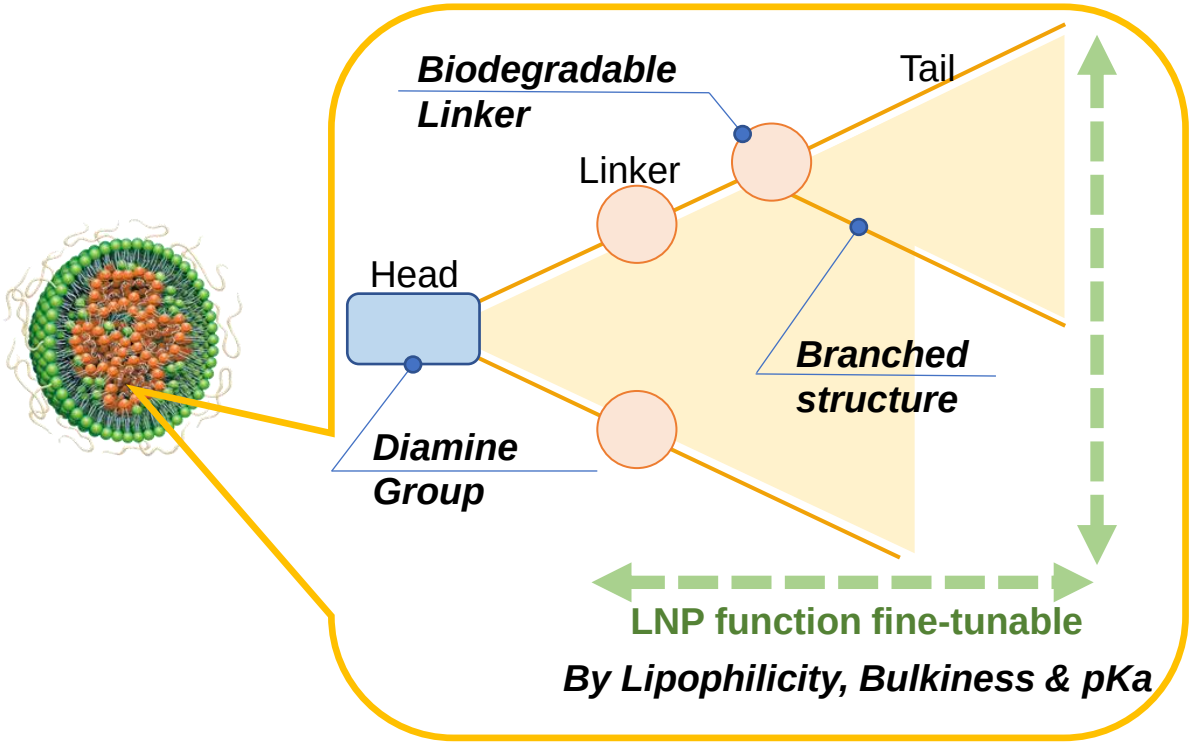
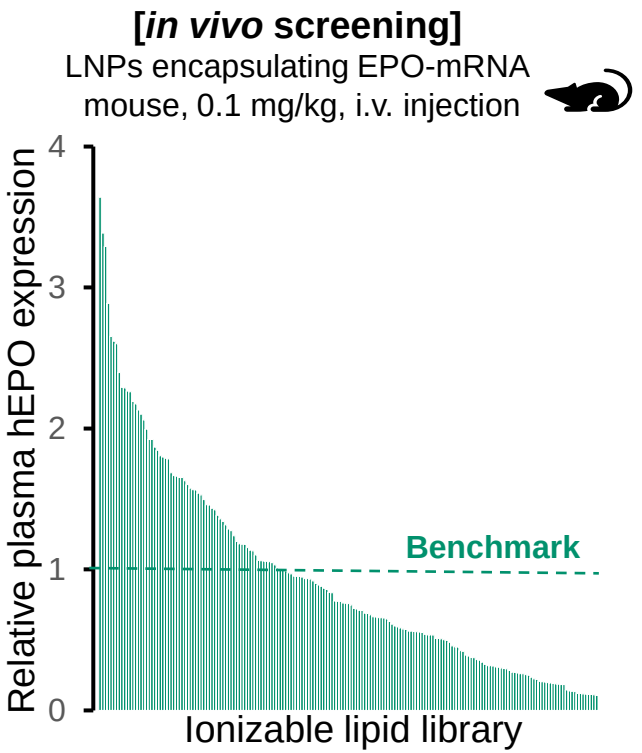
Design concept of our proprietary ionizable lipids





- **We identified multiple potent ionizable lipids through in vivo screening of over 500 newly synthesized compounds.**

Overview of FUJIFILM's lipids and LNP formulations

In Vivo

FUJIFILM Lipid	GMP Mfg.	IP Filed	Application
FL-2266	✓	✓	im vaccine
FL-0445	✓	✓	
FL-1923T		✓	im DNA immune
FL-0207T		✓	iv liver
FL-1252T		✓	
FL-1207T		✓	NHP on-going
FL-1030T		✓	iv spleen
FL-1009T		✓	iv whole-body

Main Topics

Ex Vivo

Final Goal

CAR Knock-In in human primary T cell using FUJIFILM's LNP

Current Achievement

FUJIFILM RtoU LNP	GMP Mfg.	IP Filed	Application
TR01		✓	T cell knock-out
TD01		✓	T cell pDNA delivery
FL-1009T analog-LNP		✓	ApoE independent

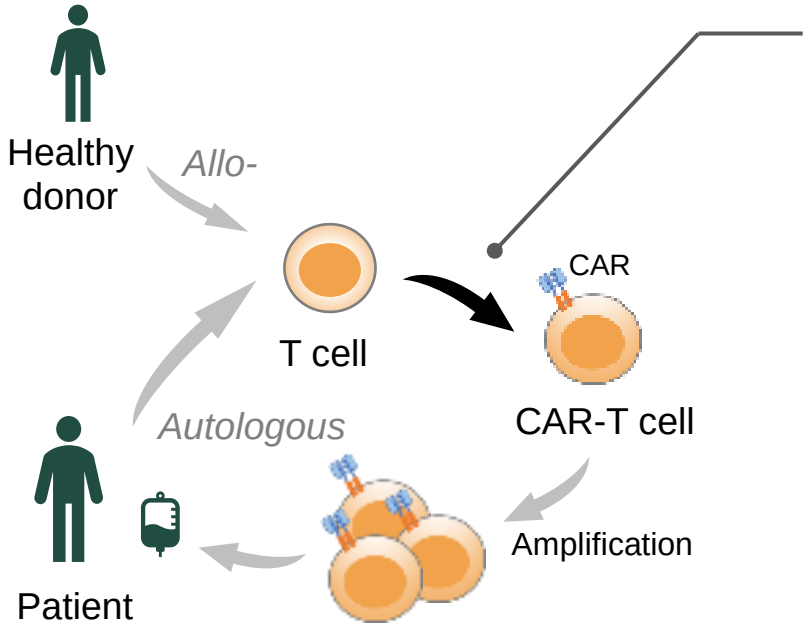
FIH: First In Human, NHP: Non Human Primates, RtoU: Ready-to-Use

➢ **Our lipids and LNP can be evaluated under MTA.**

Outline

- Our proprietary ionizable lipids
- New Ex Vivo LNP formulations for knock-out and pDNA delivery to T Cell
- New In Vivo Extra-hepatic Lipids discovered from Ex Vivo study

Knock-in methods in CAR-T manufacture



Pharmaceutics **2024**, *16(3)*, 346.

