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Expert Pharm/BioPharm
Products & CTDMO Services

Early Formulation Screening service

Accelerating LNP-mediated Nucleic Acid Medicine via a Powerful Ionizable Lipid Library Toolbox

Dr. Eleni Samaridou

Early Formulation Screening Service
Merck KGaA, Darmstadt, Germany

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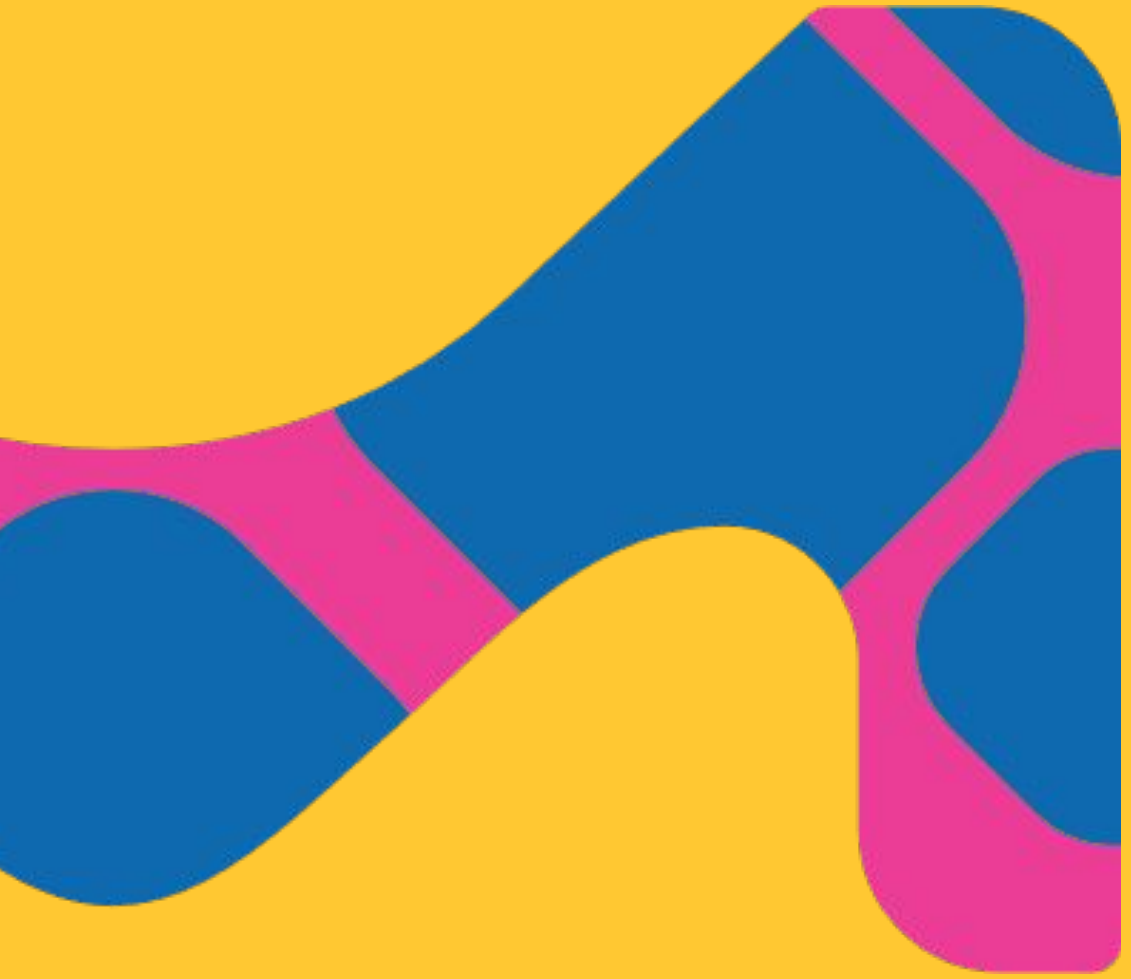

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

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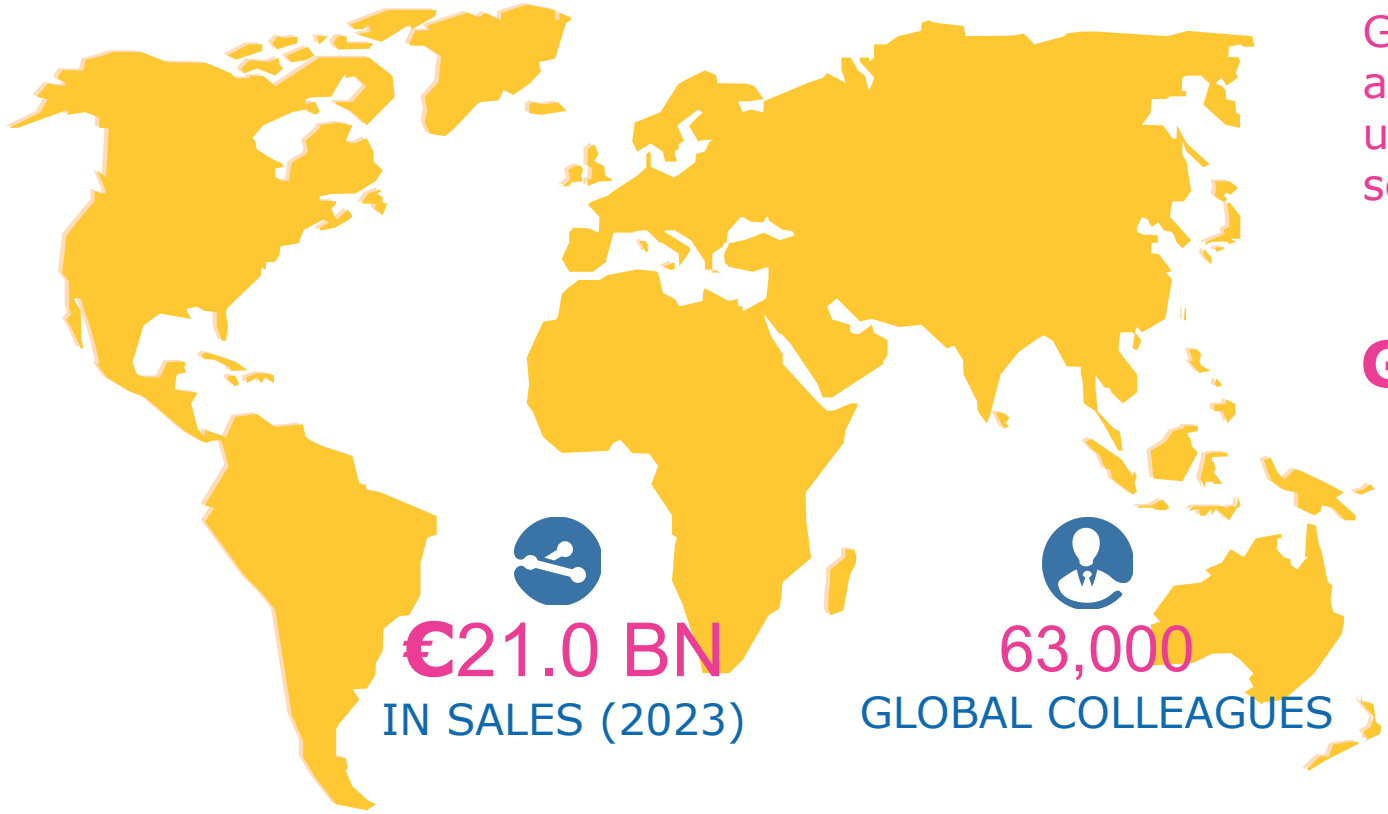
Who is
Merck KGaA, Darmstadt, Germany



