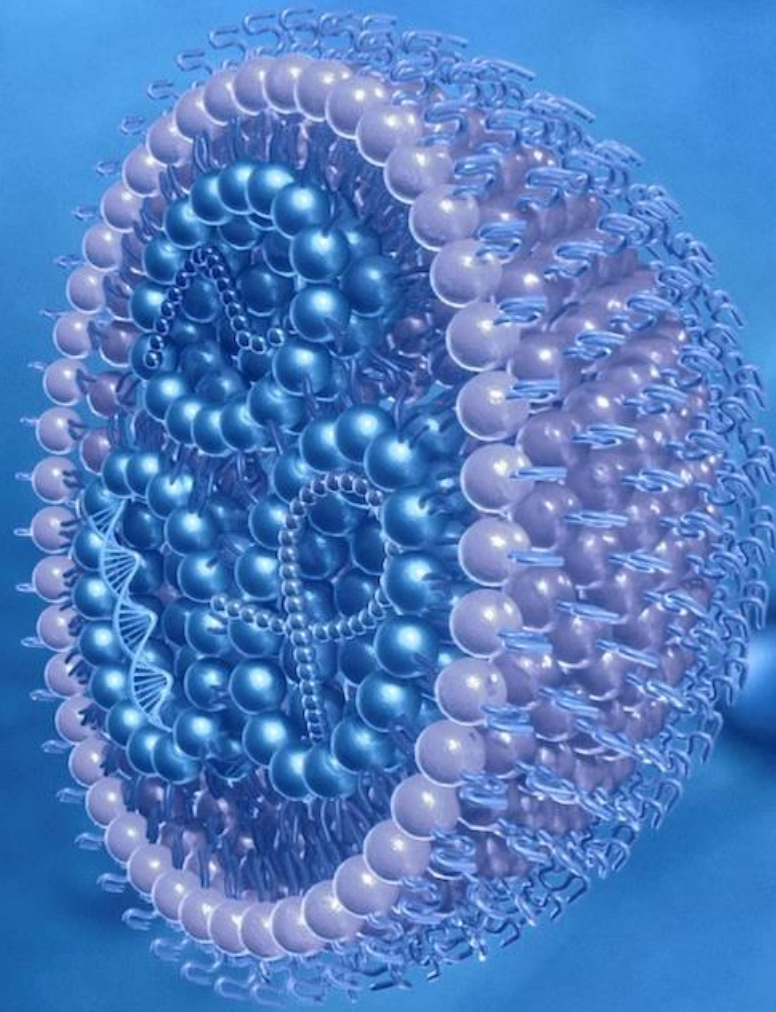
NovoArc

LIPIDS FOR
INNOVATION



NovoArc **GmbH**
www.novoarc.at

Phospholipid Research Center

09.09.2024

Pottendorfer Str. 23-25, 4, 4-1
1120 Vienna, Austria
office@novoarc.at

UNIQUE SELLING POINT

About NovoArc

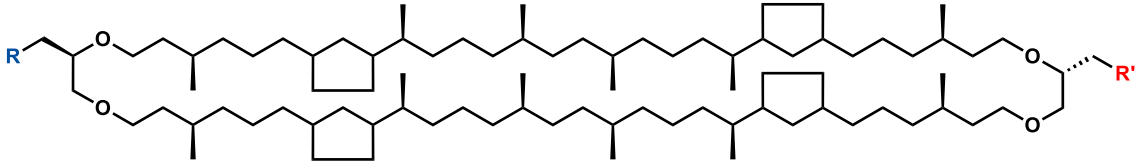
- established in **2021**, spin-off TU-Wien
- **approved production facility** in Vienna, 11 FTEs
- **financially backed** by strong private and public institutions
- **3 patents**, **several awards** - Winner of "AWS Best of Biotech"

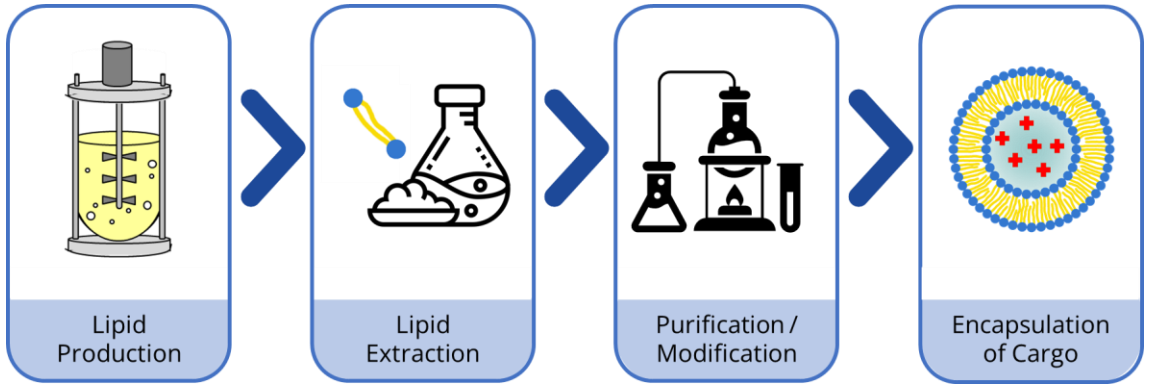
NovoArc's proprietary technology

- **Improved delivery** of pharmaceuticals
- **Library of pure semi-synthetic lipids**
- **IP protected** technology along **QbD** principles
- Controllable, reproducible and **scalable**
- Compatible with existing **CMO** plants
- Fields of application:

  oral, parenteral, topical, respiratory
- Applicable to:

  small molecules, proteins, nucleic acids





Selected partners and customers

EVONIK POWER TO CREATE · Boehringer Ingelheim · Polyplus Now Part of Sartorius · syvento BIO TECH

Oral drug delivery - Liposomes

Small molecules and peptides

TOXICOLOGY

Toxicity testing for orally administered **archaeal lipid extract** (**ALE**)

- ***In vitro* cytotoxicity** on Fibroblast Cell Line L-929 according to **ISO 10993-5:2009** at **OFI Vienna**:
  - no cytotoxic reactivity (grade 0) at 520 mg_{Lipid}/L
  - minor cytotoxic reactivity (grade 1) at 1,040 mg_{Lipid}/L

- ***In vivo* toxicity** at Institute for Medical Research and Occupational Health **IMI in Zagreb**:

acute and **repetitive dose toxicity** in Wistar **rats** by **oral gavage**

- 1 application of 2 doses (either 3 or 30 mg/kg b.w.)
- 7 applications of 2 doses (either 3 or 30 mg/kg b.w.)

animals placed in metabolic cages; animals sacrificed 24 hours after last application

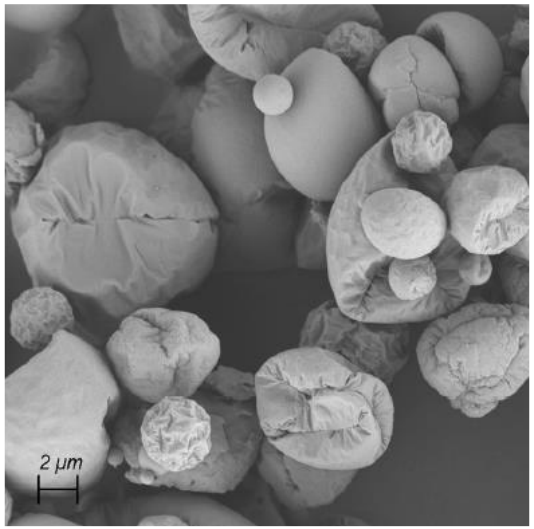
- **no** visible **changes** regarding **animal physiology and health**
- **no toxicity** for **acute** application and **repetitive applications at low dose**
- **inflammatory markers elevated** in liver and kidney at **repeated high dosage** = indication of **oxidative stress**

INSULIN

Insulin packed in **100% archaeal lipids** and tested ***in vitro*** (EE > 35%, 100 nm size, PDI 0.29, neg. Zeta potential); **Caco-2 and HT29-MTX** co-culture model in a trans-well system; liposomes were **spray-dried and lyophilized**

- **Archaeosomes** do not pass through cell layer, but stay **associated with cells** and release cargo
- **lyophilization and spray drying** without any detectable release of payload → tablets **possible**

spray-dried insulin Archaeosomes



lyophilized insulin Archaeosomes

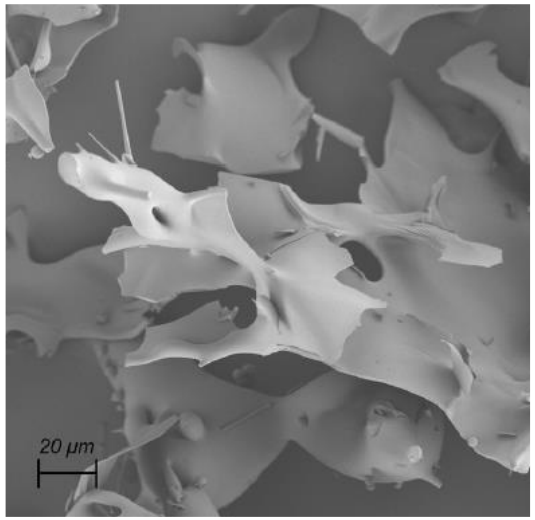


Table 4. Mean particle size and polydispersity index (PDI) of insulin-loaded archaeosomes subsequent to preparation (T_ins), after spray drying (SD) and lyophilization (LYO), respectively (T_ins SD, T_ins LYO). Initial encapsulation efficiency % EE and overall recovery % after rehydration are shown (n = 3).

