mRNA technologies: what do we know and not know?

WHO/MPP mRNA meeting, 17-21 April,
Cape Town, RSA

Drew Weissman, MD, PhD
University of Pennsylvania
Philadelphia, USA

Perelman School of Medicine
University of Pennsylvania

Conflicts of Interest

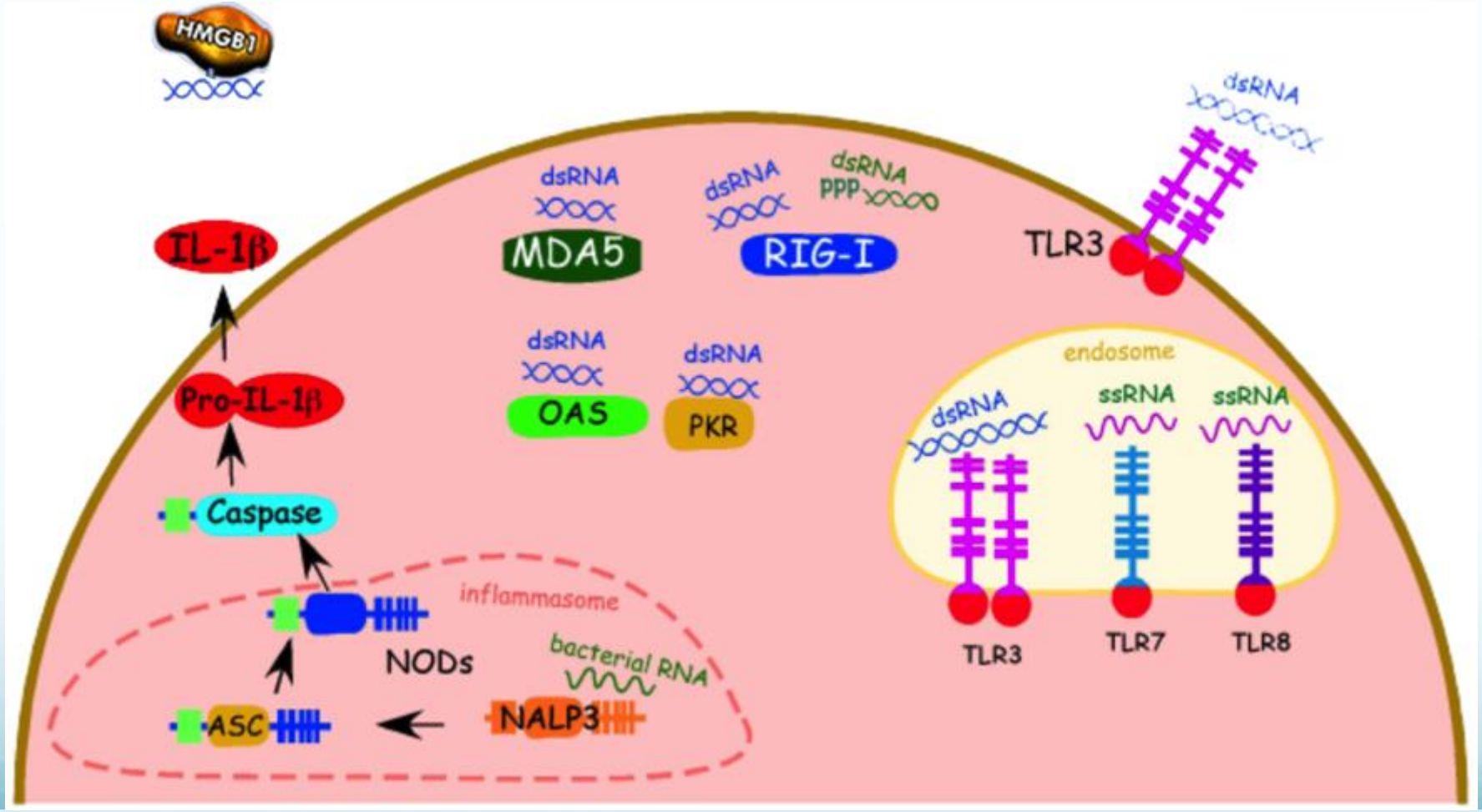
- Dr. Weissman has been issued multiple patents and has more in the process of submission covering nucleoside modified mRNA as a therapeutic, mRNA-LNP vaccines, modified mRNA delivery of cas9 gene editing systems, LNP delivery systems and other therapeutic applications of modified mRNA and LNPs.
- I am part of multiple companies developing LNPs, targeted LNPs and mRNA therapeutics.

All data being presented are confidential, please do not share or discuss with others not present at the talk.

Therapeutic mRNA background

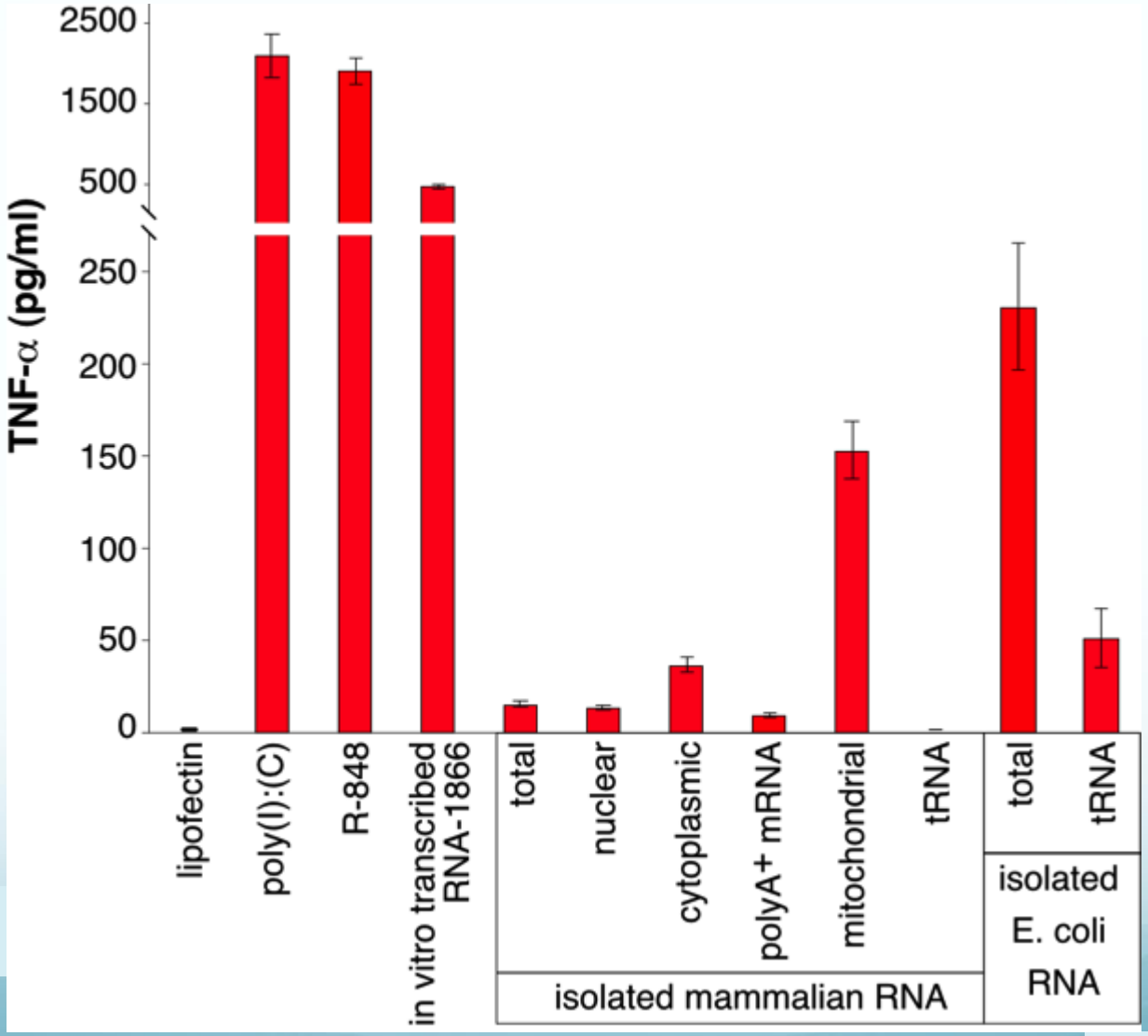
- mRNA and DNA encoding a protein were first injected into an animal in 1990. Since then, a single report of therapeutic mRNA injection into the brain was made in 1992, until recently.
- mRNA was studied as a vaccine with both ex vivo dendritic cell pulsing and in vivo injection.
- The reason why RNA was not studied is due to its complex activation of many innate immune sensors.

Intra-and extracellular mammalian RNA sensors



IFIT-2, DDX60, DHX9, DDX3, the DDX1-DDX21-DHX36 complex, RNaseL, and LRRFIP1

Natural RNAs are not equally potent activators (immunogenicity) of dendritic cells



Monocyte-derived DC (GM-CSF + IL-4)

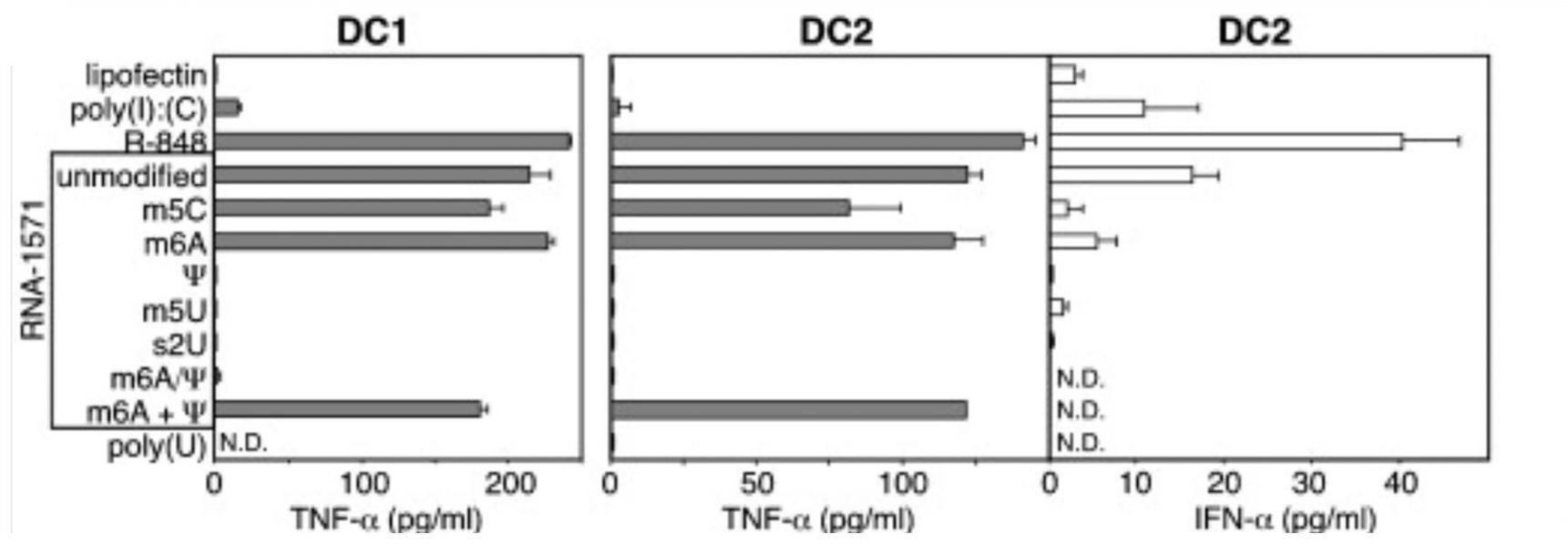
Immunity 2005, 23: 165

Natural modification of RNA alters immunogenic potential.

