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Overcoming Anti-PEG Antibody Responses to Increase Potency & Decrease Adverse Side Effects of mRNA-LNP Formulations

Randall Moreadith, MD, PhD, Chief Development Officer

July 29, 2024
4th mRNA-based Therapeutics Summit – Boston, MA

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cancer-killing toxins

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Today's Messaging:

- Anaphylaxis occurs at an unusually high incidence rate in patients receiving the approved mRNA vaccines; it is not classically characterized IgE-mediated (mast cell degranulation)
- The high titers of IgM & IgG that are boosted by the vaccines bind to LNPs, activate complement, and induce structural changes in the LNP (leak payload, allow access to serum components)
- The high titers of IgM & IgG are associated with an increased incidence of systemic reactogenicity

The Serina LNP Laboratory has identified a POZ-lipid LNP that fails to elicit an immune response to the POZ on repeat dosing

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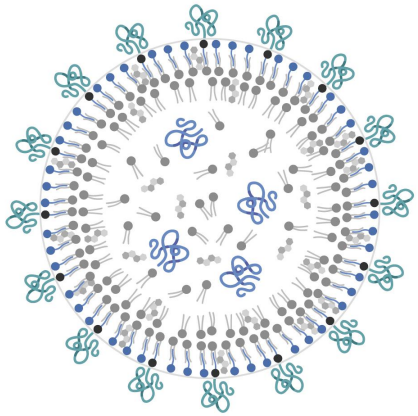
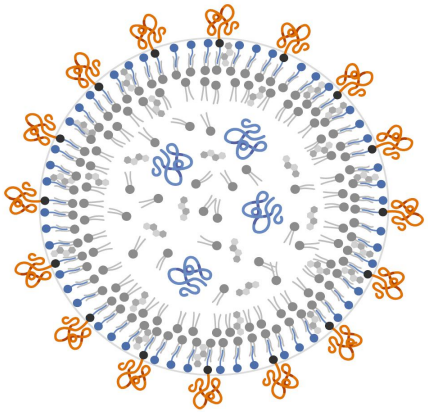
Anatomy of LNPs

What if replacing the PEG-lipid with a POZ-lipid resulted in something truly extraordinary ?



Ionizable lipid
Cholesterol
mRNA
Phospholipid
PEG-lipid



Ionizable lipid
Cholesterol
mRNA
Phospholipid
POZ-lipid

Balancing immunogenicity and reactogenicity

Review

KSBMB
Korean Society for Biochemistry and Molecular Biology

www.nature.com/emm

REVIEW ARTICLE OPEN

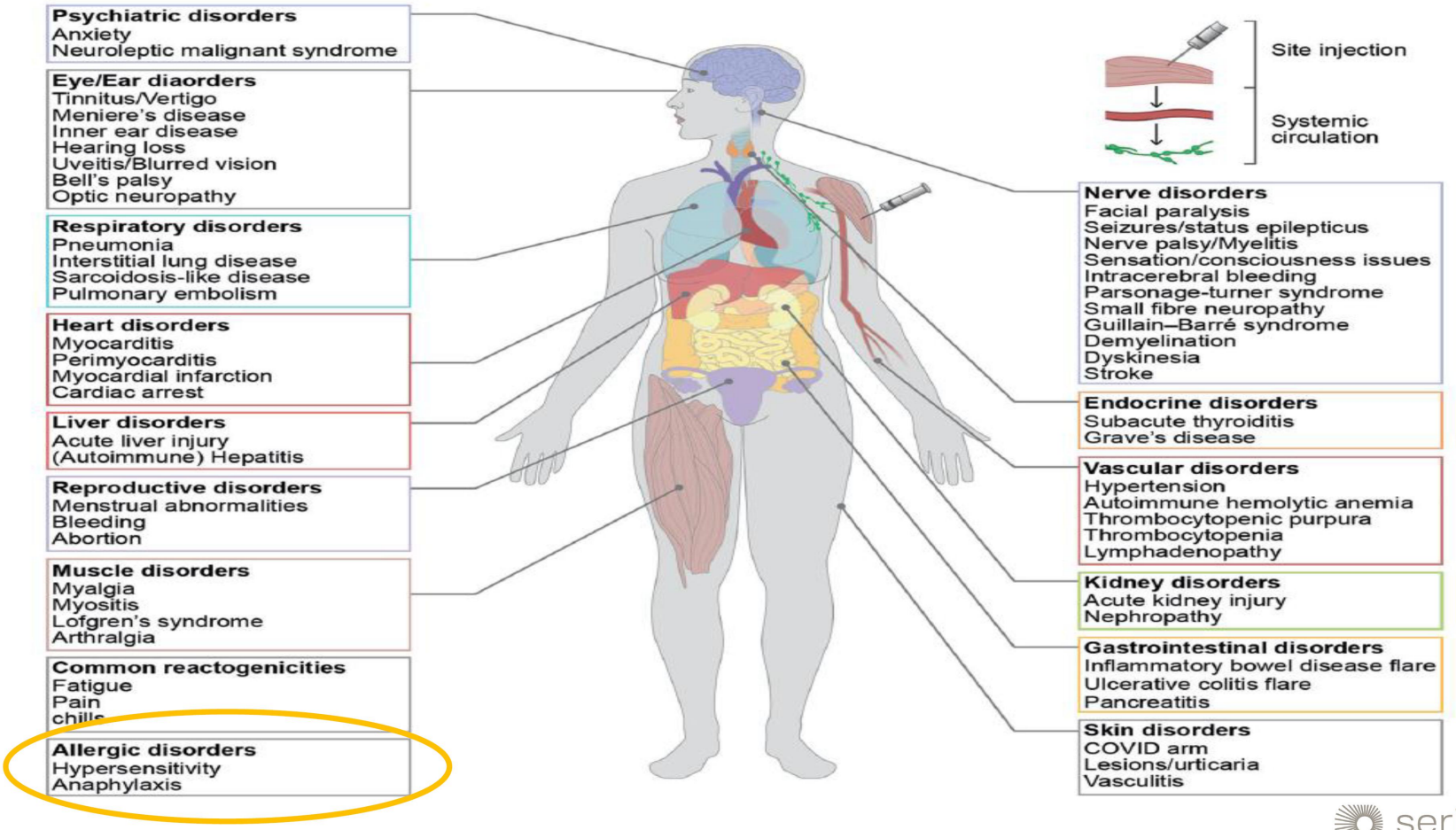
Check for updates

Knife's edge: Balancing immunogenicity and reactogenicity in mRNA vaccines

Jisun Lee[1,6], Matthew C. Woodruff[2,3,6], Eui Ho Kim[4]✉ and Jae-Hwan Nam[1,5]✉

Balancing immunogenicity and reactogenicity

List of adverse events reported with the mRNA vaccines



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In First Wave of Vaccinations Anaphylaxis Was Noted

Occurred primarily in women (~ 90%)

COVID-19

Pfizer's vaccine raises allergy concerns

Polymer in mRNA's "packaging" may cause rare anaphylactic reactions

By **Jop de Vrieze**

COVID-19 CORRESPONDENCE

Anaphylaxis to the first COVID-19 vaccine: is polyethylene glycol (PEG) the culprit?

Lene H. Garvey[1,2,*] and Shuaib Nasser[3]

[1]Allergy Clinic, Department of Dermatology and Allergy, Copenhagen University Hospital, Gentofte, Denmark, [2]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark and [3]Department of Allergy, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

Original Investigation | Allergy

Assessment of Allergic and Anaphylactic Reactions to mRNA COVID-19 Vaccines With Confirmatory Testing in a US Regional Health System

Christopher Michael Warren, PhD; Theo Thomas Snow, BS; Alexandra S. Lee; Mihir Mukesh Shah; Anja Heider, MS; Andra Blomkalns, MD; Brooke Betts, PharmD; Anthony S. Buzzanco, BS; Joseph Gonzalez, BS; R. Sharon Chinthrajah, MD; Evan Do, BS; Iris Chang, BS; Diane Dunham, BS; Grace Lee, MD; Ruth O'Hara, MD, PhD; Helen Park, PharmD; Mohamed H. Shamji, PhD; Lisa Schilling, RN, MPH; Sayantani B. Sindher, MD; Deepak Sisodiya, PharmD; Eric Smith, BS; Mindy Tsai, DMSc; Stephen J. Galli, MD; Cezmi Akdis, MD, PhD; Kari C. Nadeau, MD, PhD