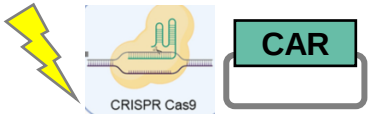
Viral vector

Pros
- High transduction efficiency
- Established manufacturing method

Cons
- Cost of Mfg and QC for viral vector
- Safety: immunogenic, mutagenesis,..

Electroporation

Gene-editor RNP, template DNA

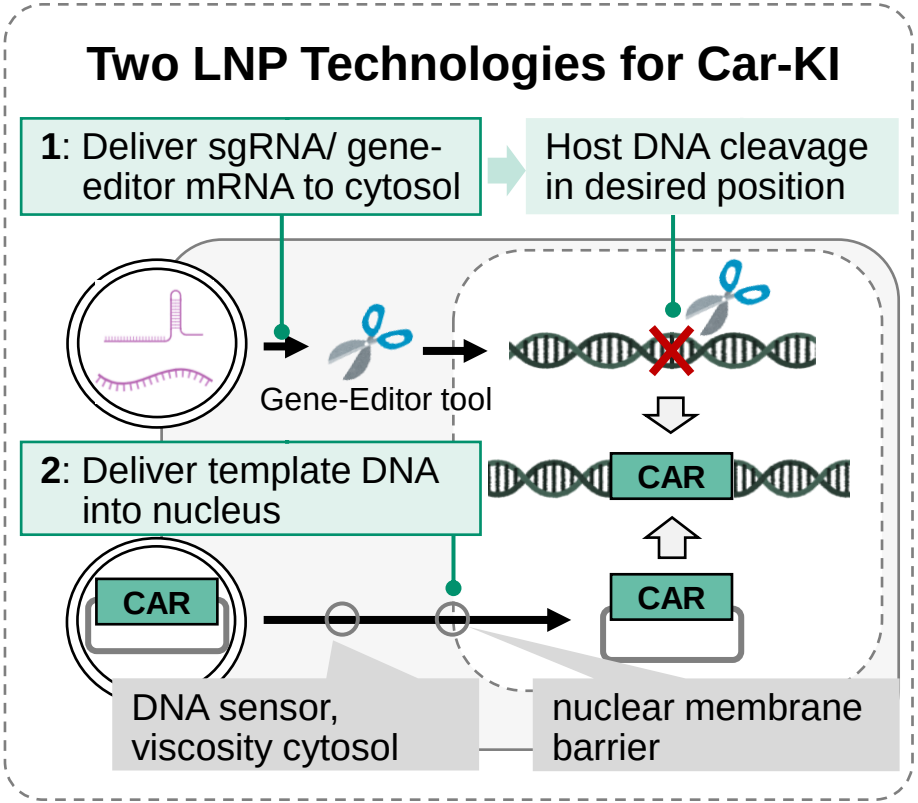


Pros
- High RNP and template DNA delivery into nucleus

Cons
- Cell damage, low cell yield

➢ **We are challenging to develop LNP formulation for Car knock-in in human T cell.**

Overview of our attempts for CAR Knock-In



➢ **For LNP CAR-KI, DNA cleavage and pDNA delivery technologies are essential.**

Overview of our attempts for CAR Knock-In

Two LNP Technologies for Car-KI

1: Deliver sgRNA/ gene-editor mRNA to cytosol

Host DNA cleavage in desired position

Gene-Editor tool

2: Deliver template DNA into nucleus

CAR

CAR

CAR

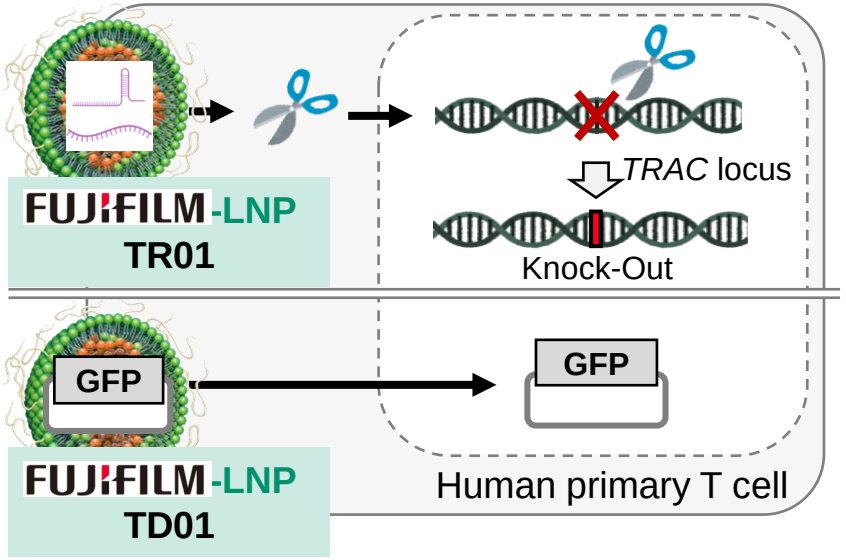
DNA sensor, viscosity cytosol

nuclear membrane barrier

Current Achievement

Study 1: Knock-out in *TRAC* locus **>80%**

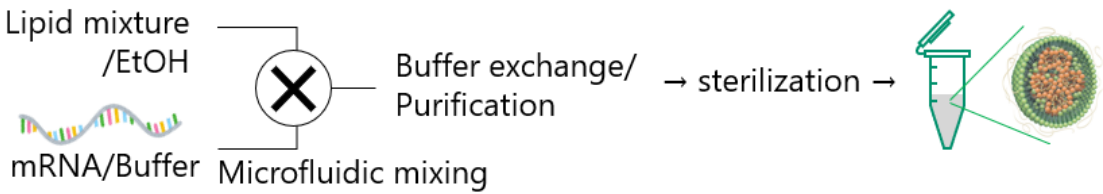
Study 2: pDNA-GFP expression **~60%**



➢ **We developed high knock-out LNP and high pDNA delivery LNP in T cell.**

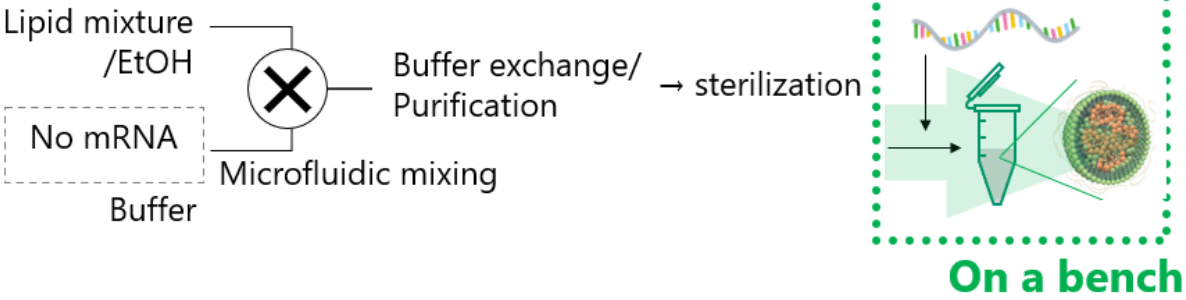
LNP preparation: Ready-to-use LNP

Conventional LNP mRNA mixed at first of the process



- ✓ **Requires mixing device equipment**
- ✓ **Loss of mRNA**
- ✓ **Takes 1-2 days for preparation**