- synthesis - basic nucleotides: ATP, CTP, GTP, UTP
- >100 different types of modifications - RNA maturation
  - isomerization
    - uridine → pseudouridine (Ψ)
  - methylation
    - ribose 2'-OH
      - Um, Am, Cm, Gm
    - bases
      - m5C, m5U, m6A, m7G,
- conserved positions
- biological function (?)
- reversible, m6A in mRNA

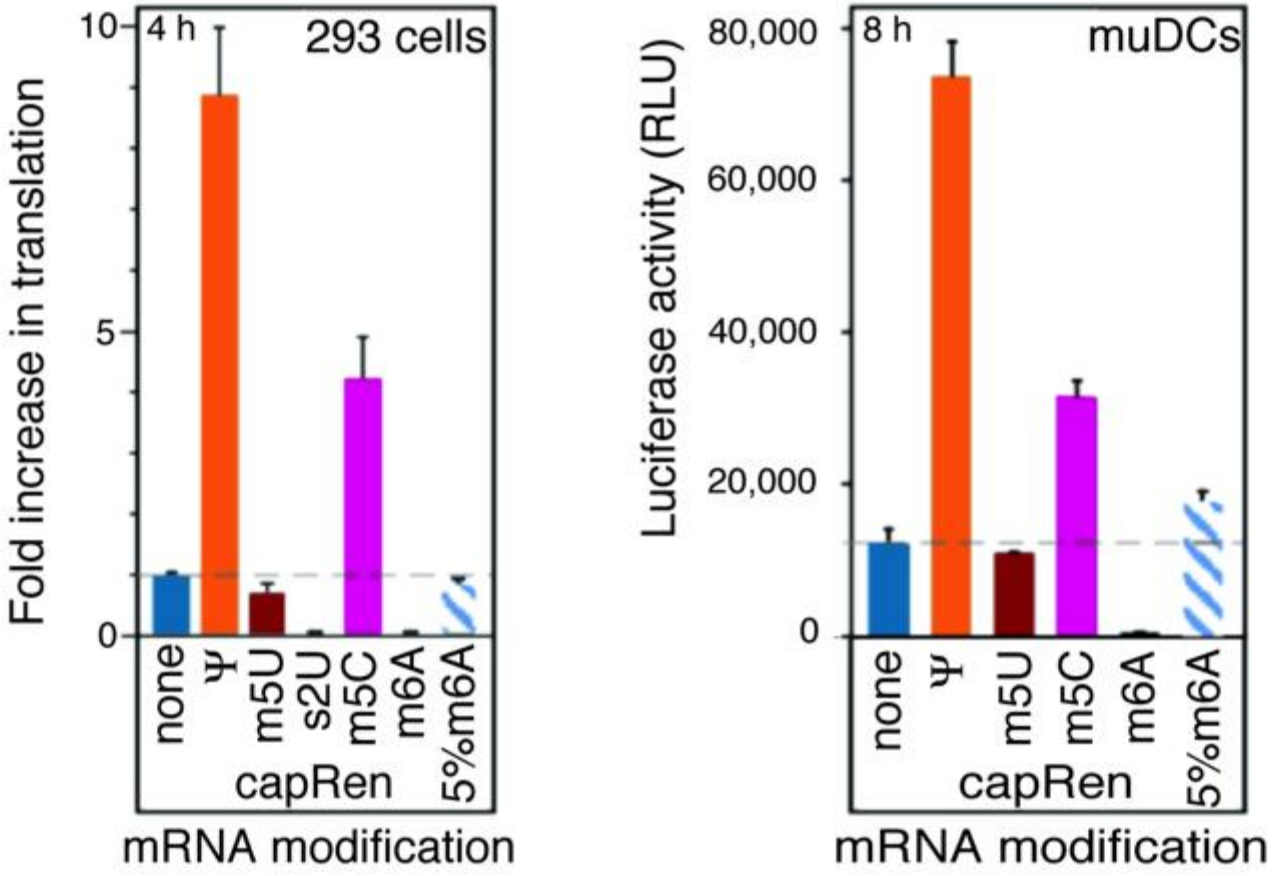
In vitro transcription

Nucleoside modification reduces the immunogenicity of RNA.



Dendritic cells purified from peripheral blood

Nucleoside-modified mRNA is translatable



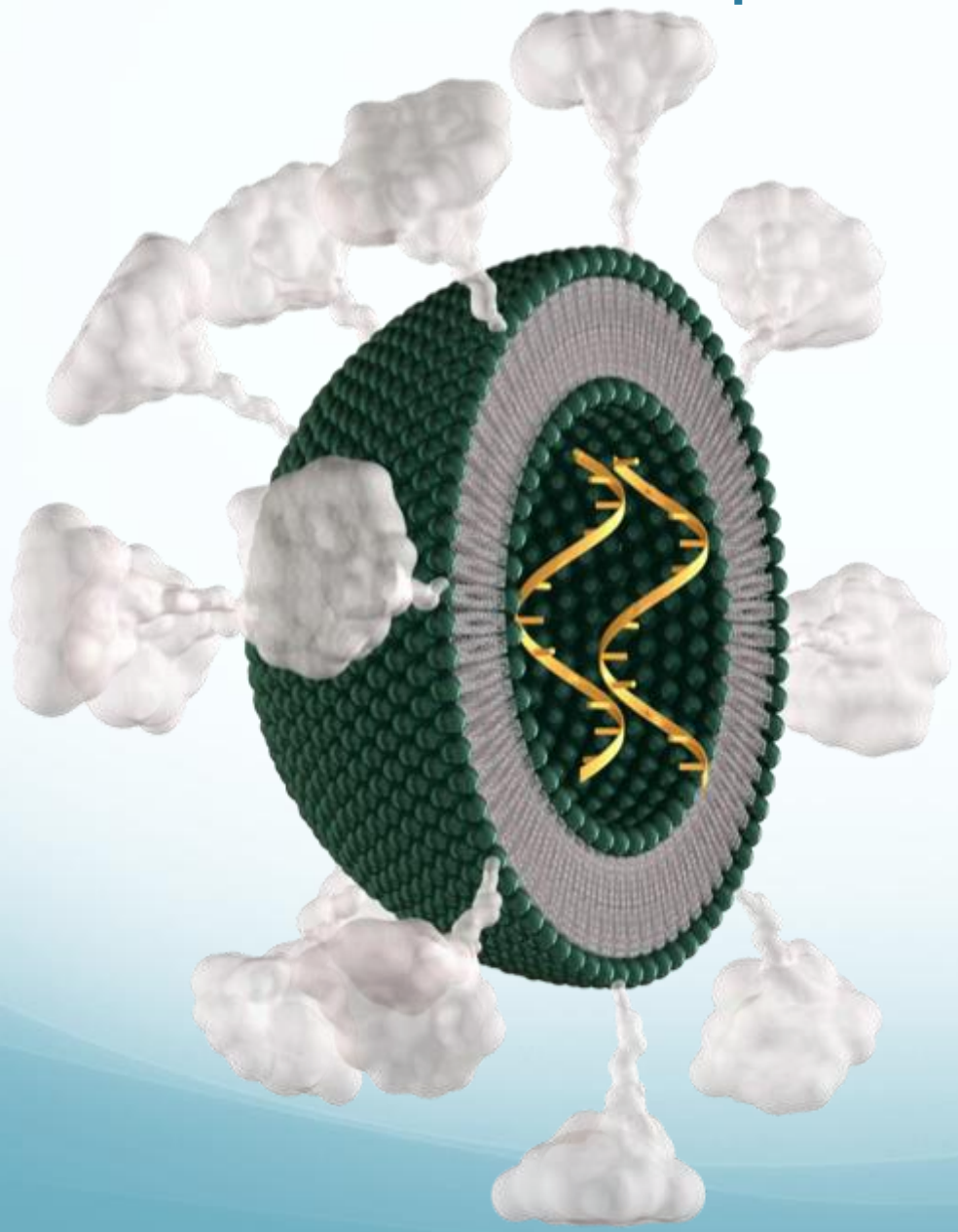
Mol Ther 2008, 16: 1833

Advantages of modified mRNA therapy

- Therapeutic protein production requires large scale cell line culture followed by purification that differs for every protein.
  - The potential for misfolding or incorrect modification has resulted in immunogenicity and adverse events.

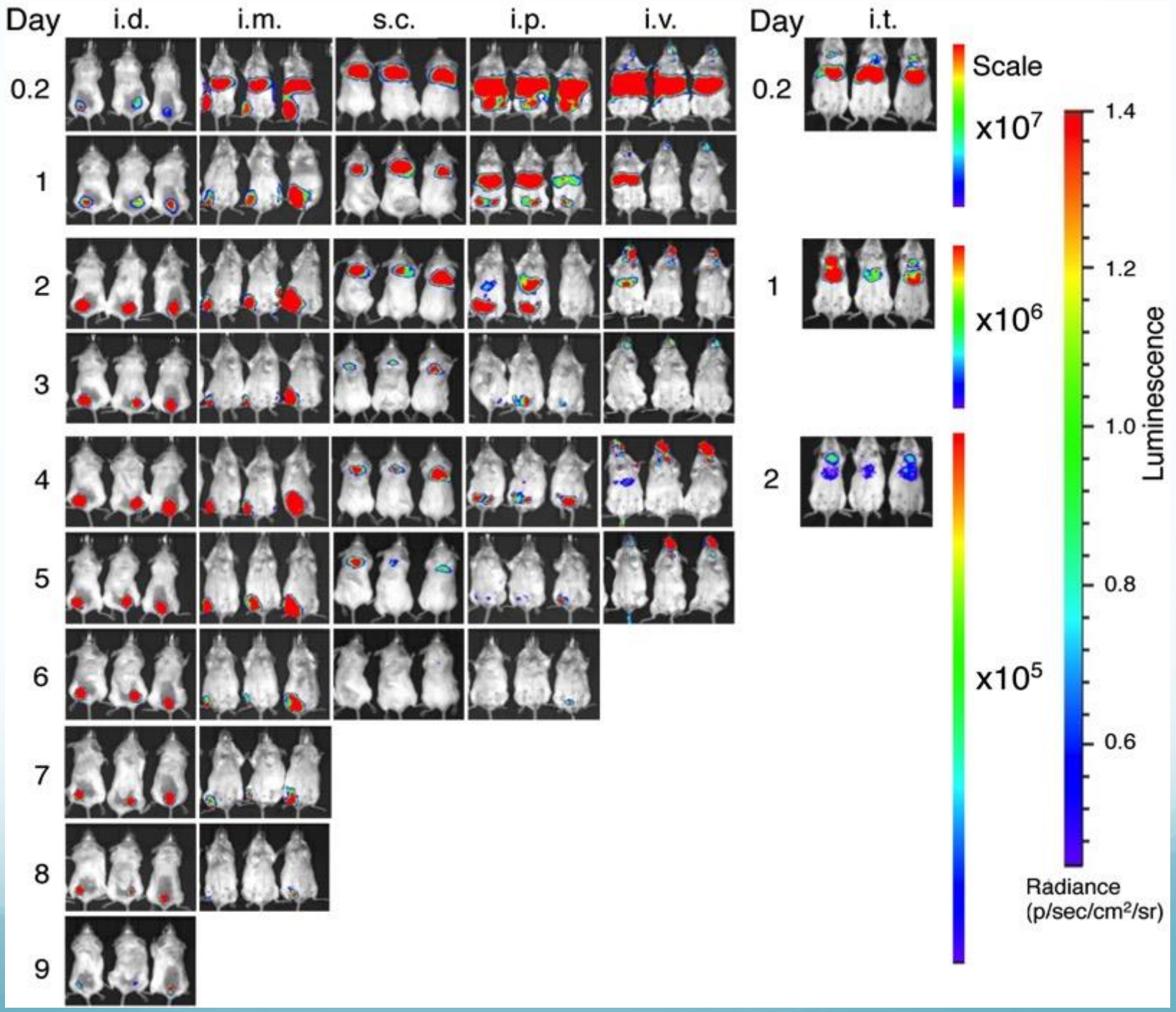
- With modified mRNA therapy, host cells produce the protein.
  - mRNA is made in a single reaction and purified in a single reaction that is the same regardless of the coding sequence.
  - Thus, modified mRNA is safer and has simplified GMP production, reduced cost, greatly increased potency, and no cold chain.

