Simplifying Progress

LipidBrick®: Innovative Cationic Lipids Fuel Next-Gen LNPs for Targeted Oncology and Advanced Cell Therapies

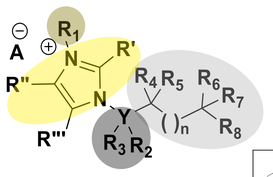
Pauline Urquia[1], Quentin Huaulmé[1], Mélodie Seiler[1], Marine Fenat[1], Margaux Briand[1], Kassandra Renaud[1], Julie Chevrier[1], Thibaut Benchimol[1], Malik Hellal[2], Claire Guéguen[1], Patrick Erbacher[1]

[1]Sartorius Polyplus, Vectura, 75 rue Marguerite Perey, 67400 Illkirch, France
[2]Sartorius Stedim FMT S.A.S. Zone Industrielle les Paluds, Avenue de Jouques, 13400 Aubagne, France

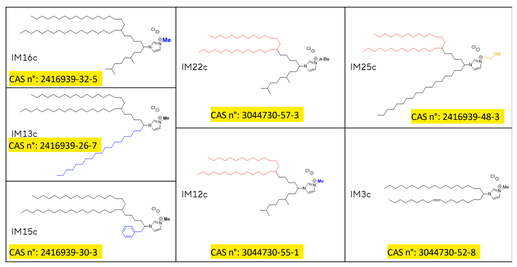
Lipid nanoparticles (LNPs) have shown promising potential in organ selective targeted -Oncology and Gene Modified Cell Therapies; however, the critical challenge lies in fine-tuning their formulation to enhance specificity, efficiency, and safety, to maximize therapeutic outcomes.
Here, we present a novel approach through the development of lipid nanoparticles (LNPs) derived from our library of Innovative cationic lipids. These LNPs demonstrate expanded extrahepatic biodistribution, improving drug delivery and cellular targeting. This addresses major challenges in cancer treatment for the development of therapeutic vaccines via targeting antigen presenting cells (APCs) and T cells in the spleen, and organ selective oncology treatments via local injection. Optimization of LNP formulations around our cationic lipids leads to localized expression without off-target effects unlike commonly used ionizable lipid-based LNPs.
In addition, we investigate ex vivo cell engineering, employing LNPs. We highlight the efficacy and versatility of LNPs based on our library of lipids, which enable the transfection of mRNA in different types of immune cells, leading to transient engineering of CAR-T and NK cells. This data suggests that LNP formulations based on our cationic lipids, with the addition of targeting ligands, could also prove effective for the generation of CAR-Ts and NKs in vivo. The data underpinning these applications were collected through a blend of proof-of-concept studies conducted both in vitro and in vivo, aimed at evaluating the efficacy and safety of imidazolium lipid-based LNP systems. Molecular assays were employed to monitor biodistribution and cellular uptake, offering real-world insights with an emphasis on safety and dosage optimization
.

LipidBrick® Library: A Range of Proprietary Cationic Lipids

Imidazolium-Based Cationic Lipids: Offering New Targeting Options and Improving Safety

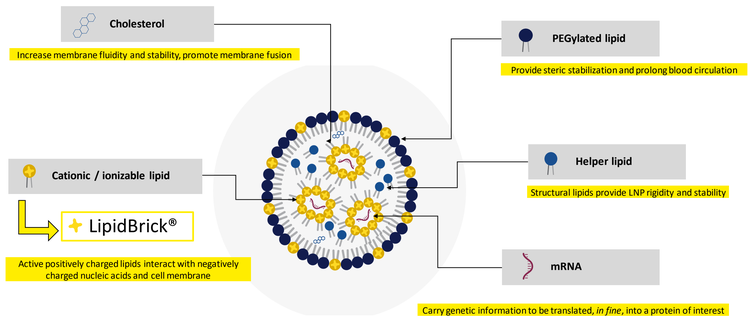


IM21.7c
CAS n°: 2416939-42-7



- New generation of cationic lipids dedicated to **LNPs or liposomes formulation**.
- Featuring an **Imidazolium polar head**, resulting in **Improved Safety** compared to traditional cationic lipids.
- Expanded into an **8-lipid library** for **greater flexibility** and **screening options**.