Ready-to-use LNP(RtoU) mRNA mixed at last at a bench

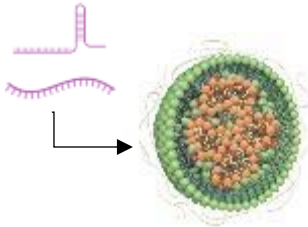


- ✓ **Just mixing by pipette**
- ✓ **No Loss of mRNA**
- ✓ **10 min for preparation**

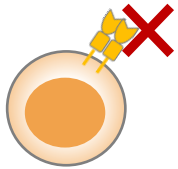
➢ **We developed RtoU LNP in study 1 and 2. We can provide RtoU LNP under MTA.**

Study 1: Knock-out screening with our lipid library

>500 LNPs with our proprietary ionizable lipid library

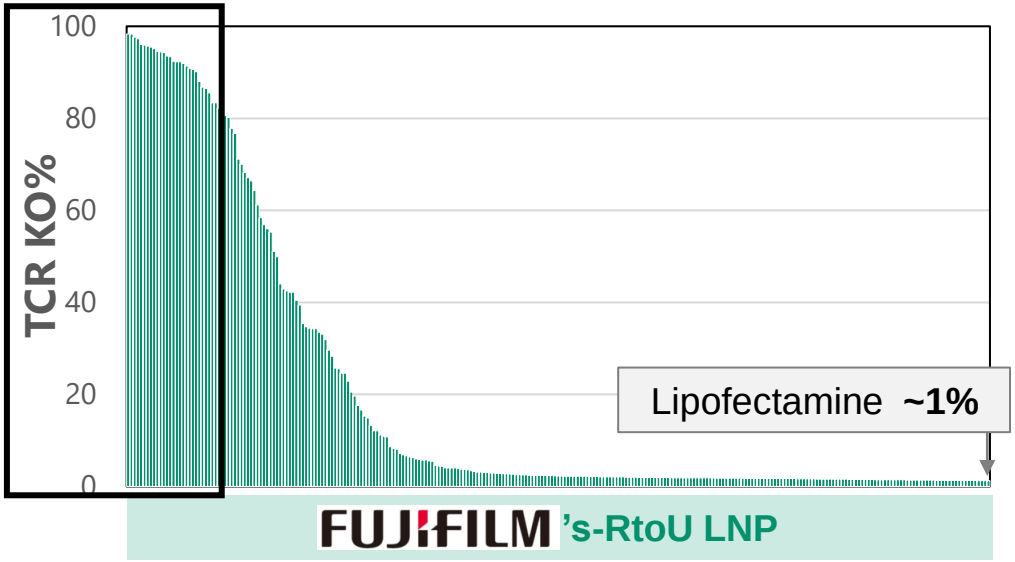


LNP encapsulating
RNA for gene-editing
(Targeting TRAC)



Activated* human primary T cells
(*aCD3/CD28 beads for 3days)

TCR Knock-out in activated T cells

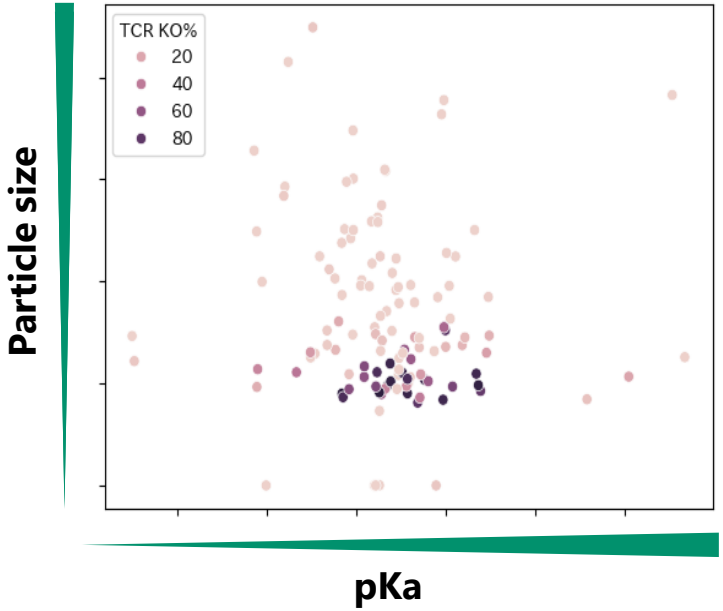


RNA 2 μg/million cells with each LNP.
TCR KO rate was examined at 4days after treatment.

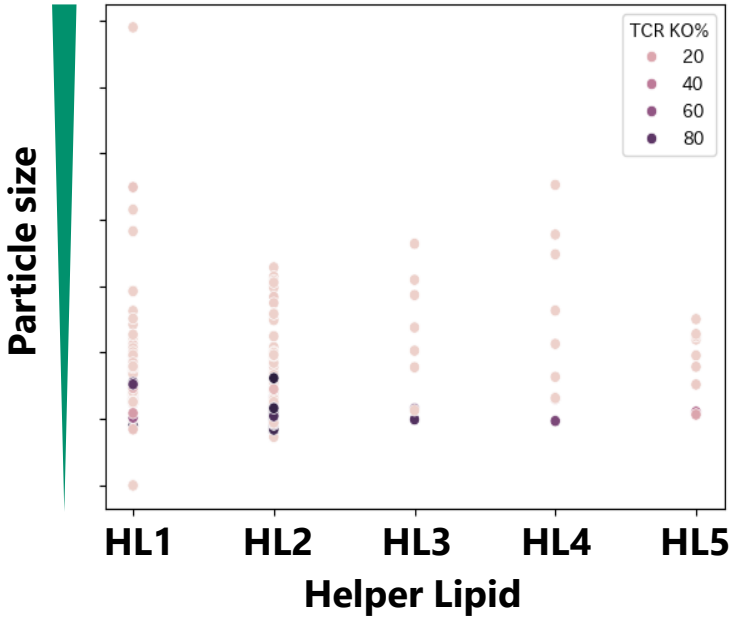
➢ **We obtained many RtoU LNP formulations with >80% TCR knock-out.**