Lipid nanoparticles (LNPs) are a new promising agent for nucleic acid delivery

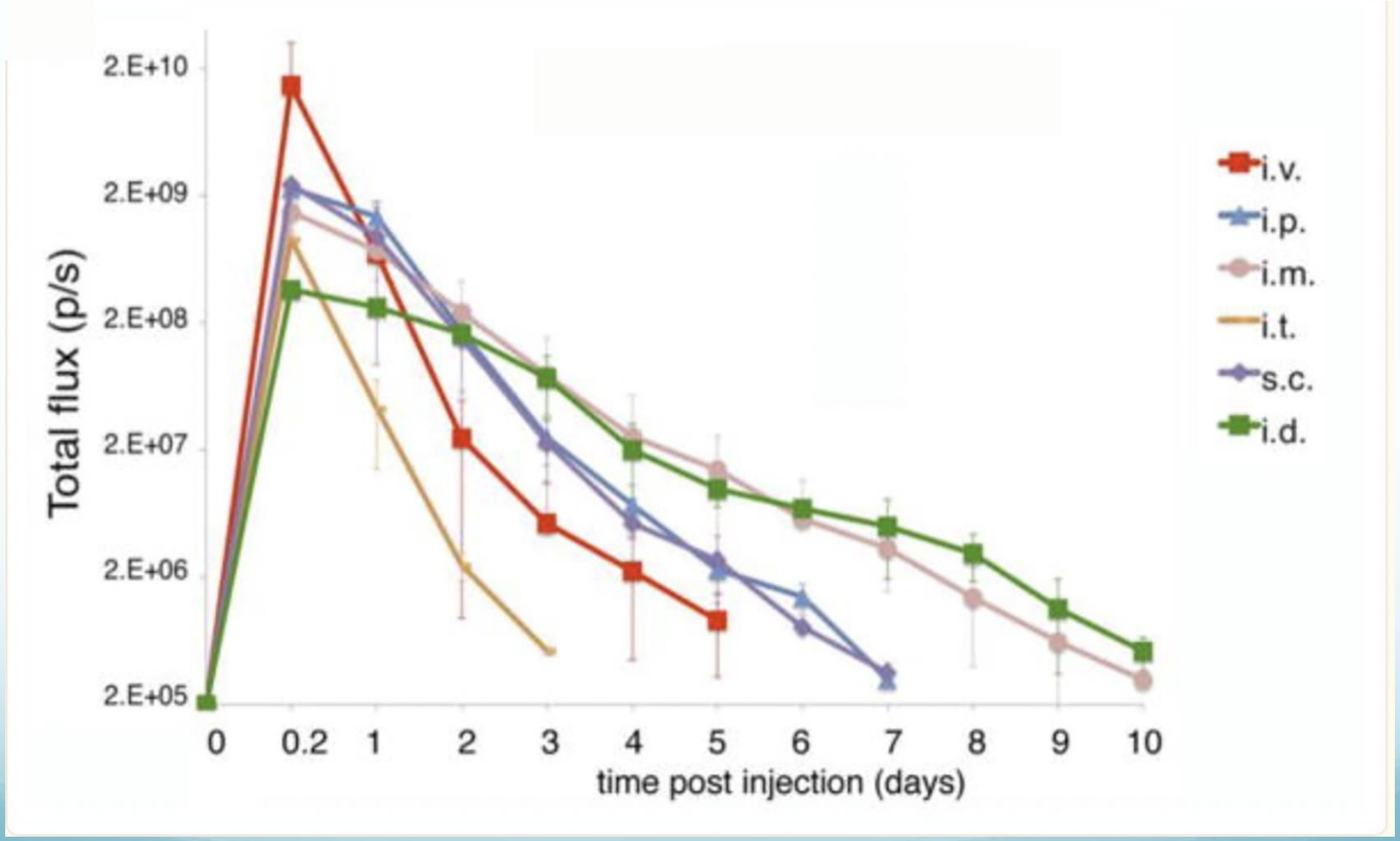


- multiple lipids (usually 4) self-assemble into 60-100 nm particles
- most LNPs are ApoE-dependent (trafficking to the liver after IV delivery) and are endocytosed by hepatocytes
- delivery of siRNA (mRNA)
- Safe, lowest dose of siRNA around 0.01 mg/kg, effective for 24-48 hours

Cartoon adapted from Acuitas Therapeutics


Day
i.d.
i.m.
s.c.
i.p.
i.v.
Day
i.t.
0.2
1
2
3
4
5
6
7
8
9
0.2
1
2
Scale
x10^7
x10^6
x10^5
1.4
1.2
1.0
0.8
0.6
Luminescence
Radiance
(p/sec/cm²/sr)

Kinetics of modified mRNA-LNP translation in vivo



Nucleoside modified mRNA-LNP vaccine platform

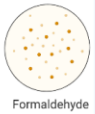
Conventional Vaccines

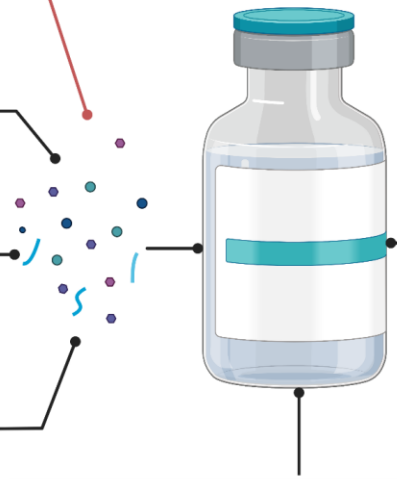
Active ingredients
Viral or bacterial antigens that directly stimulate the immune system but cannot cause disease.

Adjuvants
Aluminum salts or emulsions in small quantities that help to boost the immune response to the vaccine.

Antibiotics and Preservatives
Prevents dangerous bacterial or fungal contamination during vaccine manufacturing and storage.

Trace components
Residual inactivating ingredients (e.g., formaldehyde), and residual cell culture materials.

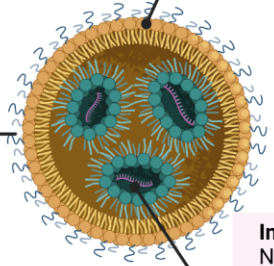




Buffers and Stabilizers
Buffers such as phosphate and stabilizers such as sugars, or polysaccharides keep the vaccine effective until it is administered to a patient.

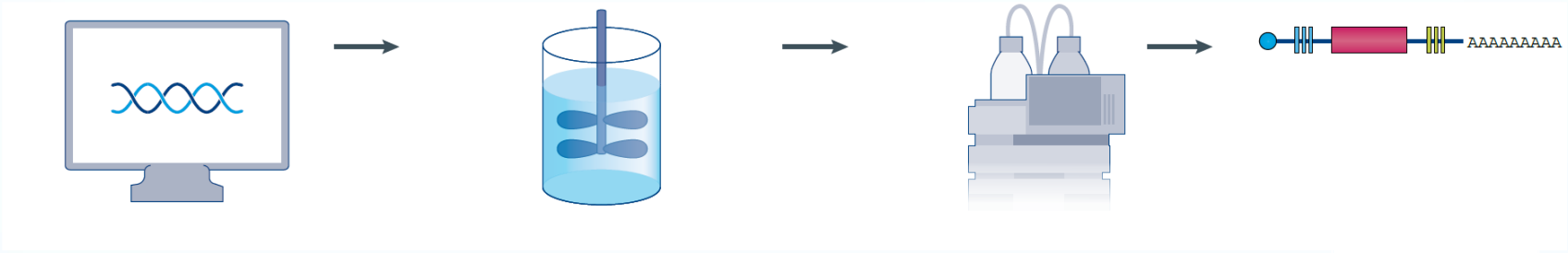
mRNA based Vaccines

mRNA-Lipid Nanoparticle (mRNA-LNP)
Mixture of lipids that encapsulate the mRNA (active ingredient), and allow its delivery to the cells. **Lipids also play an important role in the immune response**



In vitro transcribed messenger RNA
Nucleic acid molecule **encoding the necessary information to make the antigen**

Generating an mRNA Vaccine (1 of 2)



1. Sequence design

Once the genome of the pathogen is known, a sequence for the target antigen is designed and inserted into plasmid DNA

2. In vitro transcription

Plasmid DNA is transcribed into mRNA by bacteriophage polymerases

3. Purification

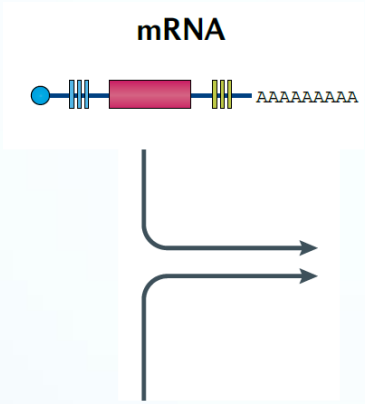
HPLC isolates mRNA transcripts and removes contaminants

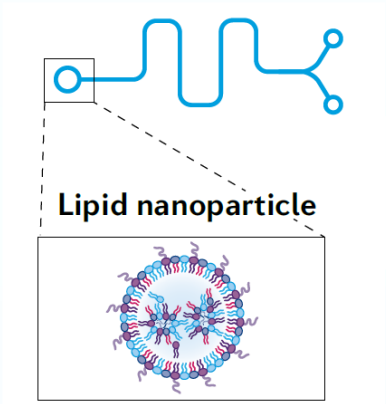
4. mRNA

The result is purified mRNA for the target antigen

Chaudhary N, et al. *Nat Rev Drug Discov.* 2021;20:817-838.