In A Consecutive Cohort Study in Japan of Pfizer/BioNTech Vaccine

Occurred primarily in women (~ 90%)

Letter to the Editor

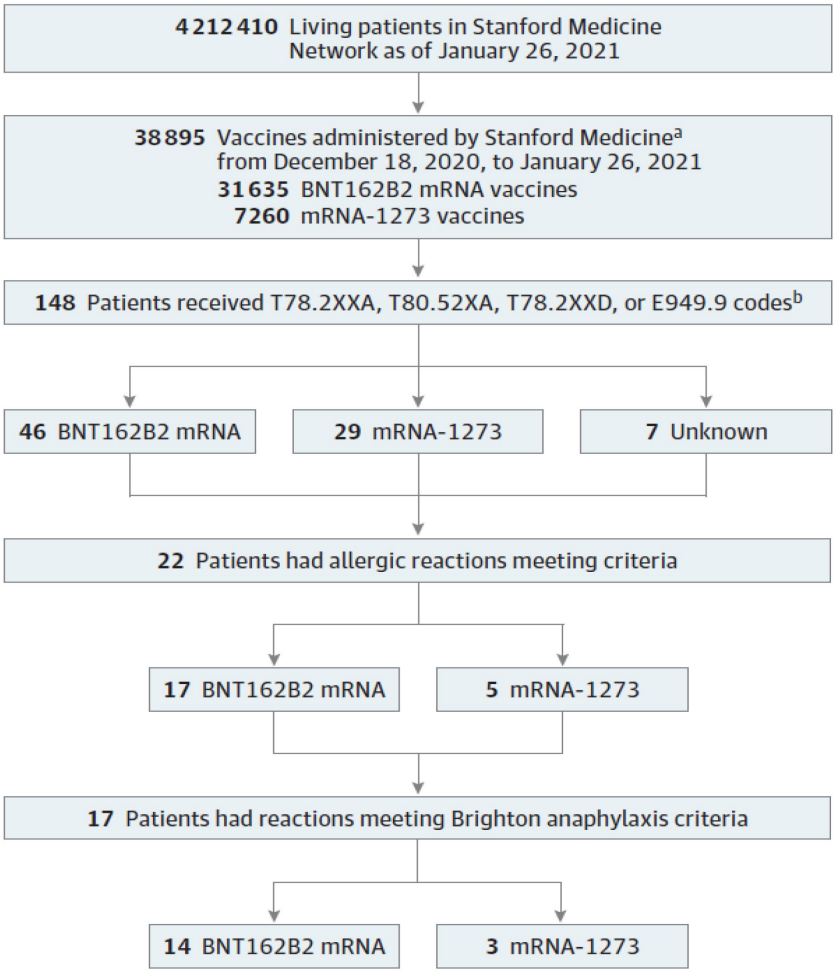
Sex differences in the incidence of anaphylaxis to LNP-mRNA COVID-19 vaccines

The incidence rate of anaphylaxis was 1:13,882

On February 17, 2021, Japan started vaccinating healthcare workers with the Pfizer-BioNTech lipid nanoparticle (LNP)-mRNA COVID-19 vaccine. Among total 79 anaphylaxis cases, 70 cases have been reported in women (89.9%) after 1,096,698 doses of the vaccine until April 4, 2021 [1]. Since the initiation of

[1] Adverse event report for COVID-19 vaccine in Japan. Accessed April 12, 2020. https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/vaccine_hukuhannou-utagai-houkoku.html.

In A Consecutive Cohort Study in Stanford Medical Center Network



Assessment of Allergic and Anaphylactic Reactions to mRNA COVID-19 Vaccines With Confirmatory Testing in a US Regional Health System. *JAMA Network Open.* 2021;4(9):e2125524.

The incidence rate of anaphylaxis meeting Brighton anaphylaxis criteria was 1:2,287

(the incidence of anaphylaxis with flu vaccine is ~ 1:2,000,000)

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Is PEG the culprit ? Yes.

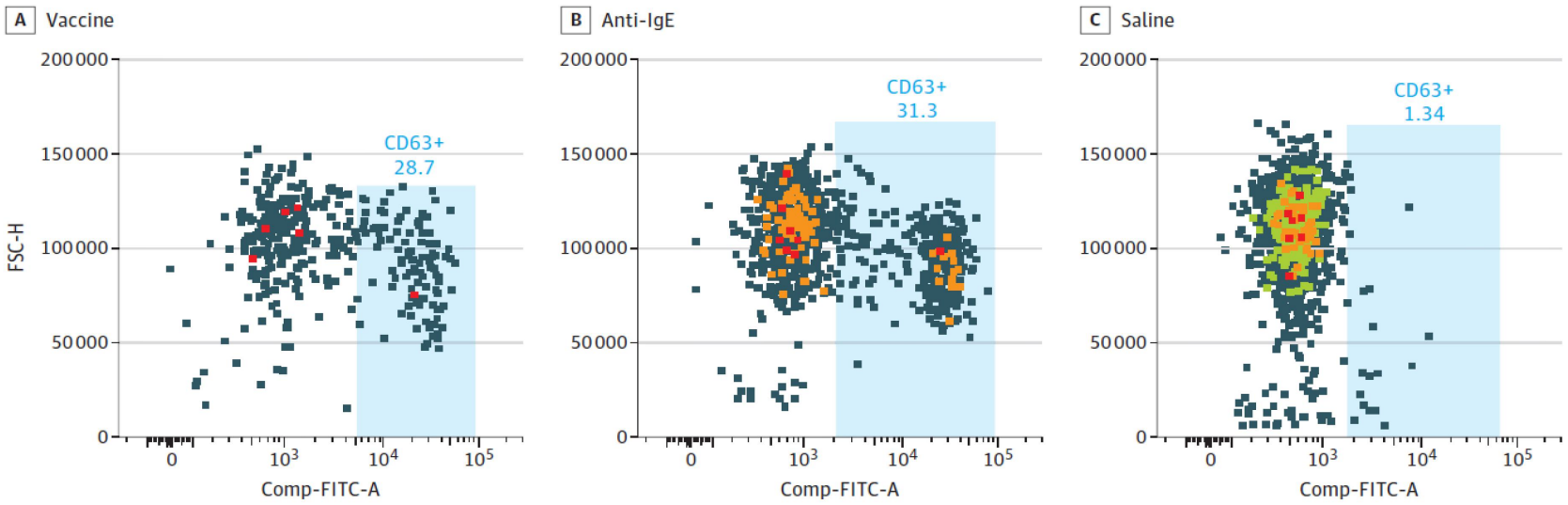
MAIN OUTCOMES AND MEASURES Allergic reactions were graded using standard definitions, including Brighton criteria. Skin prick testing was conducted to polyethylene glycol (PEG) and polysorbate 80 (P80). Histamine (1 mg/mL) and filtered saline (negative control) were used for internal validation. Basophil activation testing after stimulation for 30 minutes at 37 °C was also conducted. Concentrations of immunoglobulin (Ig) G and IgE antibodies to PEG were obtained to determine possible mechanisms.

RESULTS Of 22 patients (20 [91%] women; mean [SD] age, 40.9 [10.3] years; 15 [68%] with clinical allergy history), 17 (77%) met Brighton anaphylaxis criteria. All reactions fully resolved. Of patients who underwent skin prick tests, 0 of 11 tested positive to PEG, 0 of 11 tested positive to P80, and 1 of 10 (10%) tested positive to the same brand of mRNA vaccine used to vaccinate that individual. Among these same participants, 10 of 11 (91%) had positive basophil activation test results to PEG and 11 of 11 (100%) had positive basophil activation test results to their administered mRNA vaccine. No PEG IgE was detected; instead, PEG IgG was found in tested individuals who had an allergy to the vaccine.

10 of 11 patients had positive basophil activation tests to PEG

11 of 11 patients had positive basophil activation tests to the administered vaccine

Figure 2. Basophil Activation Testing (BAT) Assay on Example Participant Using Vaccine, Anti-Immunoglobulin E (IgE), and Saline



BAT assay on example participant with allergic reaction to the vaccine. Color indicates intensity of forward scatter and gated cells, with red being greater than orange; orange greater than green, and green greater than blue. FSC-H indicates forward side scatter-height; Comp-FITC-A, compensation–fluorescein isothiocyanate–area.

BAT assays revealed that patients with anaphylaxis gated activated CD63+ basophils in the presence of vaccine (A), PEG (not shown) - but not saline (C)

The Anti-IgE panel (B) is the positive control

Basophil degranulation releases PAF, one of the most potent anaphylotoxins known

Does vaccination induce high titers of anti-PEG antibodies ?

