The adjuvant activity of mRNA-LNP vaccines

Dr. Rein Verbeke

Lab of General Biochemistry and Physical Pharmacy
(Ghent University, Belgium)

July 24 2023 **CRS meeting**

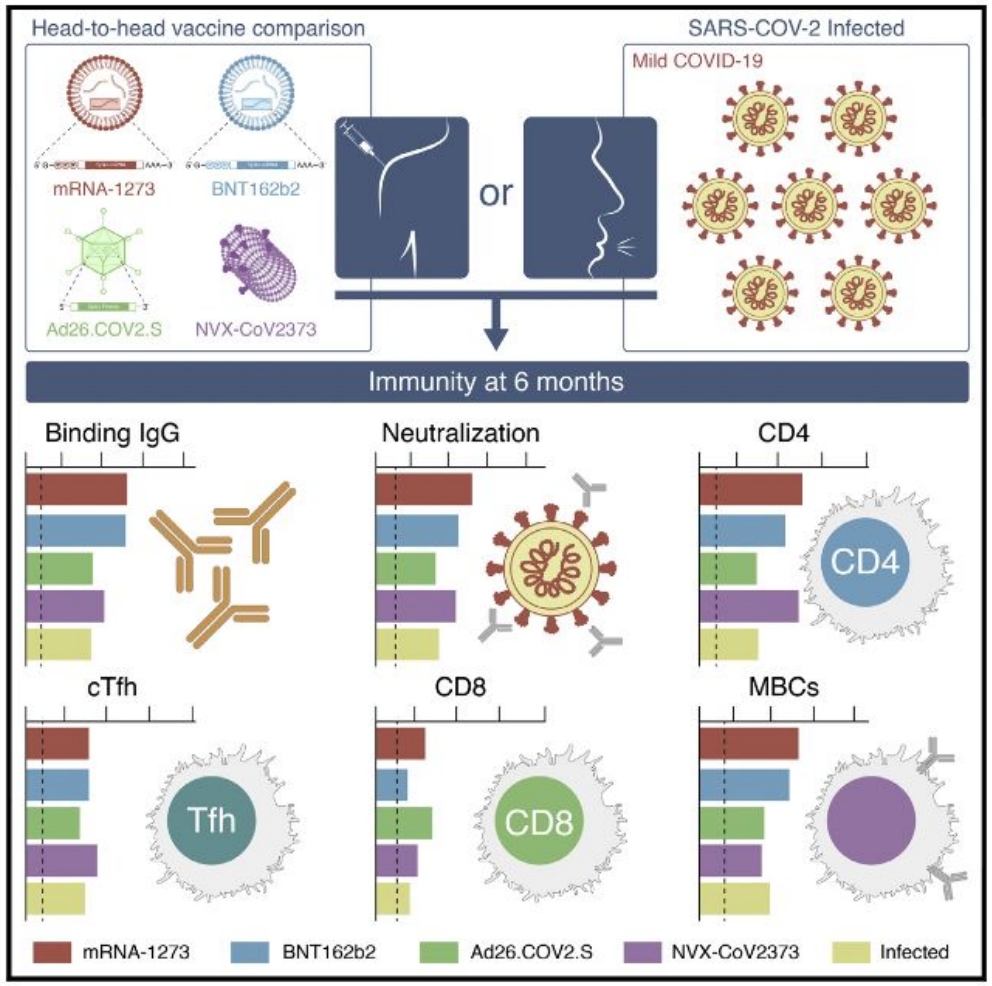
Role of formulation scientists in vaccine development,
tailoring immunogenicity through rational formulation design. - Sponsored by GlaxoSmithKline

GHENT UNIVERSITY

Laboratory for General Biochemistry and Physical Pharmacy
nanomedicines & biophotonics

CRIG

mRNA vaccines require no further addition of adjuvant to elicit robust immune responses



Zhang et al. Cell 2022

Safe and effective vaccines must stimulate the innate immune system at an appropriate level such that they achieve a balance between immunogenicity and reactogenicity

How do mRNA vaccines (need to) activate the innate immune system?

Rein Verbeke

Ghent Research Group on Nanomedicines
Visiting postdoc Pieter Cullis lab, UBC
Expertise: LNPs, mRNA vaccines ,adjuvantia

Norbert Pardi

Pardi lab, UPenn
Alum of Weissman lab
Expertise: Pioneered nucleos.-modified mRNA-LNP vaccines, influenza

Michael J. Hogan

Eisenlohr's lab, CHOP
Alum of Weissman lab
Expertise: Antigen presentation, mRNA vaccines

Karin Loré

Loré lab, Karolinska Institutet
Expertise: preclinical evaluation (mRNA) vaccines in non-human primates

Immunity
CellPress
Review

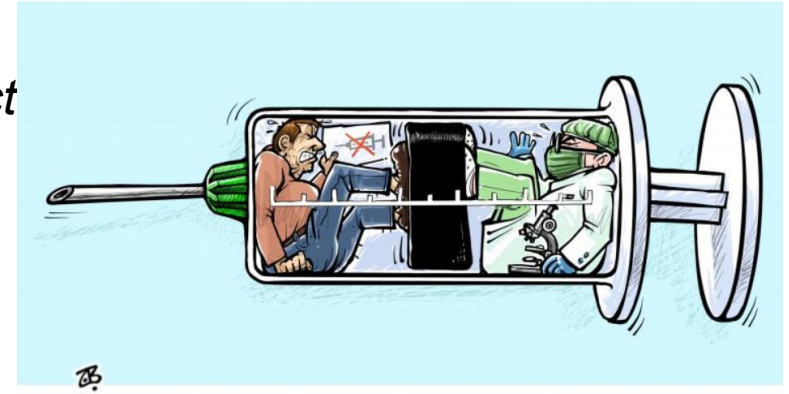
Innate immune mechanisms of mRNA vaccines

Rein Verbeke,[1,2,*] Michael J. Hogan,[3] Karin Loré,[4,5] and Norbert Pardi[6,*]

Relevance of understanding the adjuvanticity of mRNA-LNP vaccines

Billions of mRNA vaccine doses have been administered

- *Our duty as scientists to keep on investigating the mode of act and safety of mRNA vaccines and to inform the public*



Understanding the innate immune mechanisms of mRNA vaccines will be key to yield further improvements in this technology

- *Development of less reactogenic and/or more potent vaccines*
- *Tailored mRNA vaccine designs depending on its therapeutic use (e.g. immunogenic vs non-immunogenic products)*

Blame the Messenger

1950-60s: RNA (Viruses) – Infected cells make **“INTERFERON” (IFN)**

- IFN reaction inhibits viral replication – blocks viral RNA translation

Mechanism of Action of Interferon

I. Relationship with Viral Ribonucleic Acid

P. DE SOMER, A. PRINZIE, P. DENYS, JR., AND E. SCHONNE

Rega Institute for Medical Research, Department of Virology, University of Louvain, Louvain, Belgium

Accepted September 26, 1961

The mechanism of action of interferon has been investigated in two different cell systems, with concordant results. Interferon is able to suppress the one-cycle synthesis of poliovirus in embryonated eggs inoculated with infectious polio ribonucleic acid (RNA). This observation represents the basis of a sensitive method for quantitative bioassay of interferon activity. Interferon-treated chick embryo cells infected with Western equine encephalitis (WEE) virus do not yield detectable infectious viral RNA, as shown by extraction with cold phenol. It appears that interferon action occurs within the cell, after penetration of virus and before formation of mature virus particles. A close relationship with viral RNA metabolism is suggested.