Generating an mRNA Vaccine (2 of 2)





5. Nanoprecipitation

Rapid mixing of mRNA and lipids cause lipids to encapsulate the mRNA and precipitate as self-assembled nanoparticles

6. Filtration

Removes solvents and unencapsulated mRNA

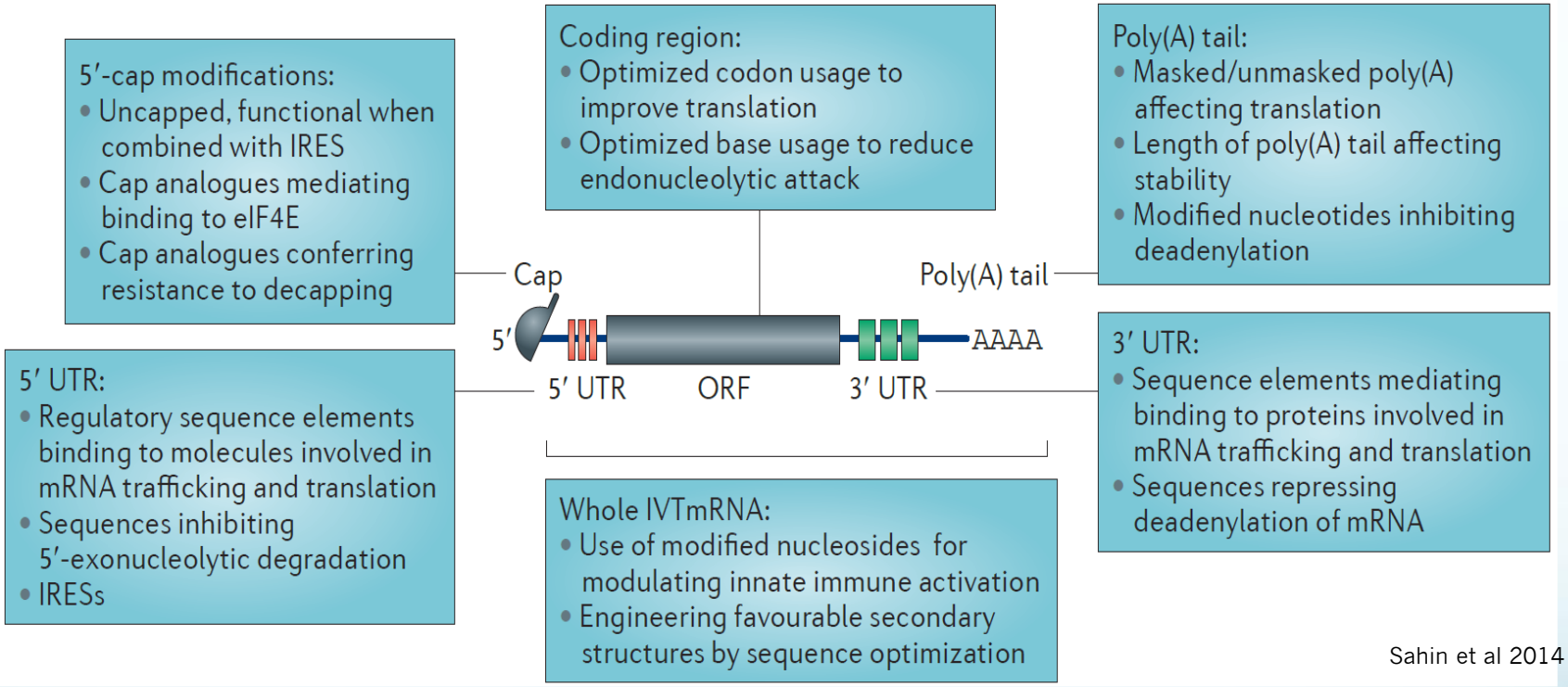
7. mRNA vaccine

Stored in sterile vials

Chaudhary N, et al. *Nat Rev Drug Discov.* 2021;20:817-838.

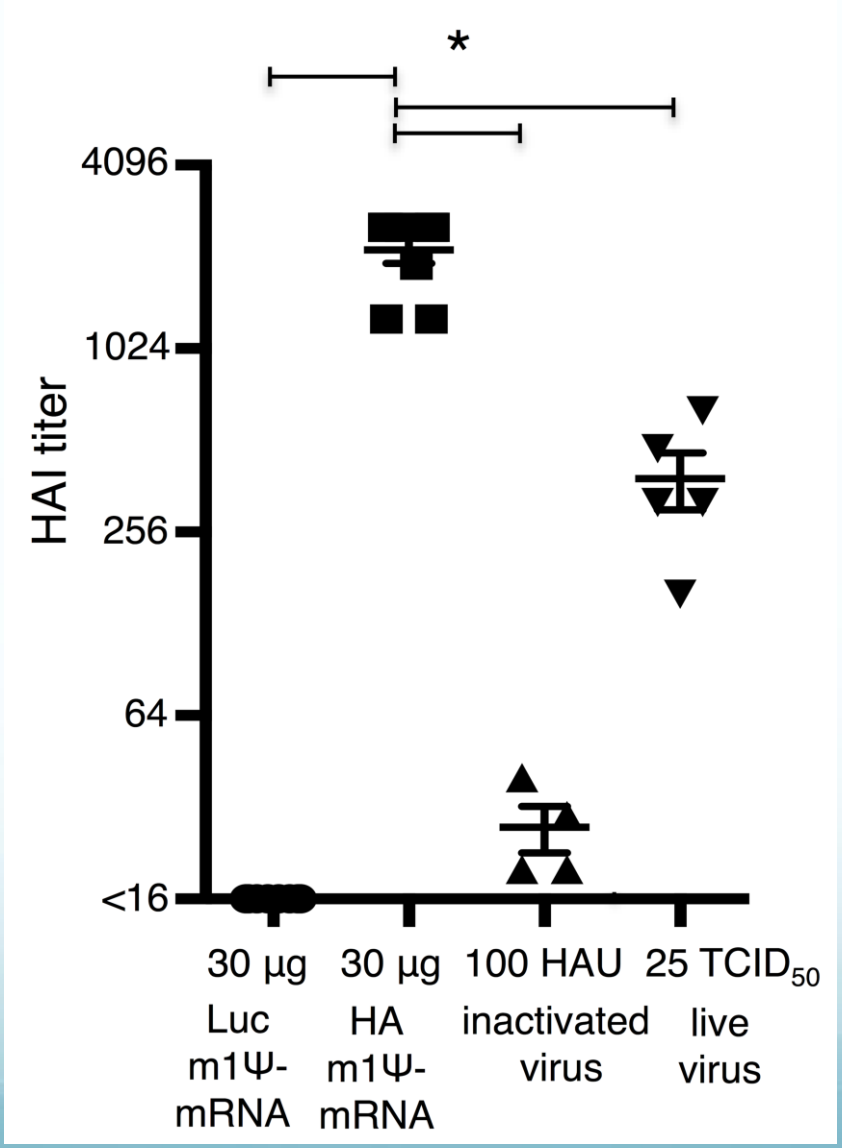
IVT mRNA can be fine tuned for prophylactic/therapeutic applications using different strategies

Fine tuning mRNA pharmacokinetics

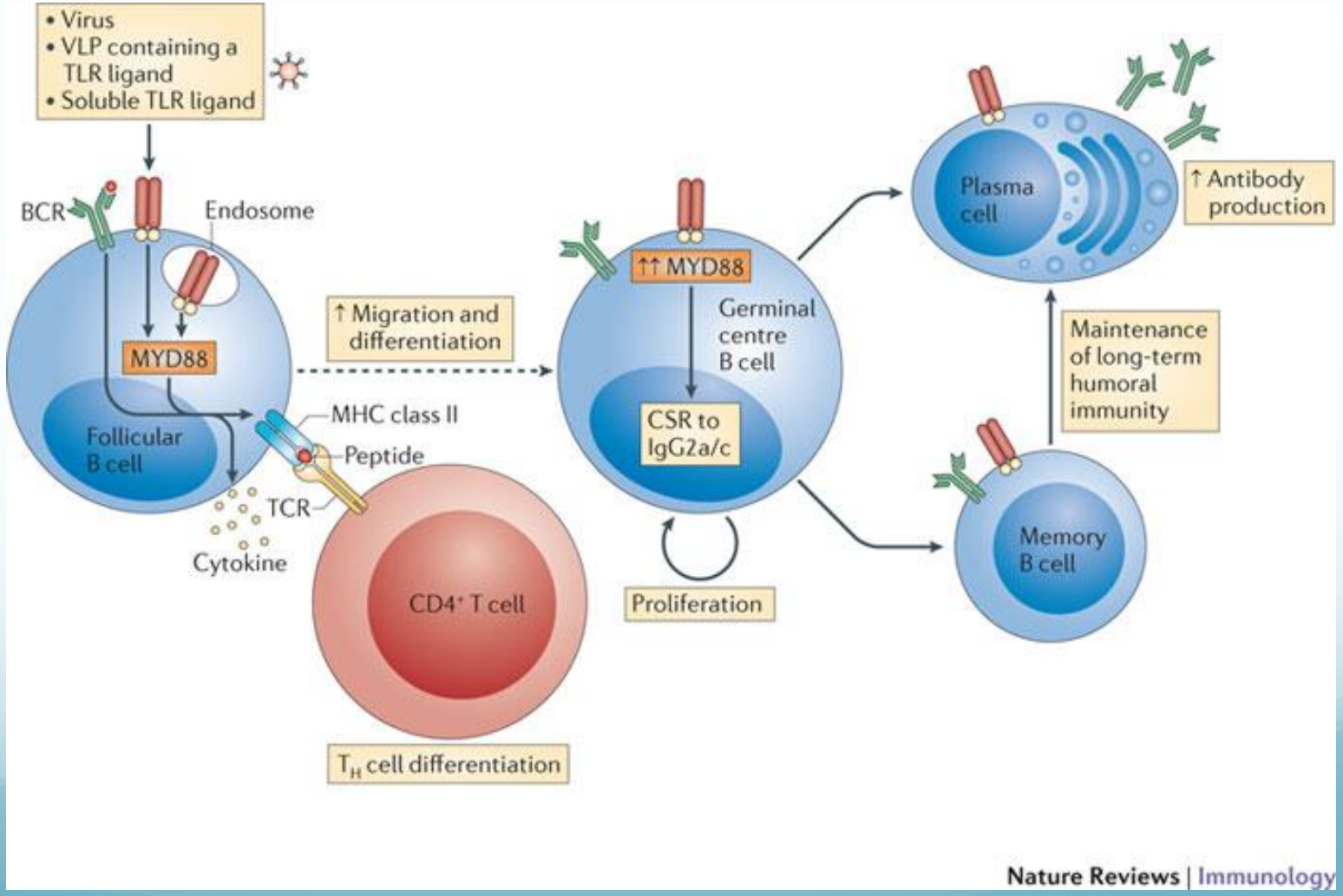


Sahin et al 2014

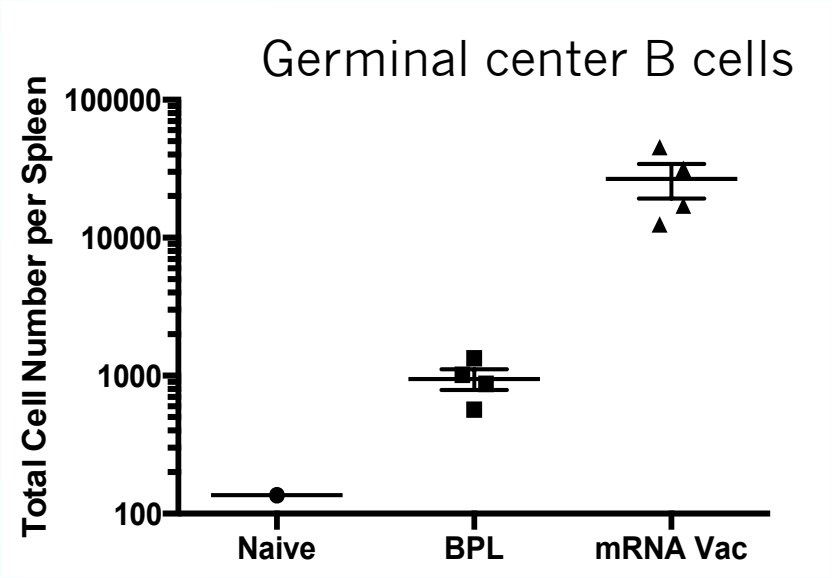
Acute infection with PR8 influenza induces lower levels of neutralization than modified mRNA-LNP vaccination