Review of recent literature

Anti-PEG Antibodies Boosted in Humans by SARS-CoV-2 Lipid Nanoparticle mRNA Vaccine

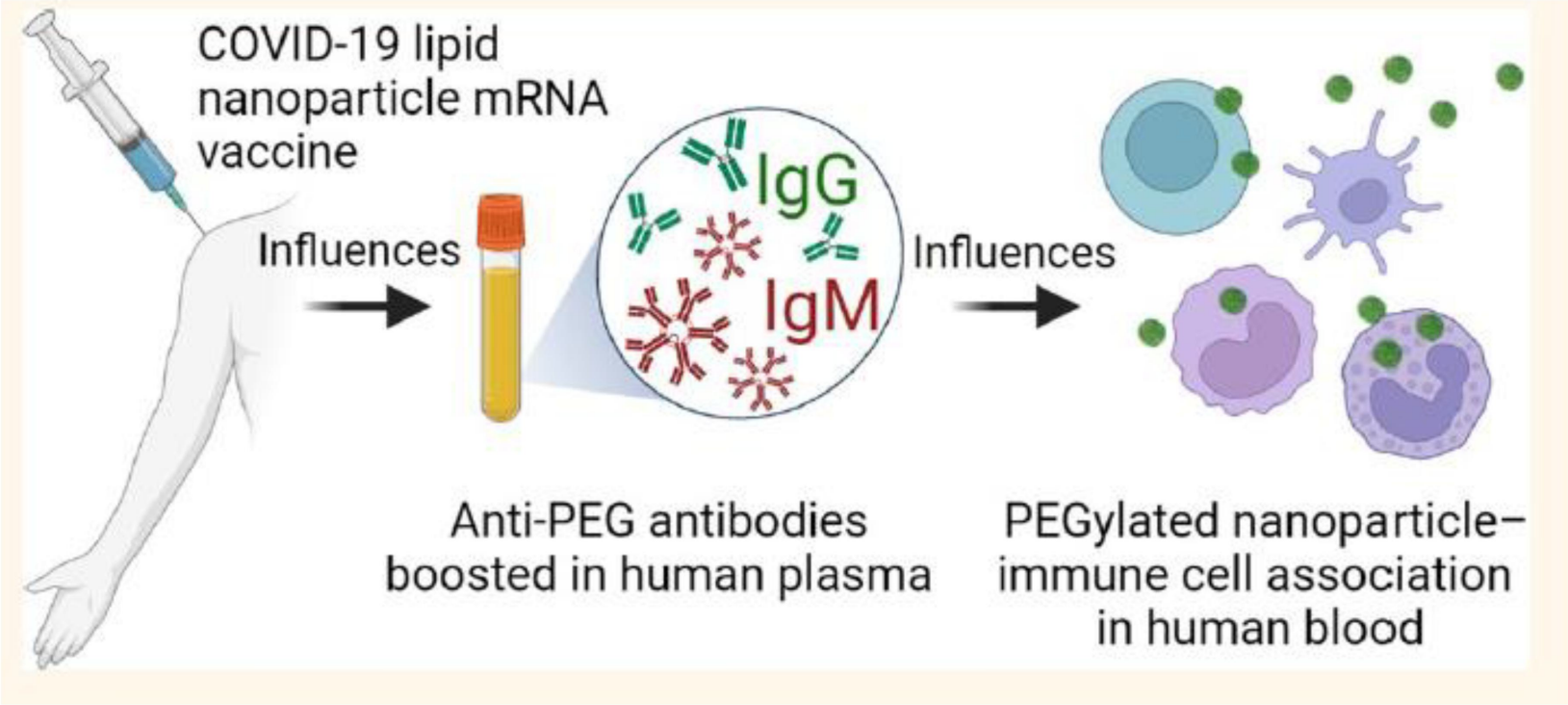
Yi Ju,* Wen Shi Lee, Emily H. Pilkington, Hannah G. Kelly, Shiyao Li, Kevin J. Selva, Kathleen M. Wragg, Kanta Subbarao, Thi H. O. Nguyen, Louise C. Rowntree, Lilith F. Allen, Katherine Bond, Deborah A. Williamson, Nghia P. Truong, Magdalena Plebanski, Katherine Kedzierska, Siddhartha Mahanty, Amy W. Chung, Frank Caruso, Adam K. Wheatley, Jennifer A. Juno, and Stephen J. Kent*

Cite This: *ACS Nano* 2022, 16, 11769–11780

Read Online

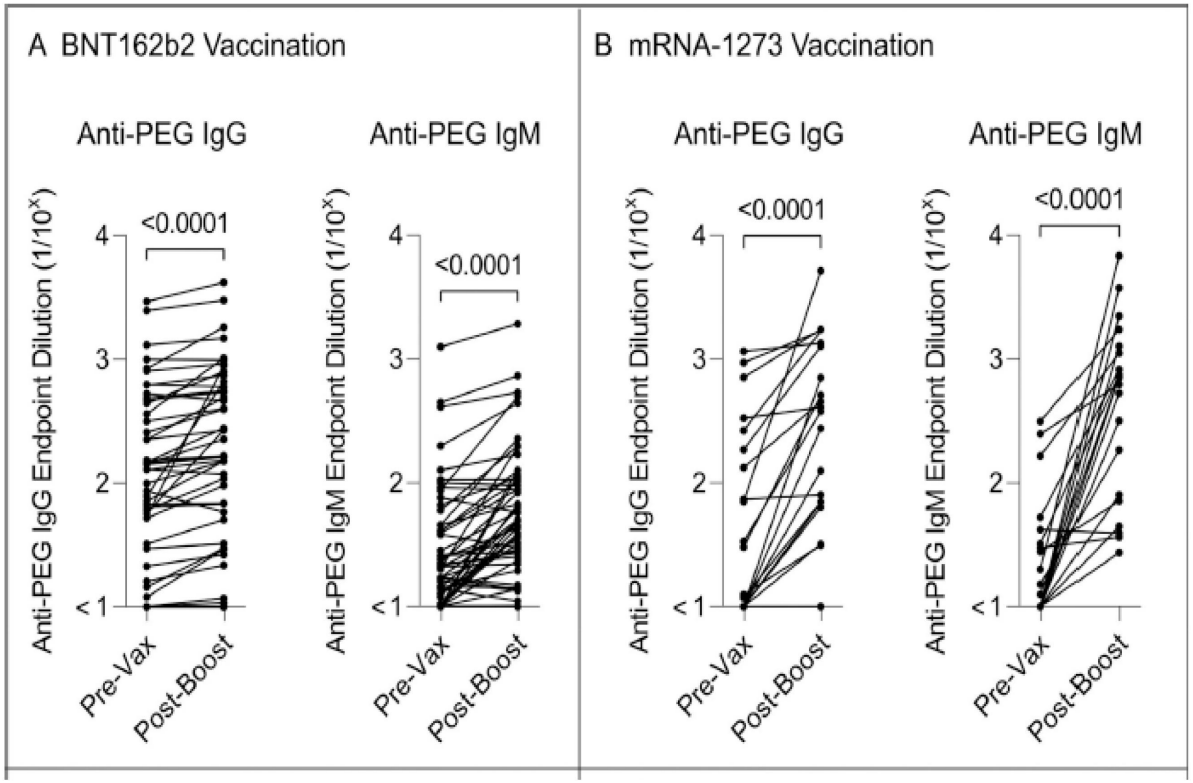
Does vaccination induce high titers of anti-PEG antibodies ?

Is this a proximate cause of reactogenicity ?



Does vaccination induce high titers of anti-PEG antibodies ?

Absolutely.