€21.0 BN
IN SALES (2023)

63,000
GLOBAL COLLEAGUES

We are
a vibrant science and technology company

Founded in 1668, Merck KGaA, Darmstadt, Germany is the world's oldest pharmaceutical and chemical company and comprises of three unique businesses focused on healthcare, life science and electronics.

GLOBALLY WE ARE...

350+
HISTORY

65
COUNTRIES

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Our integrated CTDMO offer: Customizable manufacturing from mRNA through lipids to fill & finish

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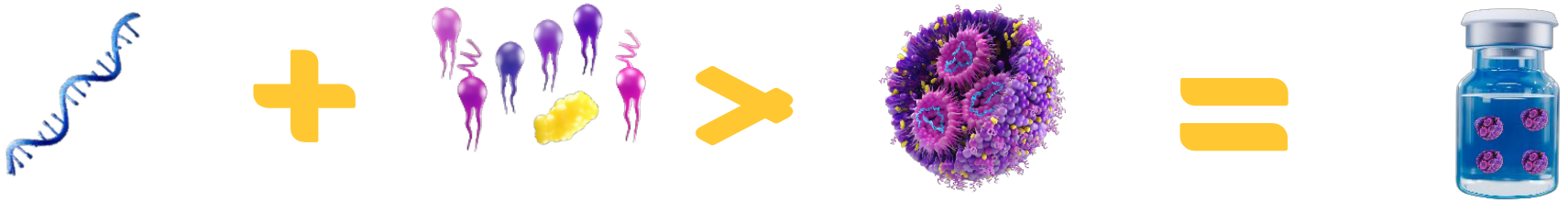
What we do

Drug substance → Excipients → Drug Product → Packaging



High quality and performance mRNA	High purity "off the shelf" and custom proprietary lipids	Formulation development & adaptable workflow to your process needs	Fill & finish: liquid and lyophilized

How we do it

1. Quality through Technical differenciation
2. Single contact point for a simplified customer experience
3. Program de-risk through Regulatory expertise

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The **Early Formulation Screening service** brings in the technical expertise supporting the **preclinical formulation development**

Preclinical

- **Formulation Screening**
- Formulation Optimization
- Early Process Development
- Analytical characterization & potency testing
- Stabilization and stability testing
- Lipid metabolism & clearance

Clinical

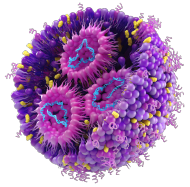
- Technology Transfer
- Process Scale-up
- Clinical GMP Manufacturing
- CMC document support for regulatory filing

Commercial

- Commercial GMP Manufacturing
- Aseptic Fill
- Quality Control
- Packaging & Inspection
- Supply Chain

Early Formulation Screening Service

Our integrated LNP CTDMO offering



Our unique approach - Formulation Screening

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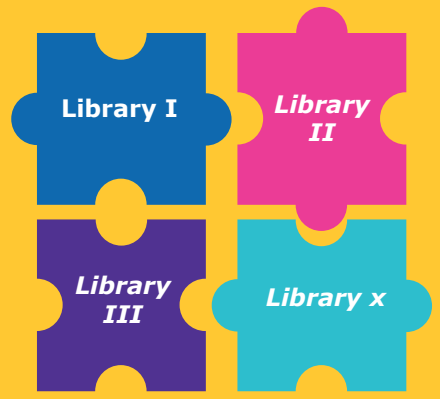
Access to a rationally designed ionizable lipid toolbox

OUR Business model

IP rights to top performing ionizable lipids – **cherry-picked from multiple patent families**

Unique combination
of state-of-the-art
ionizable lipid libraries



- ✔ **Preclinical** formulation development (incl. GLP-tox) **under existing Merck licenses** ☐ ***no license fees for clients***
- ✔ License required only when ready to enter **GMP phase**

strong lipid candidates identified
including performance confirmation in client projects

- ❑ We have **evaluated** internally & together with our lipid partners **hundreds of ionizable lipids** and have **identified the most potent-ones** for your application

Examples of Merck criteria for in-licensing selected ionizable lipids:

- ✔ ***Lipid chemistry****, purity, scalability, biodegradability & stability*
- ✔ ***FTO*** *through licensing for broad application field*
- ✔ ***In vivo efficacy & tolerability*** *– benchmarked to market LNP*
- ✔ ***Delivery to liver and beyond***

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Formulation Screening

Screening criteria selected based on customer project

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✔ **Diverse screening criteria** can be applied depending on customer project
✔ **Strong lipid candidates already identified** for diverse applications - allowing **focused screening panels per application**

Example of heatmap generated based on requested formulation screening

Selected LNP	Size	PDI	EE	pKa	Protein Expression				Cell Viability				Hemolysis	Membrane Fusion	Hit
					HepG2	C2C12	A549	RAW	HepG2	C2C12	A549	RAW			
1															✓
2															✓
3															×
4															×
5															×
6															×
7															✓
8															×
9															✓
10															✓
...															
Acceptance criteria	<100 nm	<0.3	>80%	6-7	>BM				≥BM				≤BM	≥BM	✓

PDI: polydispersity index
EE: encapsulation efficiency

Performance ≥ marketed LNP BM	
Performance < marketed LNP BM	

BM = Benchmark (marketed LNP)