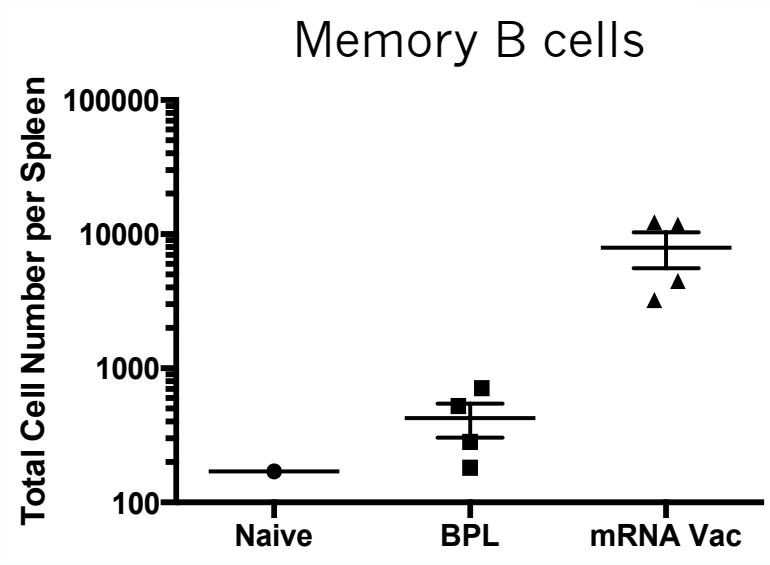


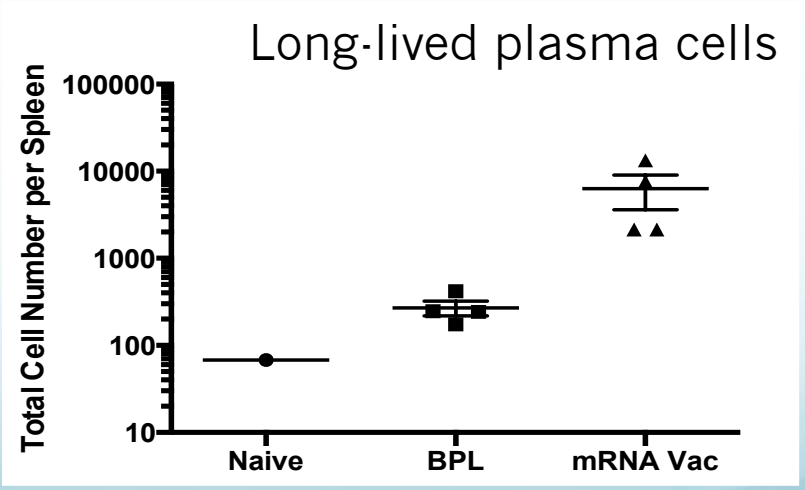
B cell response



A single immunization of PR8 HA encoding mRNA-LNPs produces HA-specific germinal center, memory, and long-lived plasma cells

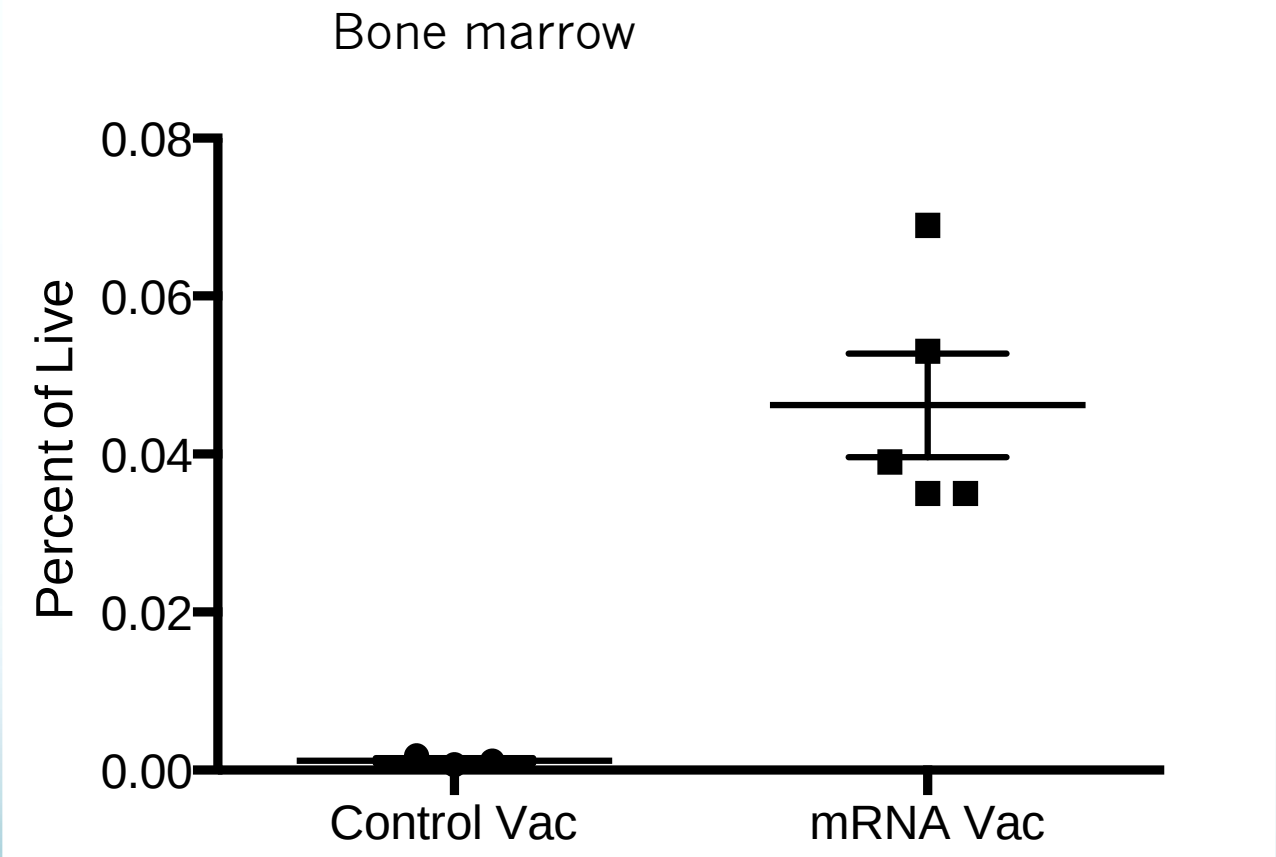






4 weeks after a single immunization with HA mRNA-LNPs

HA-specific bone marrow plasma cells 13 months after a single immunization with modified mRNA-LNPs.

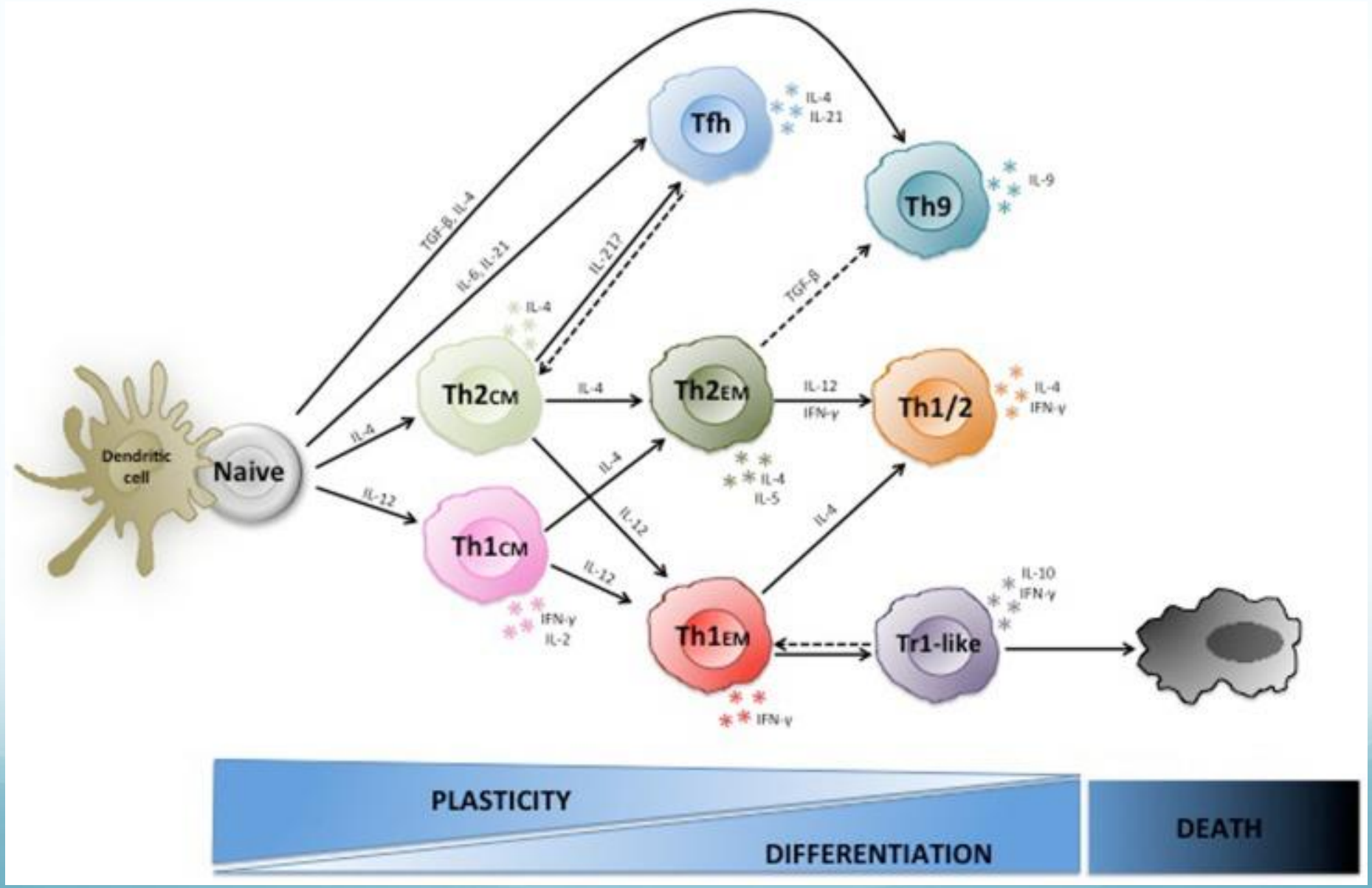


Gated on live, singlets, Dump- (CD4, CD8, F4/80, Ter119), IgD-, B220- that bound fluoresceinated influenza HA.