Anti-PEG Abs were present in 71% of patients prior to vaccination

Moderna vaccination induced a much higher titer of both IgG and IgM post-boost than the Pfizer vaccination

Moderna mean-fold change in titers were 13.1 (IgG) and 68.5 (IgM)

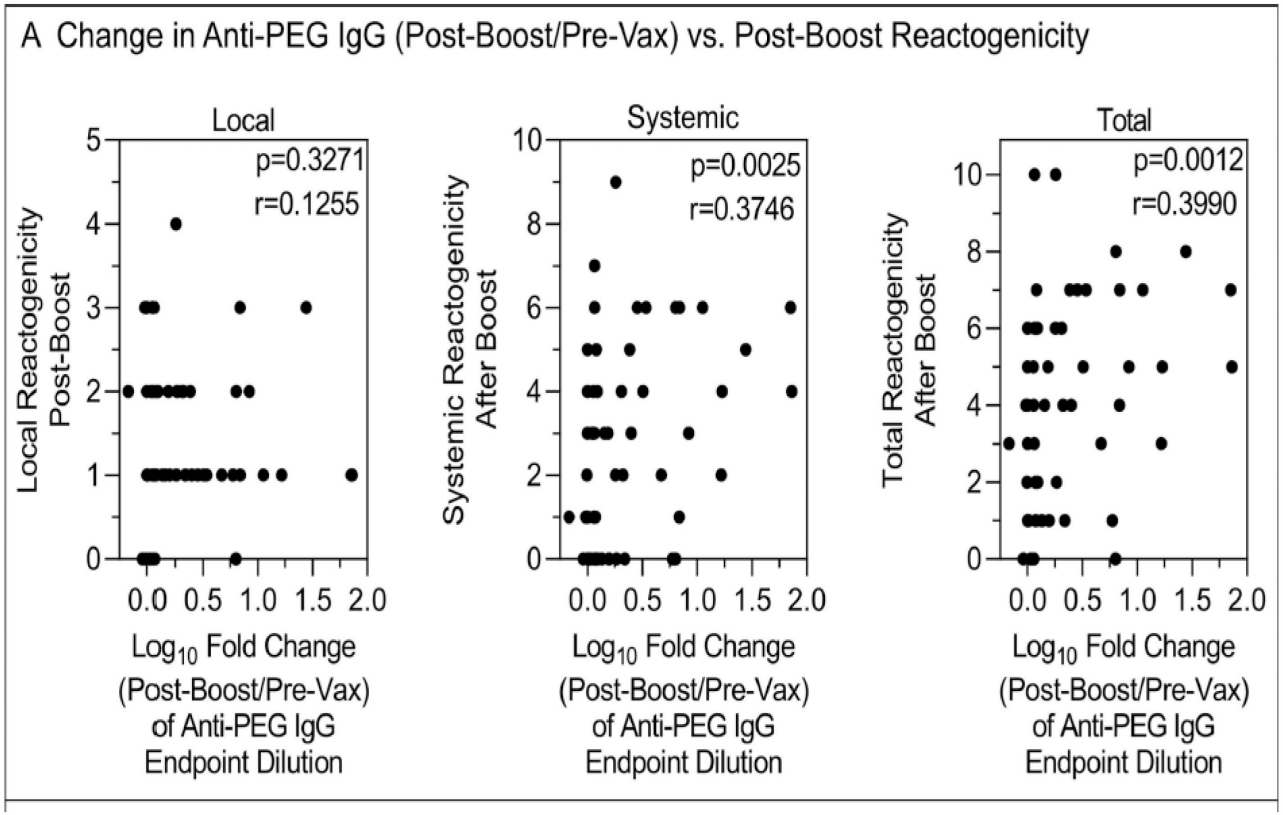
Pfizer mean-fold change in titers were 1.78 (IgG) and 2.64 (IgM)

PEG-dmg vs PEG-dma: 100 ug vs 30 ug dosing

*Note – both PEG-lipids in the vaccines have a methoxy-PEG at the terminus. Note that despite other literature to the contrary, this results in binding of C1q to antibodies boosted by the Moderna vaccine.

Does vaccination lead to higher rates of reactogenicity ?

Local (injection site) vs systemic (overall symptoms) as a function of Log_{10} titer IgG



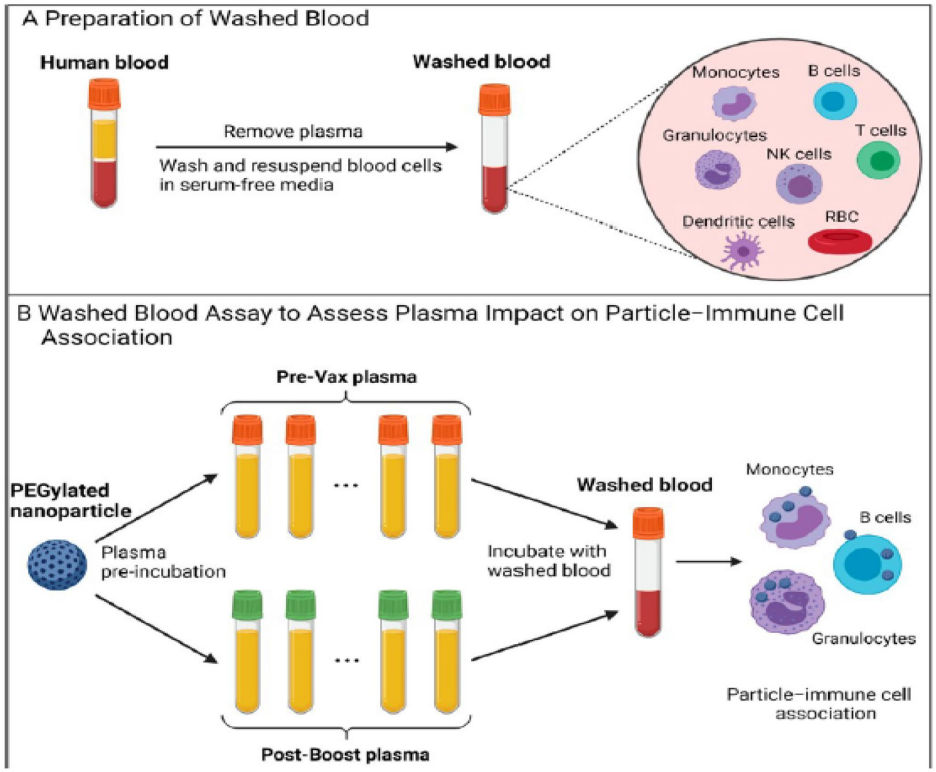
Local vs Systemic reactogenicity scores as a function of anti-PEG IgG

Higher titers of IgG post-boost correlated with higher rates of systemic reactogenicity

Does vaccination lead to binding of nanoparticles to immune cells ?

Given high titers of IgG and IgM – does this result in binding of PEG-containing nanoparticles by immune cells ?

Scheme 1. Schematic Illustration of Human Blood Assay to Assess the Impact of Plasma on PEGylated Nanoparticle Association with Human Immune Cells[a]