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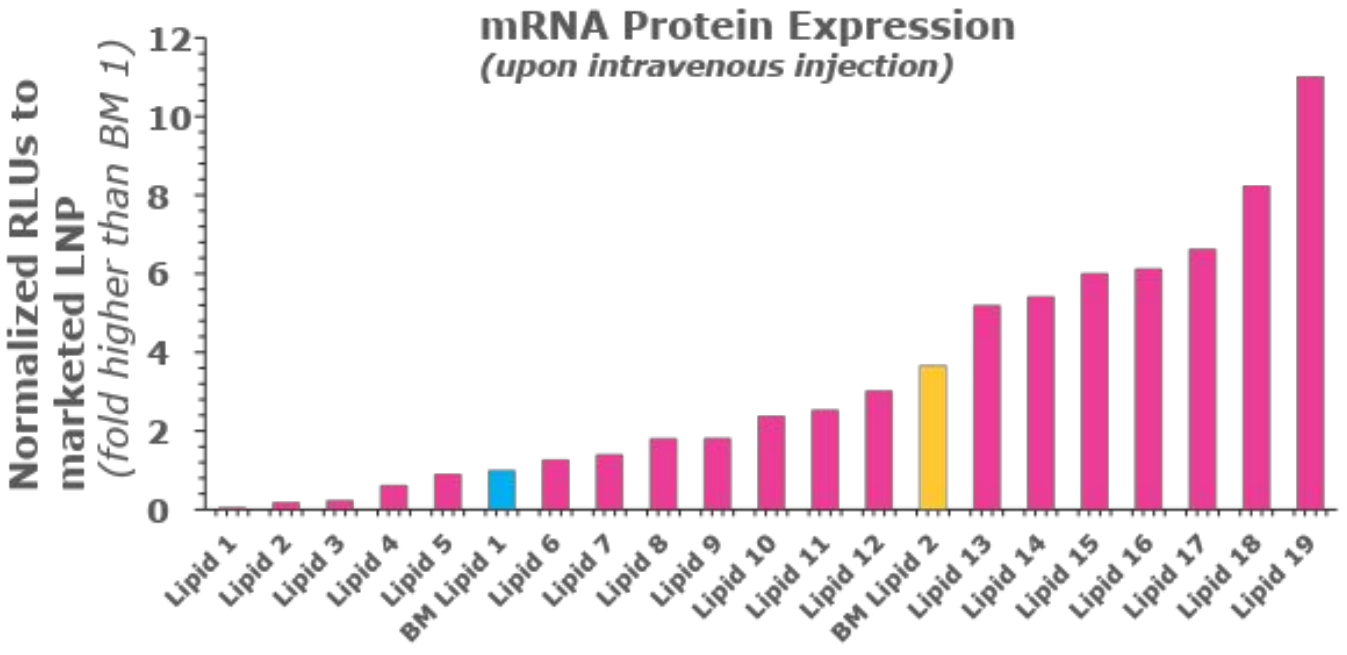
Our ionizable lipid toolbox

Selected lipids from multiple proprietary libraries – benchmarked against marketed LNP

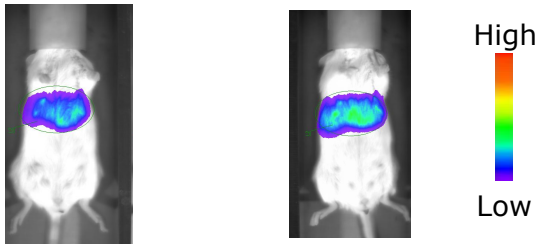


***In vivo* efficacy**
≥ marketed LNP Benchmarks (BM)

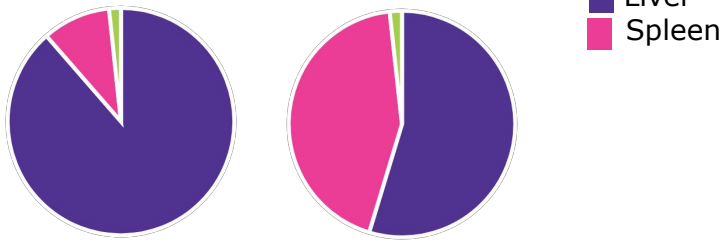
***In vivo* tissue tropism** *tuned by varying the ionizable lipid*

