tebubio

Facilitators of Life Sciences Research

How to Identify Efficient LNP Formulations for Your Model ?

Insights from Tebubio's Delivery Platform

Xavier Warnet, PhD – Project Manager

February 6th 2025

Our Team Today

Speaker

Xavier Warnet, PhD

Project Manager, Formulation Specialist

Q&A Moderator

Erica Cirri, PhD

Project Manager, RNA Specialist

Session Moderator

Frédéric Samazan

Event Manager

Webinar Agenda

1. Tebubio at a glance
2. Lipid NanoParticles basics
3. Delivery & Formulation Challenges
4. Overview of Tebubio Delivery Platform
5. Case Study: LNP formulation screening for Cancer Cells Delivery
6. Innovations through our parnters
7. Live Q&A Session

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We facilitate Life Sciences Research everyday

and contribute to a brighter future

We are Pan-European

- Founded in 1953
- Family-owned
- 100+ Employees
- Local offices across Europe

We Act for Life Sciences

- Innovation is in our DNA
- Contract Research Services Lab
- Part of EU Life Sciences ecosystems

Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA
FRANCE 2030
MUR Ministero dell'Università e della Ricerca
obn
bpifrance
ASE BIO
BIO DEUTSCHLAND

We Care

- Ethical, compliant and transparent sourcing (from OEMs only)
- Animal welfare policy
- Corporate Social Responsibility (ISO 14001 / ISO 9001, Decarbonation Program)

With Tebubio, advance your Research Projects faster

Thanks to our Holistic Range of Solutions

A large portfolio

Access to **over 1,300,000 standard** references and **non-catalogue** ones.

From trusted & ethical suppliers

Get solutions from **reputable global suppliers**, carefully selected for their **relevance**, **ethical** and **legal** compliance (e.g. Animal Welfare).

Dedicated scientific support

Our **Scientific Team** guides you to source, select and use solutions.

Based in Europe

Our **Teams** and **Contract Research Services Lab** are in Europe.

Team committed to success

A **PhD project manager** ensures the success of your project from A to Z.

Strong expertise in Life sciences

- **mRNA** production & delivery
- **Cell line** engineering & **protein** production
- **Cellular studies**
- **Biomarkers** & Biostatistics analysis

Reliable Supply Chain Management

- **IATA**
- **Human/Animal Biological Solutions**
- **Sourcing outside** existing suppliers
- **Warehousing** services

Order from a single source

Consolidate your orders with us.

Tailored agreements

From specific **one-off terms** to **procure-to-pay**, supported by **e-procurement** solutions.

Tebubio CRS : Facilitators of Life Sciences Research

What do you want to talk about?

RNA based therapeutic discovery

Production | Delivery | Expression

in vitro modeling

Organoids | Microfluidics | 2&3D Models

Biomarker mapping

Target identification | Profiling | Quantification

Data analysis

Biostatistics | NGS | Custom

Our Speaker

Xavier Warnet, PhD

CRS Laboratory Project Manager

- Xavier joined Tebubio in February 2024 as a Project Manager on the RNA based Therapeutic Discovery platform . With a robust academic background, including a Ph.D. in Biochemistry and Biophysics from University Paris Cité, he brings deep expertise in biomolecular research, analytical techniques, and innovative drug delivery technologies.
- Prior to his role at Tebubio, Xavier led the diagnostic team at LPS-BioSciences, focusing on the structural and functional characterization of Lipopolysaccharides (LPS) through advanced analytical techniques such as mass spectrometry (LC-MS-MS), LAL, and MAT assays.
- At Tebubio, Xavier now leverages his expertise to guide clients in optimizing their delivery projects, and continues to develop and expand Tebubio's LNP (Lipid Nanoparticle) delivery platform.