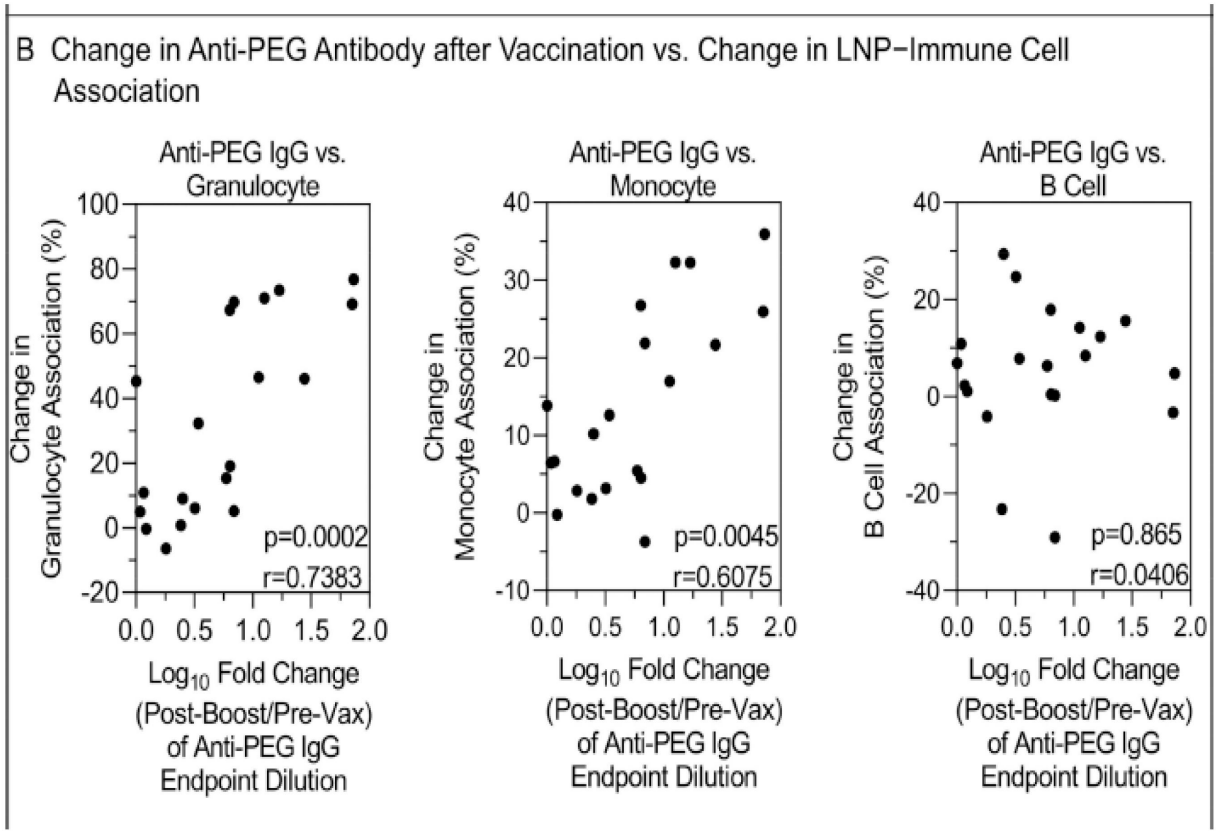


The higher titers of anti-PEG IgG and IgM induced by Moderna vaccination may lead to association of PEG-containing nanoparticles to immune cells in the plasma

The authors assessed whether the IgG / IgM binding to immune cells would lead to binding of Onpattro

Does vaccination lead to binding of nanoparticles to immune cells ?

Given high titers of IgG and IgM – does this result in binding of PEG-containing nanoparticles by immune cells ?

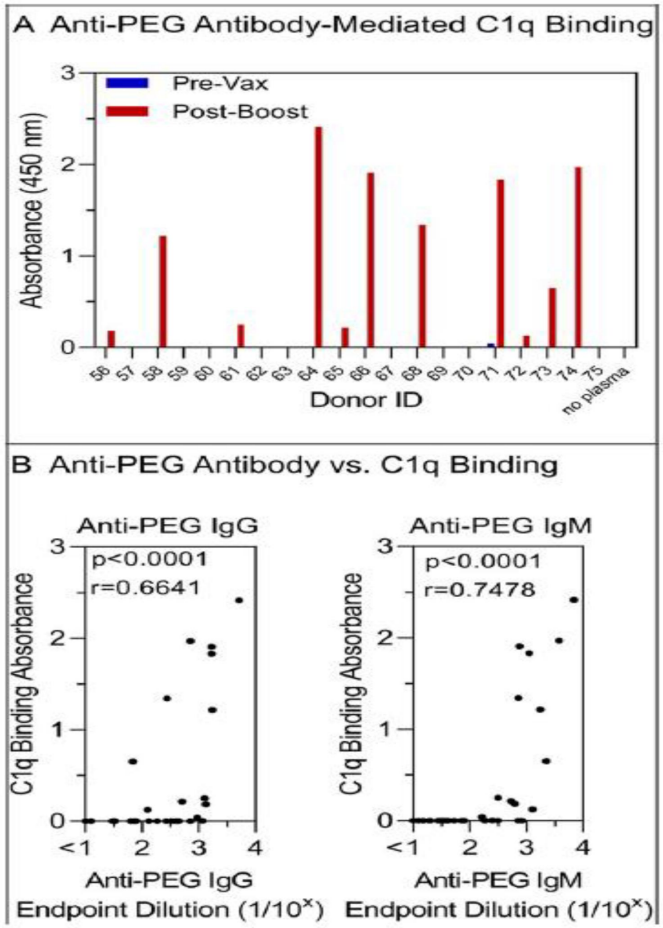


The higher titers of anti-PEG IgG induced by Moderna vaccination lead to association of Onpattro nanoparticles to the granulocyte and monocyte populations of immune cells in the plasma (Onpattro is a PEG-dmg lipid LNP)

Degranulation of granulocytes (basophils) can occur in the presence of antigen when bound by IgG on the surface of the cells

Does vaccination lead to complement activation ?

Moderna vaccination resulted in binding of C1q by anti-PEG IgG and IgM



The higher titers of anti-PEG IgG and IgM induced by Moderna vaccination (post boost) led to C1q binding

Binding of C1q may lead to complement opsonization and activation of complement pathways

Does vaccination lead to complement activation ?

It is clear that the anti-PEG antibodies bind complement … but do they compromise the LNP (study 2022) ?

Anti-PEG antibodies compromise the integrity of PEGylated lipid-based nanoparticles *via* complement

Mariona Estapé Senti [a,b,g,1], Caroline A. de Jongh [a,b,1], Kim Dijkxhoorn [a], Johan J.F. Verhoef [b], Janos Szebeni [c,d,e], Gert Storm [b,f], C. Erik Hack [a], Raymond M. Schiffelers [g], Marcel H. Fens [b,1,*], Peter Boross [a,1]

In this study, we investigated the consequences and mechanisms of complement activation by anti-PEG antibodies interacting with different types of PEGylated lipid-based nanoparticles. By using both liposomes loaded with different (model) drugs and LNPs loaded with mRNA, we demonstrate that complement activation triggered by anti-PEG antibodies can compromise the bilayer/surface integrity, leading to premature drug release or exposure of their mRNA contents to serum proteins. Anti-PEG antibodies also can induce deposition of complement fragments onto the surface of PEGylated lipid-based nanoparticles and induce the release of fluid phase complement activation products.

Substitution of PEG-dmg with PEOZ-dmg

Studies performed in collaboration with the James Dahlman Laboratory (2021-2023)

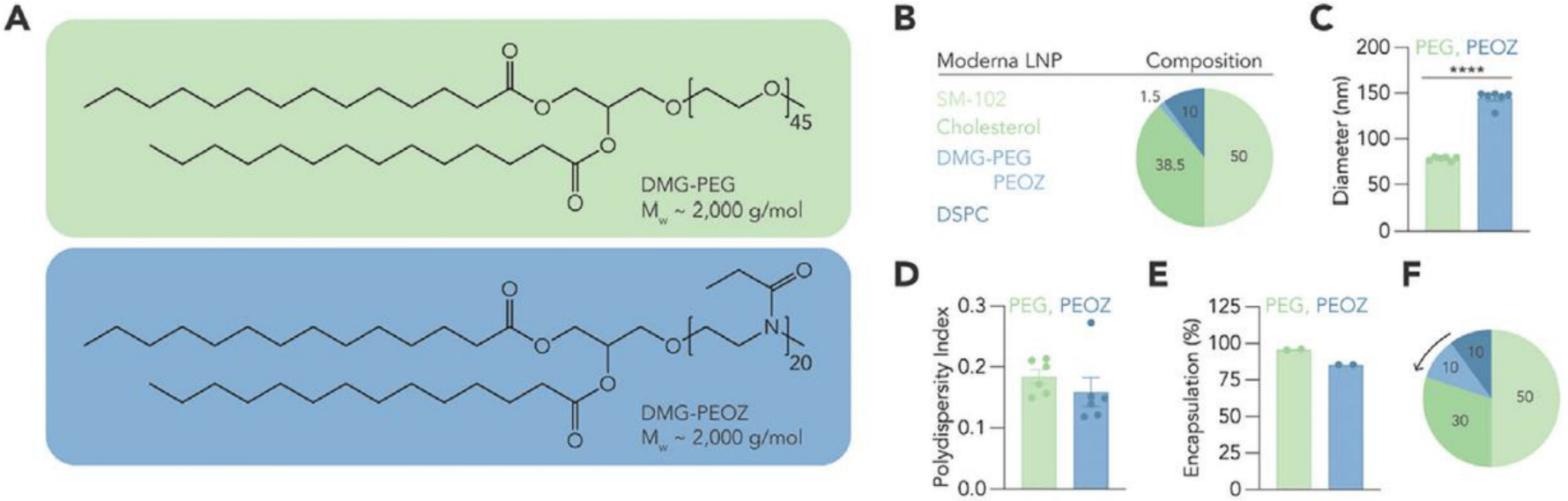
Substituting Poly(ethylene glycol) Lipids with Poly(2-ethyl-2-oxazoline) Lipids Improves Lipid Nanoparticle Repeat Dosing

*Alejandro J. Da Silva Sanchez, David Loughrey, Elisa Schrader Echeverri, Sebastian G. Huayamares, Afsane Radmand, Kalina Paunovska, Marine Hatit, Karen E. Tiegreen, Philip J. Santangelo, and James E. Dahlman**

Advanced Healthcare Materials (2024)