Study 1: Knock-out screening with our LNP formulations

LNP physical properties



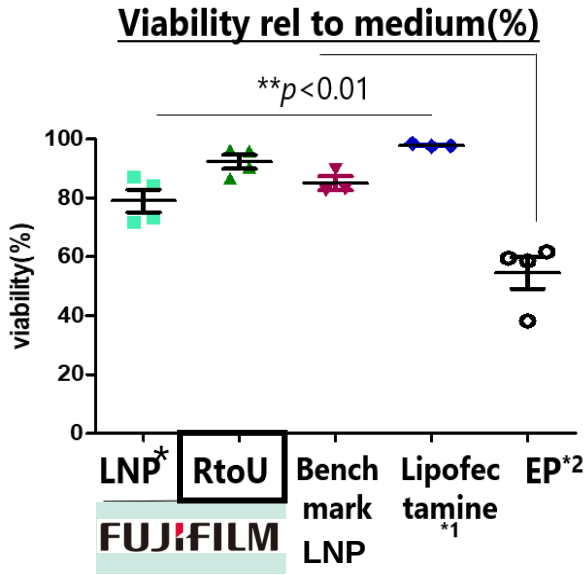
Helper lipid species

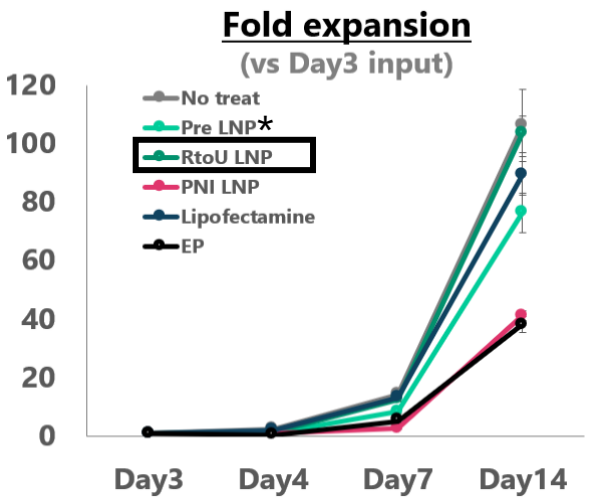


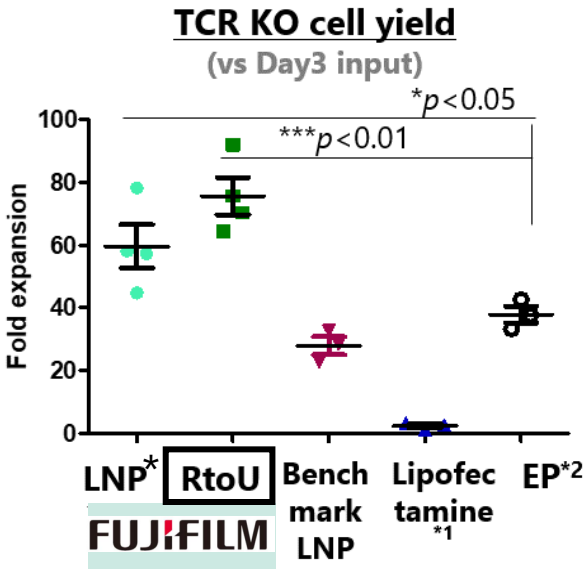
➢ **We found appropriate pKa range, particle size and compatible helper lipid.**

Study 1: Performance of **TR01** formulation

By using a prototype (**TR01**: KO 80%), we evaluated more detail using three donors.







*Conventional method preparation, *1 Lipofectamine™ MessengerMAX™, *2 4D-Nucleofactor(Lonza)/Ribonucleoprotein complex was used.

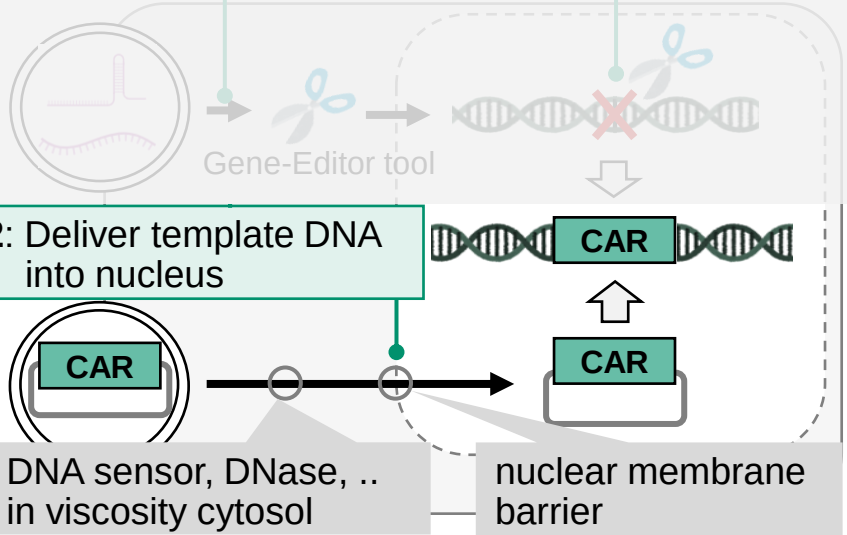
➢ **Our RtoU LNP is appropriate for host DNA cleavage process in T cells.**

Overview of our attempts for CAR knock-in

Elements for Car Knock-In by LNP

1: Deliver sgRNA/ gene-editor mRNA to cytosol

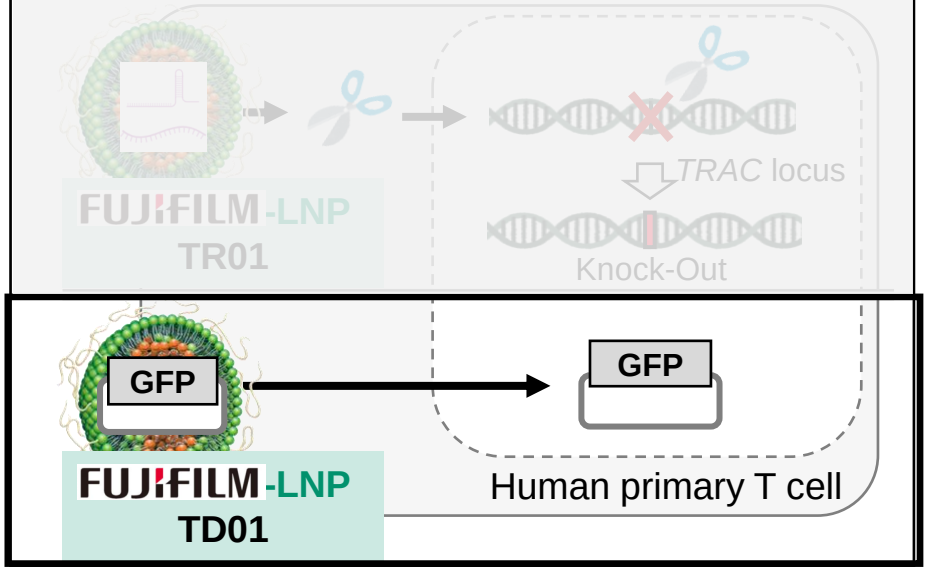
Host DNA cleavage in desired position



Current Achievement

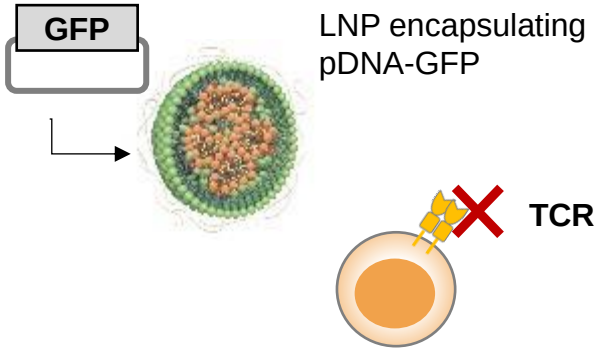
Study 1: Knock-out in *TRAC* locus **>80%**