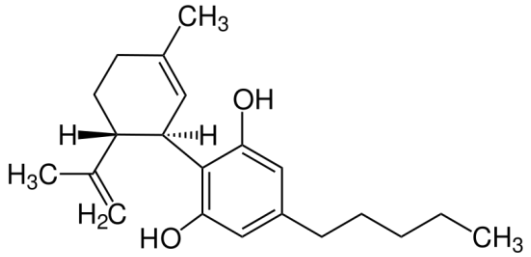
	Insulin	T_ins	T_ins SD *	TEL_ins LYO *
Mean particle size [nm]		97.5 ± 0.5	103.1 ± 1.2	105.9 ± 1.4
PDI		0.285 ± 0.003	0.348 ± 0.009	0.324 ± 0.004
Insulin [µg/mL]	10,261 ± 35	3631 ± 13	2045 ± 17	2120 ± 12
% EE		~35		
Overall recovery %			~20	~21

* Redissolved.

Archaeosomes can be formulated to stable dry powders

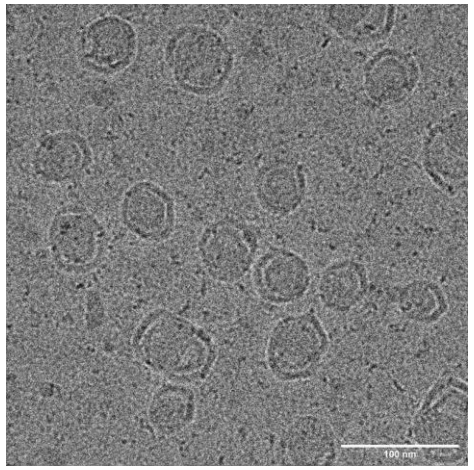
Scientific Paper published https://doi.org/10.3390/pharmaceutics16060694

CANNABIDIOL (CBD)

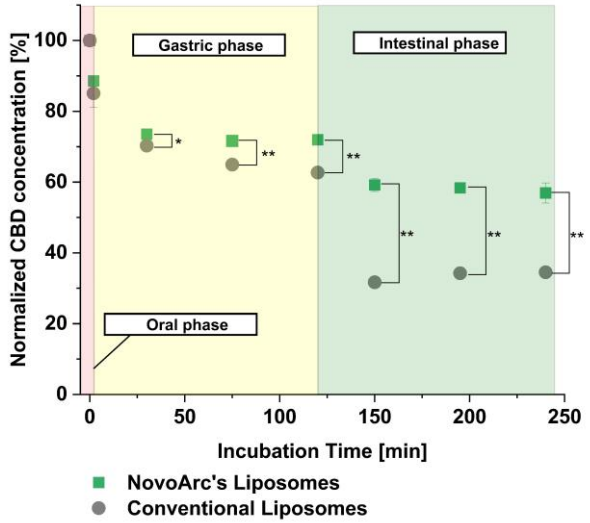


current oral administration: **bioavailability** of CBD **only 6%** due to degradation in GI tract, first-pass metabolism and low water solubility

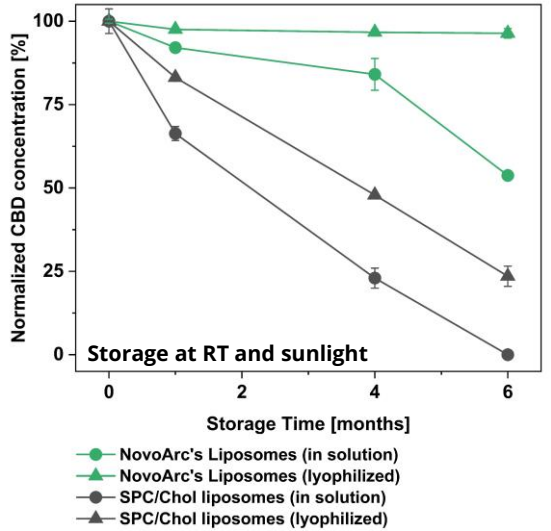
CBD packed in **conventional liposomes** (phospholipon® 90 G (SPC):cholesterol = 3:1) or **100% archaeal lipids** and tested ***in vitro*** (EE > 90%, 80-90 nm size, PDI 0.04 – 0.14, neg. Zeta potential)



Spherical particles with a monolayer thickness of 5 nm



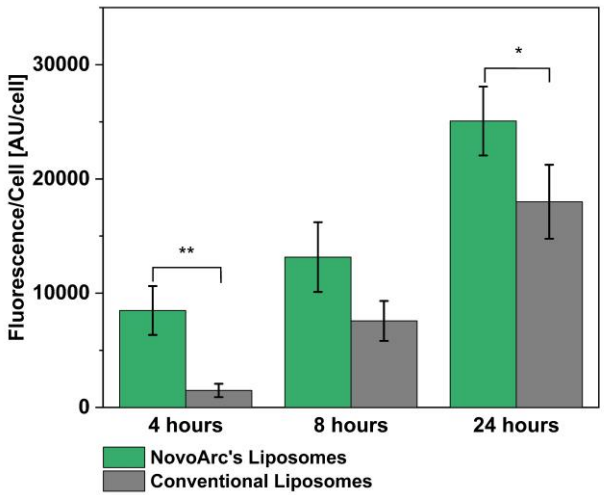
Higher stability in simulated GI tract (1.7-fold)



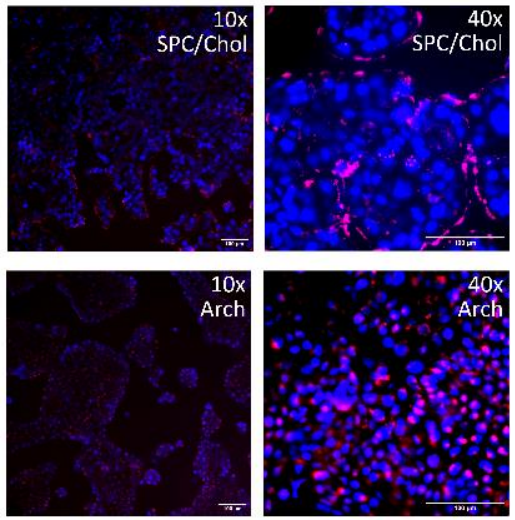
Protective effect during long term storage

Scientific Paper published https://doi.org/10.1016/j.ijpharm.2023.123434

CANNABIDIOL (CBD)



Lyophilized formulations stored for 6 months



International Journal of Pharmaceutics 645 (2023) 123434

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ELSEVIER

Archaeosomes facilitate storage and oral delivery of cannabidiol

Viktor Sedlmayr [a,1], Christina Horn [b,1], David Johannes Wurm [b], Oliver Spadiut [a], Julian Quehenberger [a,b,*]

[a] TU Wien, Institute of Chemical, Environmental and Bioscience Engineering, Vienna, Austria
[b] NovoArc GmbH, Vienna, Austria

ARTICLE INFO

Keywords:
Archaeosomes
Cannabidiol
Oral drug delivery
Liposomes
Storage stability

ABSTRACT

Cannabidiol (CBD) has received great scientific interest due to its numerous therapeutic applications. Degradation in the gastrointestinal (GI) tract, first-pass metabolism, and low water solubility restrain bioavailability of CBD to only 6% in current oral administration. Lipid-based nanocarriers are delivery systems that may enhance accessibility and solubility of hydrophobic payloads, such as CBD. Conventional lecithin-derived liposomes, however, have limitations regarding stability in the GI tract and long-term storage. Ether lipid-based archaeosomes may have the potential to overcome these problems due to chemical and structural uniqueness. In this study, we compared lecithin-derived liposomes with archaeosomes in their applicability as an oral delivery system of CBD. We evaluated drug load, storage stability, stability in a simulated GI tract, and *in vitro* particle uptake in Caco-2 cells. Loading capacity was 6-fold higher in archaeosomes than conventional liposomes while providing a stable formulation over six months after lyophilization. In a simulated GI tract, CBD recovery in archaeosomes was 57 ± 3% compared to only 34 ± 1% in conventional liposomes and particle uptake in Caco-2 cells was enhanced up to 6-fold. Our results demonstrate that archaeosomes present an interesting solution to tackle current issues of oral CBD formulations due to improved stability and endocytosis.