The Accelerated Blood Clearance (ABC) Phenomenon

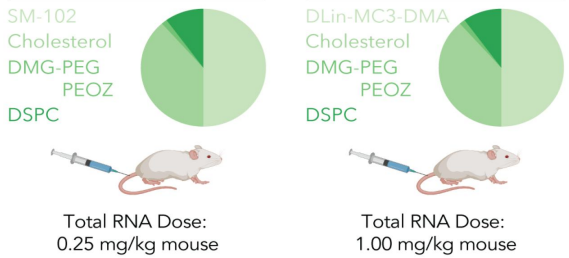
Studies performed in collaboration with the Dahlman Laboratory (2021-2023)
Biophysical properties (size, polydispersity, EE) are similar – but not identical



POZ-lipid LNPs Have Unique Properties – IV Infusion

LNPs which incorporate PEOZ-dmg selectively express the payload in macrophage (M) and dendritic (D) cells

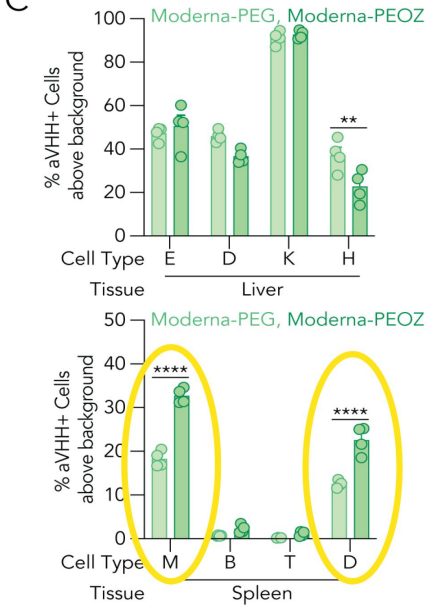
A



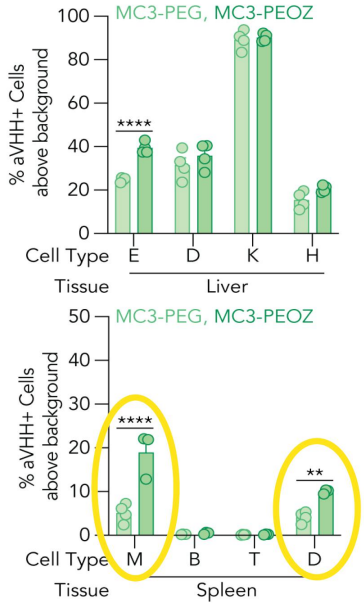
B

Cell Types
Endothelial (E): CD31+
Dendritic (D): CD45+, CD11c+
Macrophages (M): CD45+, CD11b+
Kupffer (K): CD45+, CD68+
B cells (B): CD19+
T cells (T): CD3+
Hepatocytes (H): CD31- / CD45-

C



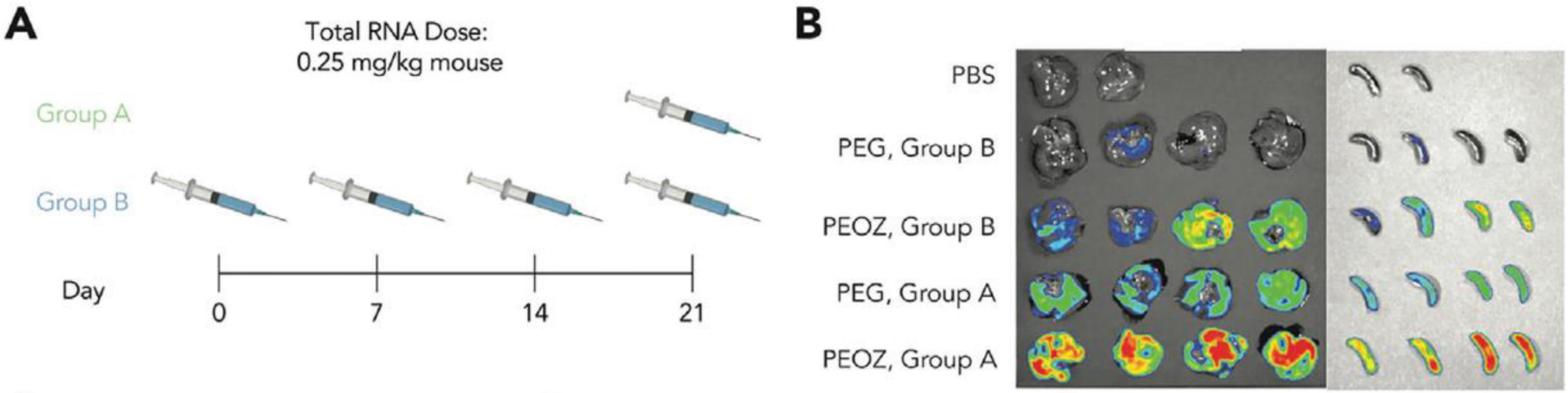
D



- In vivo results are *reproducible* and *generalizable* to other LNP formulations
  - The initial observation that a PEOZ-lipid LNP selectively targets antigen-presenting cells (APC) gave nearly identical results on repeat study (Panel C)
  - When you change the LNP formulation from Moderna's cocktail (SM 102, DSPC, PEG-dmg, cholesterol, mRNA) to Onpattro's formulation (MC 3, DSPC, PEG-dmg, cholesterol, siRNA) and compare it to PEOZ-dmg – selective targeting of APCs is still present (Panel D)
- Selective targeting of APCs *in vivo* with a POZ-lipid vs PEG-lipid LNP is a novel observation

The Accelerated Blood Clearance (ABC) Phenomenon

Studies performed in collaboration with the Dahlman Laboratory (2021-2023)
Weekly dosing of PEG-dmg vs 2K PEOZ-dmg with a luciferase payload



Weekly dosing of PEG-dmg LNPs results in ABC in mice (replicates Moderna lab data)
Weekly dosing of a PEOZ-dmg LNP also results in ABC (with production of an IgM directed to the 2K PEOZ-dmg) – but to a much lesser extent in both liver & spleen

Evaluation of Pfizer/BioNTech LNP in rat - PEG-dma

Rat immunogenicity study (The LNPs in this study employed ALC-0315 and ALC-0159 (Acuitas) at mol% for the published vaccine)

npj | vaccines

www.nature.com/npjvaccines

ARTICLE OPEN

Check for updates

Polyethylene glycol (PEG)-associated immune responses triggered by clinically relevant lipid nanoparticles in rats

Haiyang Wang[1,2,5], Yisha Wang[1,2,5], Changzheng Yuan[3,5], Xiao Xu[4], Wenbin Zhou[1,2], Yuhui Huang[3], Huan Lu[1,2], Yue Zheng[1,2], Gan Luo[1,2], Jia Shang[4] and Meihua Sui[1,2]✉

Nature Vaccines (2024)