Ionizable Lipid 1 | Ionizable Lipid 2

***In vivo* Luciferase activity**
at 6h post dose *(intravenous injection)*



***Ex vivo* Luciferase activity**
at 6h post dose *(intravenous injection)*



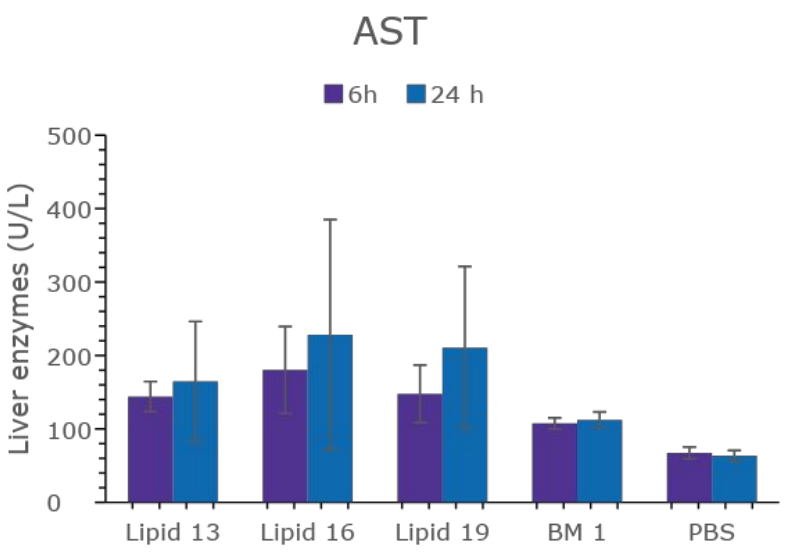
**BM = Benchmark (marketed LNP)*

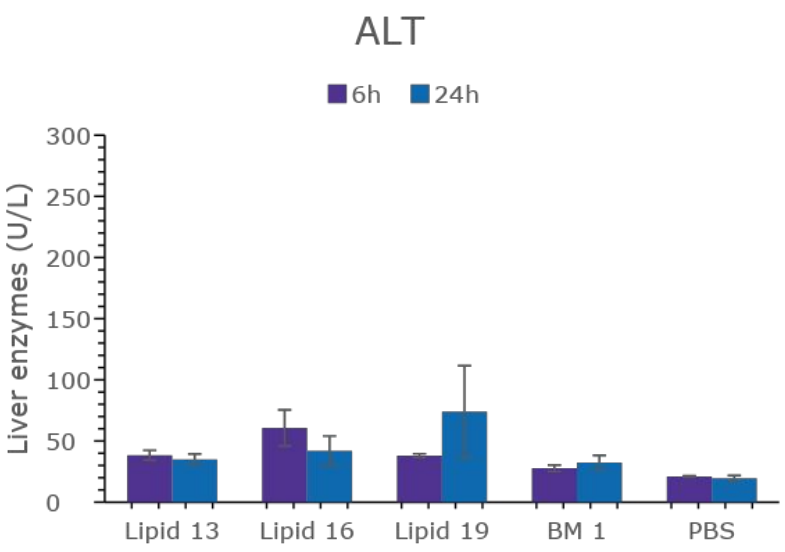
Our ionizable lipid toolbox

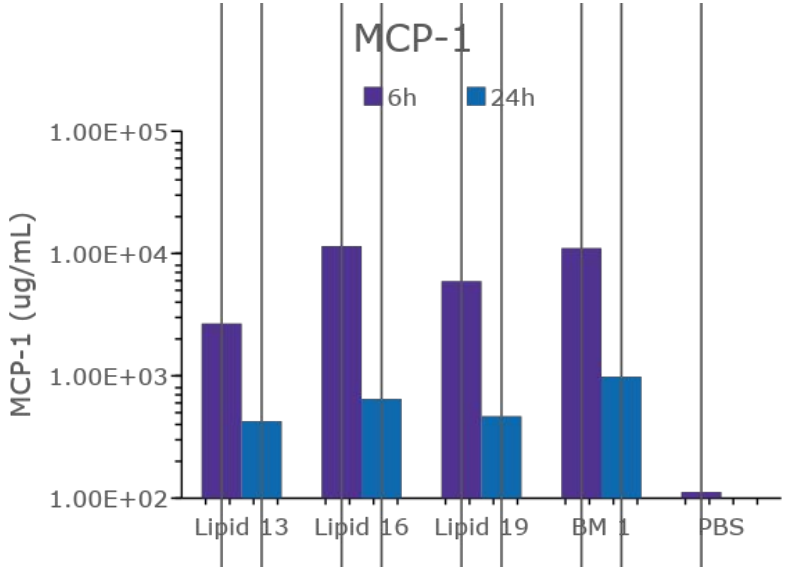
Prioritizing safety upon administration

In vivo tolerability study

Hepatic enzymes & cytokine levels measured in blood samples at 6 and 24 hours after injection



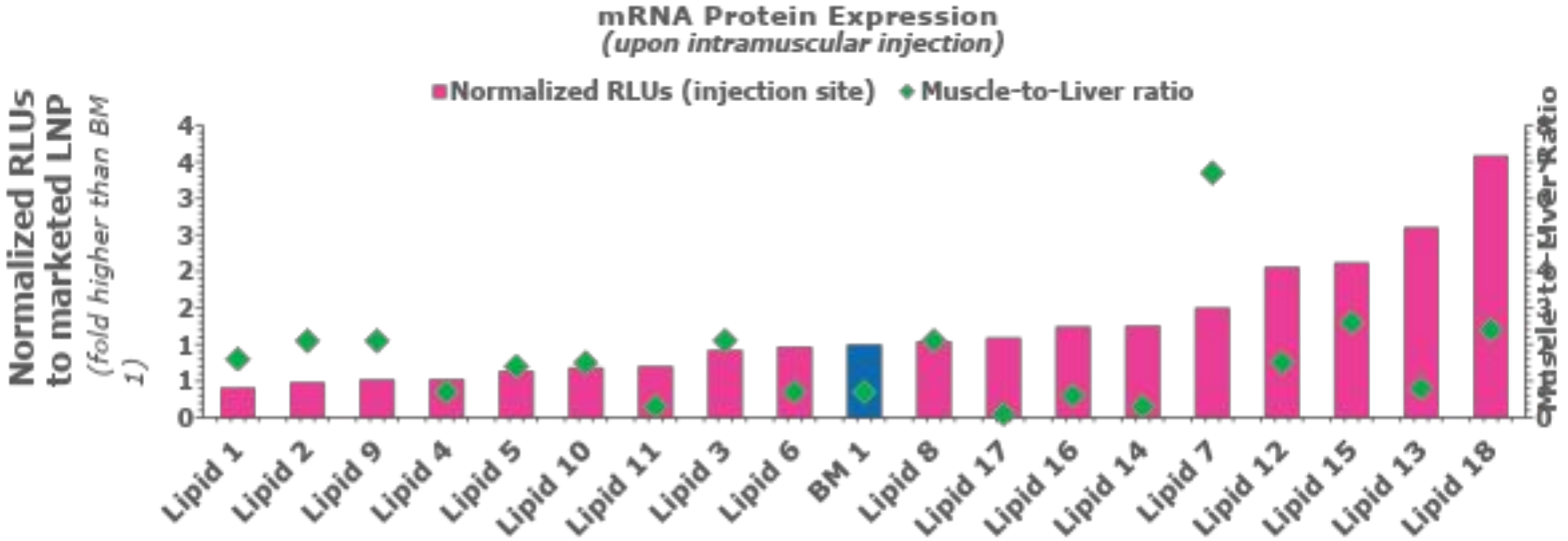




In vivo tolerability comparable to marketed LNP benchmarks

Our ionizable lipid toolbox

Lead lipids identified for mRNA vaccine applications – benchmarked against marketed LNP

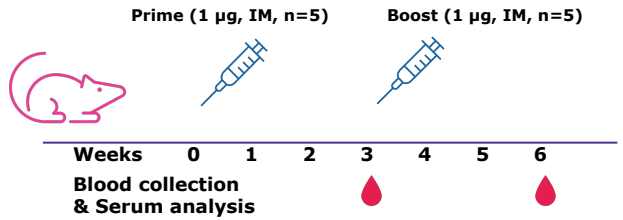


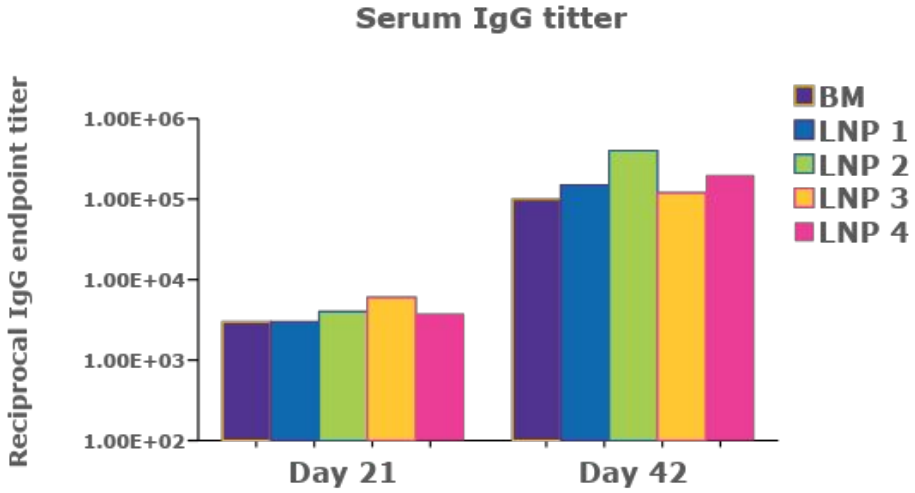
✔ *In vivo* efficacy ≥ marketed LNP Benchmarks (BM)
✔ Muscle-to-Liver ratio > 1