Blame the Messenger

1950-60s: RNA (Viruses) – Infected cells make **“INTERFERON” (IFN)**

- IFN reaction inhibits viral replication – blocks viral RNA translation

Since 1990s: Discovery of **PATHOGEN RECOGNITION RECEPTORS** involved in **RNA sensing**

- **Double stranded (ds)RNA** is recognized by TLR3, RIG-I, MDA-5, PKR, OAS etc.
- **Uridine-containing RNA** is recognized by TLR7(/8 in humans)
- **Cap1 structure** is needed to avoid recognition by IFIT1 (in ssRNA) & RIG-I (in dsRNA)

PROBLEM : Anti RNA-response can jeopardize the translation, safety & efficacy of mRNA vaccines/therapeutics

Balance? The type I IFN response can **limit the antigen availability**
It can also be a potent driver of immunity - **self-adjuvant properties**

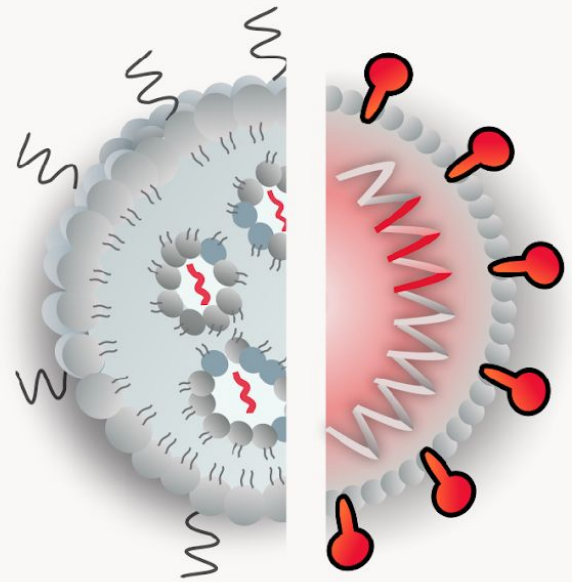
SOLUTION?

2005-… : **MODIFIED URIDINES** or uridine depletion reduce RNA sensing and improve translation

- Avoids recognition by TLR7/8, PKR, OAS… (Karikó, Weissman et al.)
- Even so, that nucleoside-modified mRNA is (was) claimed to be non-immunogenic

CASE I: Different COVID-19 mRNA vaccine candidates

All using iLNP carriers



Uridine-modified

Moderna - Spikevax
Dose: 100 µg
Efficacy*: ~95%

Pfizer/BioNTech - Comirnaty
Dose: 30 µg
Efficacy: ~95%

Unmodified

CureVac - CVnCoV
Dose: 12 µg
Efficacy: ~47%
2nd generation

Providence - PTX-COVID19-B
Dose: 40 µg
Non-inferior to Comirnaty
in Phase 2 trial

Self-amplifying

Arcturus- ARCT-154
Dose: 5 µg
Efficacy: ~55%

Imperial - LNP-nCoVsaRNA
Dose: 0.1-10 µg
Phase 1: Low immunogenicity

*Prevention against symptomatic COVID-19 disease

CASE II: Seemingly similar mRNA products for different applications

moderna

Uridine-modified-LNP	Viral diseases
mRNA-1273 - Spikevax Dose: 100 µg IM Efficacy*: ~95%	**Prevention of disease/infection** • Neutralizing antibodies • T cell responses

Uridine-modified-LNP	Cancer vaccines
mRNA-4157 (Merck) Dose: 1000 µg IM Phase 2 stage	**Destroy tumors/ memory for relapse** • CD8 & CD4 T cell responses • NK cells …

Uridine-modified-LNP	Therapeutics
mRNA-3927 Dose: 600 µg/kg IV Phase 2 stage	**Protein production** • Avoid innate immunity as it jeopardize safety & function

BNT162b2 is still capable of inducing a transient type I IFN response

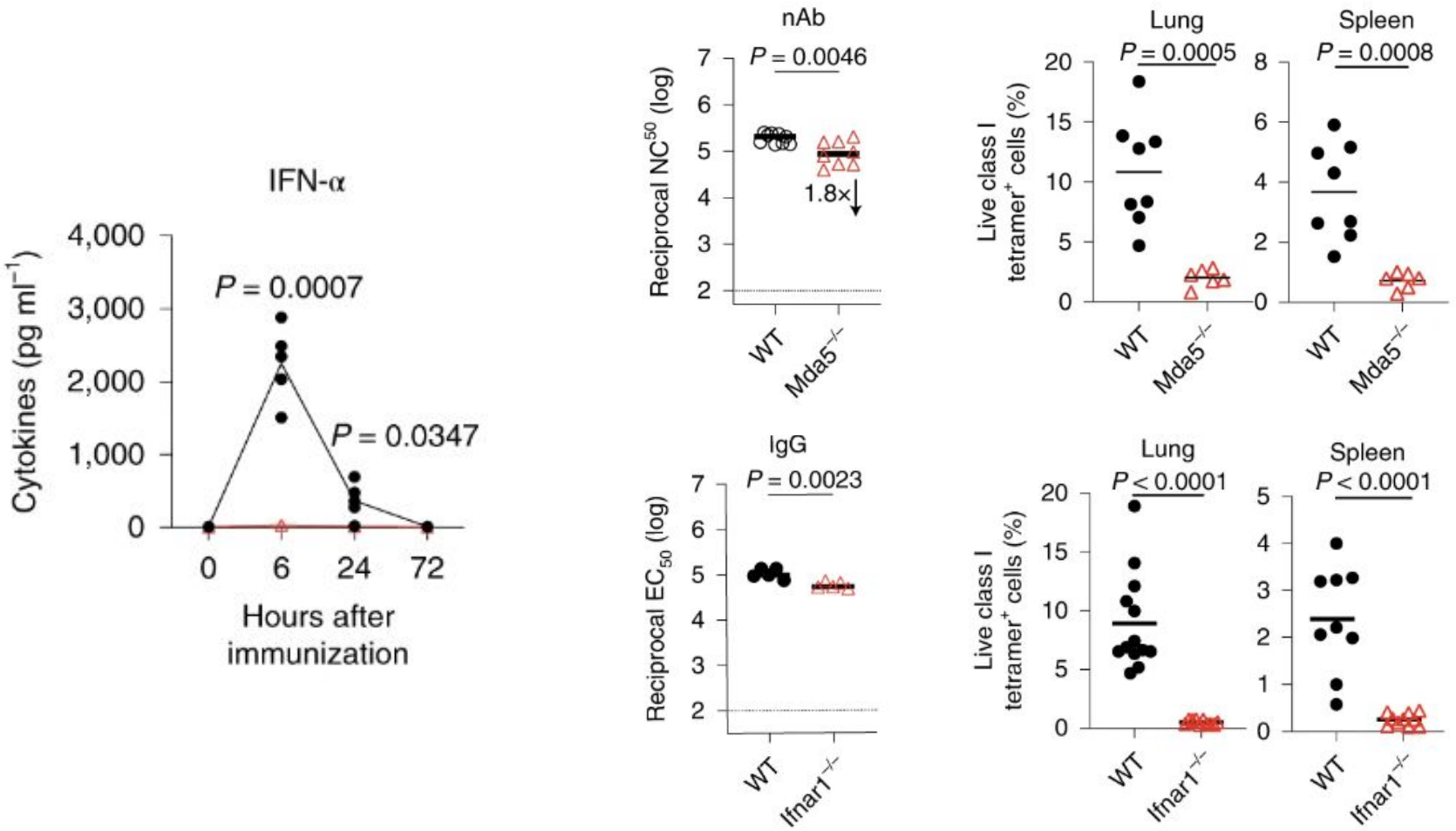


Fig. 7: MDA5–IFNAR1 axis is important for BNT162b2-induced CD8^{+} T cell response.