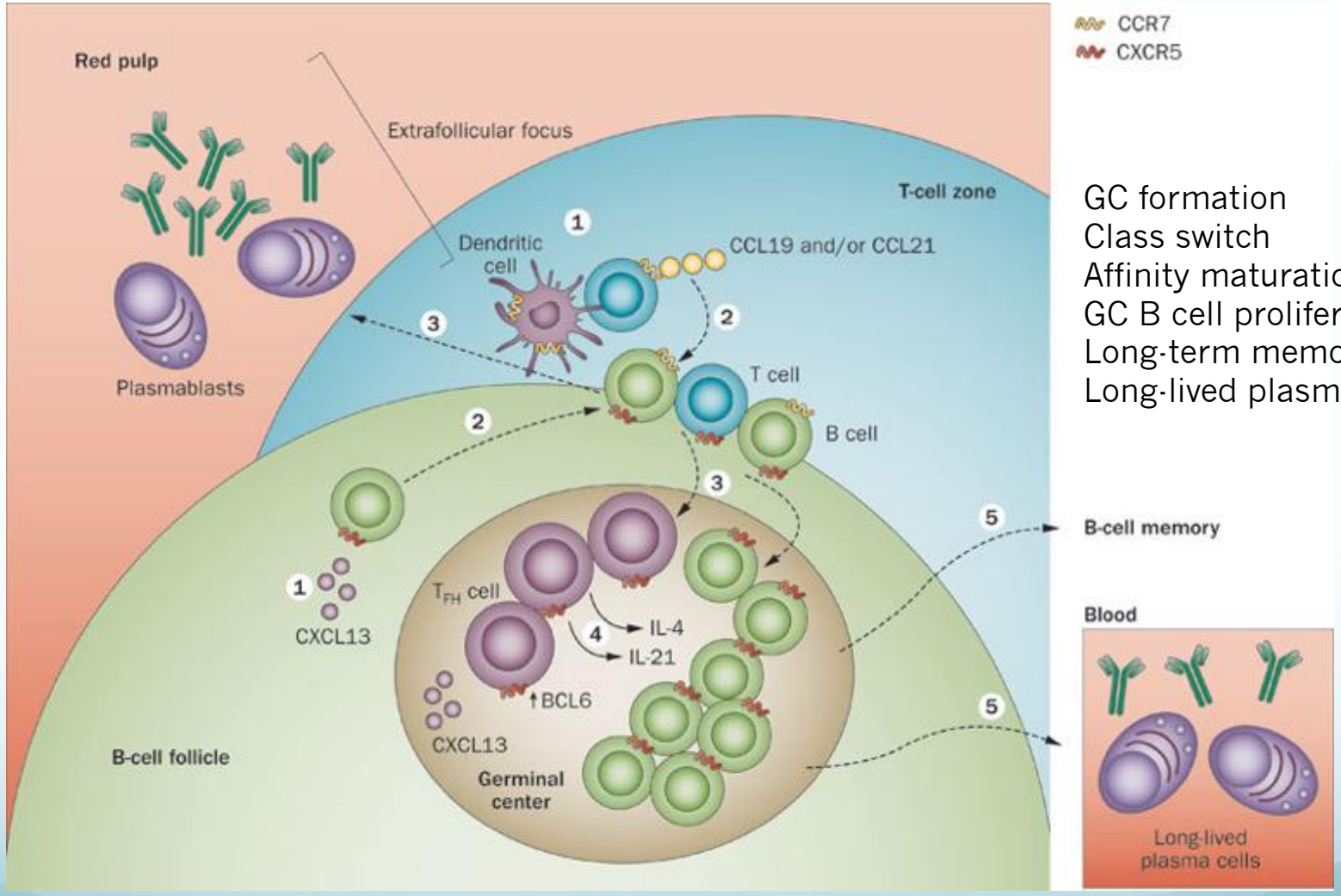
Vaccines mechanisms

- Vaccines use adjuvants to increase immune responses and reduce amount of immunogen needed.
- All currently developed adjuvants signal innate immune receptors, TLRs, NODs, helicases, inflammasomes, to induce inflammation and activate typically Th1, Th2, or Th17 responses.

CD4+ T cell differentiation and effector function.



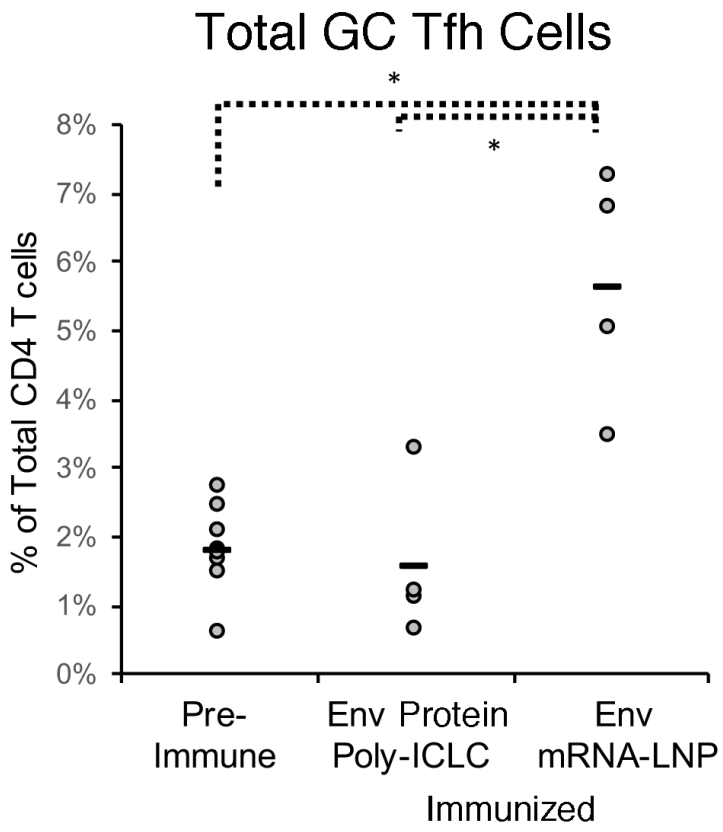
T follicular helper cells

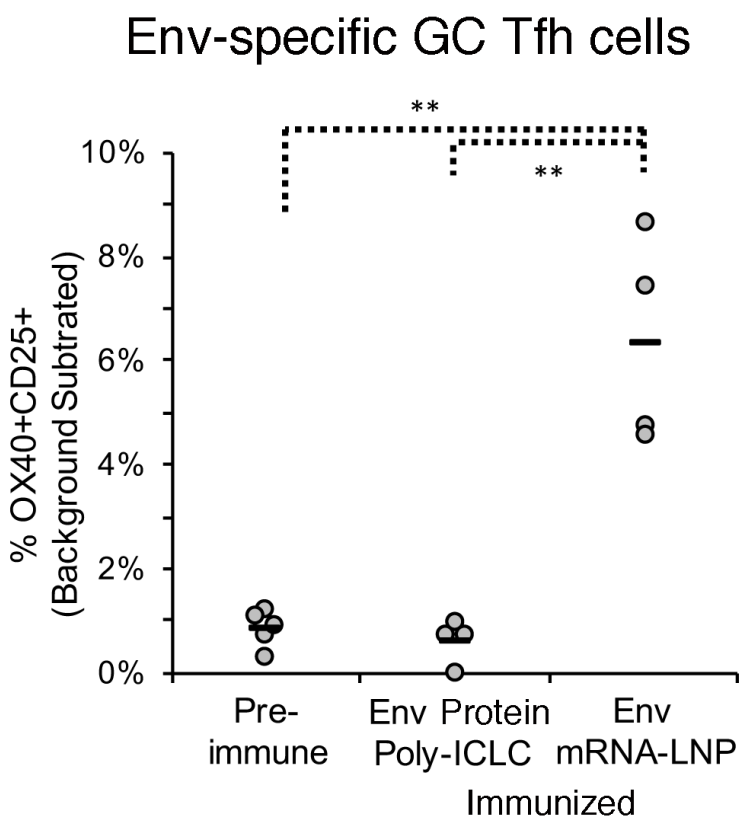


GC formation
Class switch
Affinity maturation
GC B cell proliferation
Long-term memory
Long-lived plasma cells

They are critical in driving B cell responses and memory and are the subject of many vaccine-adjuvant studies.

Frequencies of total and Env-specific germinal center (GC) T follicular helper (Tfh) cells in CH505 T/F immunized macaque lymph nodes