Study 2: pDNA-GFP expression **~60%**

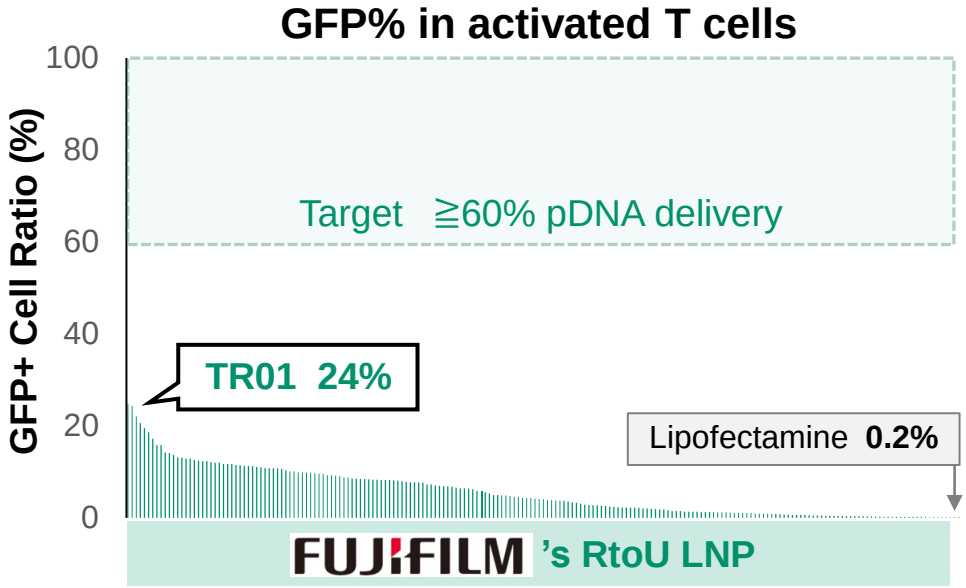


Study 2: pDNA delivery 1st screening using lipid library

>500 LNPs with our proprietary ionizable lipid library



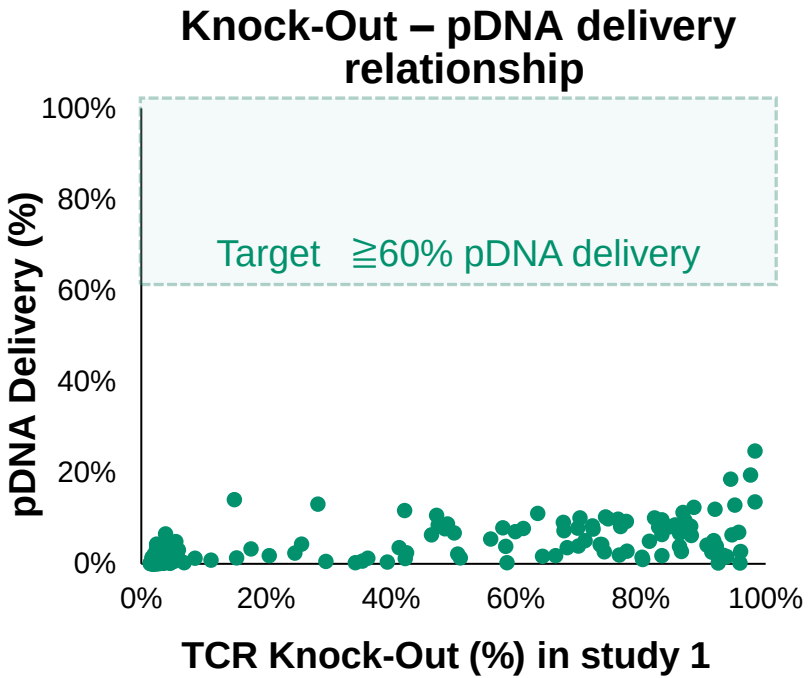
Activated* human primary T cells
(*aCD3/CD28 beads for 3days)

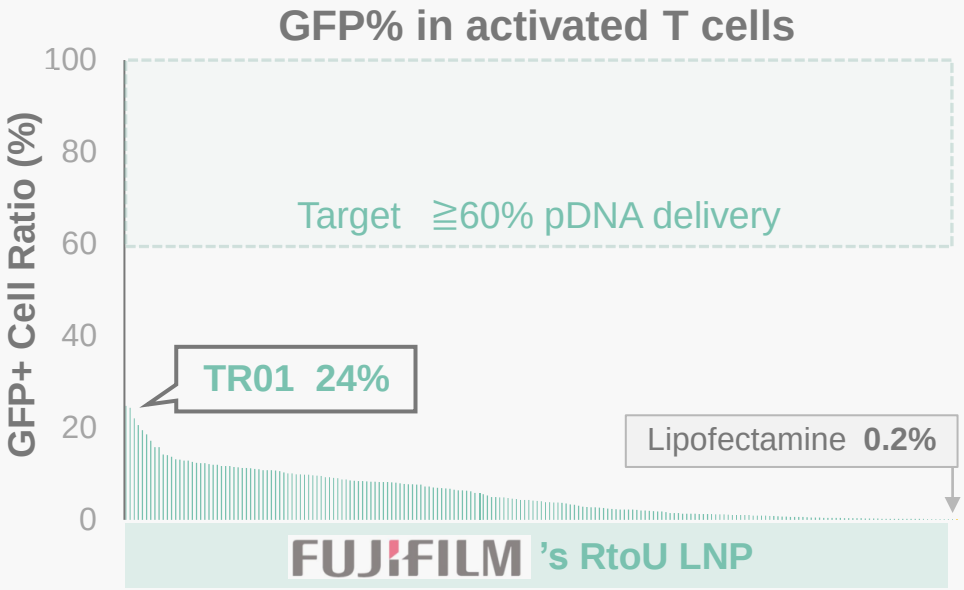


pDNA 1.8 μg/million cells with each LNP.
GFP expression ratio was examined at 1day after treatment.

➢ **pDNA delivery 24% was higher than Lipofectamine but not sufficient for knock-in.**

Study 2: pDNA delivery 1st screening using lipid library





pDNA 1.8 μg/million cells with each LNP.
GFP expression ratio was examined at 1day after treatment.