Our ionizable lipid toolbox

Strong candidates for mRNA vaccines

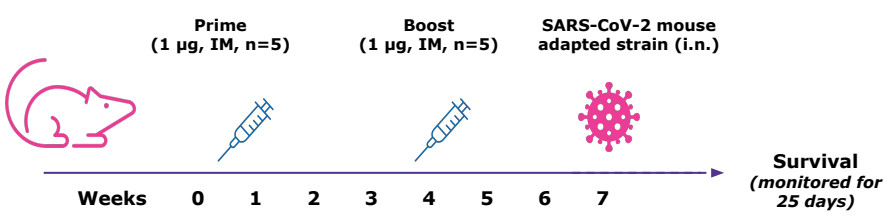
Immunogenicity studies – SARS Cov-2 vaccine

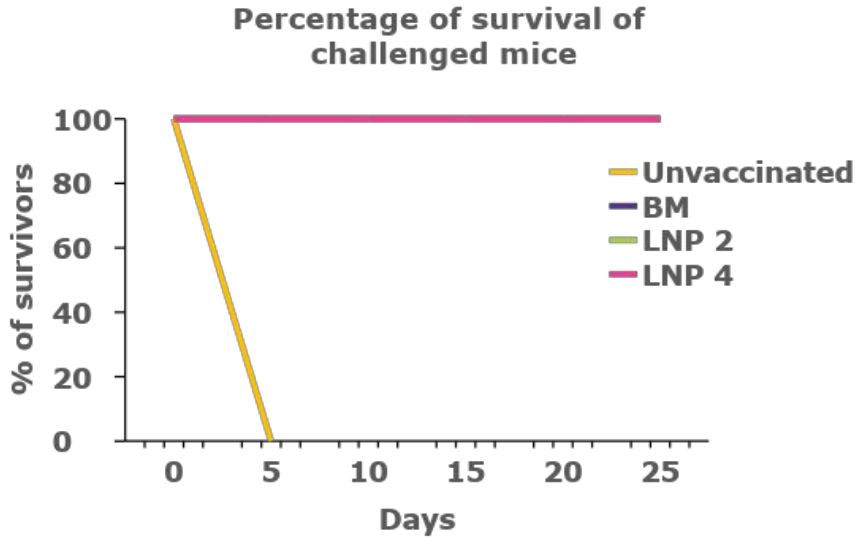




*BM = Benchmark (marketed LNP)

Challenge Study – SARS Cov-2 vaccine





Data provided by lipid partner

Our ionizable lipid toolbox

Validated for multiple nucleic acid payloads

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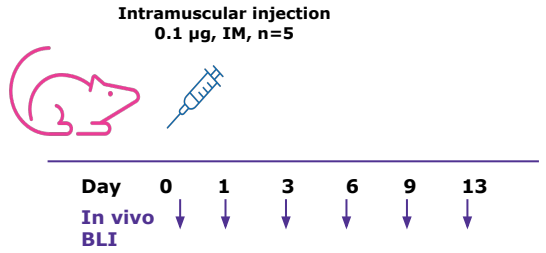
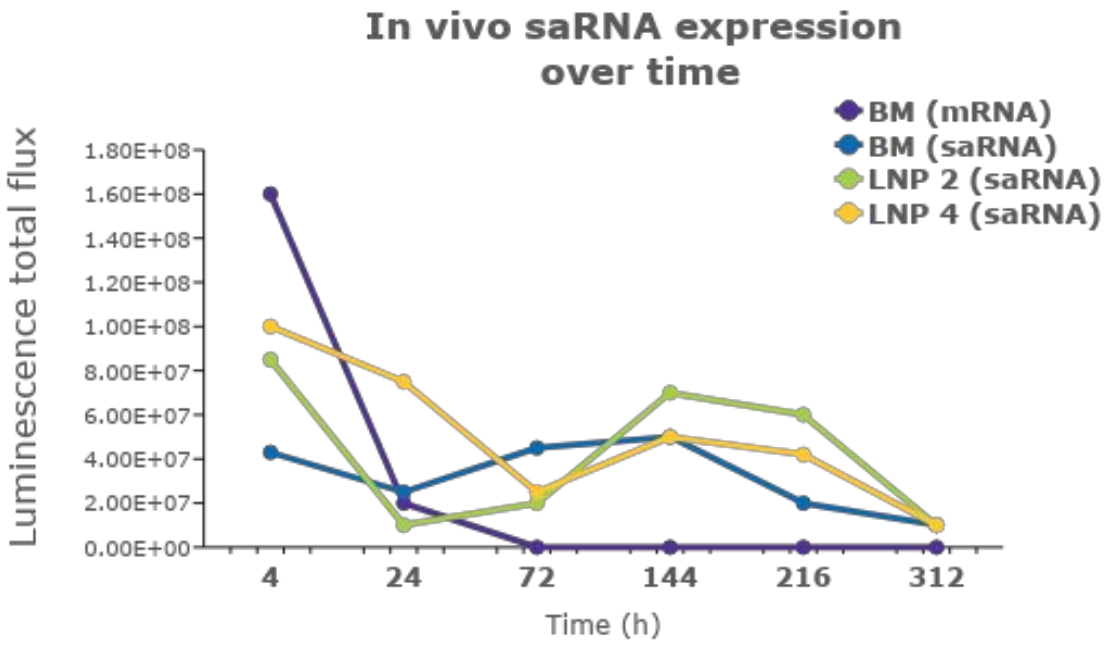
Self-amplifying RNA (saRNA)