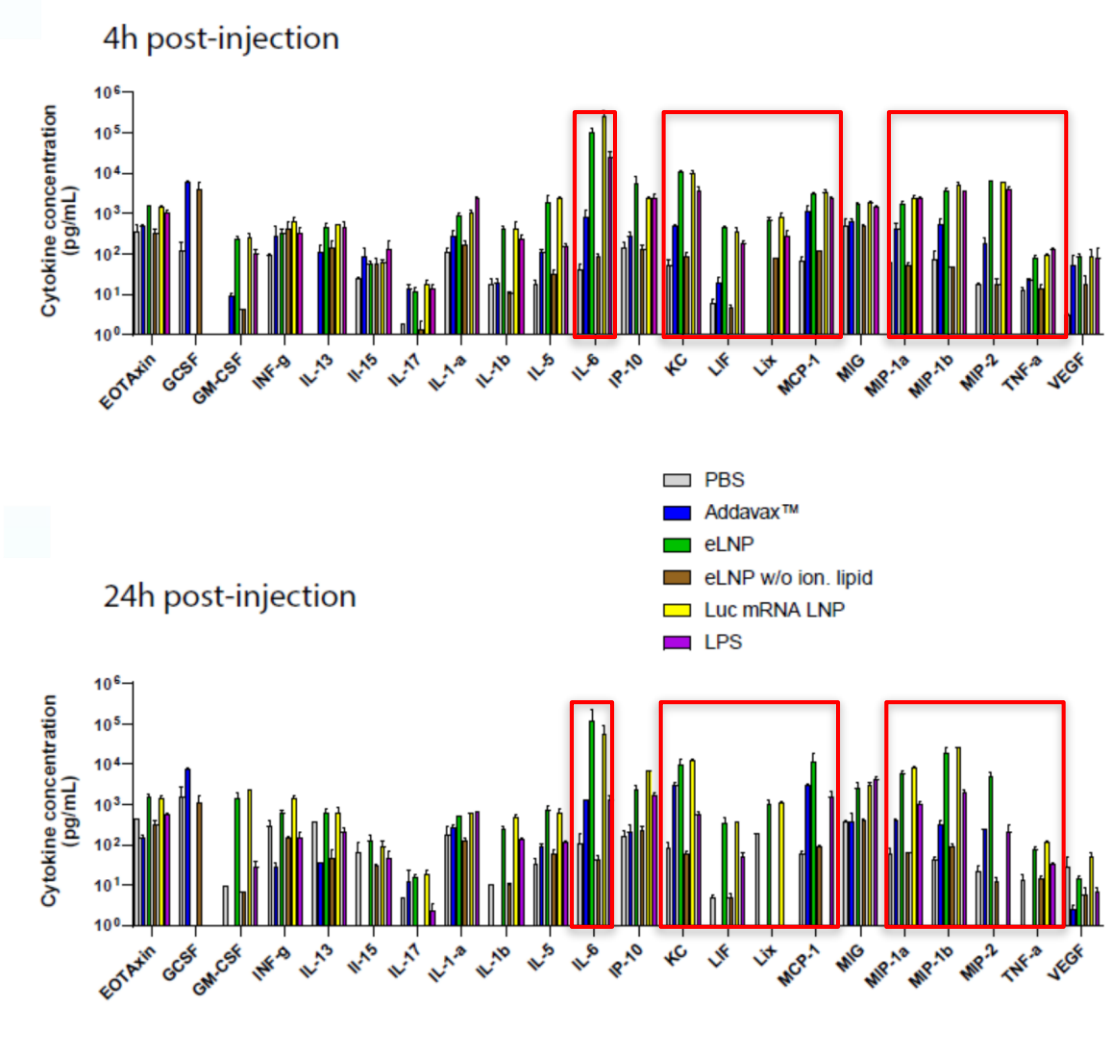


CHAVI-ID
Duke Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery

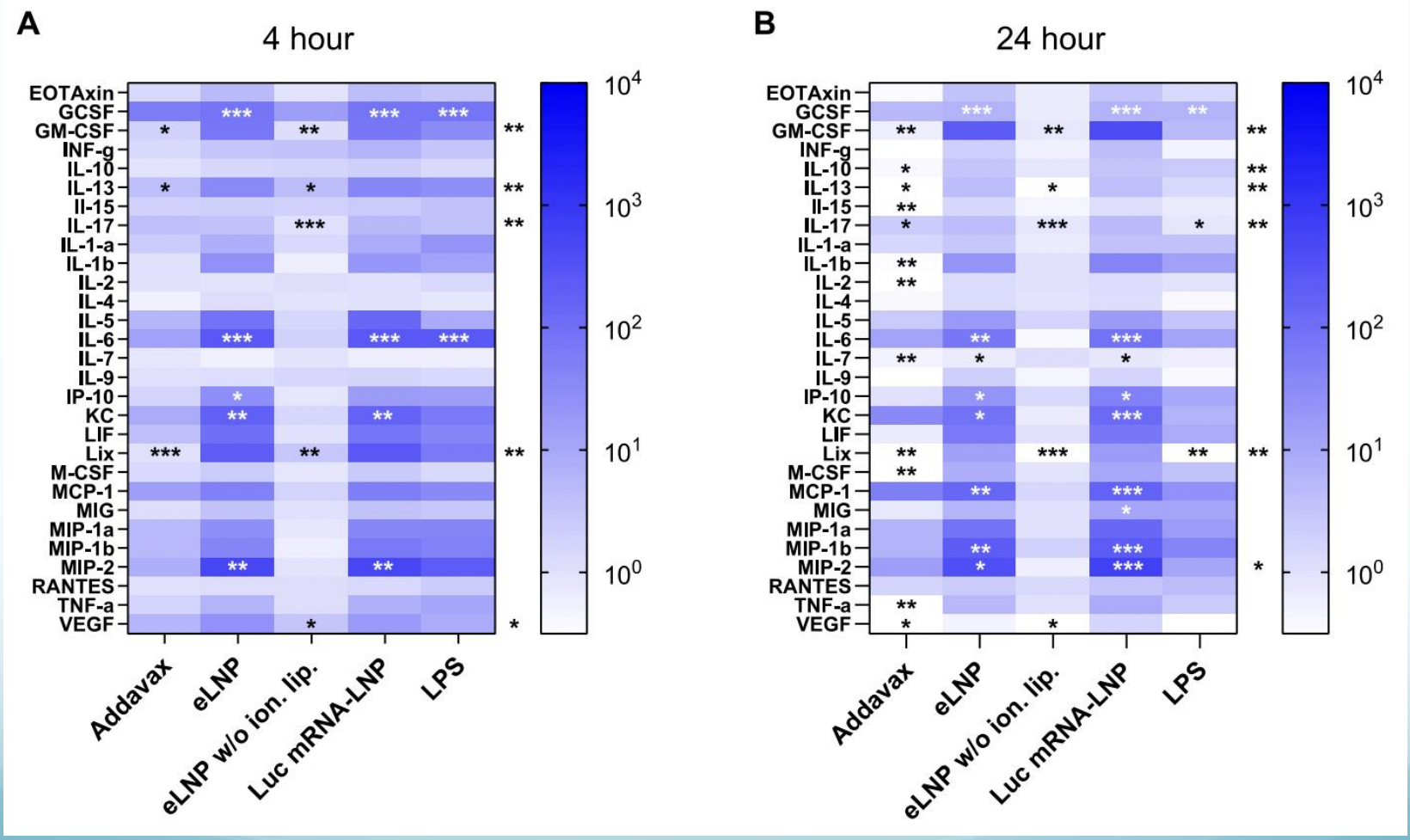
Mechanisms of potent immune response induction by LNPs

LPS activates TLR4
dsRNA activates TLR3
CpG oligos activate TLR9
RNA activates TLR7-8, Rig-I, NOD2, PKR, others
Alum activates inflamasomes

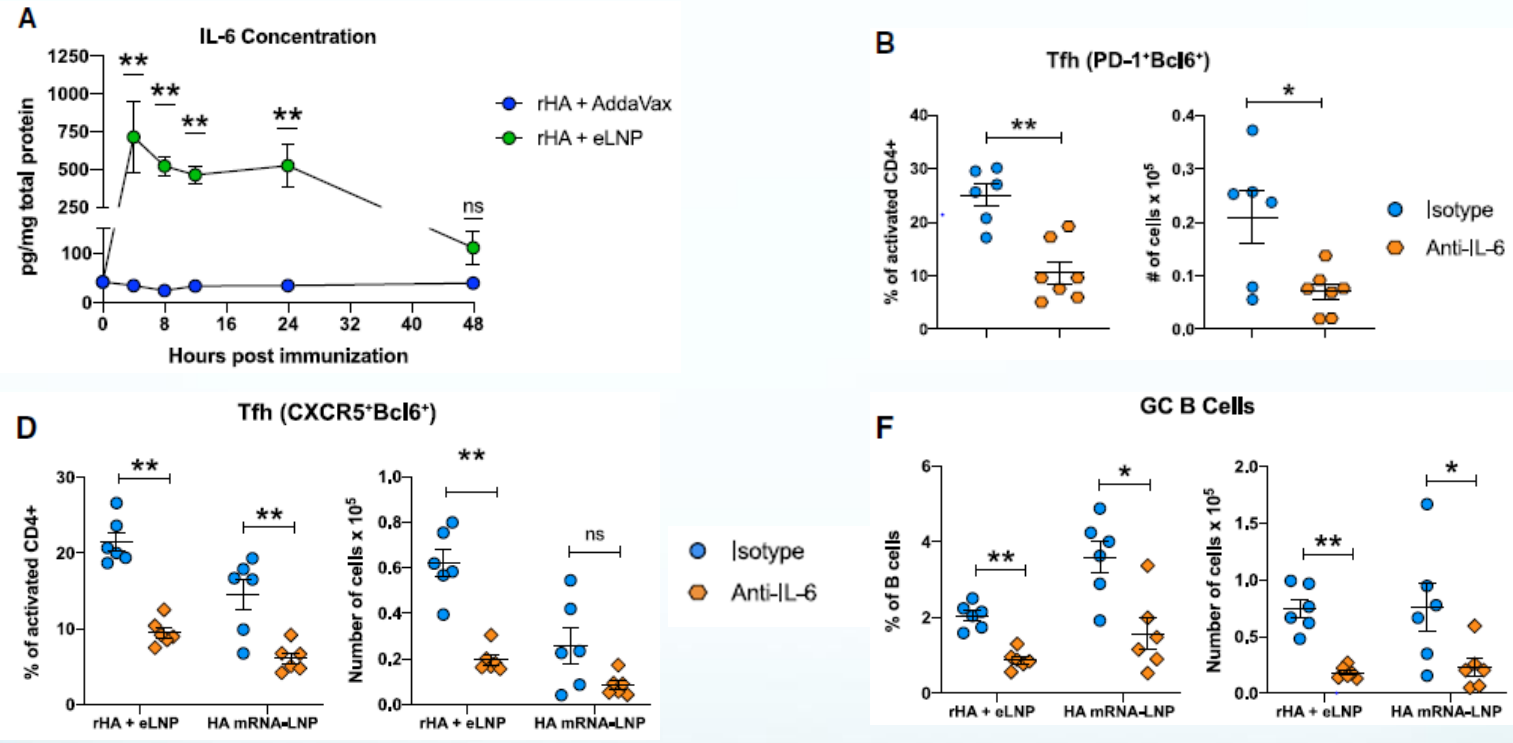
eLNP and mRNA LNP induce high levels of IL-6, KC and chemokines in dLN, and in circulation



LNPs induce IL-6 and chemokines and no type 1 IFNS.