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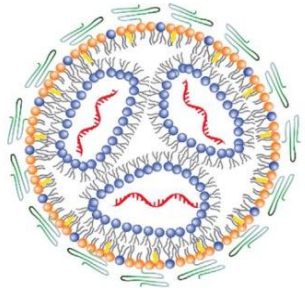
Delivery or Formulation Challenges

Some Basics on LNPs

- **Approved by the FDA for mRNA delivery.**
- **Biocompatible by nature.**
- **"Easy" to synthetize.**
- **Encapsulation of various cargos**
  (miRNA, siRNA, mRNA, …)

LNPs are **composed of (at least) 4 different "classes" of lipids**:

- **Ionizable/cationic lipid** (~50%):
- **Sterol** (~38%):
- **Structural lipid** (~10 %):
- **PEG-lipid** (~1-2%):



Lipid nanoparticle (LNP)

Hald Albertsen C. et al., Advanced Drug Delivery Reviews (2022)

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Main obstacles for efficient LNP preparation and Delivery

OR

Delivery Services

mRNA preparation

- Inadequate sequence (UTRs, polyA, etc)
- Suboptimal modifications (capping, UTPs)
- mRNA solution pH

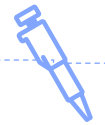
Lipids Composition & Mixing

- Lipids proportions
- Type of ionizable lipids
- Proportions of lipids to mRNA (N/P)
- Mixing

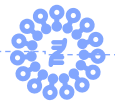
LNP Formation

- LNP stability remains an issue (addition of cryoprotectant possible)
- LNP sizes and homogeneity crucial

- Low translation/expression.
- Poor expression and toxicity.
- Poor encapsulation efficiency.



- Heterogeneous LNP (polydisperse)
- Poor encapsulation efficiency.
- Poor transfection and/or expression in vitro and *in vivo.*



- Loss of LNPs structure.
- mRNA degradation.
- Poor transfection and translation *in vivo.*

Formulation screening remains essential

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We can Support You Throughout Your Entire mRNA Workflow

More insights on our Delivery Platform

Why choose Tebubio Contract Research Services?

- **Different** entry points
- **Flexibility** : from formulation screening, to larger batch encapsulation for in vivo studies.
- **Accessibility** to a wide variety of off-the-shelf formulation and lipids.

We can Support You Throughout Your Entire mRNA Workflow

What about custom mRNA production.

Want more information on our mRNA production platform and capabilities ?

Ask to receive the recording of our last webinar

A complete solution for preclinical research

Our microfluidic devices

For screening and initial optimization …

Minimum amounts of reagents

Encapsulation from 10µg

Spark NanoAssemblr
Precision NanoSystems, Cytiva

… To advanced optimization and validation

Fine tuning of particles properties

Encapsulation to few mg RNA

Tamara
Inside Therapeutics

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Case Study: LNP formulation screening for Cancer Cells Delivery

Project

Identify the optimal LNP-based formulation to ensure efficient delivery of the candidate RNA to a spheroid cancer cell model (HCT116).

Solution

- **15 different formulations** were settled, thanks to our large library of lipids, to identify the most efficient combination
- **Small scale screening** were performed, to identify a primary **selection of potent formulation specific** to the customer cellular models, including:
  - Quality control of LNPs physicochemical parameters : Encapsulation efficiency, average particle size and polydispersity index
  - Control of LNP Internalization and RNA translation efficiency : Fluorescent lipids and eGFP-mRNA.

Customer pain point

- Finding the adapted lipids and formulation parameters.
- Characterize, select and optimize the best formulation.
- Assess transfection efficiency

Case Study: LNP formulation screening for Cancer Cells Delivery

LNP Formulation Design and Encapsulation

15 different formulations built

- Combining the most relevant lipids for the HCT 116 model
- Selection from our library and partners
- Formulations composed by:
  - **Mix of 5 different ionizable/cationic lipids**
  (ALC-0315, SM-102, LipidBrick IM21.7c, CP-LC-0741, DODMA)
  - **Mix of different structural lipids**
  (DSPC, DOPE)
  - **7 formulations with custom fluoresecent lipids**
  (Cy3-1,2-Dipalmitoyl-sn-glycero-3-phosphoethanolamine)

Encapsulation

- 5-15 µg of mRNA used for each formulation
- eGFP mRNA produced and optimised by Tebubio

Case Study: LNP formulation screening for Cancer Cells Delivery

Quality control of LNPs physicochemical parameters

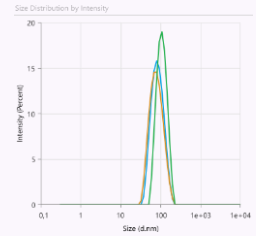
Quality control

- **Encapsulation efficiency (EE)** analysis (%) by Ribogreen Assay
- **Average particle size (z-average)** by DLS
- **Polydispersity index (P)** by DLS