delivery





***In vivo* saRNA expression ≥ marketed LNP Benchmarks** with tunable pharmacokinetics and peak expression

***BM = Benchmark (marketed LNP)**

Data provided by lipid partner

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Formulation Optimization

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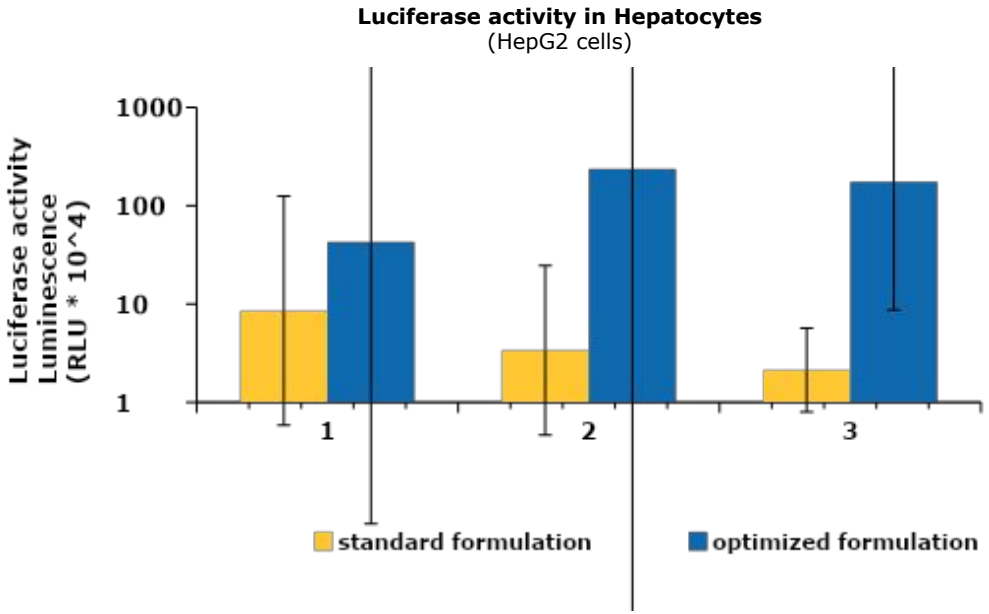
Increased efficacy by customizing the formulation composition

Technical expertise & know-how to modify the formulation composition in order to **tune the formulation properties, biological performance & tissue tropism**

***In vitro* efficacy**

increased by ~40times

by composition optimization



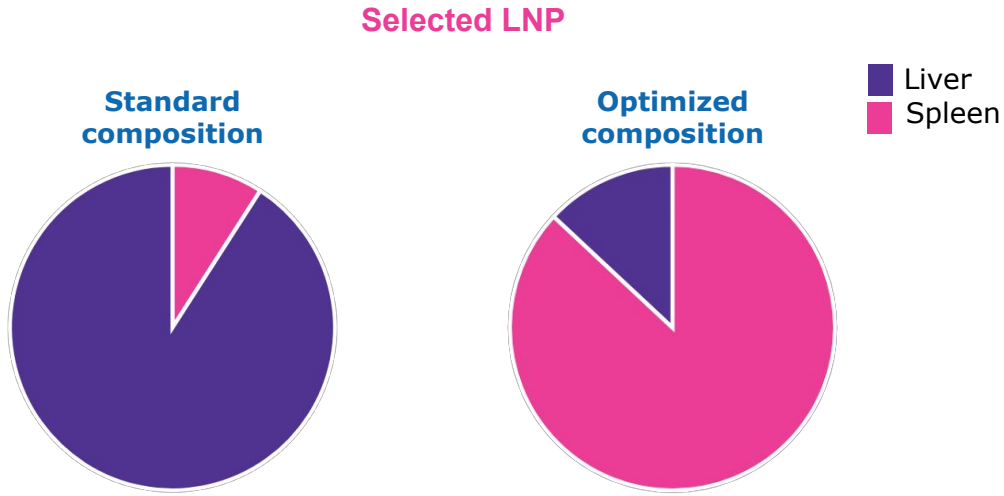
***In vivo* tissue tropism**

tuned from liver to spleen

*by composition optimization**

***Ex vivo* Luciferase activity**

at 6h post dose *(intravenous injection)*



***Focusing on the mol% of one specific lipidic excipient**

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Formulation Scale-up

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Early-stage process development & in-house technology transfer to large-scale GMP manufacturing

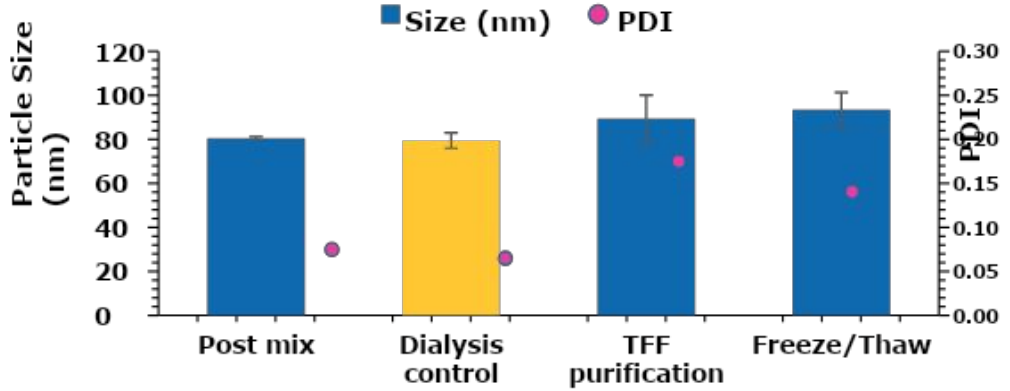
Processes in place for **transition from small to large-scale mixing approaches**

Mixing type	Scale increase	Size (nm)	PDI	EE%
Microfluidics (Toroidal mixer)	1-fold	63 ±3	0.18 ±0.04	93 ±5
Microfluidics (High throughput mode / SHM)		67 ±4	0.20 ±0.04	94 ±4
T-junction mixer	100-fold	80 ±4	0.07 ±0.01	94 ±0
Impingement Jet mixer		75 ±1	0.07 ±0.02	97 ±1

SHM = Staggered herringbone mixer
PDI = polydispersity
EE = encapsulation efficiency

- ✔ **Wide variety of mixing technologies** for different LNP scales **to ensure scalability**
- ✔ **Manufacturing equipment & processes aligned with our GMP site – technology transfer templates in place**

Strong expertise and know-how for efficient **process development – transferable to large scale GMP manufacturing**



Potency in selected cell line *(normalized)*	
Freshly produced LNP *(purified by dialysis)*	1.00
Frozen LNP *(purified by TFF)*	1.08

IJM = Impingement Jet mixer
TFF = Tangential Flow Filtration

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Lyophilized mRNA-LNP

No loss of *in vivo* performance upon lyophilization

Strict ultra-cold chain supply *is a significant challenge for RNA-based medicines*

- ***increased costs & inconvenience for the end-users and for distribution worldwide***

Lyophilization of RNA-loaded LNP enables the storage and shipment of LNP formulations at logistically convenient temperatures.