From: Mechanisms of innate and adaptive immunity to the Pfizer-BioNTech BNT162b2 vaccine

Li et al. Nat. Immunol.. 2022

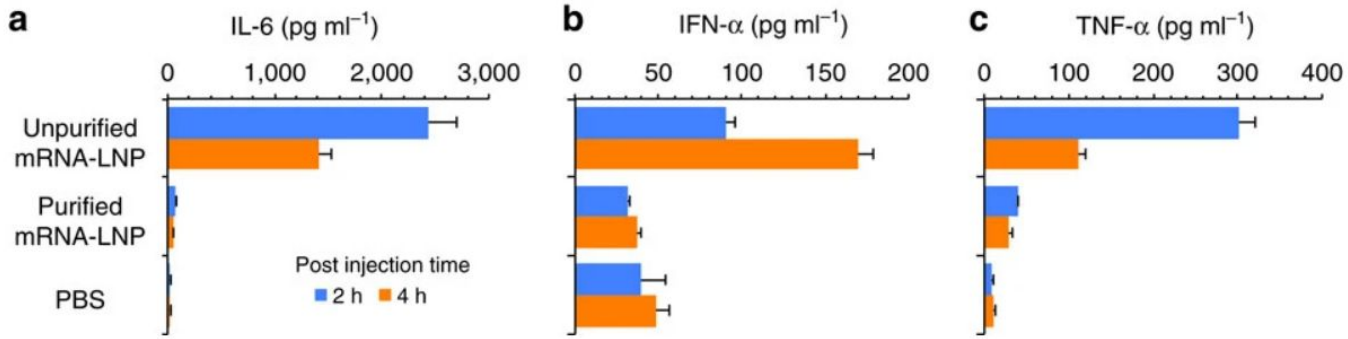
Keep it clean or a bit dirty

T7 polymerase gives rise to **CONTAMINANTS** in the form of short and long **double stranded RNA (dsRNA)**

- dsRNA can strongly contribute to the innate immune response
- Need for additional **purifications methods**: HPLC, Cellulose purification, RNase III
- **Alternative**: optimization of T7 IVT reaction (e.g. Moderna published on T7 mutant)

Figure 3: Analysis of innate immune activation by mRNA-LNPs.

From: Administration of nucleoside-modified mRNA encoding broadly neutralizing antibody protects humanized mice from HIV-1 challenge



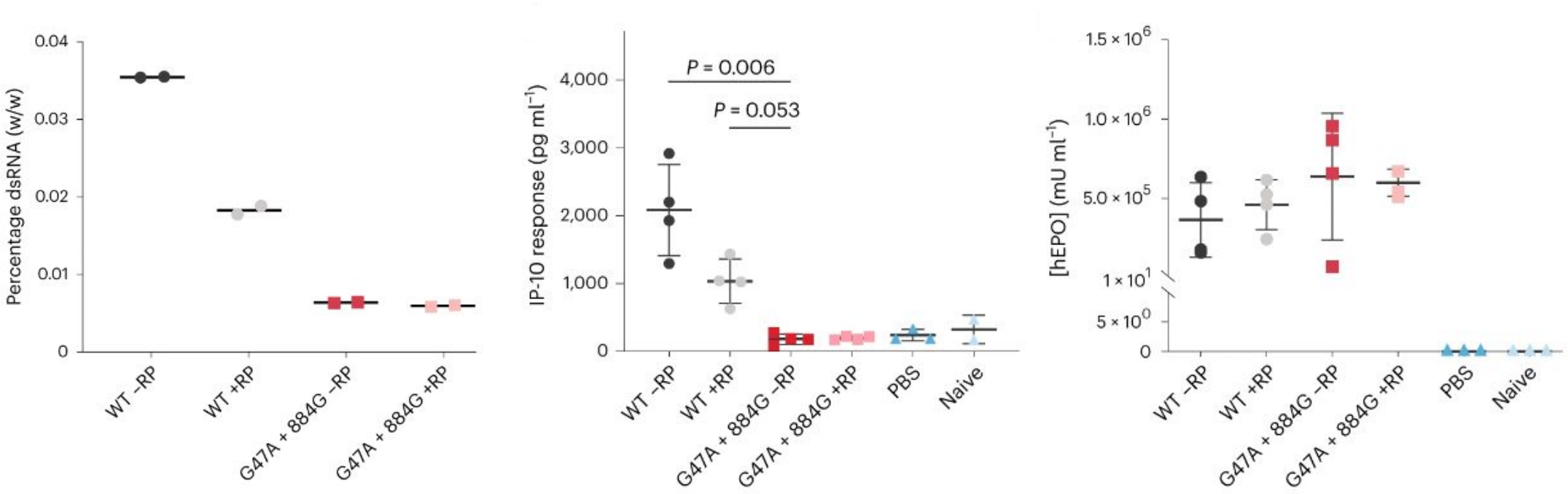
C57Bl/6 mice were i.v. injected with a 1 mg kg^{-1} dose of nucleoside-modified, FPLC-purified Luc mRNA-LNPs; unpurified, nucleoside-modified Luc mRNA-LNPs (1 mg kg^{-1}) (positive control) and phosphate buffered saline (PBS) (negative control). Animals were bled 2 and 4 h post injection and interleukin-6 (**a**), IFN-α (**b**) and tumour necrosis factor-α (**c**) levels were measured in plasma by Luminex assay. Error bars are s.e.m. Statistical analysis: one-way analysis of variance with Bonferroni correction, $P<0.01$ in comparisons of PBS to non-purified mRNA-LNPs and non-purified mRNA-LNPs to purified mRNA-LNPs. Group size is five animals.

Pardi et al. Nat. Commun. 2017

Impact of dsRNA content on mRNA-iLNP therapeutics

Fig. 5: In vitro and in vivo performance of mRNAs synthesized by WT and G47A + 884G T7 RNAPs.

From: An engineered T7 RNA polymerase that produces mRNA free of immunostimulatory byproducts



Dousis et al. Nat. Biotechnol. 2022

Keep it clean or a bit dirty

Impact of dsRNA in mRNA products/vaccines

(Covid-19) Vaccines: Presence of dsRNA content?

Dirty secret ? Explanation for adjuvant effects, and/or side effects?