	1	2 *	3 *	4	5	6	7 *	8
EE (%)	98,2	62,5	41,3	63,3	99,6	100,0	43,4	87,7
z-average (nm)	84,4	75,0	72,1	74,8	120,1	109,8	77,4	65,6
PI	0,23	0,21	0,15	0,14	0,23	0,20	0,15	0,17

	9 *	10 *	11	12	13	14	15
EE (%)	99,6	99,8	90,3	84,6	82,9	87,2	91,1
z-average (nm)	95,4	100,4	90,7	104,3	129,9	100,1	95,7
PI	0,29	0,24	0,20	0,07	0,06	0,07	0,09

Formulations incorporating **fluorescent lipids.**



Analysis of physicochemical parameters provide already some insights

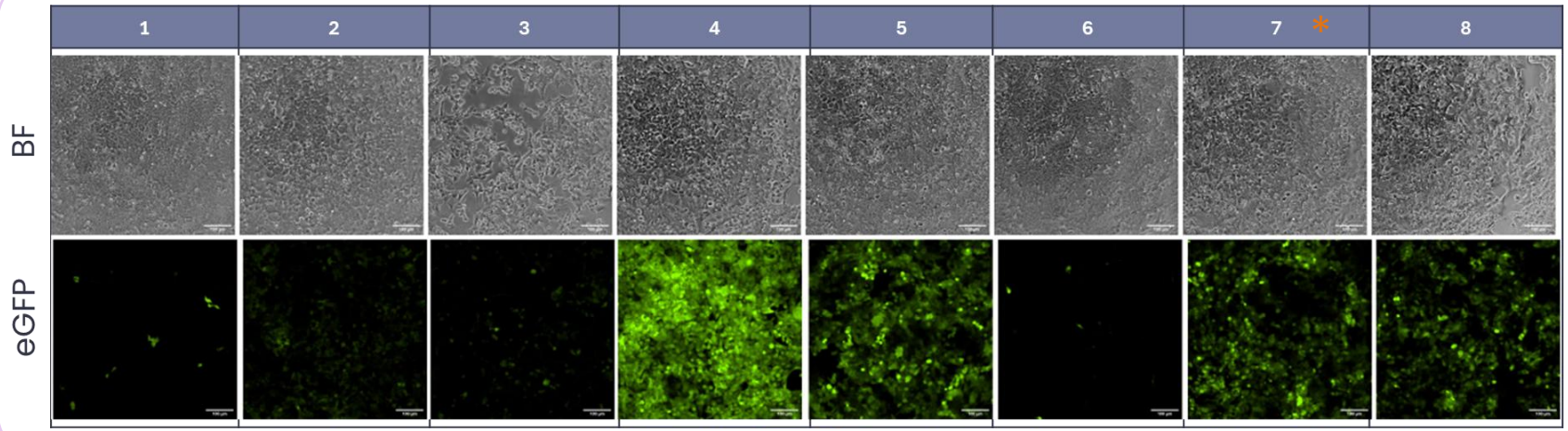
- Poor encapsulation efficiency (*) : #3 #7 #2 & #4 ➔ If those formulations proved to be efficient, more optimization would be required to achieve proper encapsulation.
- High heterogeneity (*): #9 &#10 ➔ which would be a problem for *in vivo* transfections but should have less of an impact for *in vitro* studies.

Case Study: LNP formulation screening for Cancer Cells Delivery

Control of internalization and RNA expression

Quality Control

- **Transfection into HCT116 2D** model
- **200ng of RNA used**
- **Analysis by microscopy :** Brightfield (BF) (cell viability) and Fluorescence (translation level)



Expression analysis allow us to perform a first selection of efficient formulations

- All the formulations tested led to protein expression.
- From this first screening, it was possible to select the most promising formulations : #4 #5 #7 & #8).
- It should be noted that poor encapsulation efficiencies is not necessarily connected to low protein expression (i.e #7, 43% encapsulation).