Enabling Formulation of Suitable 4- and 5-lipid Lipid Nanoparticles



- LipidBrick® Library is **composed of permanently charged cationic lipids** that play a key role in LNP composition, functioning as the active lipid that electrostatically binds with the genetic payload to enable **improved encapsulation efficacy, cellular uptake** and a **broadened biodistribution beyond the liver.**

Powering Tailored LNP Formulations Across Cell Types

Screening conditions:

LNP Composition	Molar ratio range (%)
LipidBrick® **IMXXc**	20 – 50
Phospholipid (DOPE or DSPC)	10 – 40
Cholesterol	28.5 – 48.5
DMG-PEG2k	1.5

Performance of best LNP Formulation for each LipidBrick® lipid:

LipidBrick®	IM3c	IM12c	IM13c	IM15c	IM16c	IM21.7c	IM22c	IM25c
HepG2								
HEK-293								
C2C12								
Huh7								
A-498								
Caco-2								
Primary T cells								

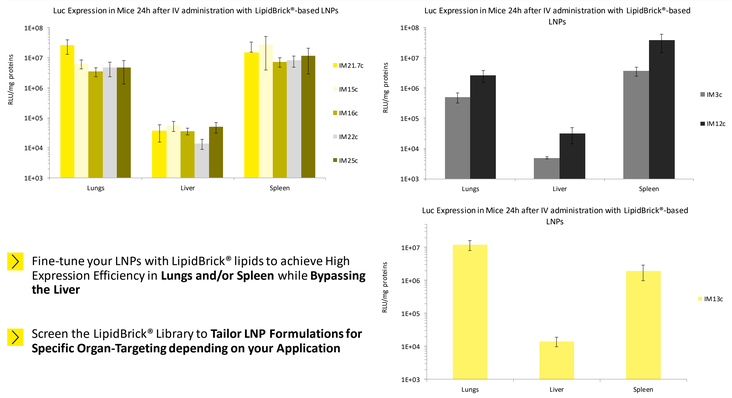
RLU
10^6 10^7 10^8 10^9 10^{10}

High-Throughput Screening of Diverse LipidBrick® Lipid-Based LNPs for Encapsulation and Delivery of Fluc mRNA: 25-100 ng of mRNA-LNPs were applied per well in 96-well plates, with luciferase expression evaluated 24- or 48-hours post-transfection across various cell types in vitro.

- Each application requires **tailored LNP formulations** to achieve optimal results (one size ≠ fit all!) .**LipidBrick® Library offers diverse options** with varied chemical structures, **facilitating high-throughput screening (HTS).**

Targeted Oncology: LipidBrick® Library for Targeted Cancer Therapies

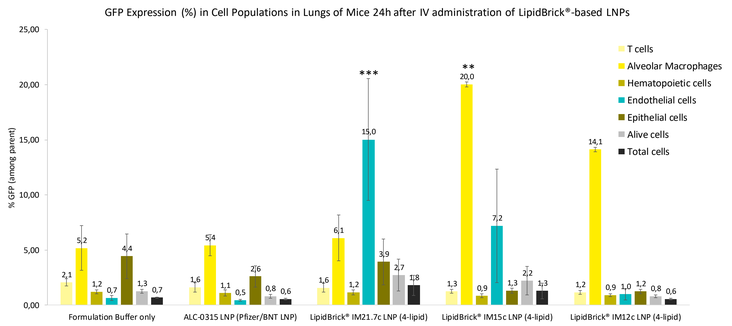
Tailored Formulations: Providing to modulate Biodistribution



- Fine-tune your LNPs with LipidBrick® lipids to achieve High Expression Efficiency in **Lungs and/or Spleen** while **Bypassing the Liver**
- Screen the LipidBrick® Library to **Tailor LNP Formulations for Specific Organ-Targeting depending on your Application**

10µg of mRNA encoding Fluc were injected through retro-orbital (RO) injection in mice. Luciferase expression was assessed 24 hours post-injection.

Empowering RNA Therapies: Cell-Specific Targeting with LipidBrick®-based LNPs

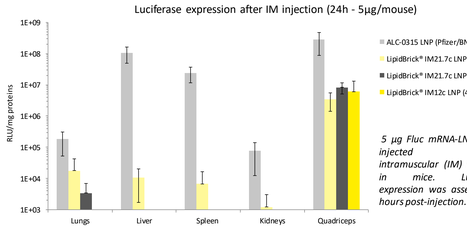


*10 µg CleanCap EGFP 5moU mRNA-LNPs at N/P ratio of 6.8 were injected through retro-orbital (RO) injection in mice. Lungs were harvested 24 hours post-injection, dissected and labelled before being analyzed by flow cytometry. One-way ANOVA, Tukey's multiple comparison vs. Formulation buffer only, ** p<0.01 and *** p<0.001.*

- Screen the LipidBrick® Library to Fine-Tune your LNP Formulations for **Specific Cell-Targeting** depending on your application.
- LipidBrick®-based LNPs present a promising approach for **high transfection efficacy in endothelial cells and/or alveolar macrophages**, **key targets for innovative lung disease therapies, including lung cancer**.