➢ **Even in LNP, which showed high knock-out in study 1, pDNA delivery % was low.**
→ **We worked on further improvement**

Study 2: pDNA delivery improvement from three perspectives

Topics

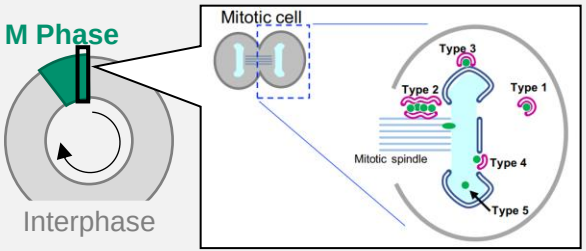
Cell Manipulation

Cellular environment change

Medium, Cytokine species,
LNP addition timing, etc
(*not disclosed*)

Reference

Transfected pDNA enters into nucleus during Telophase in proliferating cells

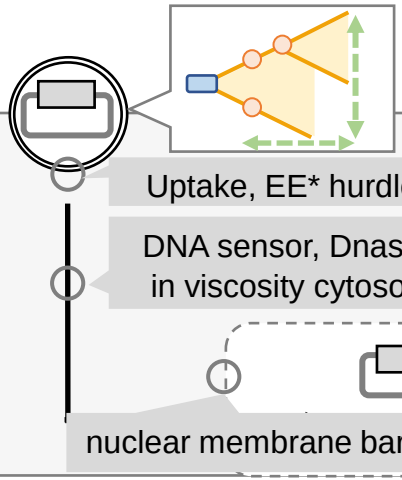


Communications Biology **2022**, *5*(1), 78

Lipid Design

New design and synthesis

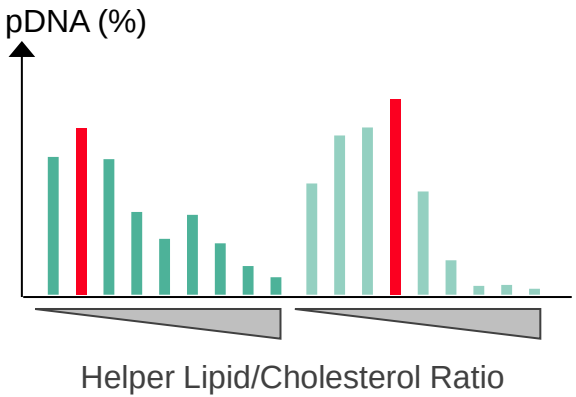
Multiple regression analysis
→ Extract key parameters
→ Re-design of structure



LNP Formulation

LNP optimization

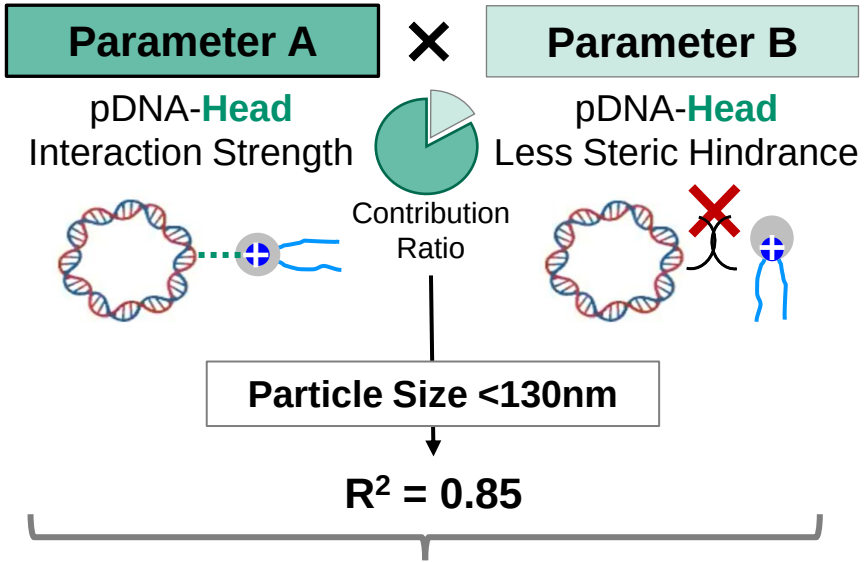
Helper species, four types of lipids ratio, etc
according to ionizable lipid

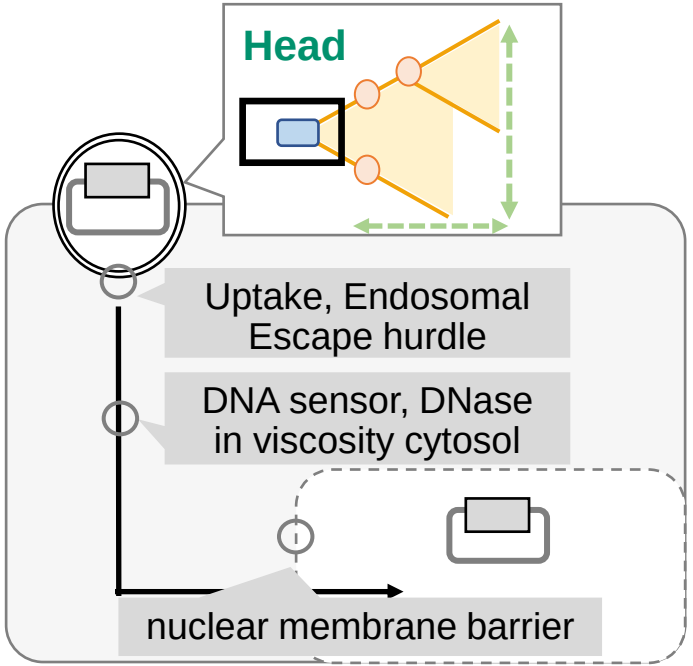


*EE: Endosomal Escape

Study 2: Parameters for new lipid design

Through multiple regression analysis of one part of 1st screening results, correlation was observed between **Parameter A×B** and pDNA delivery%.





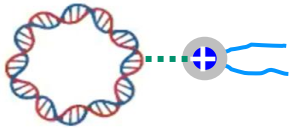
- **Strong interaction of pDNA to head-group of our proprietary lipids was efficient.**

Study 2: New lipids for pDNA delivery

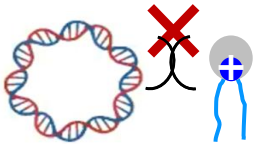
After that, we obtained another **Parameter C,** having both **Parameter A** and **B**,
and we designed new **Head** structures with **Parameter C** value↑, then actually synthesized.