Therapeutics: **Get rid of dsRNAs !!!**

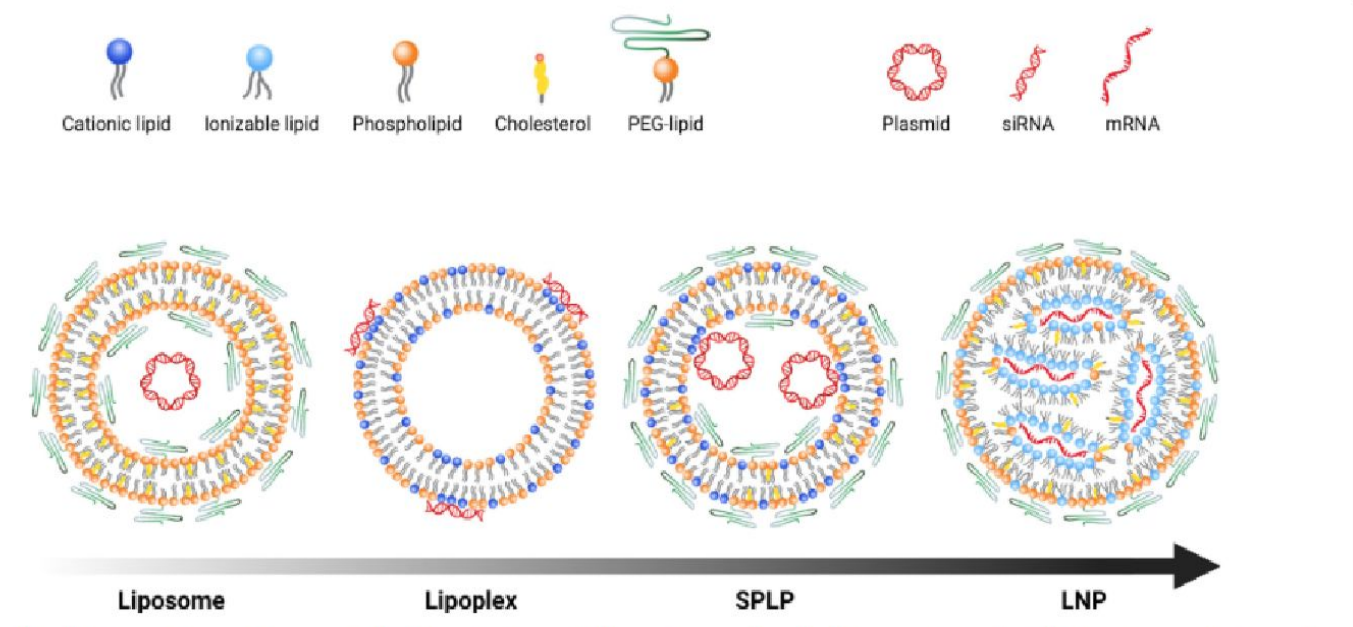
JEM Journal of Experimental Medicine

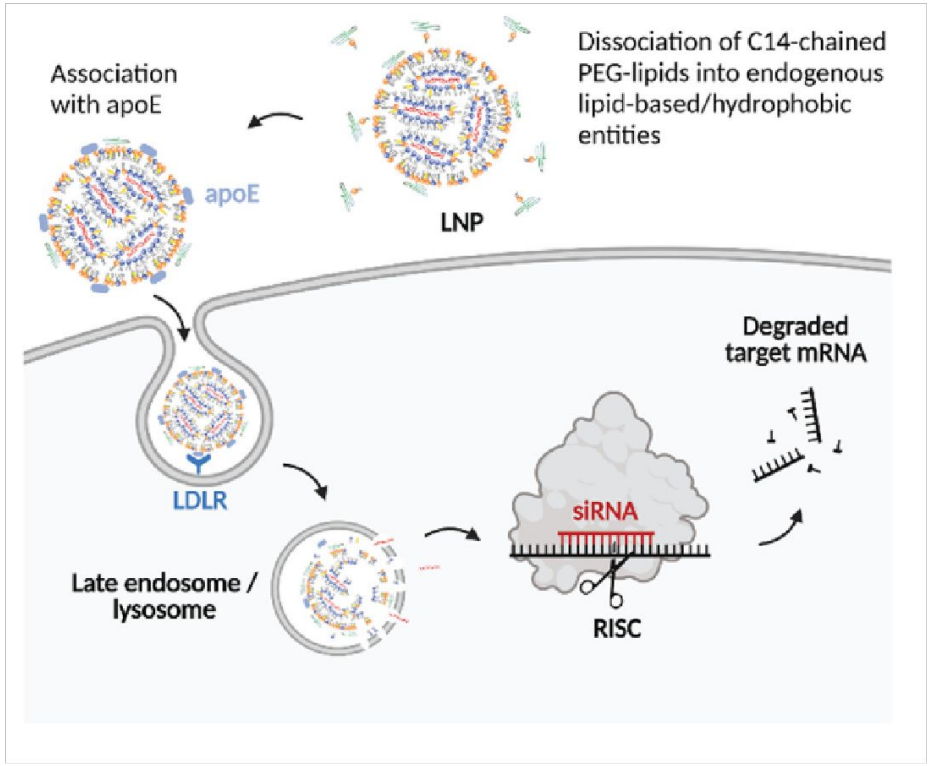
ARTICLE

Human type I IFN deficiency does not impair B cell response to SARS-CoV-2 mRNA vaccination

Aurélien Sokal[1*], Paul Bastard[2,3,4,5*], Pascal Chappert[1,6**], Giovanna Barba-Spaeth[7**], Slim Fourati[8,9], Alexis Vanderberghe[6,10], Pauline Lagouge-Roussey[6,10], Isabelle Meyts[11], Adrian Gervais[2,3], Magali Bouvier-Alias[8,9], Imane Azzaoui[6,10], Ignacio Fernández[7], Andréa de la Selle[1], Qian Zhang[2,3,5], Lucy Bizien[2,3], Isabelle Pellier[12,13], Agnès Linglart[14], Anya Rothenbuhler[14], Estelle Marcoux[15], Raphael Anxionnat[16], Nathalie Cheikh[16], Juliane Léger[17], Blanca Amador-Borrero[18], Fanny Fouyssac[19], Vanessa Menut[20], Jean-Christophe Goffard[21], Caroline Storey[22], Caroline Demily[23], Coralie Mallebranche[12,13], Jesus Troya[24], Aurora Pujol[25], Marie Zins[26], Pierre Tiberghien[27,28], Paul E. Gray[29,30,31], Peter McNaughton[31,32], Anna Sullivan[31,32], Jane Peake[31,32,33], Romain Levy[2,3,34], Laetitia Languille[10], Carlos Rodiguez-Gallego[35,36], Bertrand Boisson[2,3,5], Sébastien Gallien[37], Bénédicte Neven[34], Marc Michel[10], Bertrand Godeau[10], Laurent Abel[2,3,5], Felix A. Rey[7], Jean-Claude Weill[1], Claude-Agnès Reynaud[1], Stuart G. Tangye[31,38,39], Jean-Laurent Casanova[2,3,4,5,40], and Matthieu Mahévas[1,6,10]