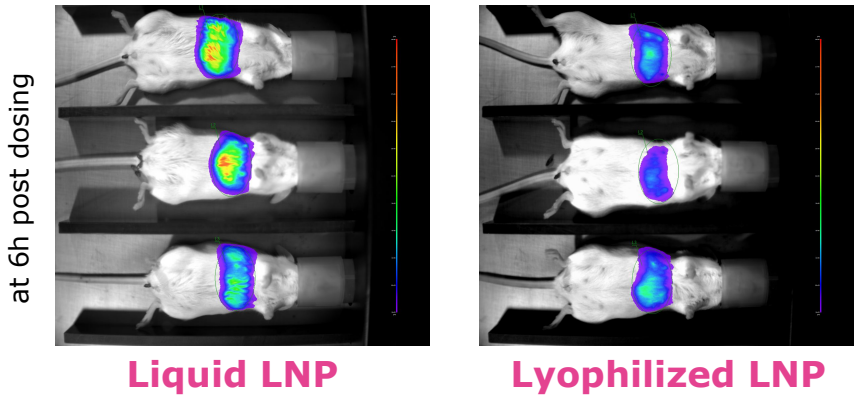
- *Developing such process remains challenging because of the physical stresses imposed by freezing and drying of the product*

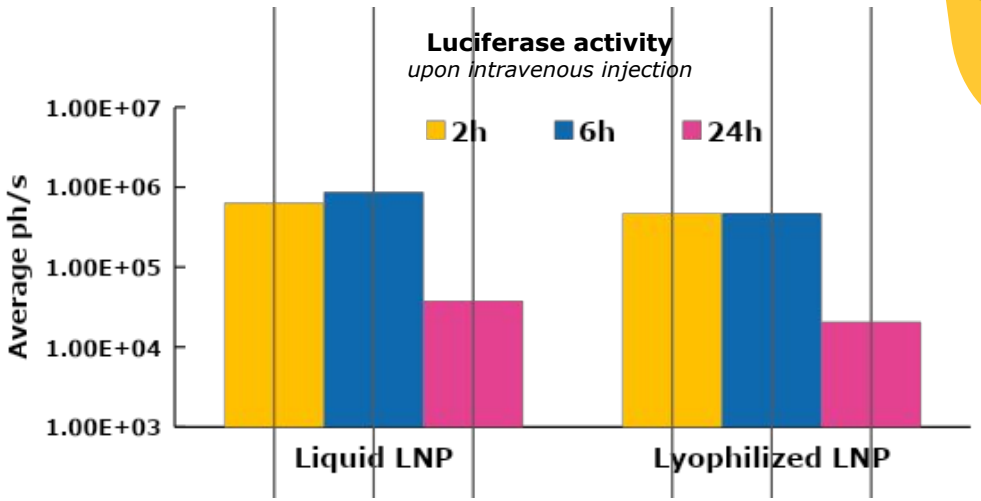
High quality **Emprove®** Excipients for optimal lyoprotectant matrix

Optimized lyophilization **process in place**
transferable to GMP

State-of-the-art lyophilization & **analytical equipment**

In vivo performance of lyophilized mRNA-LNP maintained based on our optimized lyophilization process





Confirmed also with siRNA loaded LNP – Data not shown

Lipidomics Service

Shedding light on metabolism & clearance of novel LNP excipients

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Why?

- ✔ Assess LNP formulations' pharmacokinetics and biodegradability after administration
- ✔ Lipid metabolism and clearance key information related to lipid safety and tolerability
- ✔ Fulfill regulatory requirements to advance to clinical testing

New LAUNCH

Crucial role of **lipid metabolism/clearance** on the **safety** and **tolerability** of LNP therapies & vaccines

moderna

Mol Ther. 2017 Jun 7;25(6):1316-1327. doi: 10.1016/j.ymthe.2017.03.035. Epub 2017 Apr 27.

Preclinical and Clinical Demonstration of Immunogenicity by mRNA Vaccines against H10N8 and H7N9 Influenza Viruses

Kapil Bahl [1], Joe J Senn [2], Olga Yuzhakov [1], Alex Bulychev [2], Luis A Brito [2], Kimberly J Hassett [1], Michael E Laska [2], Mike Smith [2], Örn Almarsson [2], James Thompson [2], Amilcar Mick Ribeiro [1], ...

Mol Ther Nucleic Acids. 2019 Apr 15; 15: 1–11. PMCI
Published online 2019 Feb 7. doi: 10.1016/j.omtn.2019.01.013

Optimization of Lipid Nanoparticles for Intramuscular Administrat mRNA Vaccines

Kimberly J. Hassett,[1] Kerry E. Benenato,[1] Eric Jacquinet,[1] Aisha Lee,[1] Angela Woods,[1] Olga Yuzh Sunny Hima... Iain McFady... Giuseppe C...

Original Article

A Novel Amino Lipid Series for mRNA Delivery: Improved Endosomal Escape and Sustained Pharmacology and Safety in Non-human Primates

Staci Sabnis [1], E. Sathyajith Kumarasinghe [1], Timothy Salerno [1], Cosmin Mihai [1], Tatiana Ketova [1], Joseph J. Senn [1], Andy Lynn [1], Alex Bulychev [1], Iain McFadyen [1], Joyce Chan [1], Örn Almarsson [1], Matthew G. Stanton [1,2], Kerry E. Benenato [1] ✉

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https://doi.org/10.1016/j.ymthe.2018.03.010 Get rights and conten

Molecular Therapy

CellPress

Volume 21, Issue 8, August 2013, Pages 1570-1578

Original Article

Biodegradable Lipids Enabling Rapidly Eliminated Lipid Nanoparticles for Systemic Delivery of RNAi Therapeutics

Martin A Maier [1], Muthusamy Jayaraman [1], Shigeo Matsuda [1], Ju Liu [1], Scott Barros [1], William Querbes [1], Ying K Tam [2], Steven M Ansell [2], Varun Kumar [1], Jane Qin [1], Xuemei Zhang [1], Qianfan Wang [1], Sue Panesar [1], Rento Hutabarat [1], Mary Coriaty [1], Julia Hettinger [1], Pachamuthu Kandasamy [1], David Butler [1], Kallanthottathil G Rajeev [1], Bo Pang [1], ...Akin Akinc [1] ✉ ✉

Alnylam PHARMACEUTICALS

Regulatory guidance documents highlight the requirement of **lipidomics data** for novel lipids

- ***FDA Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation*** *(April 2018)*
- ***FDA Guidance for Industry: Safety Testing of Drug Metabolites*** *(Feb 2008)*
- ***FDA Draft guidance for Industry: In vitro Metabolism and Transporter-Mediated Drug-Drug Interaction studies*** *(Oct 2017)*
- ***EMA: Guideline on the Investigation of Drug Interactions*** *(Jan 2013)*
- ***EMA: Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product*** *(Feb 2013)*