Fine-Tuning LNPs with LipidBrick® Library to Enhance Targeting Specificity

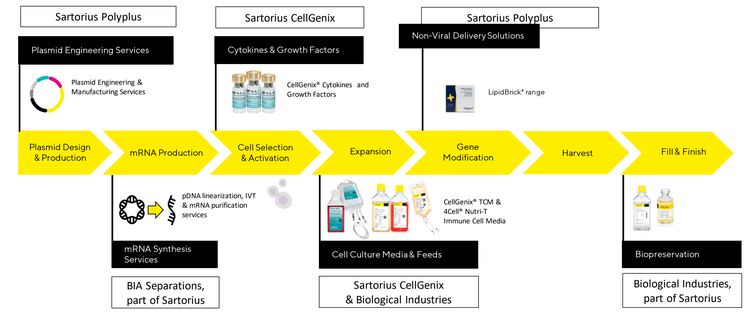
- **Precise targeting** via local administration
- **Reduced risks of side effects**
- LipidBrick®-based LNPs present a **promising approach intratumoral delivery for solid tumor treatment, local gene editing, protein replacement or tissue regeneration**



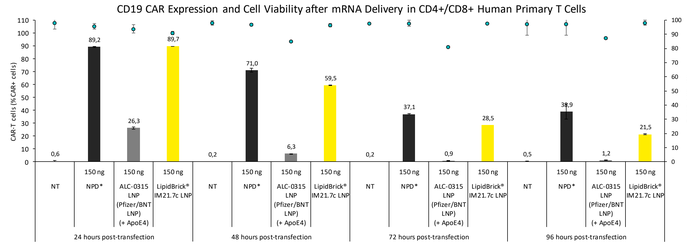
5 µg Fluc mRNA-LNPs were injected through intramuscular (IM) injection in mice. Luciferase expression was assessed 24 hours post-injection.

Advanced T Cell Therapy: LipidBrick® for Non-Viral Delivery

Comprehensive Portfolio for Non-Viral Gene-Modified Cell Therapy Solutions



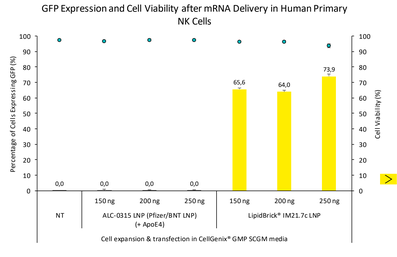
Enhanced & Durable CD19 CAR mRNA Expression in T Cells with LipidBrick®-based LNPs



Human Primary T cells were isolated from were PBMCs and frozen. Cells were thawed and cultivated in CellGenix® GMP TCM medium supplemented with CellGenix® recombinant human IL-7 (10 ng/mL) and IL-15 (10 ng/mL) at a density of 1.5x10⁶ cells/mL for 4 hours before being activated with T Cell TransAct™ for 2 days. On the day of transfection, T cells were washed and resuspended in CellGenix® GMP TCM medium supplemented with CellGenix® recombinant human IL-7 (10 ng/mL) and IL-15 (10 ng/mL). 187 500 cells were then plated per well of a 96-well plate and 150 ng of CD19 CAR mRNA-LNPs were applied per well. For ALC-0315 LNPs, each well was supplemented in ApoE4 to enable the transfection. After 4 hours, each well was supplemented with 175 µL CellGenix® GMP TCM medium containing cytokines. Percentage of CAR+ T cells and Cell Viability were then assessed by Flow cytometry 24h, 48h, 72h and 96h post-transfection. NPD: New Product in Development.*

- LipidBrick®-based LNPs present a **promising Non-Viral Delivery approach for *ex vivo* Gene-Modified Cell Therapy** applications, such as **CAR-T Cell Therapies**.
- Data suggests LipidBrick®-based LNPs with **targeting ligands** could be **effective for the generation of *in vivo* CAR-Ts and NKs**

Achieve Promising Transfection Efficacy in Primary Human NK Cells



Human Primary NK cells were isolated from were PBMCs and frozen. Cells were thawed and cultivated in CellGenix® GMP SGCM medium supplemented with human AB serum and a cocktail of cytokines at a density of 2x10⁶ cells/mL for 10 days. 187 500 cells were then plated per well of a 96-well plate and 150 to 250 ng of GFP mRNA-LNPs were applied per well. For ALC-0315 LNPs, each well was supplemented in ApoE4 to enable the transfection. After 4 hours, each well was supplemented with 175 µL CellGenix® GMP SCGM medium containing cytokines. Percentage of GFP+ cells and Cell Viability were then assessed by Flow cytometry 24h post-transfection.

- LipidBrick®-based LNPs demonstrate **High Transfection Efficacy** while Maintaining **High Cell Viability** onto **Diverse Primary Immune Cell Types.**