iLNPs were first optimized for i.v. delivery of siRNA to hepatocytes

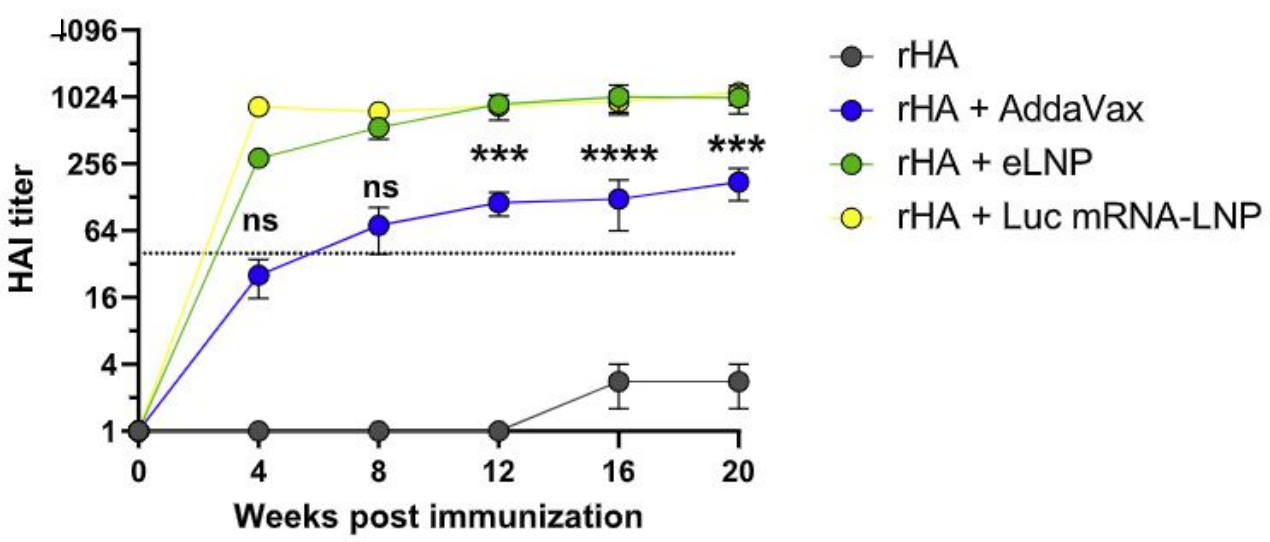


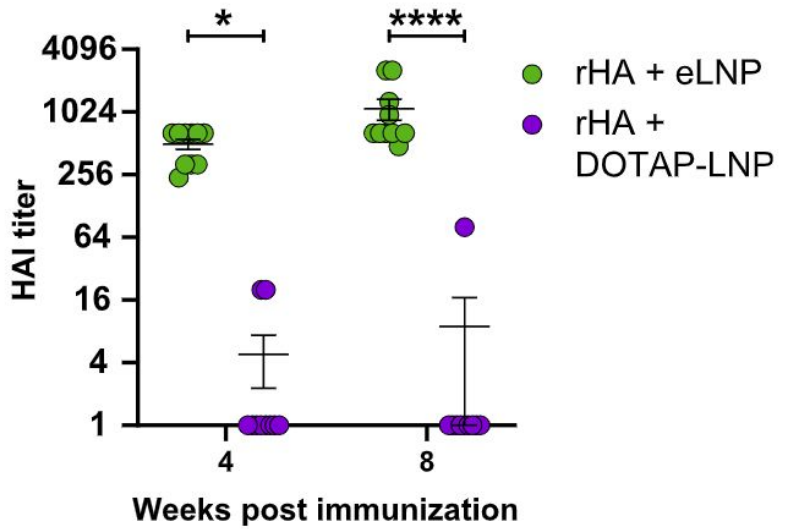


Albertsen G.H. et al. Adv. Drug. Deliver.. 2022

Empty iLNPs (can) provide a strong adjuvant activity to (mRNA) vaccines

Combination of protein antigen with iLNPs as adjuvants





Alameh et al. Immunity 2021

Empty iLNPs (can) provide a strong adjuvant activity to (mRNA) vaccines

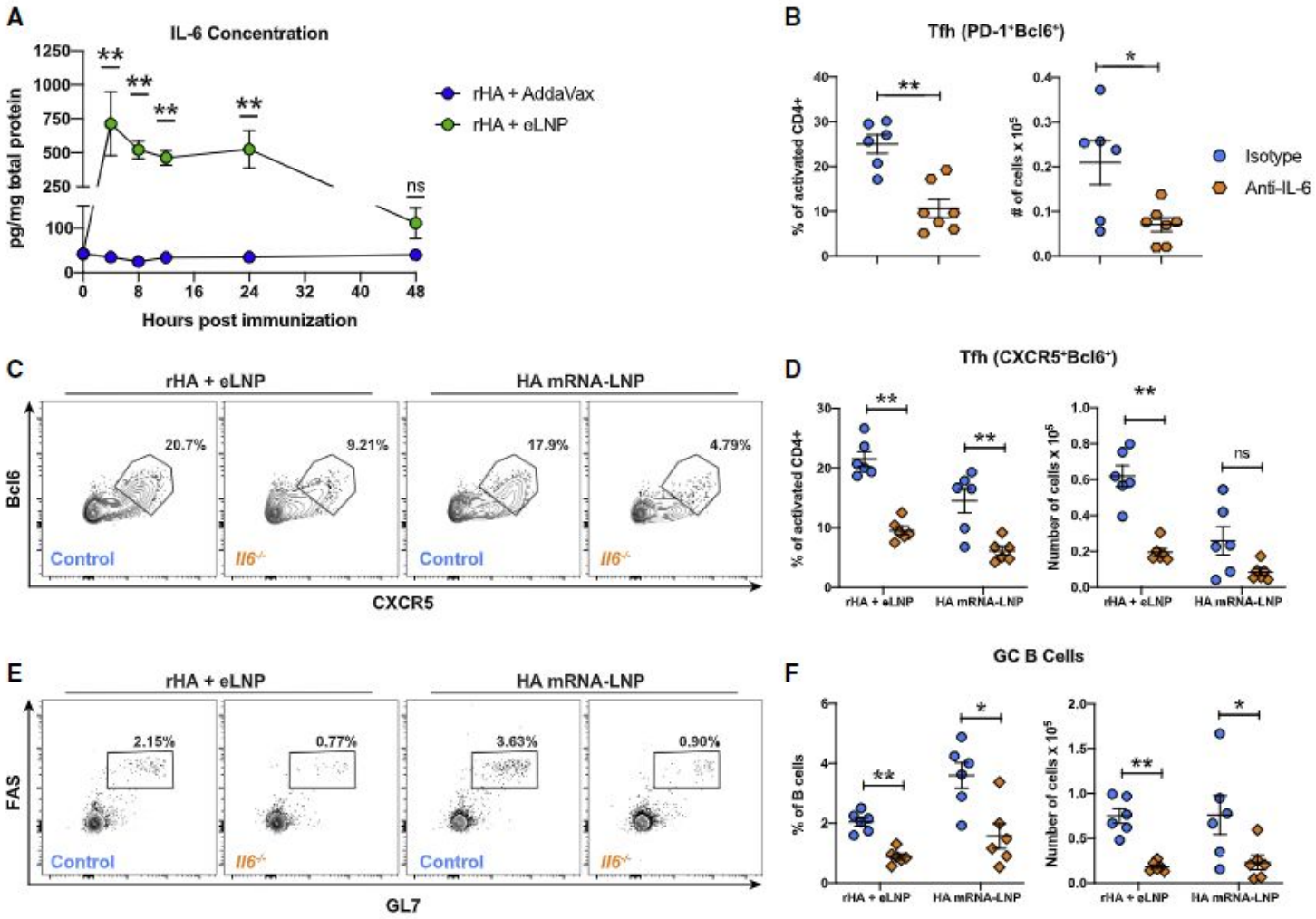


Figure 7. IL-6 is crucial for LNP-induced GC reactions

(A) BALB/c mice received a single IM immunization with 10 μg rHA adjuvanted with eLNP or AddaVax.