6-fold increased endocytosis by colonic cell line

No endocytosis of conventional liposomes
Successful uptake of NovoArc's liposomes

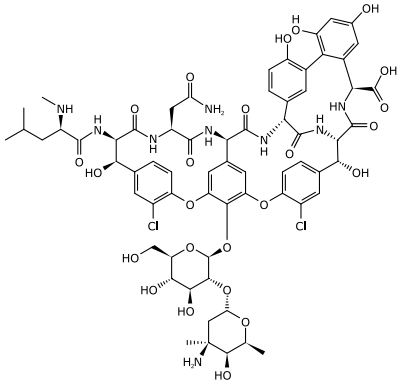
Scientific Paper published

Archaeal lipids protect cargo and deliver it to small intestine

Scientific Paper published

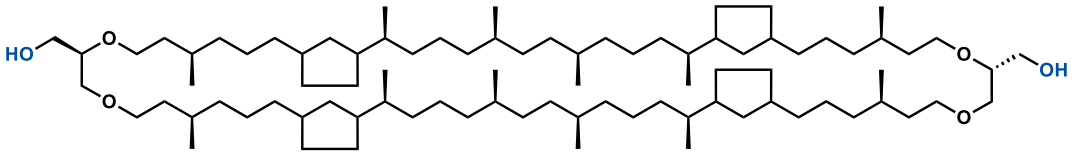
https://doi.org/10.1016/j.ijpharm.2023.123434

VANCOMYCIN



current administration intravenously, no oral bioavailability

Vancomycin packed in different **mixtures** of **lecithin, cholesterol** and **archaeal lipid extract** or purified archaeal lipid species **GDGT** (EE 40-50%)



GDGT

	Size [nm]	PDI	Zeta potential [mV]
TEL-liposomes (5 mol-%)	107.47 ± 2.20	0.122 ± 0.028	-3.24 ± 0.21

	Size [nm]	PDI	Zeta-potential [mV]
GDGT-liposomes (5 mol-%)	107.60 ± 2.25	0.159 ± 0.013	-4.04 ± 0.74

archaeal lipid amount ↑ : size and polydispersity ↑, Zeta potential ↓

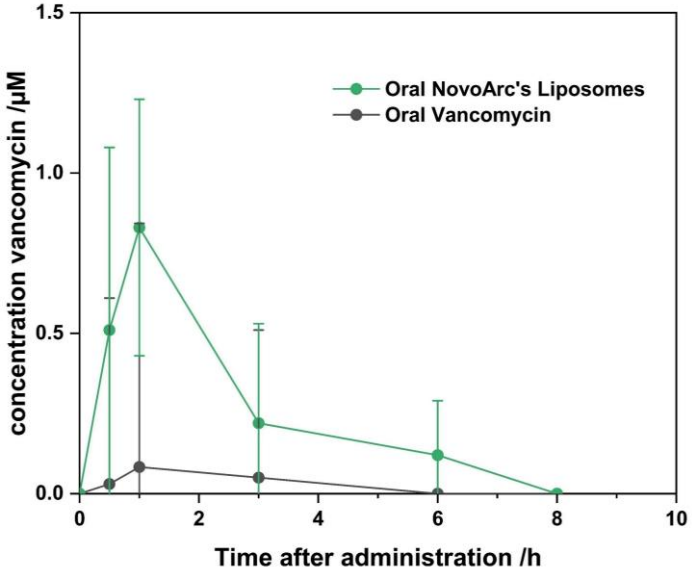
Scientific manuscript in preparation

VANCOMYCIN

Vancomycin packed in mixture of lecithin, cholesterol and **5 mol% GDGT**

	Size [nm]	PDI	Zeta-potential [mV]
GDGT-liposomes (5 mol-%)	107.60 ± 2.25	0.159 ± 0.013	-4.04 ± 0.74

No signs of toxicity



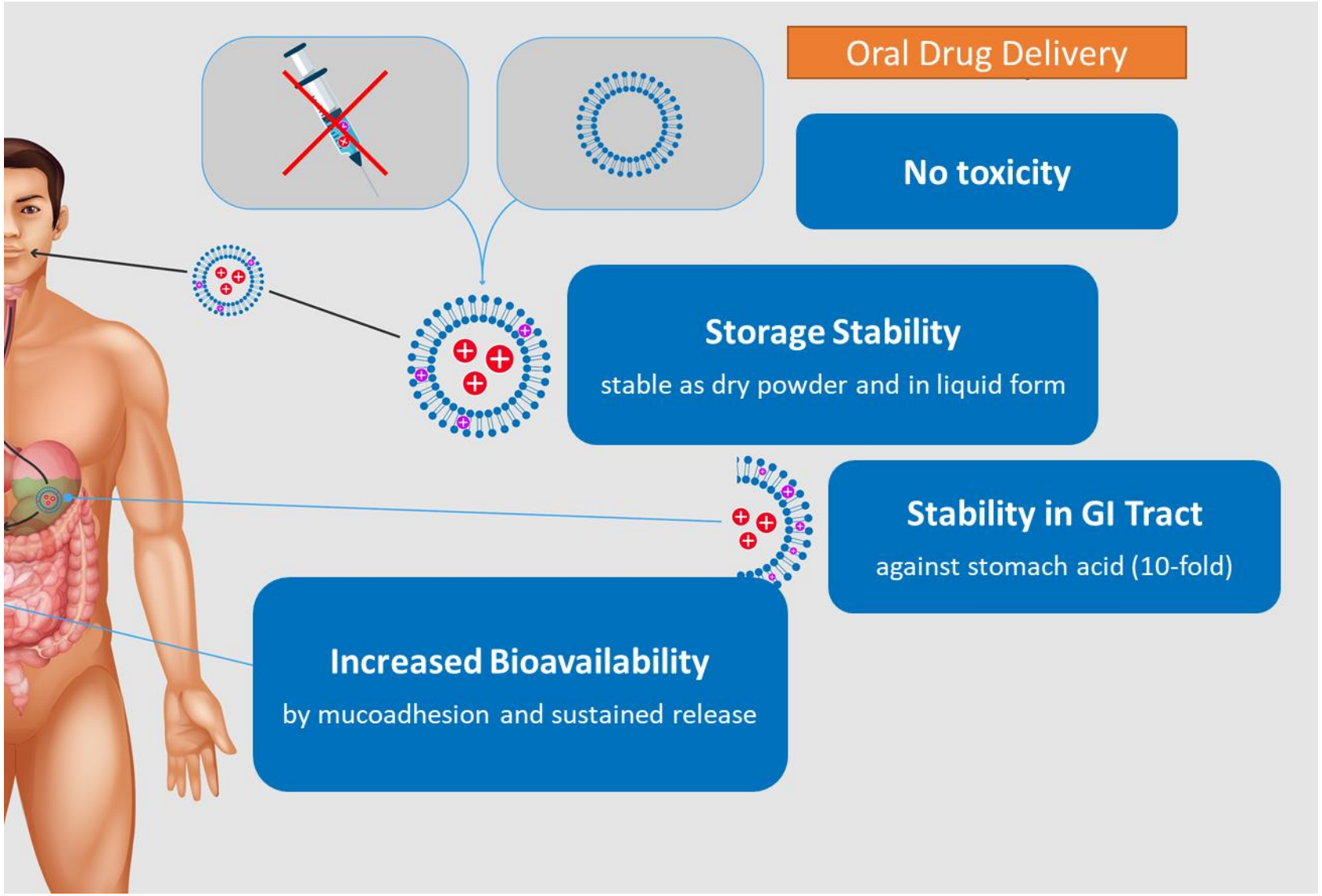
Route of administration	Description	AUC [µM/h]	relative Bioavailability
i.v.	Free	8.09	100%
oral	Free	0.244	3.0%
oral	**NovoArc's Liposomes**	**2.14**	**26.5%**

GDGT protects cargo in stomach and delivers it to small intestine

9-fold boosted bioavailability *in vivo*

Scientific manuscript in preparation

BENEFITS OF NOVOARC'S TECHNOLOGY

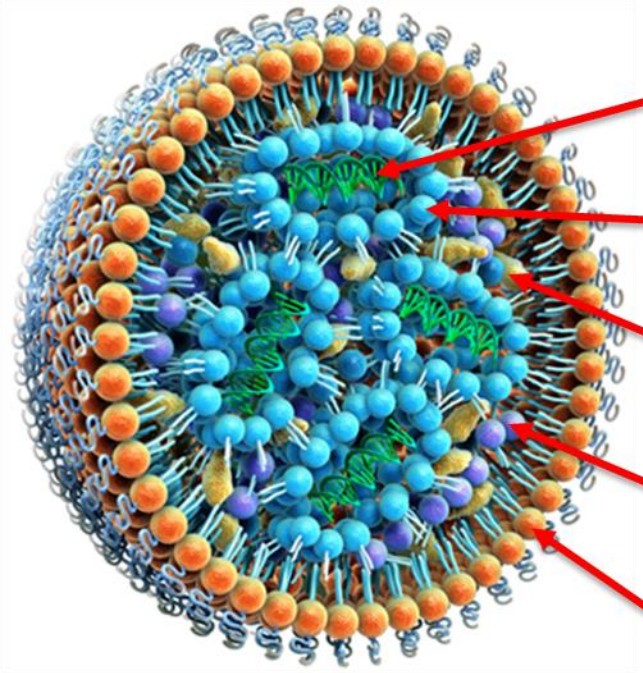


Delivery of mRNA via LNPs

in vitro case studies (parenteral + oral)

CASE STUDY mRNA

Components of an LNP



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Nucleic acid: cargo

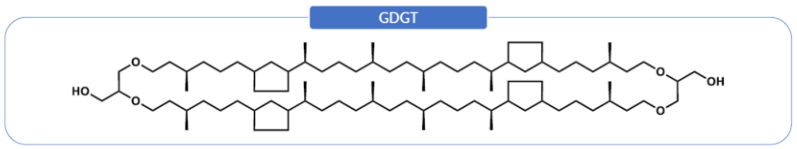
Ionizable lipids: packaging of negative cargo RNA — 45-60 mol%

Cholesterol: endosomal uptake, membrane fusion — 25-35 mol%

Helper lipids: structure building, stability — 8-15 mol%

PEG-lipids: prevents aggregation & enzymatic degradation — 1-2 mol%

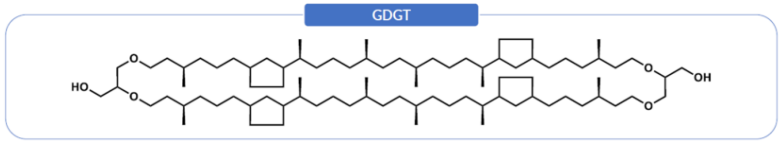
→ **Substitution with NovoArc's native Lipids**



CASE STUDY mRNA

Native TELS: archaeal lipid extract (**ALE) and GDGT as helper lipids** in LNP formulations, eGFP mRNA