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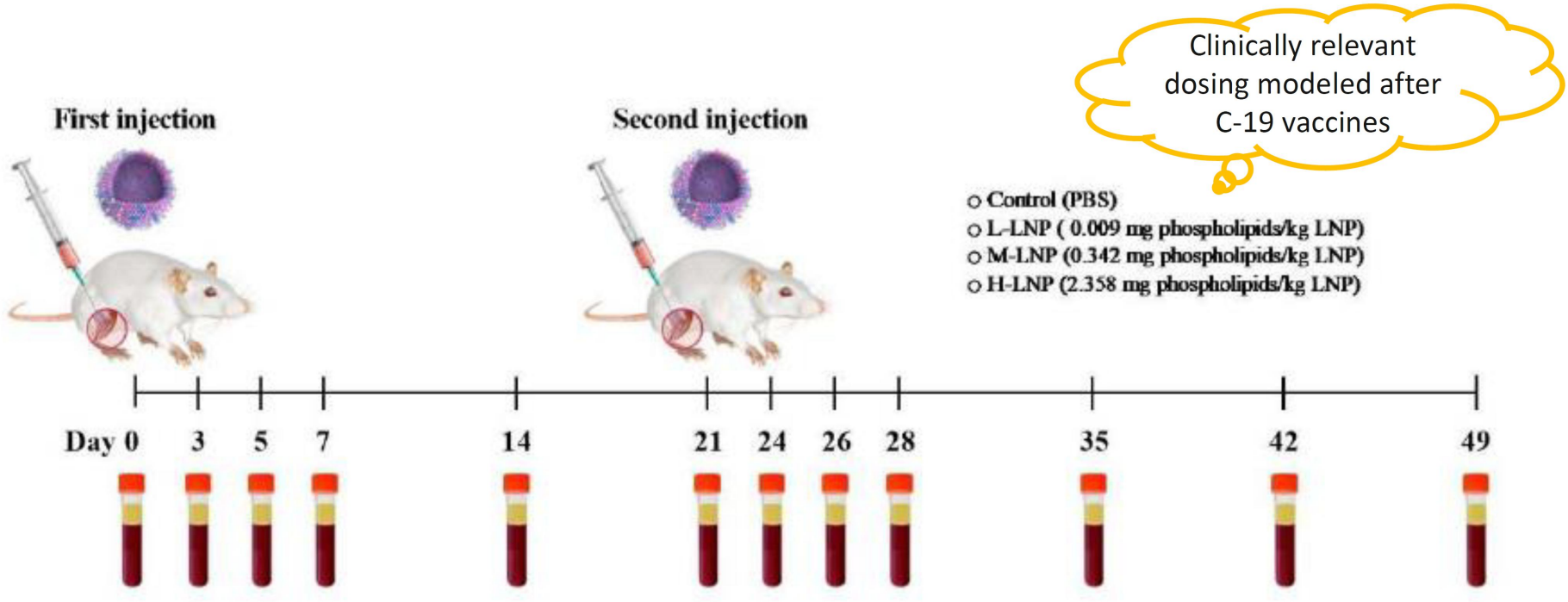
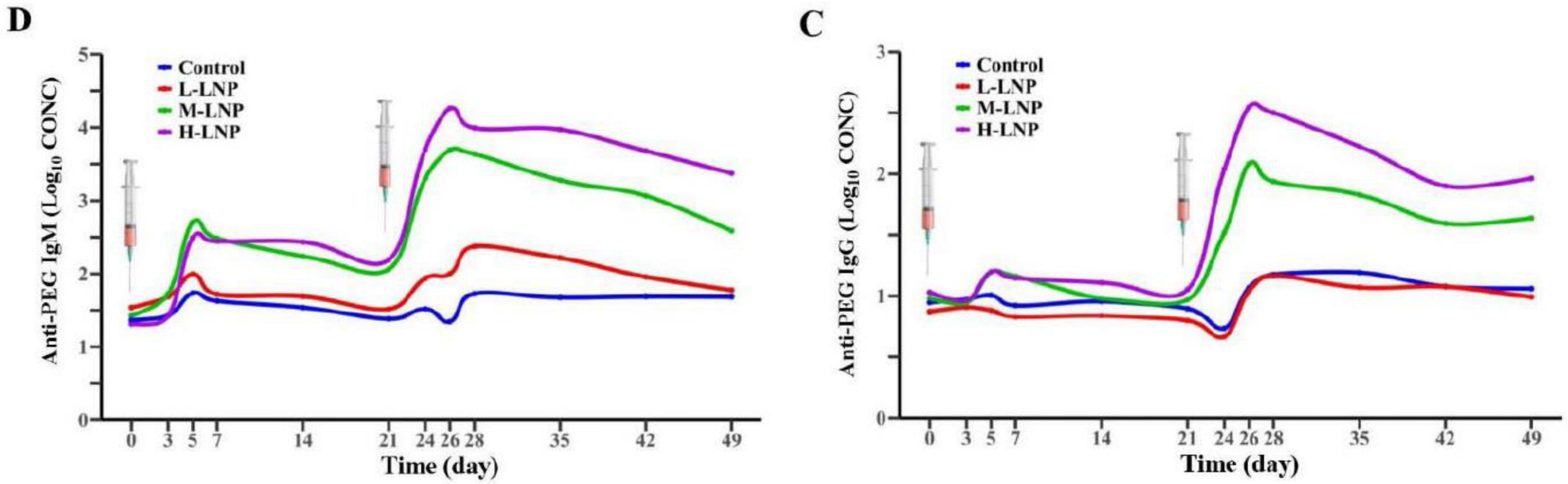
Does vaccination with clinically relevant LNPs lead to anti-PEG Abs

Rat immunogenicity study (Pfizer/BioNTech vaccine formulation)



Anti-PEG antibody induction in rat

Boosted anti-PEG antibodies - IgM and IgG are both boosted (similar to human data)

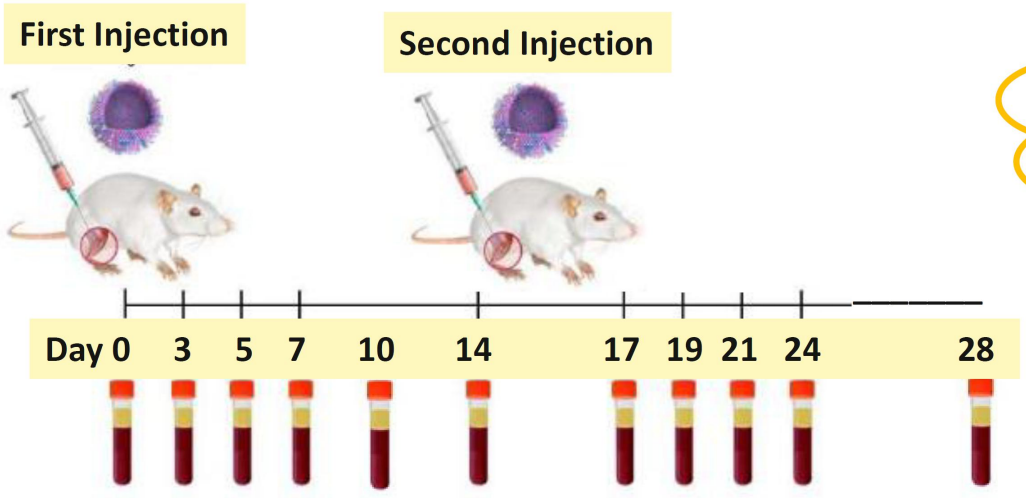


Both anti-PEG IgM and IgG antibodies are induced in the rat following vaccination of relevant doses of LNPs
Low levels of antibodies are produced by the Low Dose
Higher levels of anti-PEG antibodies are induced – and boosted – by the Mid Dose and High Dose

The boosted levels of IgM (~1.8) and IgG (~2.5) are remarkably consistent with the human data

Does vaccination with PEOZ-dma vs PEG-dma lead to different anti-PEG response

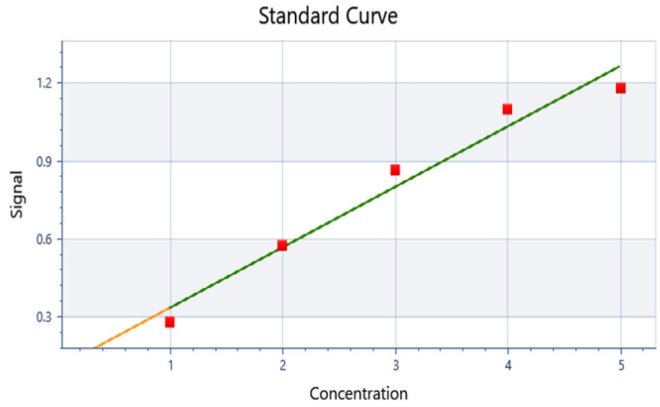
Serina study design to evaluate PEOZ-dma and PEG-dma



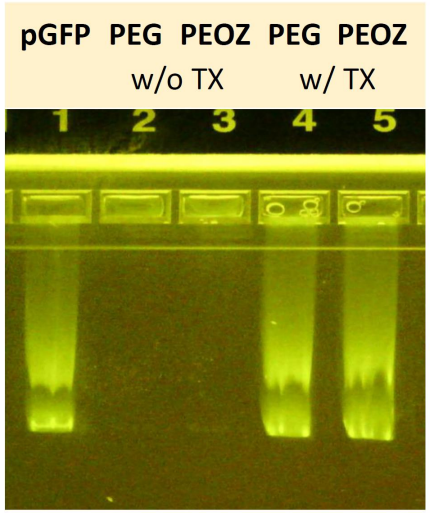
The High Dose – 2.0 mg phospholipid / kg body weight
The Mid Dose – 0.2 mg phospholipid / kg body weight

This study design dosed rats with two injections (two doses – mid & high) at a 14 day interval
The LNPs contained 1.6 mol% of the following polymer-lipids (a) PEOZ-dma **or** (b) PEG-dma (ALC-0159) with a DNA payload (GFP) that was evaluated in vitro for LNP biophysical properties (size, PDI, EE, transfection efficiency, osmolality)
Blood samples were taken at the indicated time points for CBC (day 0, 14 & 28), LFTs (day 0, 14 & 28) and ELISA determinations (all other days)