Parameter A × **Parameter B**

pDNA-**Head** Interaction Strength

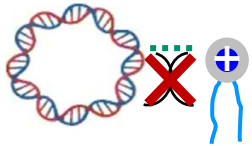


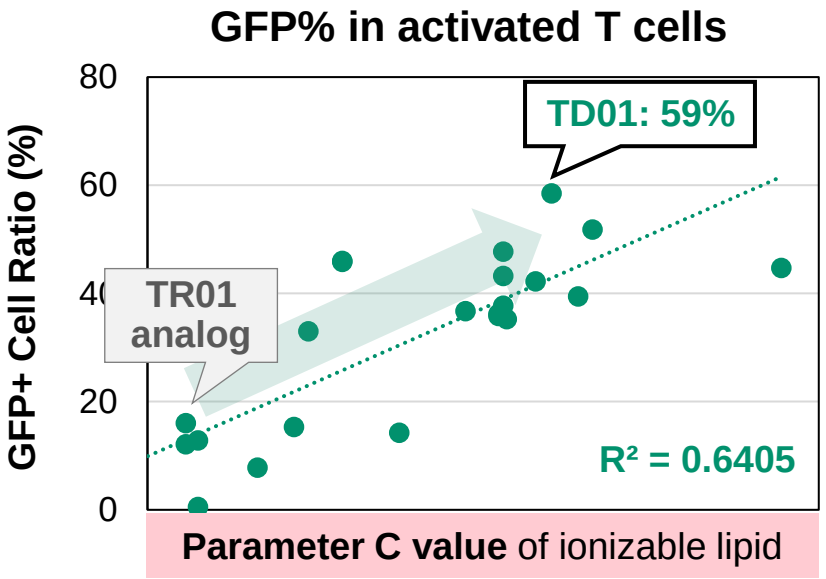
pDNA-**Head** Less Steric Hindrance



Parameter C

pDNA-**Head** Electrostatic Interaction

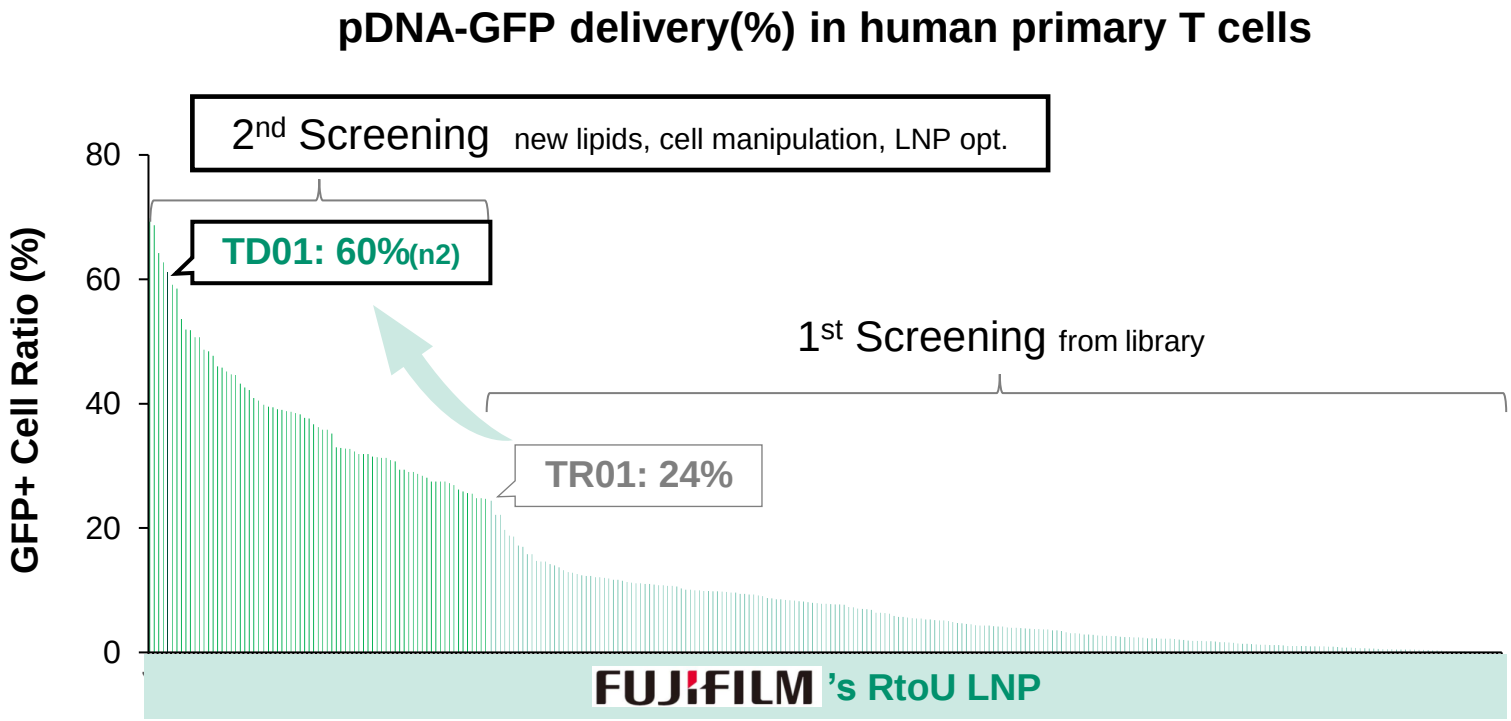




※LNPs with size <130nm

➢ **We developed RtoU LNP TD01 for pDNA delivery ~60%**

Study 2: Summary of pDNA-GFP Delivery



- **RtoU LNP TD01 can be evaluated under MTA.**

Outline

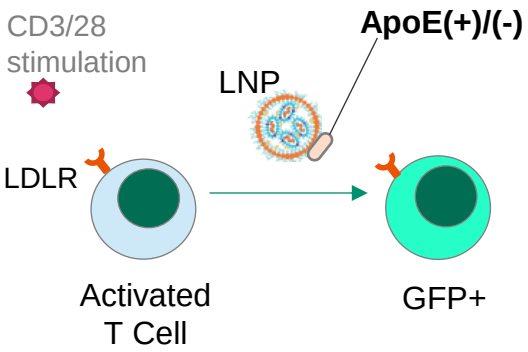
- Our proprietary ionizable lipids
- New Ex Vivo LNP formulations for knock-out and pDNA delivery to T Cell
- New In Vivo Extra-hepatic Lipids discovered from Ex Vivo study

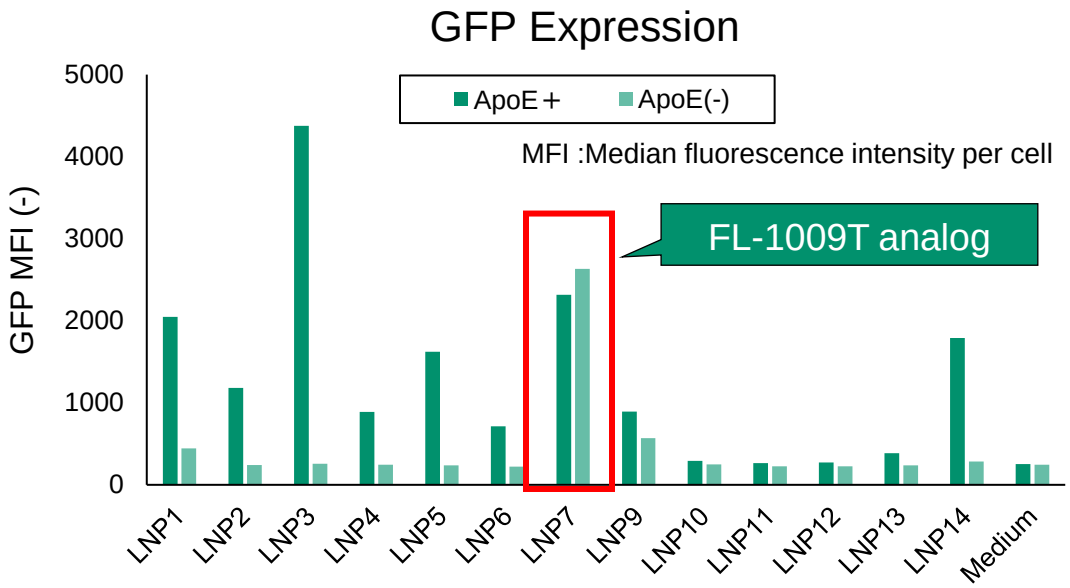
Opportunity: ApoE non-mediated uptake in ex vivo