In vitro assay on Human skeletal muscle myoblasts (**HSMM**) and Murine skeletal muscle myoblasts (**C2C12**)



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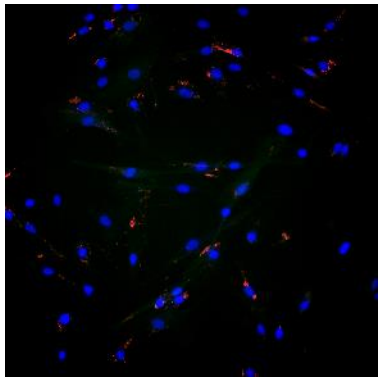
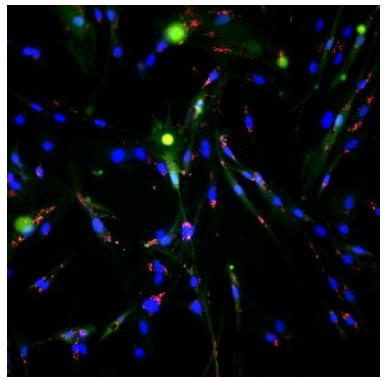
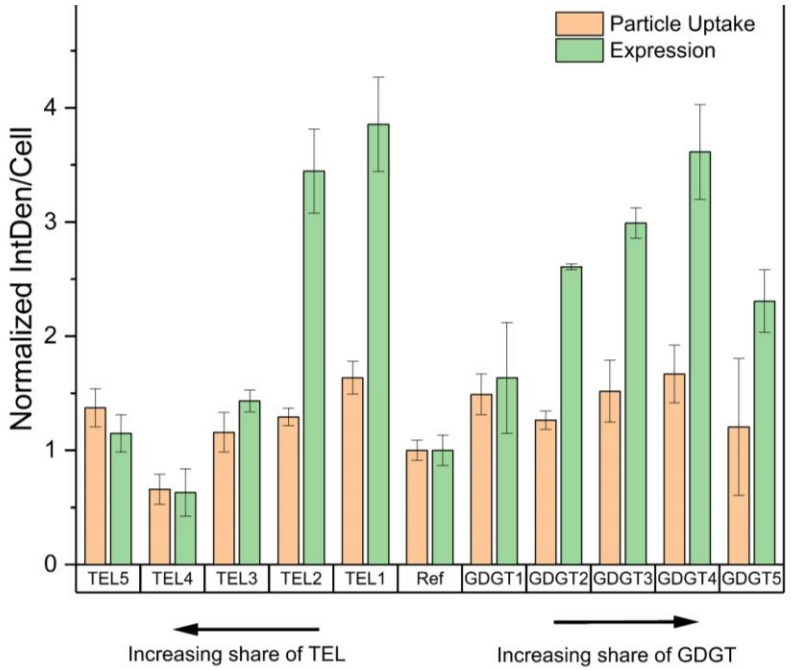


GDGT





3.9 x higher expression in **HSMM** after 24 hours

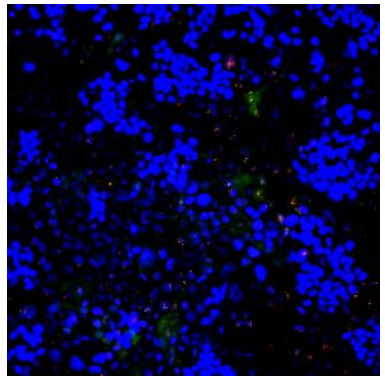
1.7 x higher expression in **C2C12** after 24 hours

CASE STUDY ORAL mRNA

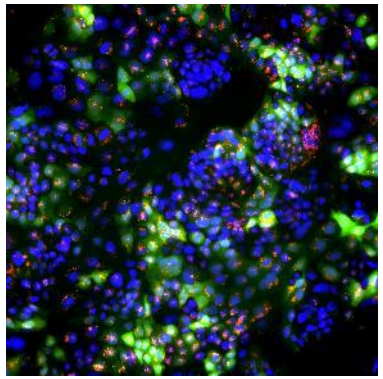
Native TELS: archaeal lipid extract (**ALE) and GDGT as helper lipids** in LNP formulations, eGFP mRNA

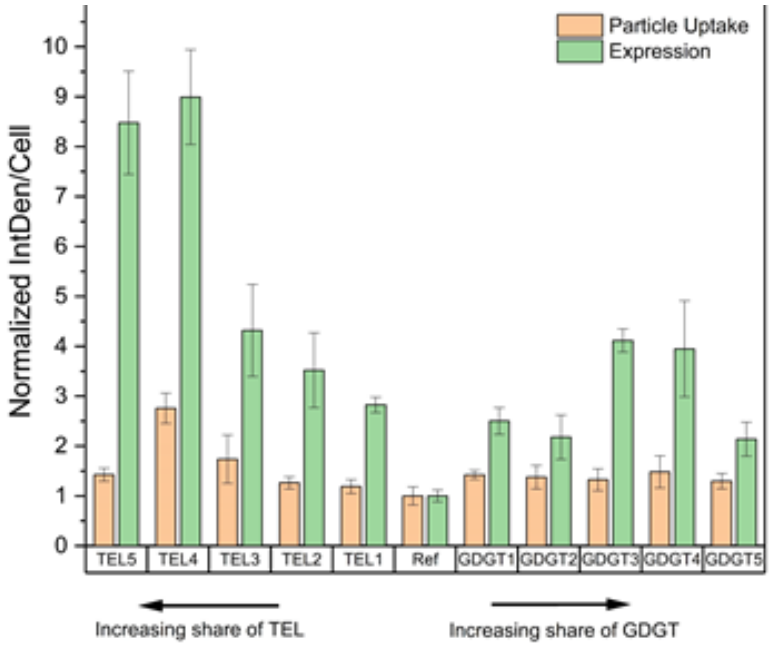
In vitro assay for parenteral mRNA delivery on **Caco-2** cells

Reference



GDGT

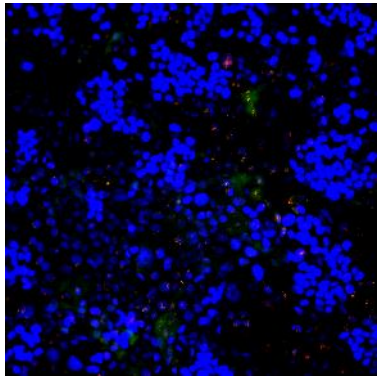


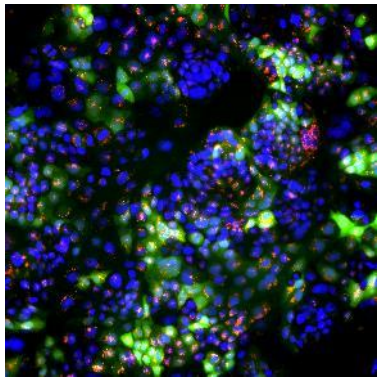


9 x higher expression in **Caco-2** after 24 hours

CASE STUDY mRNA

GDGT boosts transfection efficiency up to 9-fold





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journal homepage: www.elsevier.com/locate/ejpb

Archaeal ether lipids improve internalization and transfection with mRNA lipid nanoparticles

Viktor Laurin Sedlmayr [a], Silvia Schobesberger [b], Sarah Spitz [b], Peter Ertl [b], David Johannes Wurm [c], Julian Quehenberger [a,c], Oliver Spadiut [a,*]

[a] Research Division Integrated Bioprocess Development, Institute of Chemical, Environmental and Bioscience Engineering, TU Wien, Gumpendorfer Straße 1a, Vienna 1060, Austria
[b] Research Division Organic & Biological Chemistry, Institute of Applied Synthetic Chemistry, TU Wien, Getreidemarkt 9/163, Vienna 1060, Austria
[c] NovoArc GmbH, Pottendorfer Straße 23-25, Vienna 1120, Austria

ARTICLE INFO

Keywords:
Lipid nanoparticles
mRNA
Vaccine
Endosomal escape
Archaeosomes
Tetraether lipids

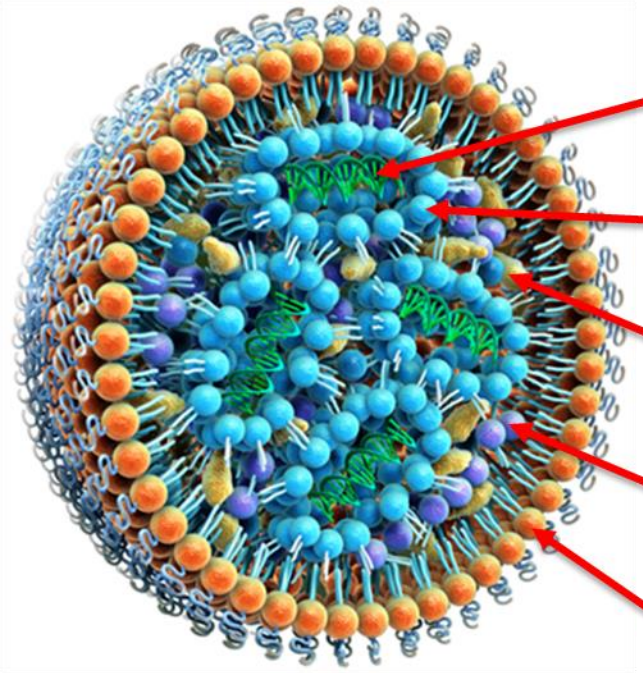
ABSTRACT

Neutral and positively charged archaeal ether lipids (AEL) have been studied for their utilization as novel delivery systems for pDNA, showing efficient immune response with a strong memory effect while lacking noticeable toxicity. Recent technological advances placed mRNA lipid nanoparticles (LNPs) at the forefront of next-generation delivery systems; however, no study has examined AELs in mRNA delivery yet. In this study, we investigated either a crude lipid extract or the purified tetraether lipid caldarchaeol from *Sulfolobus acidocaldarius* as potential novel excipients for mRNA LNPs. Depending on their molar share in the respective LNP, particle uptake, and mRNA expression levels could be increased by up to 10-fold in *in vitro* transfection experiments using both primary cell sources (HSMM) and established cell lines (Caco-2, C2C12) compared to a well-known reference formulation. This increased efficiency might be linked to a substantial effect on endosomal escape, indicating fusogenic and lyotropic features of AELs. This study shows the high value of archaeal ether lipids for mRNA delivery and provides a solid foundation for future *in vivo* experiments and further research.