**not exhaustive list*

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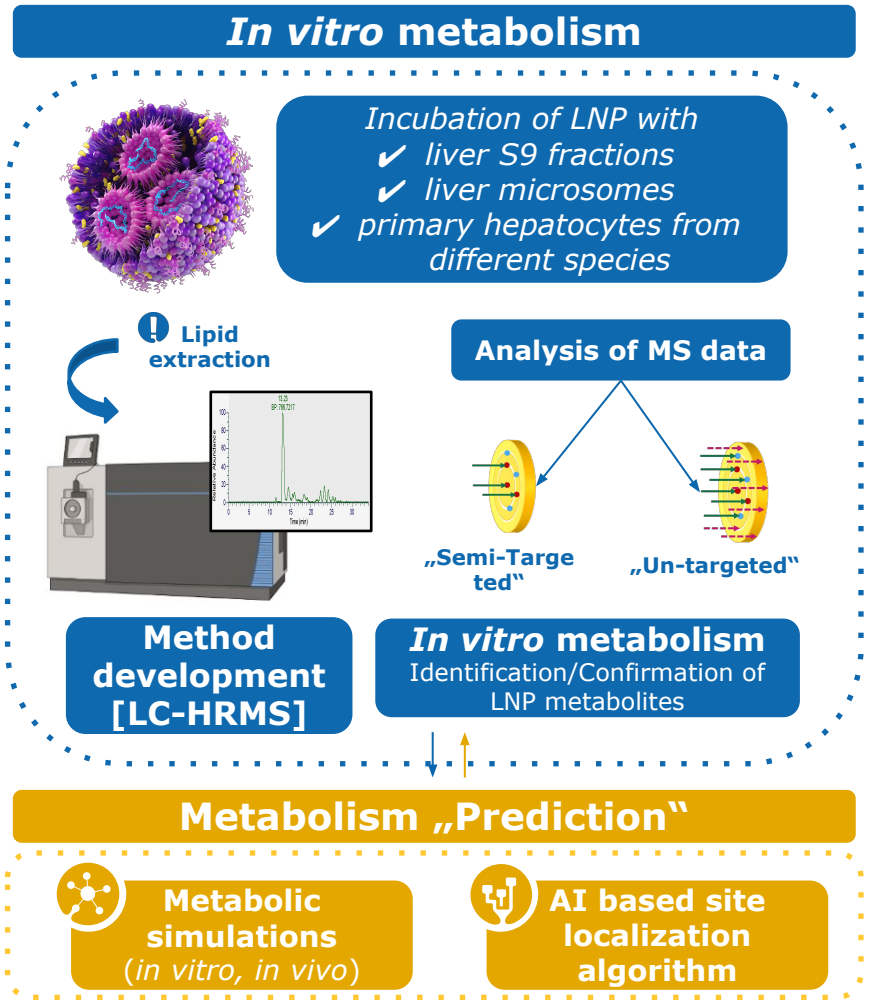
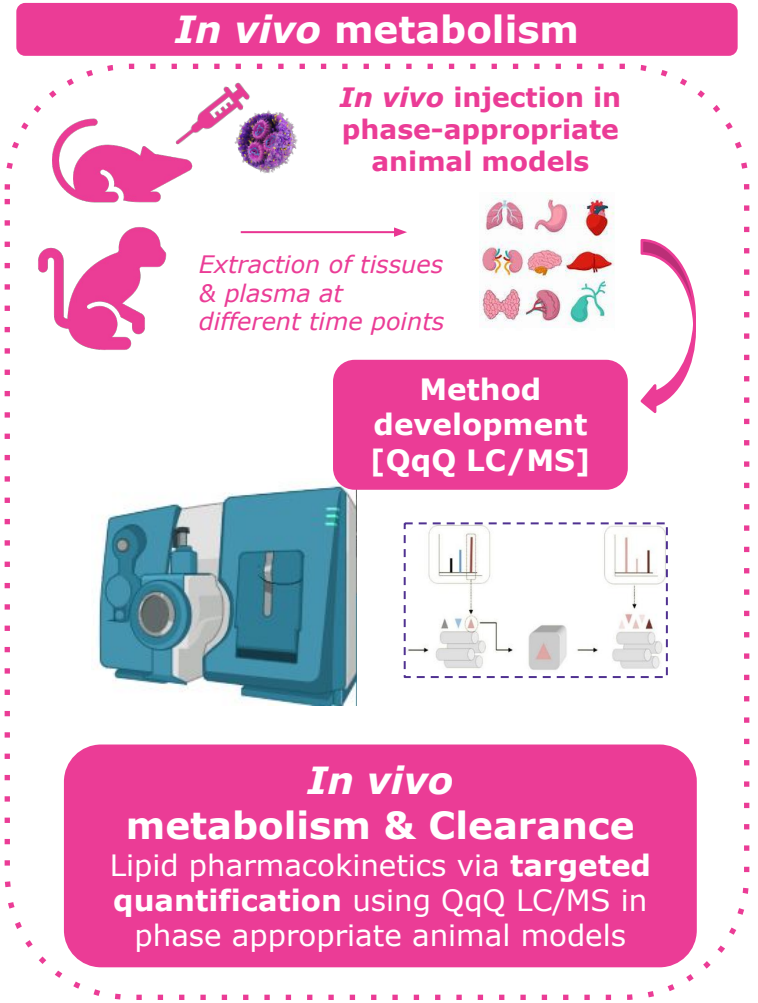
Lipidomics Service

Shedding light on metabolism & clearance of novel lipidic excipients in LNP





Metabolism simulation - *in vitro* and *in vivo* metabolism & clearance

- ✔ **Strong expertise** and **state-of-art equipment** for accurate quantitation of both **parent lipids & metabolites** (*in vitro/in vivo*)
- ✔ **Semi-targeted** (via LC-HRMS) **& targeted approaches** (via QqQ MS)

* *LC-HRMS: Liquid chromatography–high resolution mass spectrometry*

QqQ MS: triple quadrupole mass spectrometry

Powering life-changing medicines

Your integrated partner for mRNA, lipids and LNP CTDMO Services

- **Best-in class technical support** through **30+ years** of expertise in multiple nucleic acid modalities, lipids and LNPs
- Access to **cherry-picked ionizable lipids** from multiple libraries
- **Large panel of analytical capabilities** (including lipidomics)
- Expertise in LNP **stabilization** and **state-of-art lyophilization** capabilities
- **In-house technology transfer to GMP** manufacturing of LNP

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Thank you
for your attention

We look forward to supporting you

Eleni Samaridou, PhD
Eleni.Samaridou@merckgroup.com

For more information come meet our experts at booth #68

Technical differentiation

Simplified customer experience

Regulatory expertise