Phospholipid content, Particle size, Polydispersity, Zeta Potential & Encapsulation efficiency

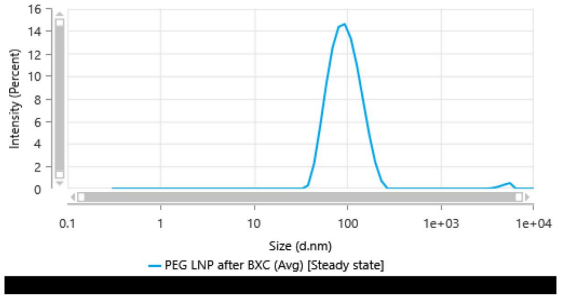


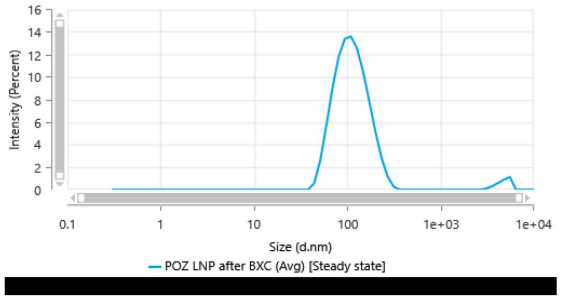
Phospholipid Standards



After Buffer Exchange in Tris-HCl, pH 7.1

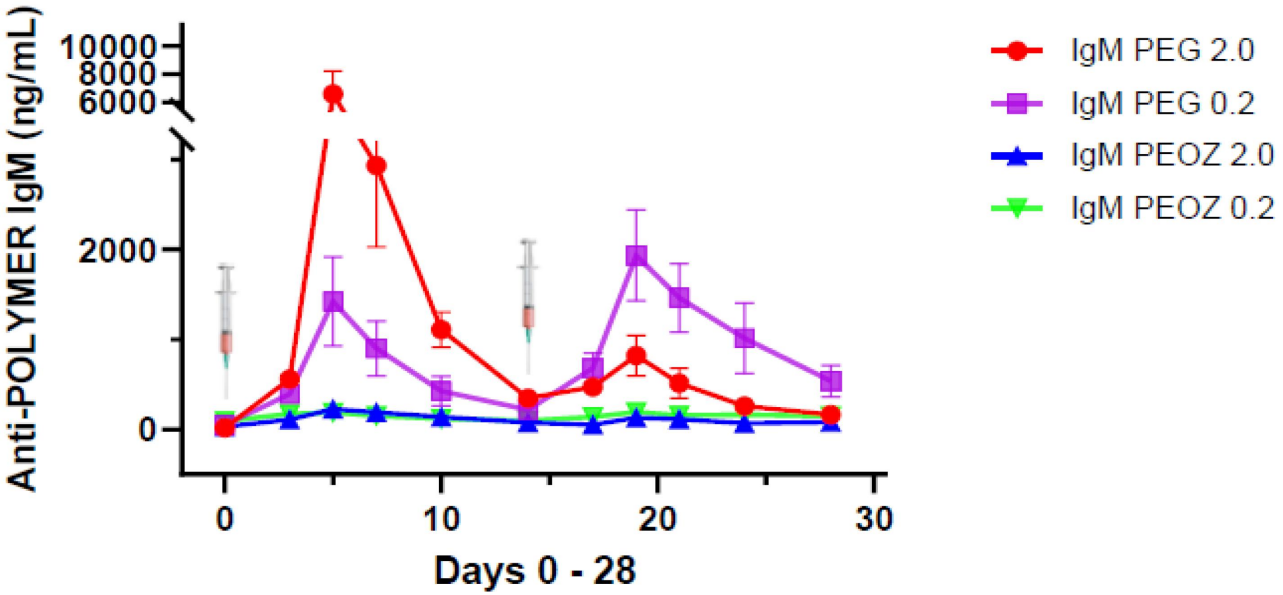
Sample Name	Z-Average (nm)	Polydispersity Index (PI)	Zeta Potential (mV)	Encapsulation Efficiency
PEG LNP after BXC (Avg)	88.26	0.1592	-16.43	>98%
PEOZ LNP after BXC (Avg)	106.3	0.2099	-10.97	>98%





Anti-IgM antibodies to PEG-dma vs PEOZ-dma

Serina LNP Laboratory

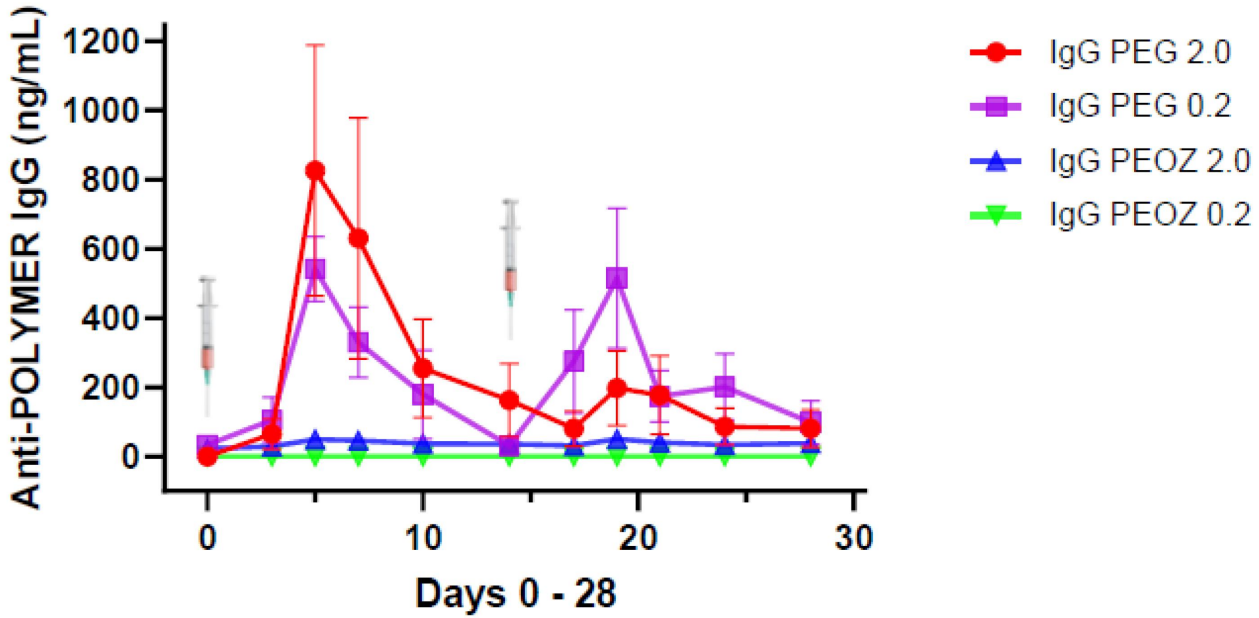


Anti-IgM antibodies are readily detected, and boosted, at the High Dose and Mid Dose when LNPs employing PEG-dma are injected (similar to published data)

LNPs prepared with PEOZ-dma failed to elicit an IgM response at either the High Dose or Mid Dose

Anti-IgG antibodies to PEG-dma vs PEOZ-dma

Serina LNP Laboratorv



Anti-IgG antibodies are readily detected at both the High Dose and Mid Dose when LNPs employing PEG-dma are injected (similar to published data)

LNPs prepared with PEOZ-dma failed to elicit an IgG response at either the High Dose or Mid Dose

Today's Messages:

- Anaphylaxis to the vaccines appears to be due to basophil degranulation, likely the result of high titer IgG (possibly IgM) to the PEG in the formulation
  - Now recognized as an uncommon mechanism of anaphylaxis, first described clinically ~ 15 years ago
- The high titers of IgM & IgG are associated with an increased incidence of reactogenicity (possibly other AEs)
- The Serina LNP Laboratory has identified PEOZ-dma as a component for LNP formulations that is virtually identical in biophysical properties to the PEG-dma LNP (Pfizer/BioNTech formulation)
- **PEOZ-dma LNPs fail to elicit an IgM or IgG immune response on repeat dosing**

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Thank You

Balancing immunogenicity and reactogenicity

Serina's technology is poised to advance v2.0 & v3.0 vaccines