https://doi.org/10.1016/j.ejpb.2024.114213

CASE STUDY mRNA

Components of an LNP



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Nucleic acid: cargo

Ionizable lipids: packaging of negative cargo RNA — 45-60 mol%

Cholesterol: endosomal uptake, membrane fusion — 25-35 mol%

Helper lipids: structure building, stability — 8-15 mol%

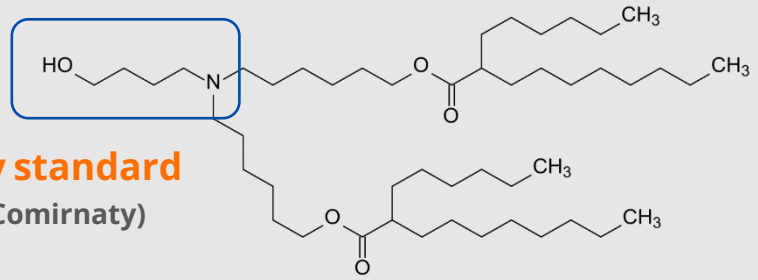
PEG-lipids: prevents aggregation & enzymatic degradation — 1-2 mol%

→ **Library** of semisynthetic **ionizable Tetraether Lipids**

NOVOARC'S PRODUCTS

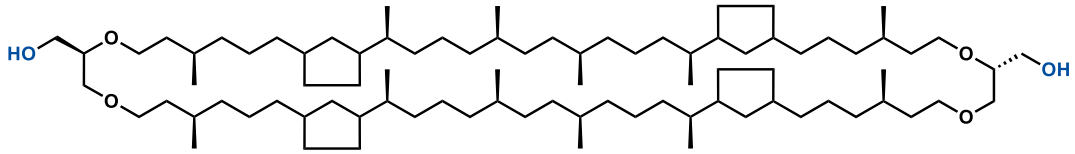
Industry standard

ALC0315 (Comirnaty)



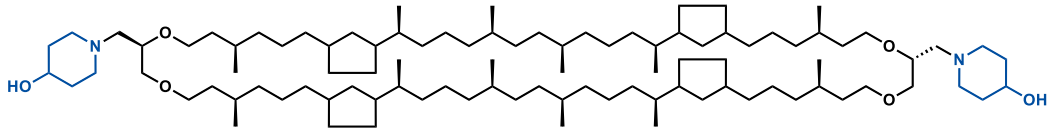
NovoArc's native lipid

GDGT

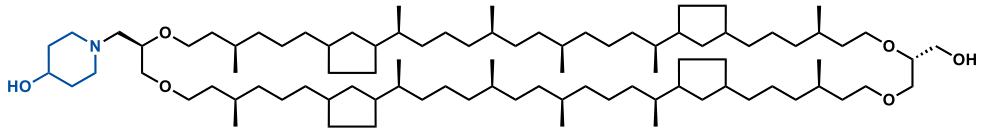


NovoArc's modified lipids (ionizable)

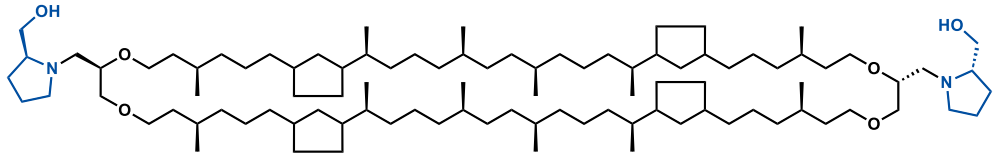
OHPIPD- GDGT



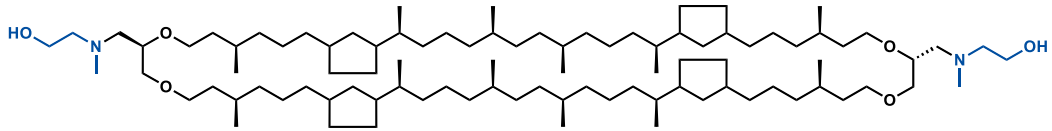
mono - OHPIPD- GDGT



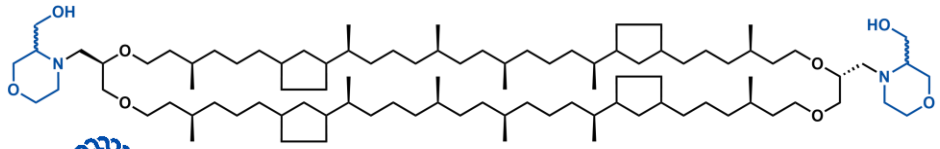
LPRO - GDGT



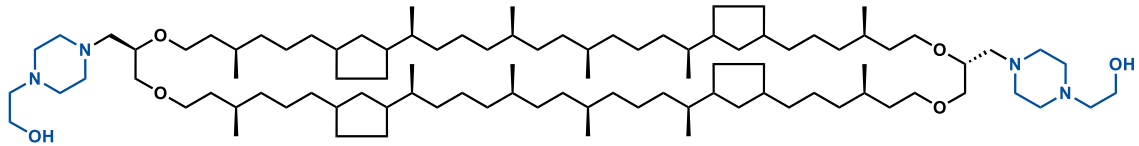
NMEA - GDGT



MORPHO - GDGT



HEPIPA - GDGT

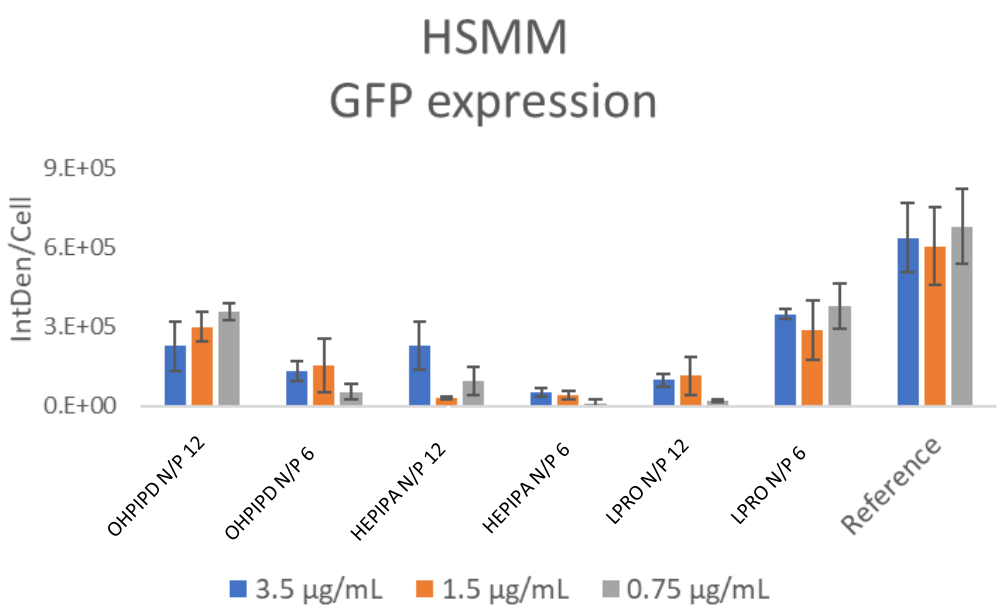


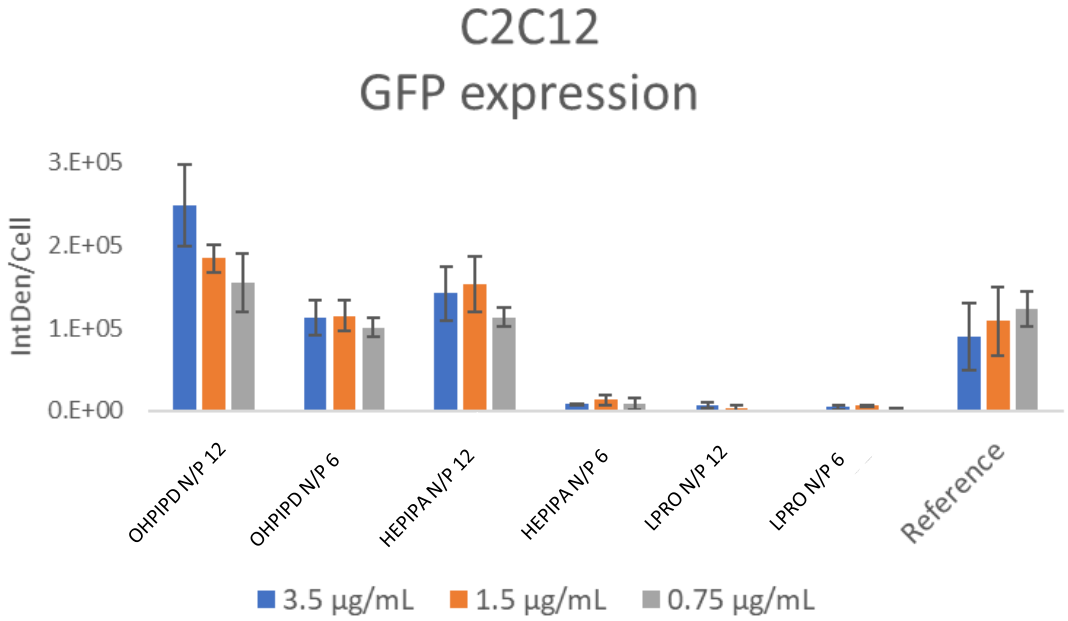
NovoArc

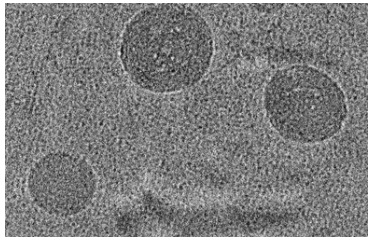
CASE STUDY mRNA

Different ionizable GDGTs (**iGDGTs**) used in mRNA LNP formulations with varying N/P ratios; eGFP mRNA; EE > 95%, 60-95 nm size, PDI < 0.1, negative Zeta potential