Alameh et al. Immunity 2021

Impact of ionizable lipid on pro-inflammatory response

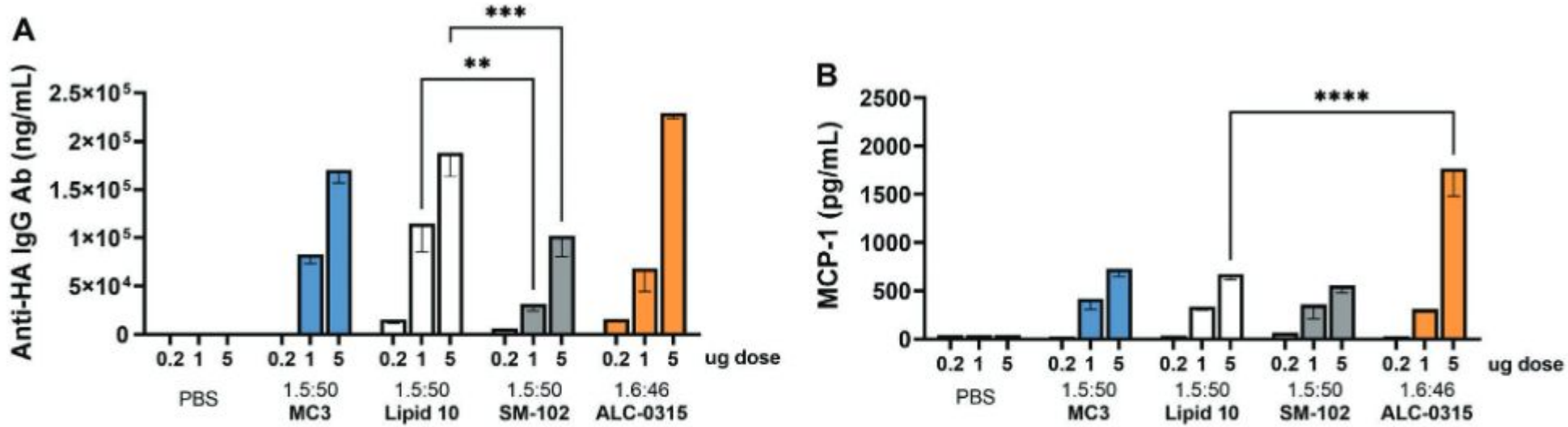
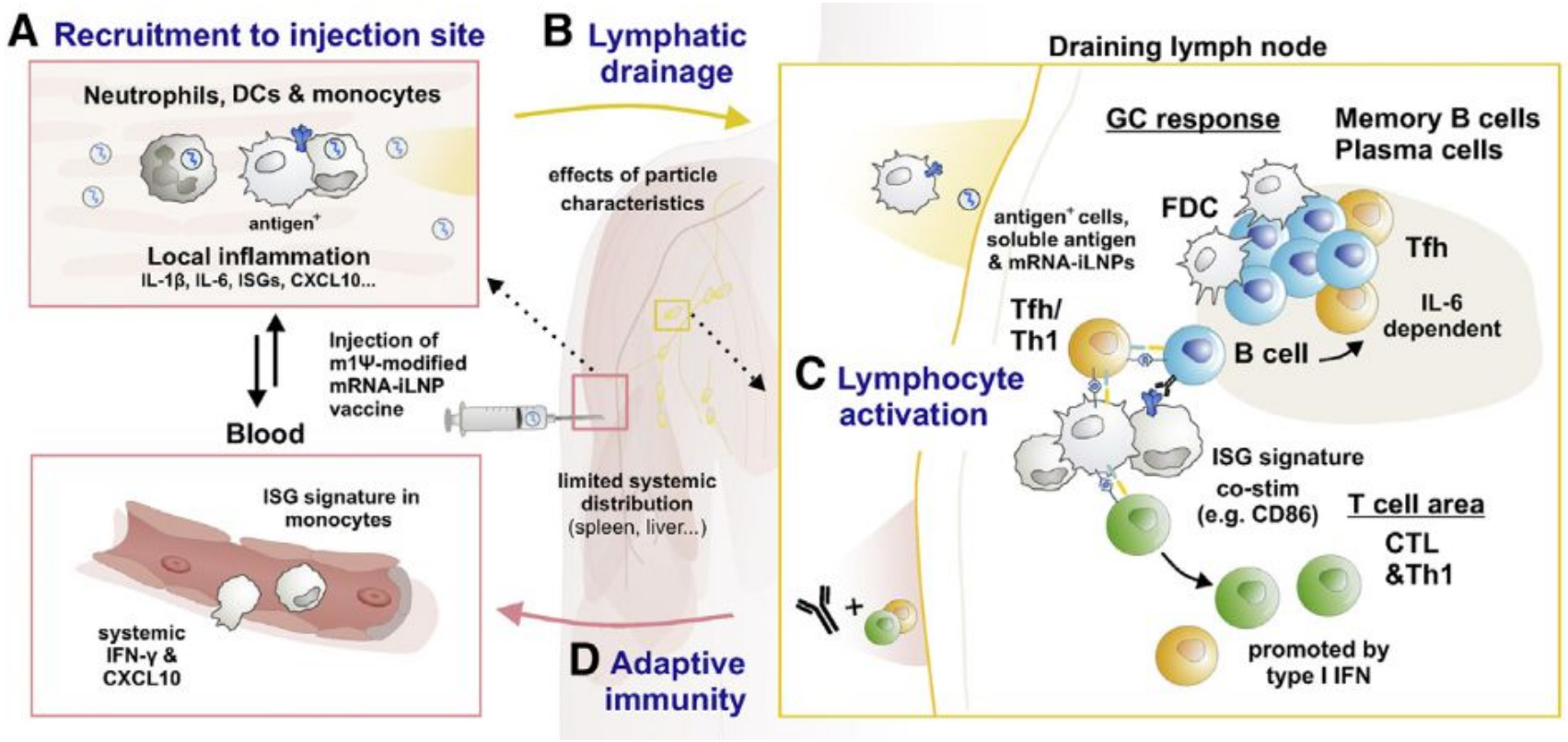


Figure 5. Evaluation of ionizable lipids following IM administration in HA vaccine model. Mice injected IM with 0.2, 1, or 5 µg dose of HA mRNA LNP on Day 0. A) Anti-HA IgG antibody levels on Day 28 post-dose were measured by ELISA. B) Quantification of plasma MCP-1 at 6 h post-dose by ELISA. $N = 4$ BALB/c mice per group. Error bars are S.E.M. $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$ based on two-way ANOVA with Tukey adjustment for multiple testing correction.

Lam et al. Adv. Mat. 2023

Innate immune cell dynamics upon administration of mRNA-iLNP vaccines



- How are mRNA-LNPs taken up by immune cells?
- Which innate immune cell types are responsible for antigen presentation ?
- How are iLNP sensed by the innate immune system ?
- Which innate immune pathways contribute to reactogenic versus immunogenic effects?

Verbeke, R., Hogan, M.J., Loré, K., Pardi, N. et al. Immunity 2022

The path to next generation mRNA-iLNP vaccines

Many variables can affect the reactogenicity and immunogenicity of mRNA vaccines

Need for standardization

- **mRNA**: Modified vs unmodified, method of purification, dsRNA content, Cap structure etc.
- **Lipid carrier**: LPX ≠ LNP, LNP1, LNP2… (disclose identity of lipids, molar ratios…)

Opportunity

- Multiple possible paths to develop mRNA vaccines that hit the sweet spot of immune activation (*that can hit a sweeter spot*)

Could mRNA vaccines benefit from the addition of adjuvantia?

- To improve durability of adaptive immune responses?
- To provide dose-sparing effects?
- To empower cellular immunity?

… TEASER Wednesday

4:30 PM – 6:30 PM US PST Tech Session 2: Immuno Delivery

Location: Champagne 1 & 2

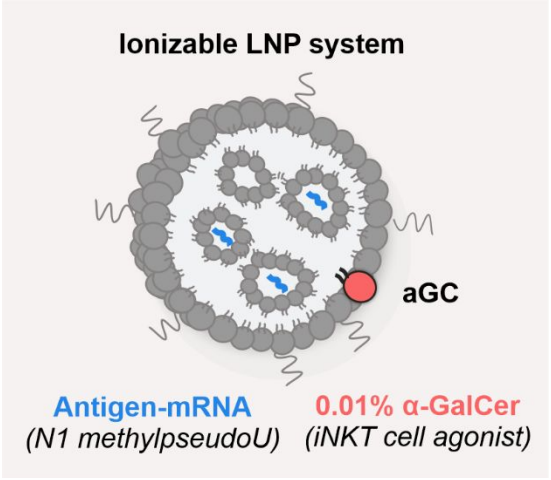
Galsome approach to strengthen mRNA vaccines

Lipid nanoparticle (LNP) for co-delivery of:

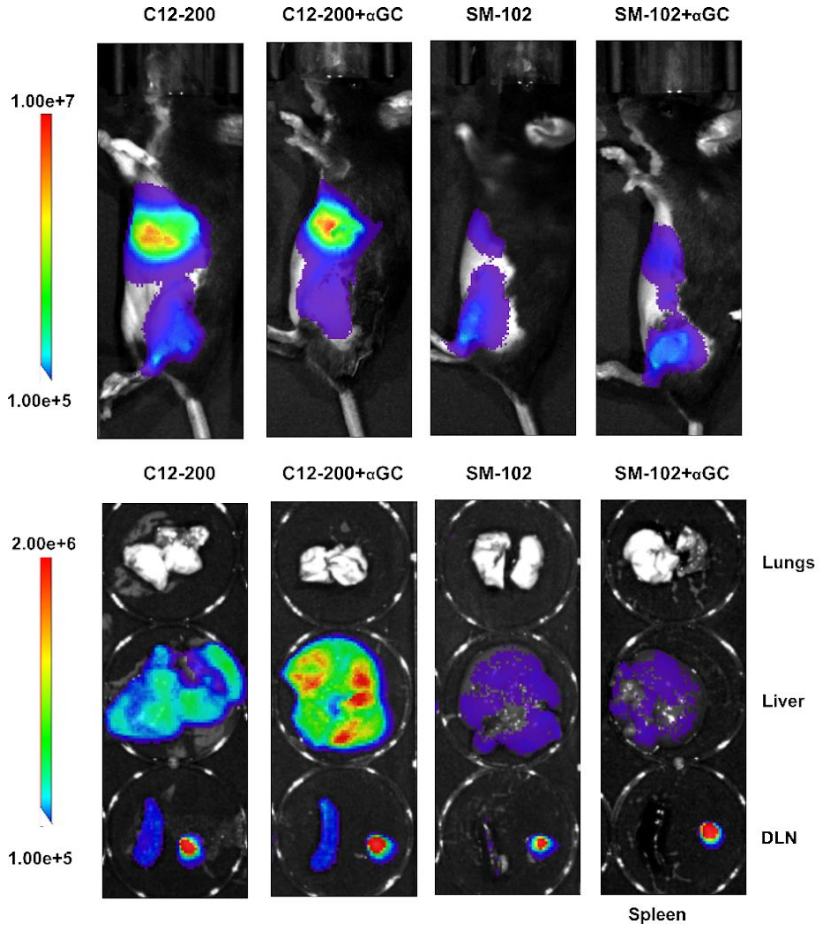
- **Nucleoside-modified mRNA encoding antigens**
  immunosilent -> Limited type I IFN activity
  Better tolerability and translation

- **α-Galactosylceramide (adjuvant)**
  Anchored in lipid carrier (0.01 mol%)

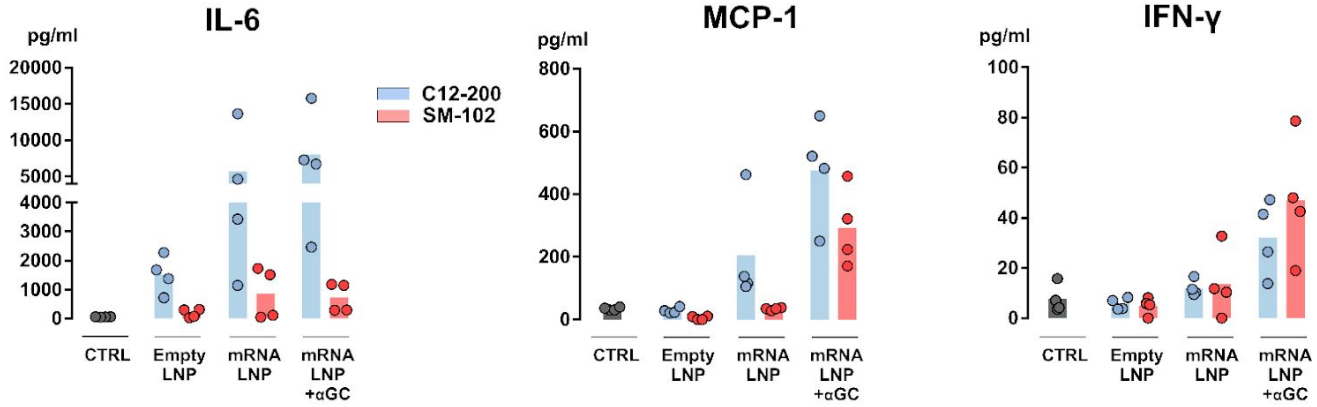
Verbeke R. et al. ACS Nano 2019
WO2020/058239

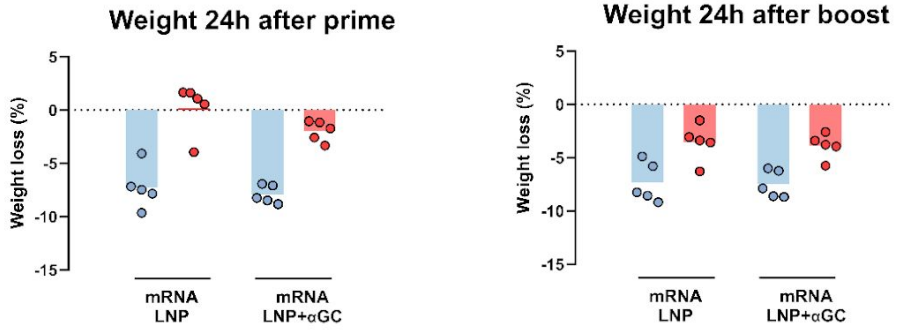


Benchmark study with SM-102 mRNA LNPs



(6h post-injection of 2.5µg luciferase mRNA)