Case Study: LNP formulation screening for Cancer Cells Delivery

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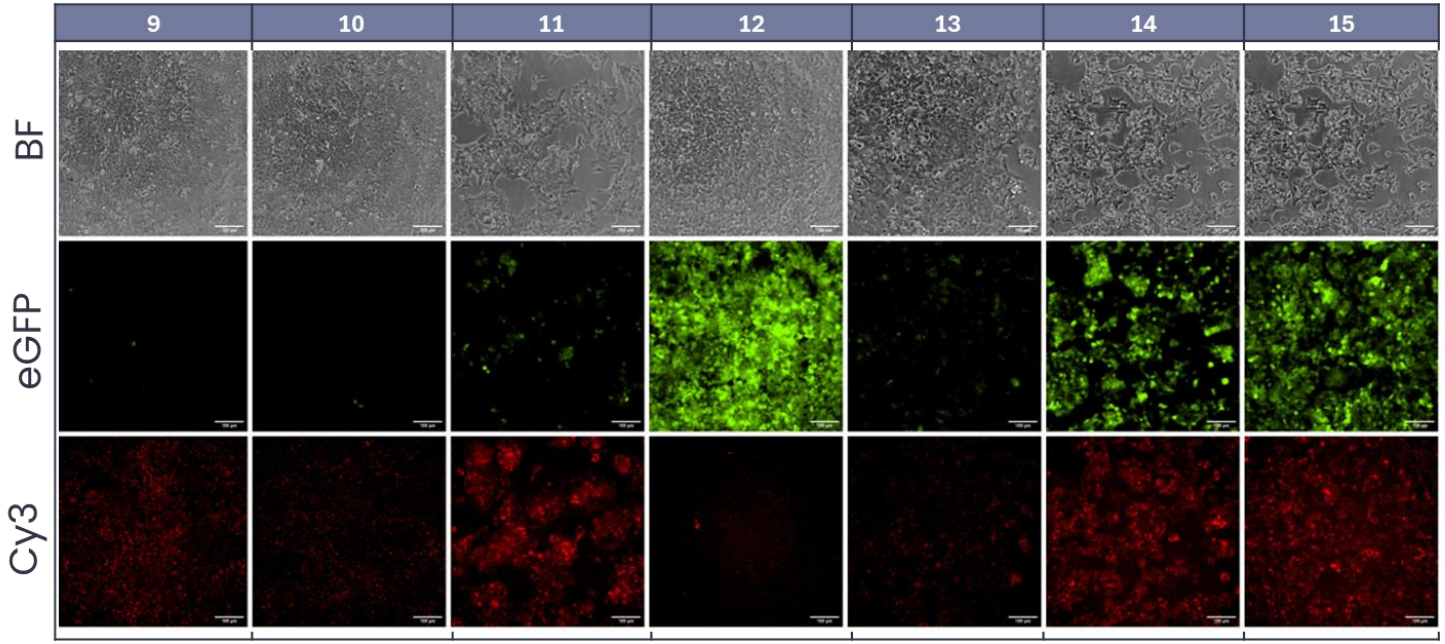


<u>Expression analysis allow us to perform a first selection of efficient formulations</u>

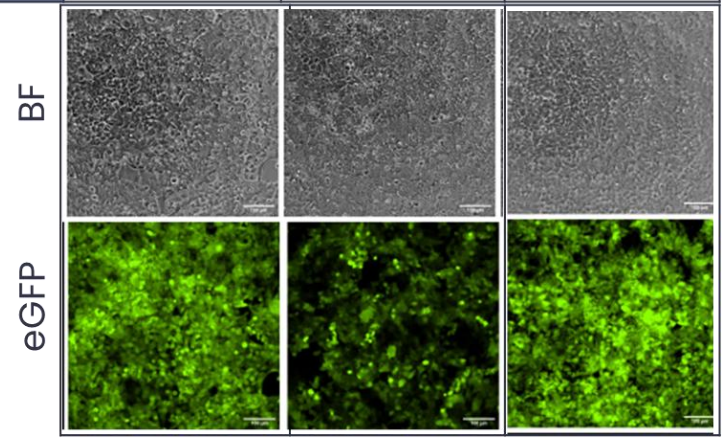
- Fluorescent LNPs offer a complementary analysis.
- Membrane fusion **appears** to work for all of them (Cy3) which would indicate that the low expression level obtained for some of them (#11 and #9) might be related to poor endocytosis.