In vitro assay for parenteral mRNA delivery on Human skeletal muscle myoblasts (**HSMM**) and Murine skeletal muscle myoblasts (**C2C12**)







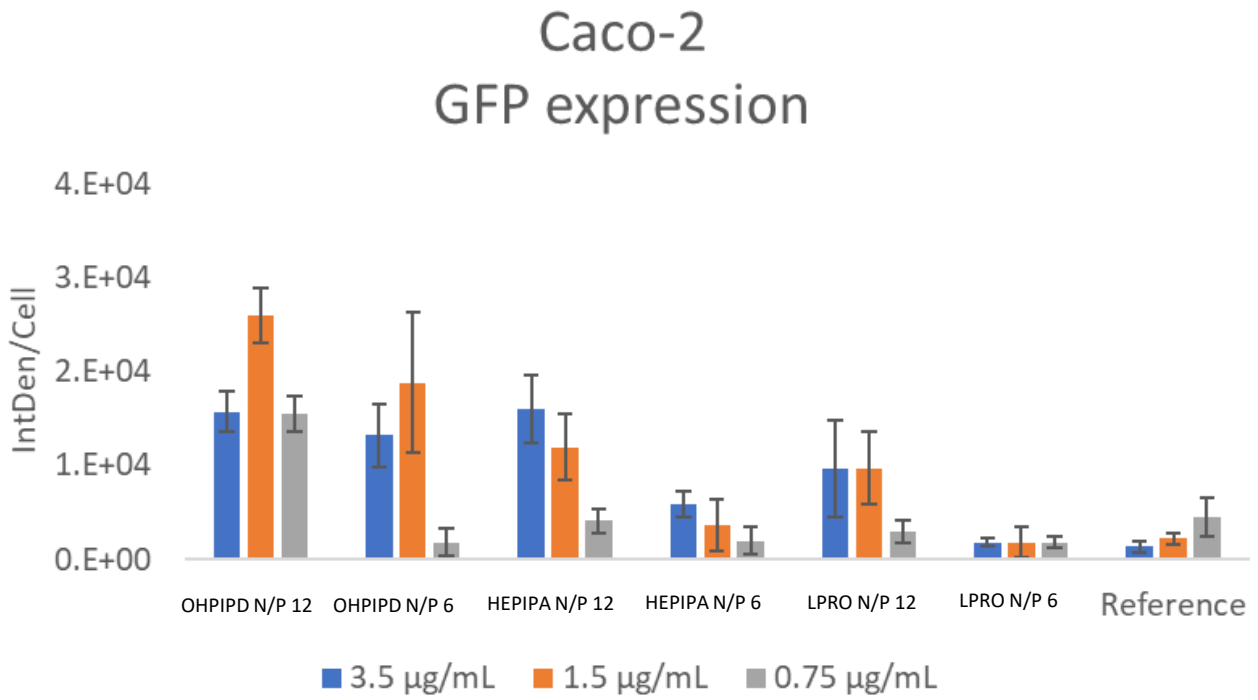
2 x lower expression in **HSMM** after 24 hours

2.5 x higher expression in **C2C12** after 24 hours

CASE STUDY mRNA

Different **ionizable GDGTs (iGDGTs)** used in LNP formulations with varying N/P ratios; eGFP mRNA; EE > 95%, 60-95 nm size, PDI < 0.1, negative Zeta potential

In vitro assay for parenteral mRNA delivery on **Caco-2** cells



15 x higher expression in **Caco-2** after 24 hours

Delivery of mRNA via LNPs

in vivo study (pharmacokinetics)
i.m. application

CASE STUDY mRNA

***In vivo* pharmacokinetics (PK) study** with Erythropoetin **mRNA** and **GDGT**

Study outline:

Animal model: male Wistar rats

3 animals per formulation

Single dose i.m. (500 µL per animal)

Dose: 50 µg mRNA/kg BW

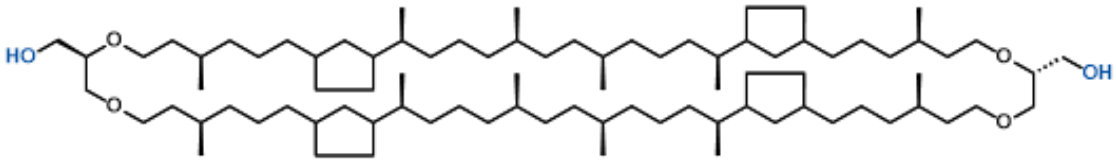
Positive control: state-of-art

GDGT 8 mol % (+ DSPC 1.4 mol %)

Negative control: empty LNP

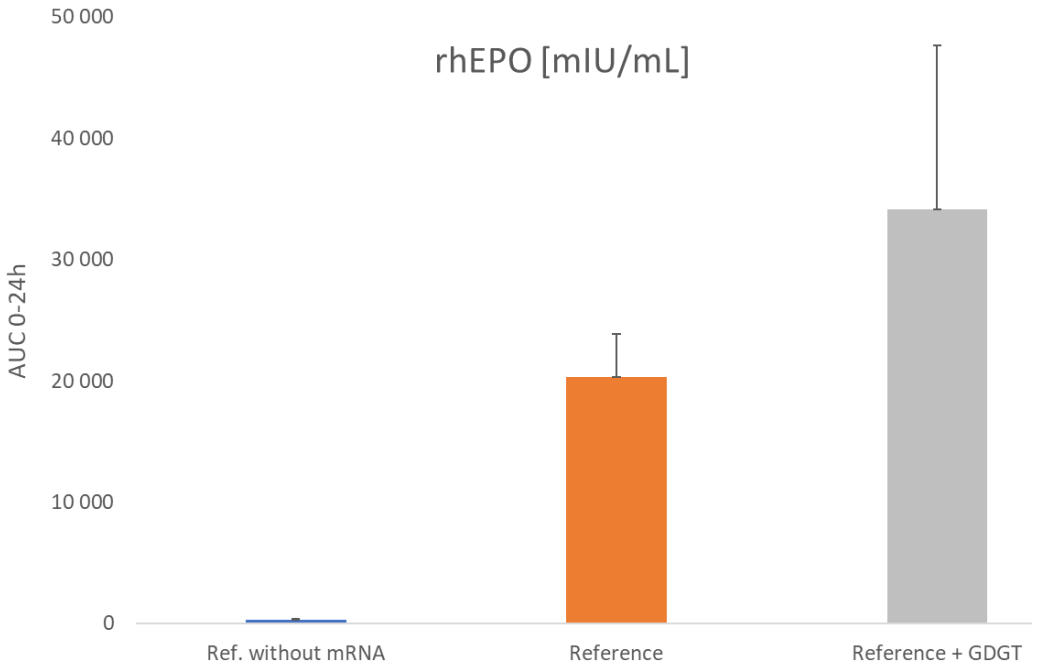
Observed parameters:

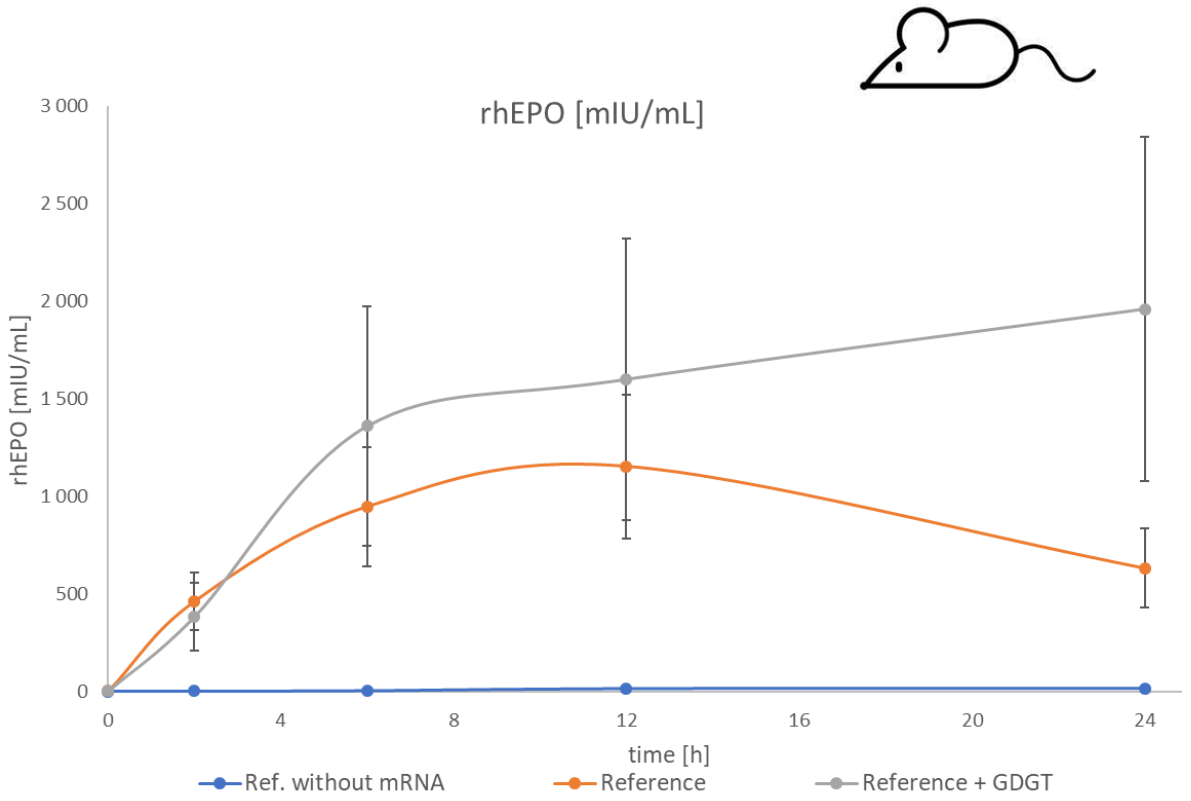
- Viability and mortality
- Clinical observations
- Body weight (pre-treatment)
- Blood parameters (pre-treatment and after 24 h)
- **Cytokine serum levels** (ELISA), after 24 h
- **EPO serum concentration** (ELISA), after 2, 6, 12 and 24 h



No signs of toxicology by addition of GDGT
No effect on cytokine release by GDGT

CASE STUDY mRNA





8 mol % GDGT increase *in vivo* bioavailability 2-fold and cause sustained protein expression after 24 h EPO level is 3.1-fold higher

CASE STUDY mRNA

***In vivo* pharmacokinetics (PK) study** with Erythropoetin **mRNA** and different iGDGT species

Study outline:

Animal model: male Wistar rats

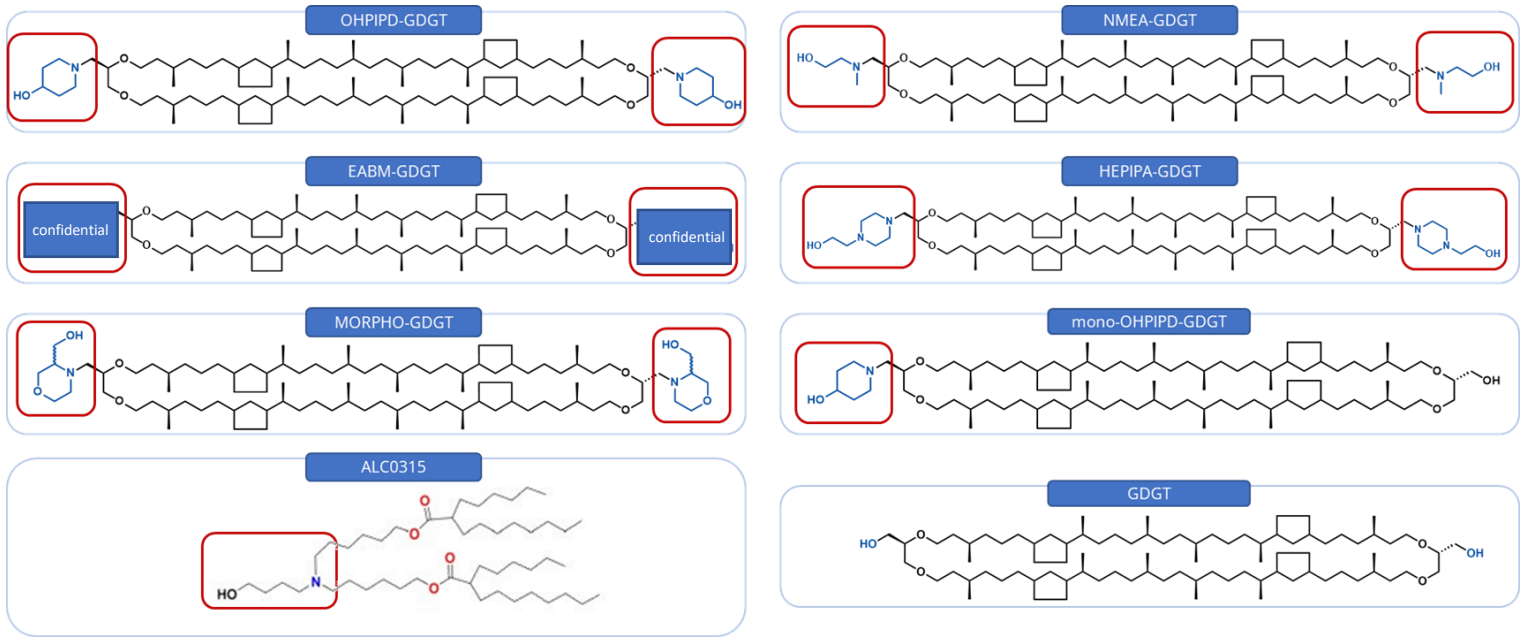
3 animals per formulation

Single dose i.m. 50 µg mRNA/kg BW

Positive control: ALC0315

Negative control: empty LNP

N/P ratios 6 and 12 tested



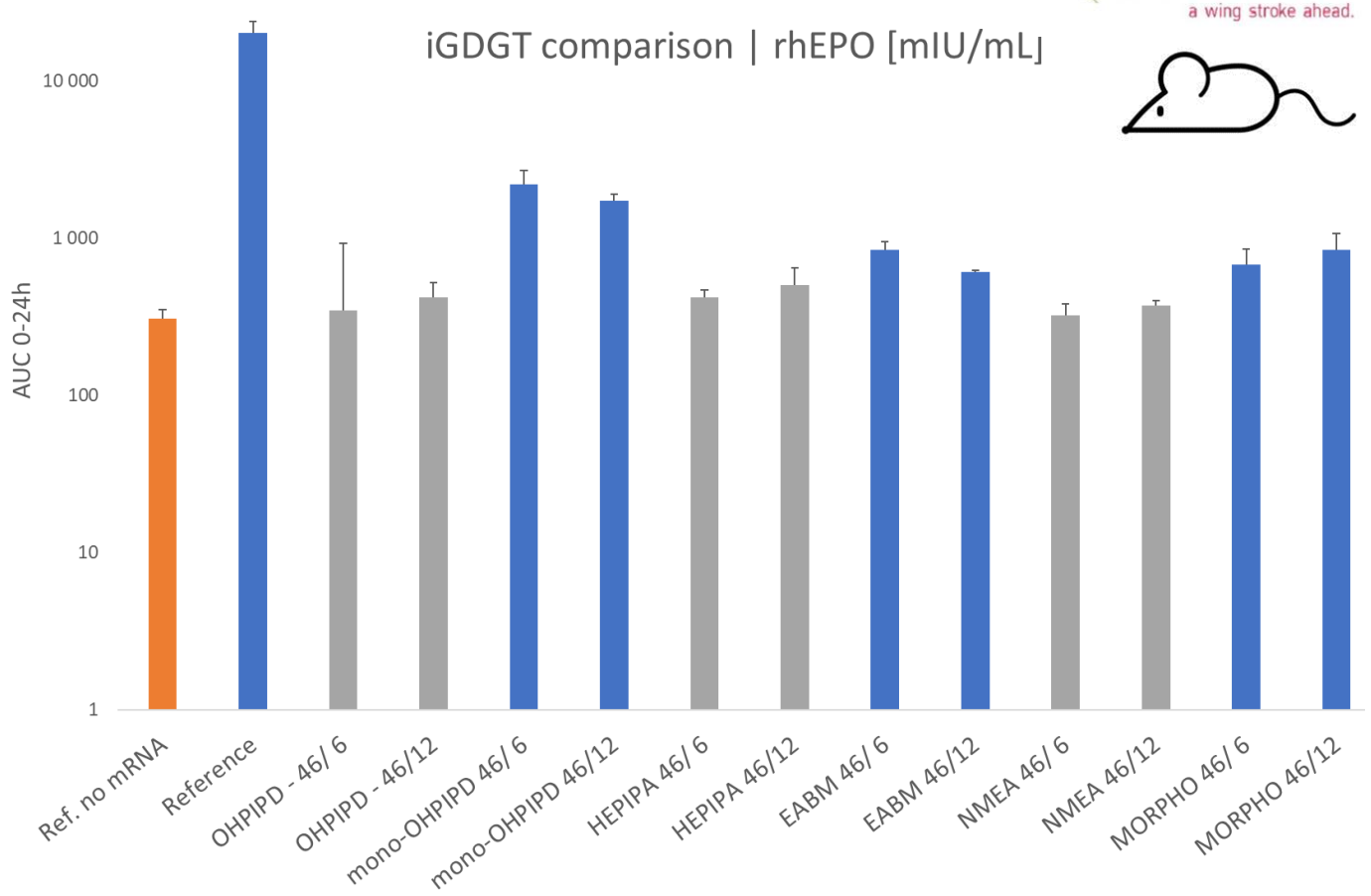
Observed parameters:

- Viability and mortality
- Clinical observations
- Body weight
- Blood parameters (pre-treatment and after 24 h)
- **Cytokine serum levels** after 24 h
- **EPO serum concentration** after 2, 6, 12 and 24 h

No signs of toxicology by addition of iGDGT
No effect on cytokine release by iGDGT

CASE STUDY mRNA

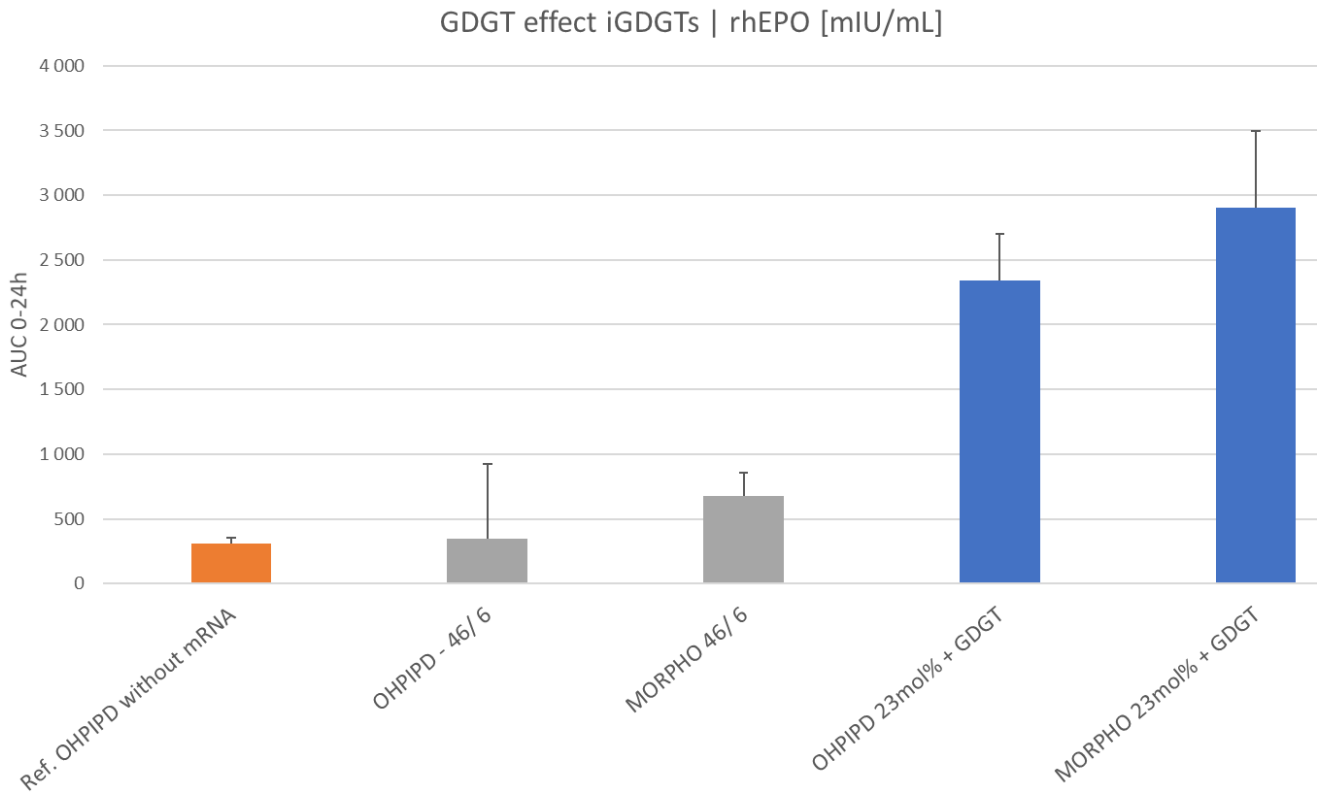
- Sucessfull transfection
- ALC0315 more efficient than iGDGT species



No effect on cytokine release by iGDGTs
Successful transfection, ALC0315 outperforms iGDGTs

CASE STUDY mRNA

Addition of GDGT to selected iGDGT formulations – can we boost production?



GDGT boosts production 5-fold
Formulations optimization for i.m. application necessary

CASE STUDY mRNA

Summary:

- **No** signs of **toxicology** by GDGT and iGDGT species
- **No** effect on **cytokine release** by GDGT and iGDGT species
- **iGDGT i.m. transfection works**, but formulation optimization necessary

  (compare in vitro studies, under certain conditions lower transfection observed)
- **GDGT improves i.m.** in vivo transfection

  (compare *in vitro* studies, up to 9x improvement)

Formulation optimization for i.m. application necessary

CASE STUDY mRNA

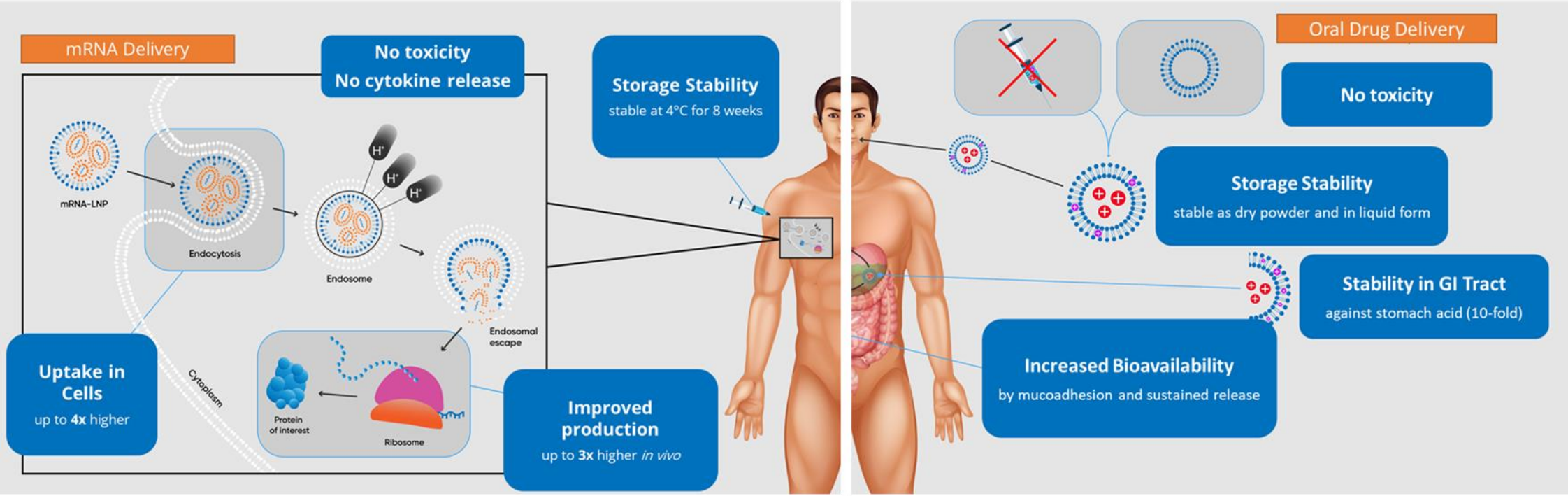
Summary:

- **No** signs of **toxicology** by GDGT and iGDGT species
- **No** effect on **cytokine release** by GDGT and iGDGT species
- **Very promising in vitro data**
  - **9x higher** transfection efficiency for **GDGT**
  - **15x higher** transfection efficiency for **iGDGT**

Transfer results from *in vitro → in vivo*

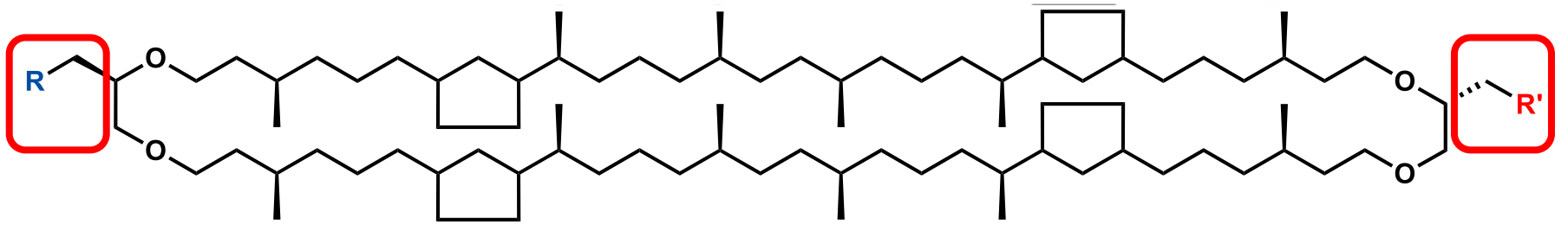
NEXT GENERATION mRNA VACCINATION

NOW it is time to **disrupt the market** with unprecedented **oral mRNA vaccination** and **novel oral medication** based on **NovoArc's enabling technology**



NEXT GENERATION LIPIDS

GDGT-DERIVATIVE



We have the technology to customize R and R´ according to specifications and requests

TEAM

Dr. David Wurm – CEO

www.novoarc.at
david.wurm@novoarc.at
+43 1 3451428 10

Dr. Julian Quehenberger – CTO

www.novoarc.at
julian.quehenberger@novoarc.at
+43 1 3451428 11

Prof. Oliver Spadiut – CPO

www.novoarc.at
oliver.spadiut@novoarc.at
+43 1 58801 166420

ADVISORY BOARD

Mag. Helmut Schmutz	Serial Entrepreneur/Investor in Biotech/Pharma
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