GFP-mRNA, LNP1～14

Ex vivo Activated T Cell transfection

ApoE (+/-)



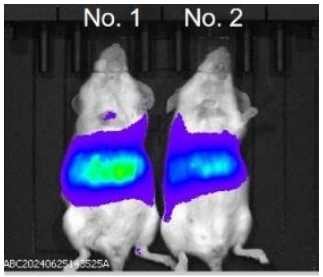


➢ **Lipid with ApoE non-mediated uptake potential was found in ex vivo study.**

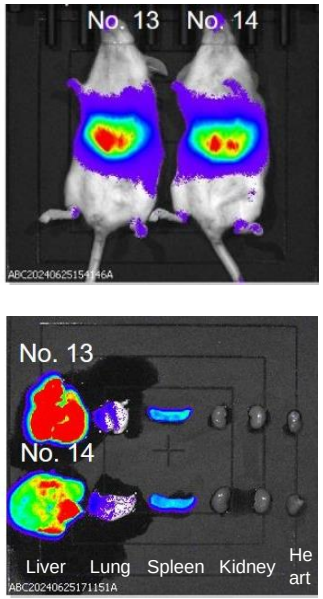
Expression Distribution in Mouse (i.v.)

LNPs encapsulating FLuc-mRNA
Experimental animal：ICR mouse (male, 5wk, N=2)
Dose：0.2 mg/kg(mRNA), i.v. 5hr(In Vivo), 6hr(Ex Vivo)

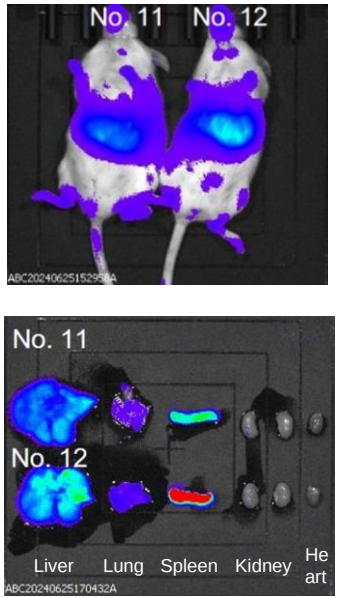
Lipid-5



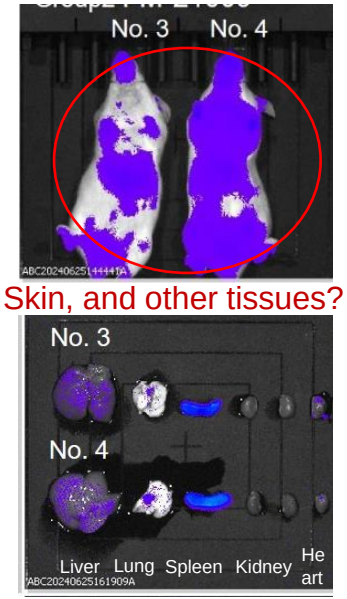
FUJIFILM-Lipid for Liver



Lipid **FL-1030T**



Lipid **FL-1009T**



Skin, and other tissues?

Further investigation is underway.

- **Lipids with different structure demonstrated different tissue tropism.**

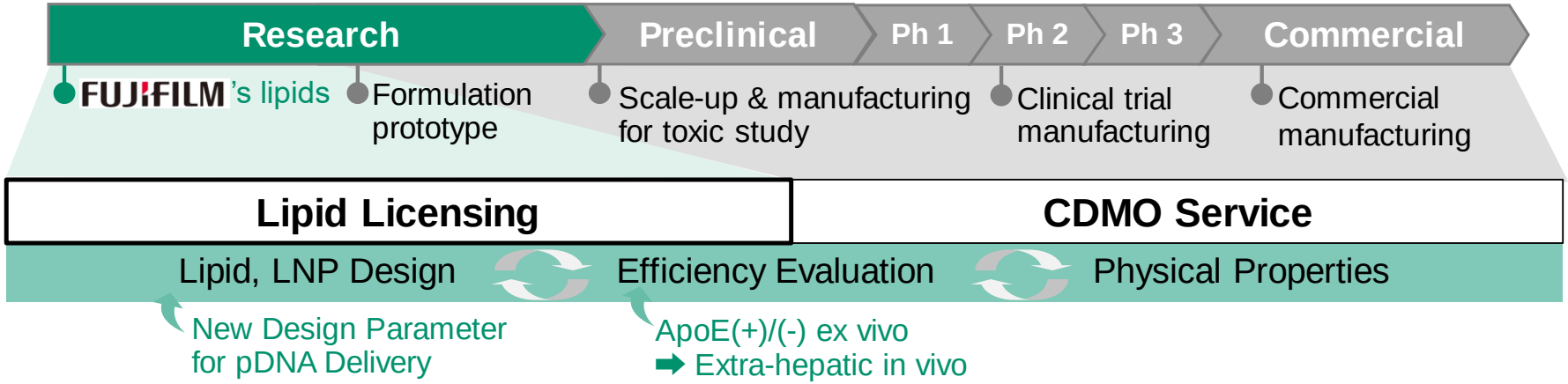
Summary

New Lipids and Delivery Technologies leading to Car-T Knock-In

- New Ex vivo LNP formulation **TR01** for host gene knock-out in *TRAC* locus **>80%**
- New Ex vivo LNP formulation **TD01** for pDNA-GFP delivery into nucleus **~60%**

New Lipids for extra-hepatic delivery in vivo

- Based on ApoE-independent in Ex Vivo, we obtained **FL-1009**(whole-body) and **FL-1030**(spleen)



Overview of FUJIFILM's lipids and RtoU LNP

Booth **No.19** FUJIFILM

In Vivo

FUJIFILM Lipid	GMP Mfg.	IP Filed	Application
FL-2266	✓	✓	im vaccine
FL-0445 FIH	✓	✓	
FL-1923T		✓	im DNA immune
FL-0207T		✓	iv liver
FL-1252T		✓	
FL-1207T		✓	NHP on-going
FL-1030T		✓	iv spleen
FL-1009T		✓	ApoE independent

Ex Vivo

Final Goal

CAR Knock-In in human primary T cell using FUJIFILM's LNP

Current Status

FUJIFILM RtoU LNP	GMP Mfg.	IP Filed	Application
TR01		✓	T cell high knock-out
TD01		✓	T cell high pDNA delivery
FL-1009T analog-LNP		✓	ApoE independent

➢ **These lipids and RtoU LNP formulations can be evaluated under MTA.**

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and many colleagues


Fujifilm Group's Purpose
Giving our world more smiles
We bring diverse ideas, unique capabilities,
and extraordinary people together to change the world.
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Value from Innovation