Case Study: LNP formulation screening for Cancer Cells Delivery

Conclusion

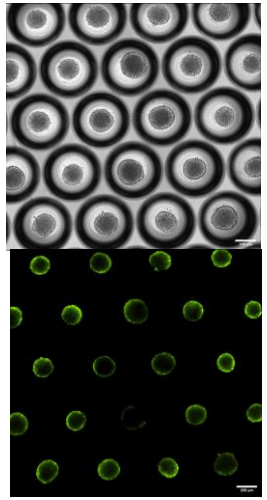
- We managed to select from 15 different formulations with different lipids compositions for their efficiency *in vitro* in HCT116 cell lines.
- Adding fluorescent lipids showed that some formulations fused with cellular membranes but did not lead to protein translation, suggesting some defects during endocytosis.
- This screening allowed us to identify the most potent formulations that would be used for transfection of cancer cells (HCT116) cells.

	4	5	12
EE (%)	63,3	99,6	84,6
z-average (nm)	74,8	120,1	104,3
PI	0,14	0,23	0,07



Added Value

- Access to different innovative lipids (ionizable, and others) and to provide ways of tracking LNPs.
- We offered an integrative approach, from LNP preparation to downstream analyses.
- These formulations constitute a cornerstone for in vivo experiments.



mRNA delivery – Our services

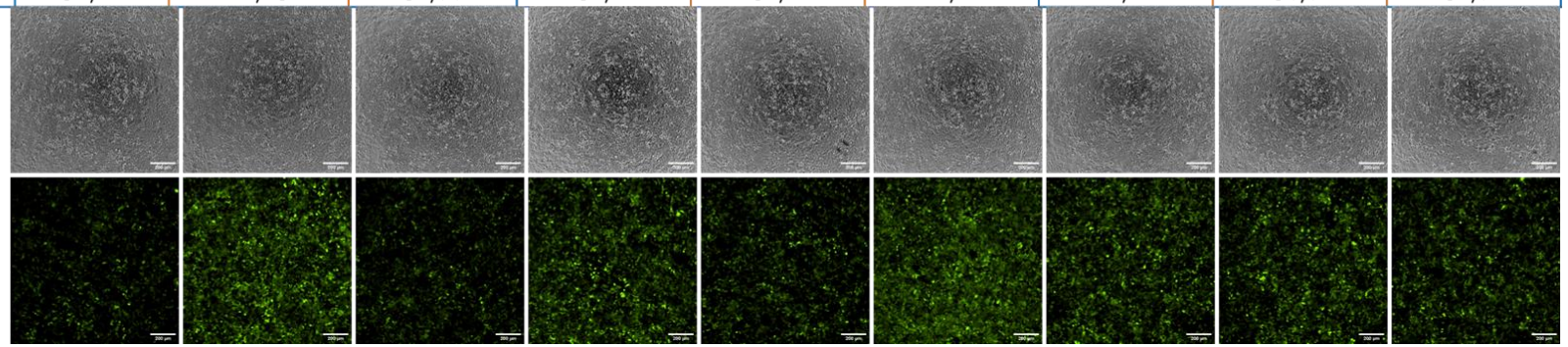
Encapsulate higher mRNA quantities

Tamara
Inside Therapeutics

Upscalling

- **We used off-the-shelf lipid mixtures** (LNP-0315, ABP Biosciences) **and eGFP mRNA** (Cap1, 120A, N1-Methyl-Pseudouridine) **for simplicity purpose.**
- **We tested different formulation parameters.**

	Flow rate ratios			N/P ratios			Total Flow rate (ml/min)		
	3	4	5	4	6	10	1	2	4
z-average (nm)	156,8	149,7	168,1	155,8	173,0	149,9	183,9	186,0	163,9
Polydispersity index	0,014	0,024	0,033	0,037	0,027	0,017	0,040	0,047	0,020
% encapsulation	89,07	74,78	82,33	87,17	87,12	70,27	77,52	83,31	87,32



- Transfection efficiency was assessed in different cell types (Fibroblasts, MCF, and HCT116) with similar results.
- The next step is to formulate higher quantities of mRNA using the formulations selected in the previous step.