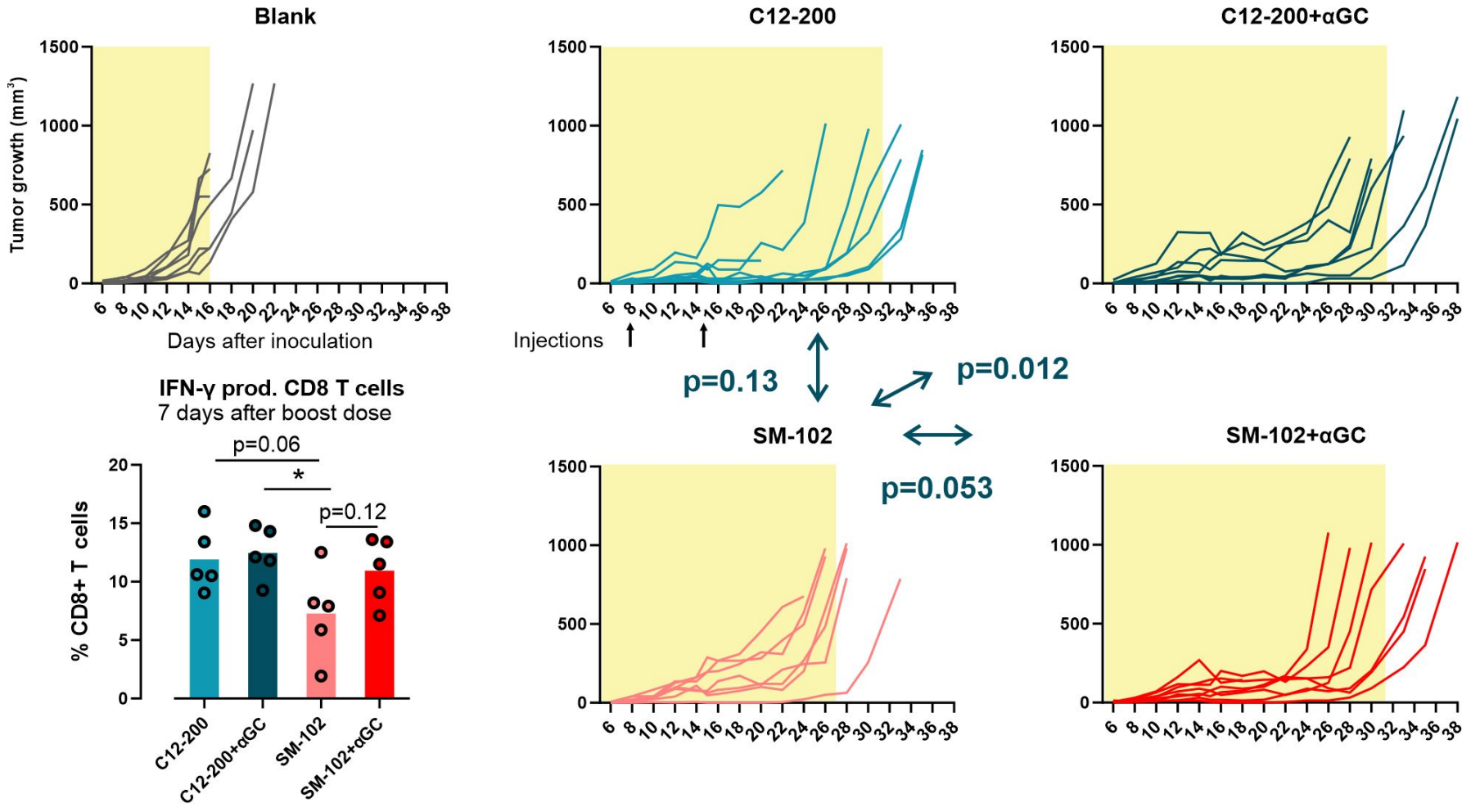




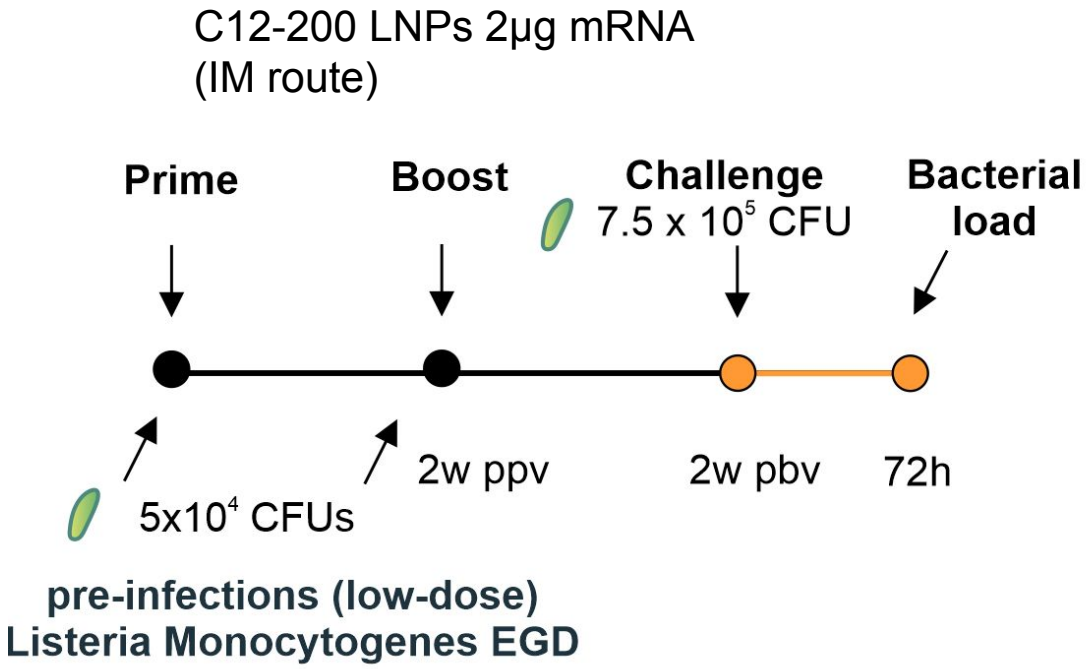
- **C12-200 is more reactogenic than SM-102 (Moderna)**
- **C12-200 is slightly better in transfection than SM-102**

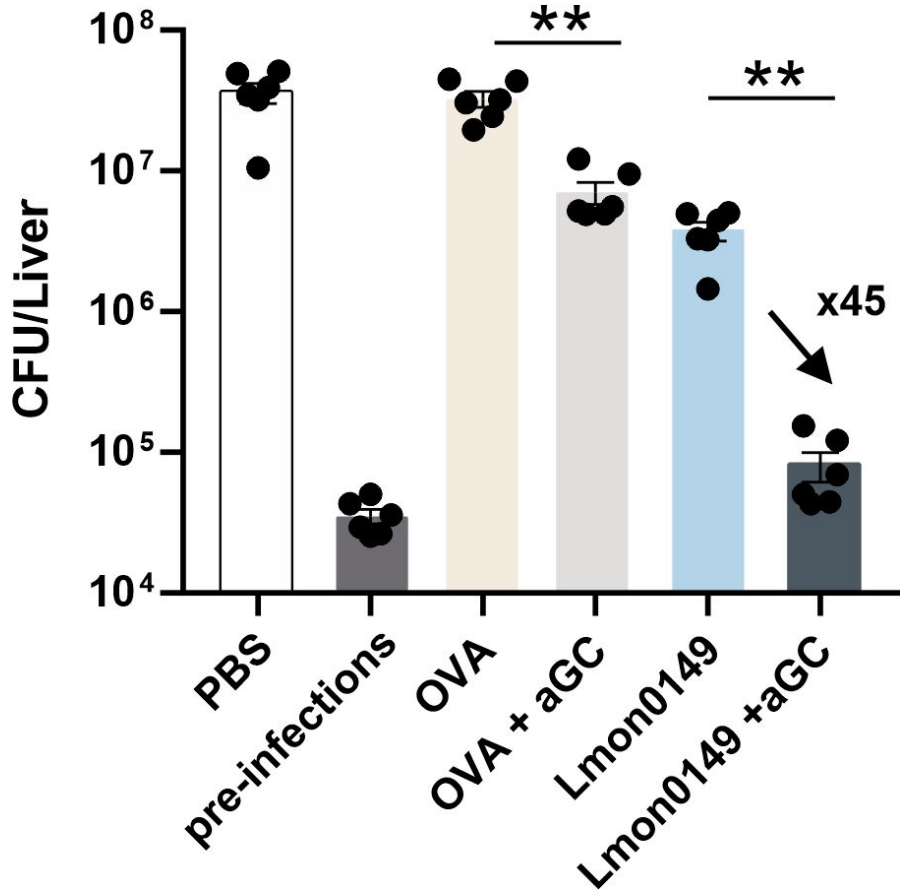
Alameh MG et al. Immunity 2021
Verbeke R. et al. Immunity 2022

Therapeutic effects in B16 melanoma model



Proof of concept of Galsome potential for intracellular bacteria





- **Synergistic protective effects of αGalCer & mRNA-LNP vaccine**

Mayer R. et al. Nat. Commun. 2022
EP22170792.0

Baxerna project - development of bacterial mRNA vaccines
(EU funded – 9 million)

Institut Pasteur

Demangel
Mycobacteria
Tuberculosis & Buruli Ulcer

Van der Henst & Muraille
Acinetobacter baumannii
AMR

VIB ULB

VIB Impens
MS Proteomics
Immunopeptidomics

VIB Martens
MS Bio-IT
Immunopeptidomics

Antigen discovery

mRNA vaccine development

Breckpot
mRNA design
immunotherapy

De Smedt
mRNA vaccines
adjuvantia

Netea
trained immunity
innate immunity

Loré
vaccine mechanisms
In vivo models

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GMP mRNA-LNP
production

Vermaelen
Pneumology
Tuberculosis

Leroux-Roels
Center for Vaccinology
Clinical trials

U4 GENT GMP unit Cell Therapy

CEVAC

clinical trial

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RheaVita — Vaccine lyophilization & storage

Quantoom Biosciences — Cheaper en local Vaccine production

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fwo Research Foundation Flanders Opening new horizons