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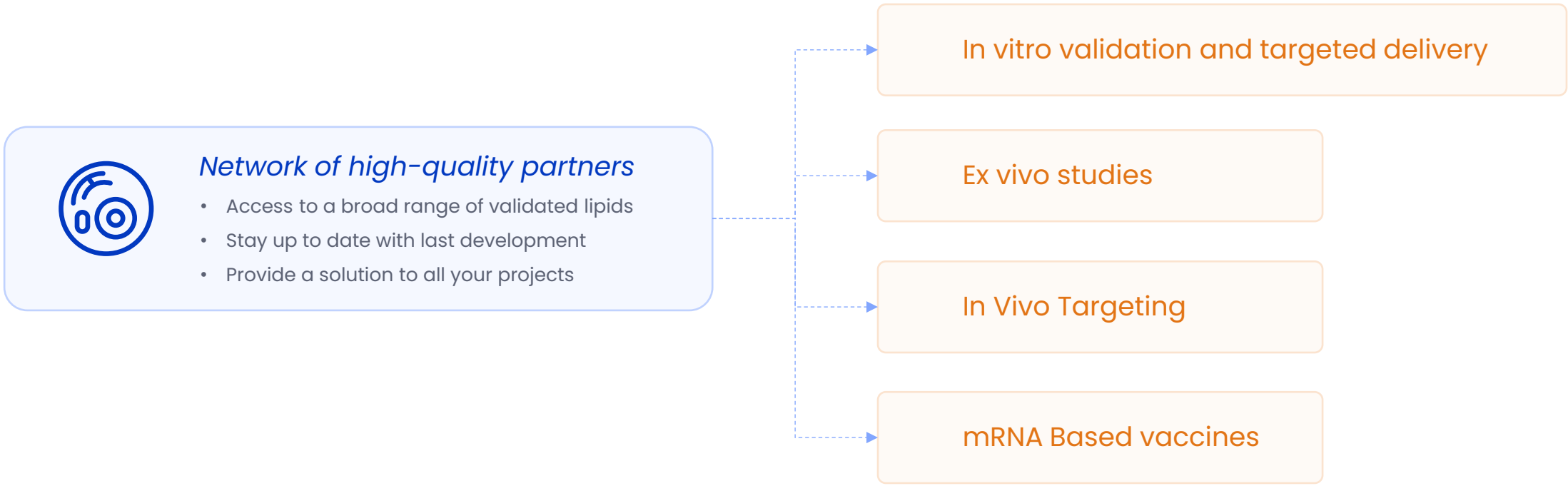
Access to a broad range of validated Lipids



Access to a broad range of validated Lipids

Network of high-quality partners

- Access to a broad range of validated lipids
- Stay up to date with last development
- Provide a solution to all your projects

In vitro validation and targeted delivery

Echelon Biosciences

BROADPHARM

SARTORIUS Polyplus

LipidBrick®

Performance of best composition per LipidBrick® lipid

	IM3c	IM12c	IM13c	IM15c	IM16c	IM21.7c	IM22c	IM25c
Hep G2								
HEK-293								
C2C12								
Huh-7								
A-498								
Caco-2								
T cells								

Extensive library of ionizable/cationic lipids from the literature.

Access to a broad range of validated Lipids

Network of high-quality partners

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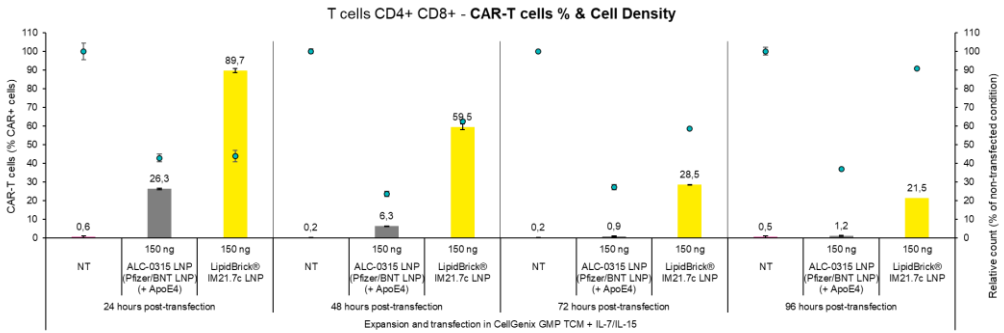
Ex Vivo Studies



LipidBrick®IM21.7c-LNPs allows Efficient & Durable CAR Expression in Primary T cells using low mRNA amount (~0.8 µg CAR mRNA/106 T cells) & without the need of ApoE supplementation.

LipidBrick® efficient tool for CAR expression

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Access to a broad range of validated Lipids

Network of high-quality partners

- Access to a broad range of validated lipids
- Stay up to date with last development
- Provide a solution to all your projects

mRNA based Vaccines

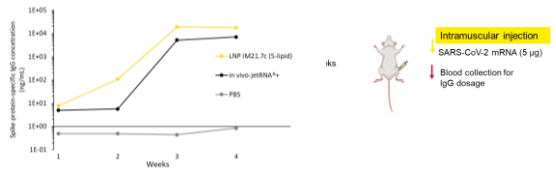
Off-the-shelf formulation (ALC-0315, SM-102)

Innovative lipids for efficient and safe vaccines solutions

LipidBrick®

SARTORIUS

Polyplus



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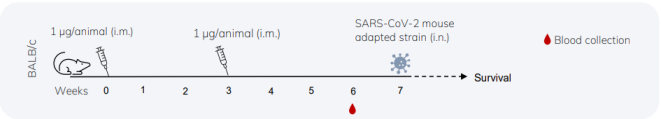

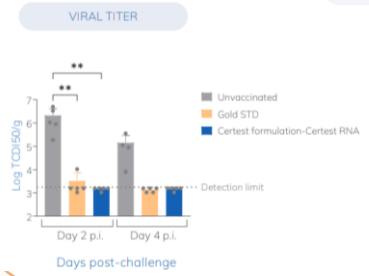

Our partners have performed extensive analyses of their lipids efficiency for vaccines *in vivo.*

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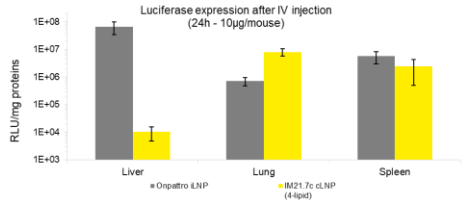
Access to a broad range of validated Lipids

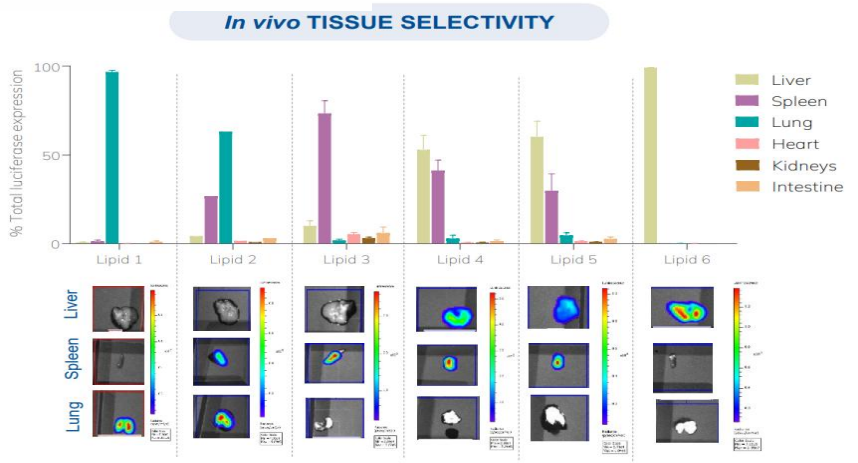
Network of high-quality partners

- Access to a broad range of validated lipids
- Stay up to date with last development
- Provide a solution to all your projects

In Vivo Targeting

LipidBrick® efficient lipid for extra-hepatic targeting, in itself or with other ionizable lipids.





All Certest lipids have been validated *in vivo*

Support you to identify the best solution for your project

Take home message

Propose multiple answer to your projects

Thanks to our extensive lipids' library : off-the-shelf formulations, "classical" ionisable lipids, innovative ionisable/cationic lipids, conjugated lipids, etc.

Flexibility around nucleic acids encapsulation

Whether you want to find the right formulation or want to validate in vivo your targets, we can offer adapted solutions.

Finding the right formulation for your projects

through our established partners, we have access to multiple approaches for *in vivo*, *in vitro*, *ex vivo* or mRNA-based vaccines applications

Comprehensive Services

Our experts will help you to move from proof-of-concept studies to validation of your strategies and find the solution to your problematic.

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Any Questions ?

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