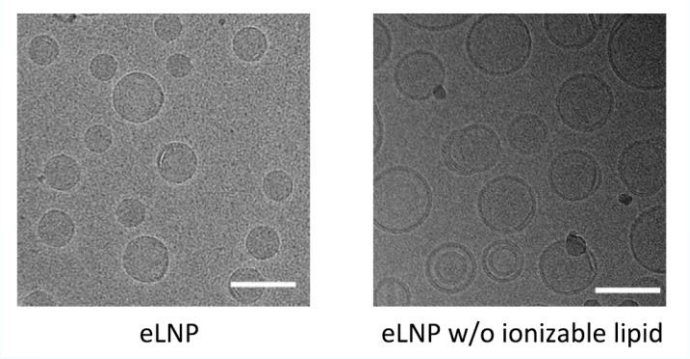


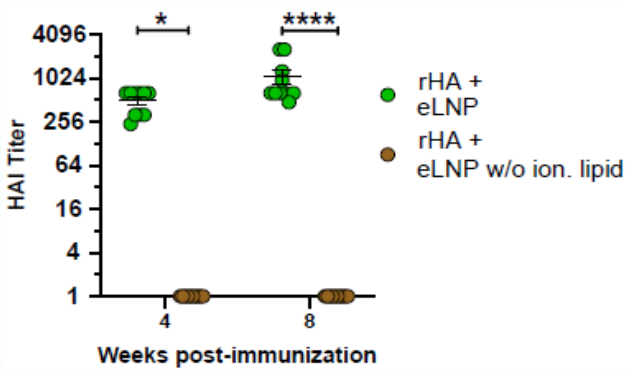
LNPs induce robust IL-6 production and poor type 1 IFNs, which is required for efficient induction of T_{FH} cells

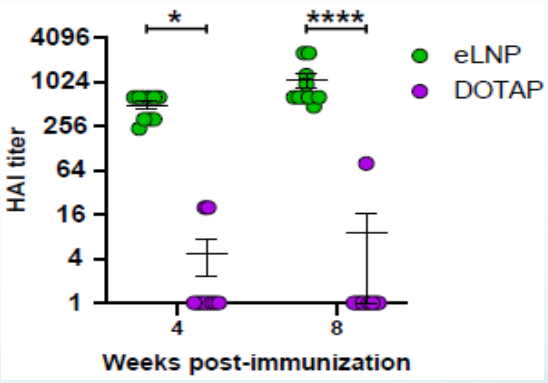


Alameh, MG, Weissman, D, Pardi, N et al. Immunity 54: 2877-2892, 2021

The ionizable lipid is responsible for the adjuvant activity of the LNP formulation (eLNP = empty LNP)

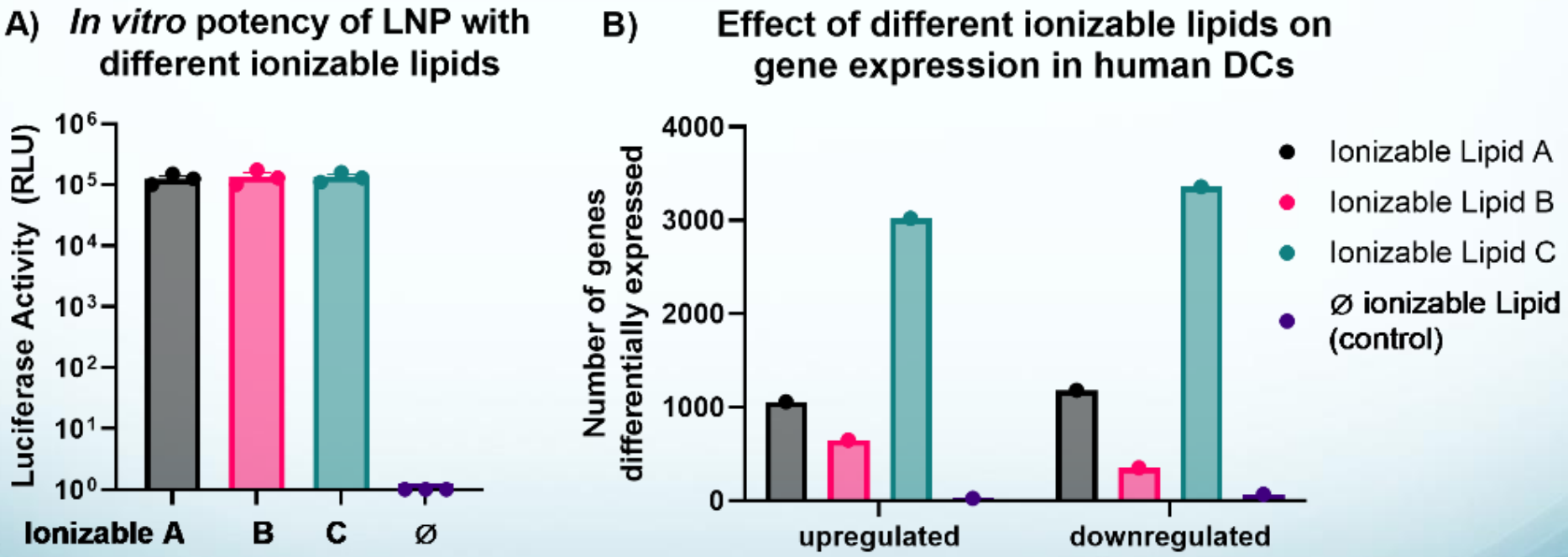






Alameh, MG, Weissman, D, Pardi, N et al. Immunity 54: 2877-2892, 2021

Different ionizable lipids induce different gene expression profiles

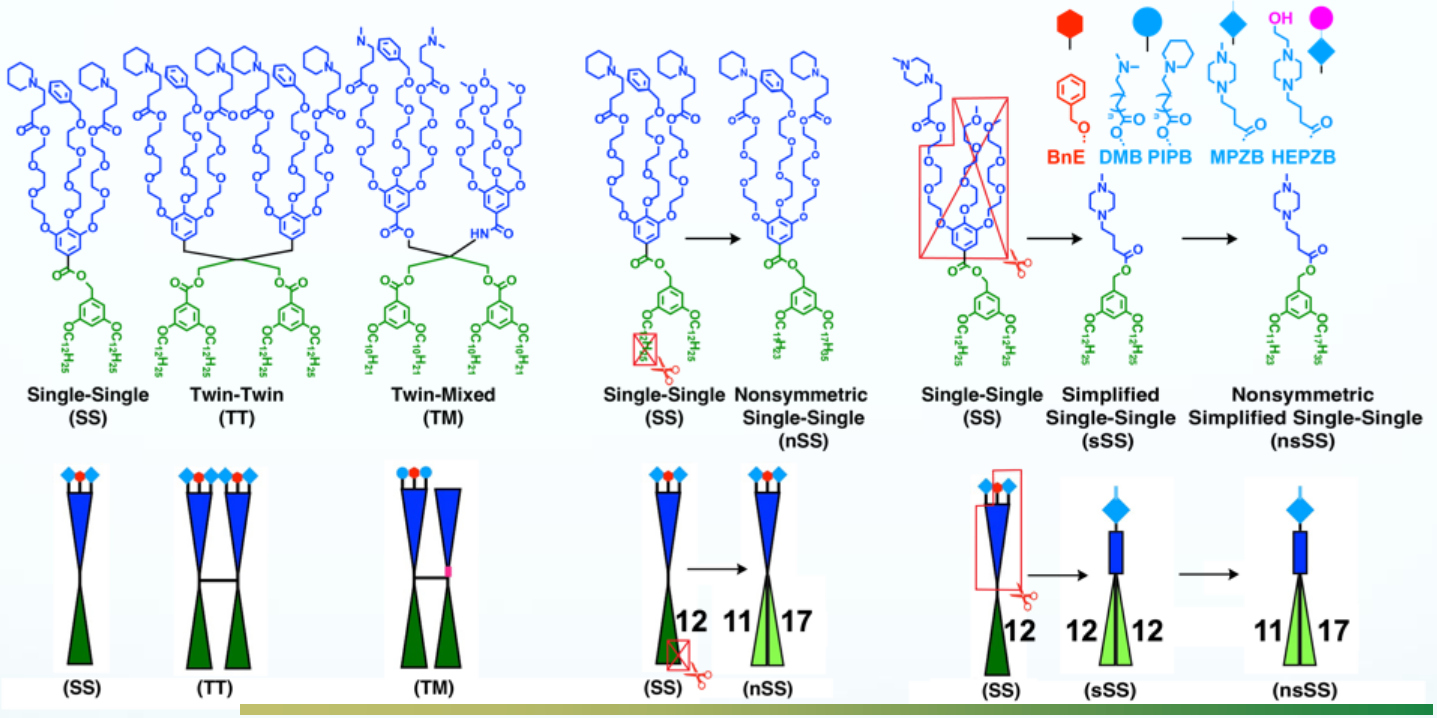


Conclusions – How do modified mRNA-LNP vaccines induce potent responses.

- Prolonged immunogen expression, which leads to GC loading
- LNP is a specific adjuvant that induces a Th1-biased Tfh response.

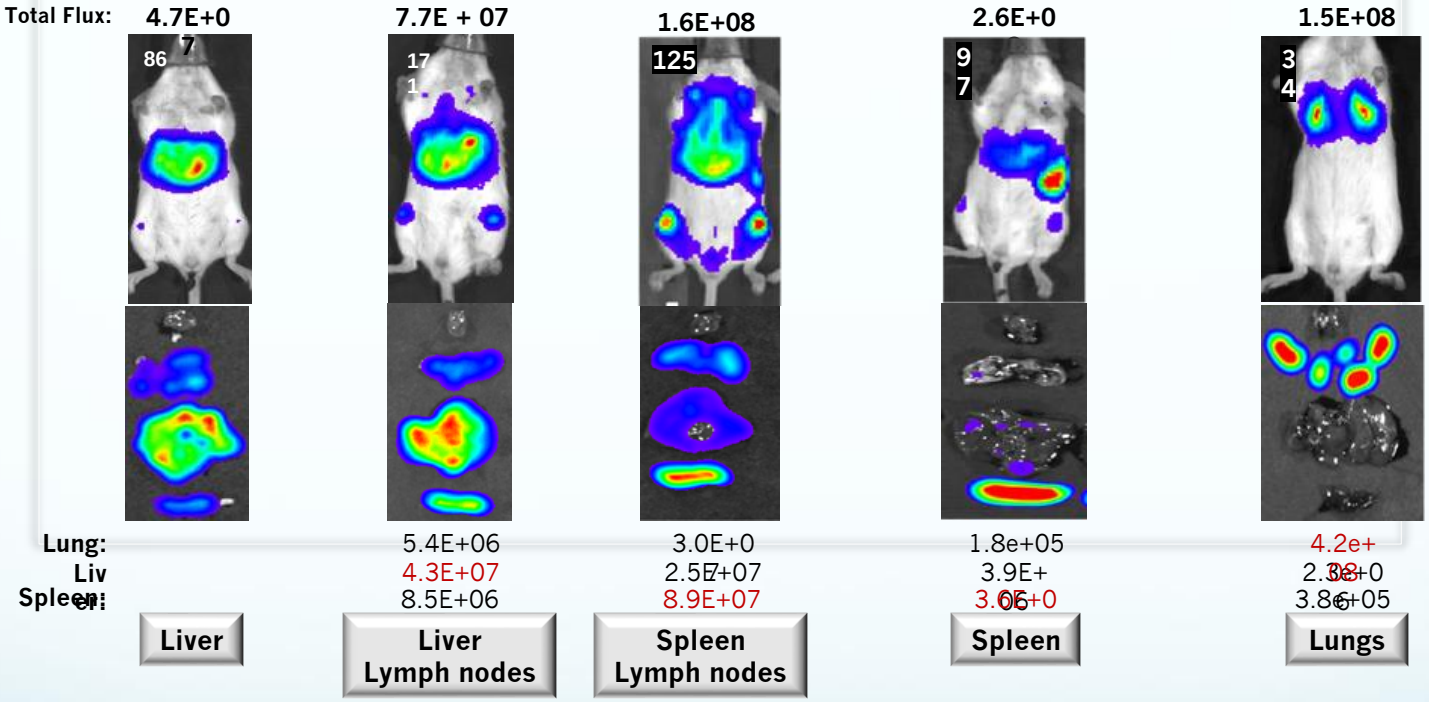
One component nanoparticles

Design Strategy Principles for Accelerated Modular Orthogonal Synthesis of Rational Libraries of Programmed Sequence-Defined IAJDs

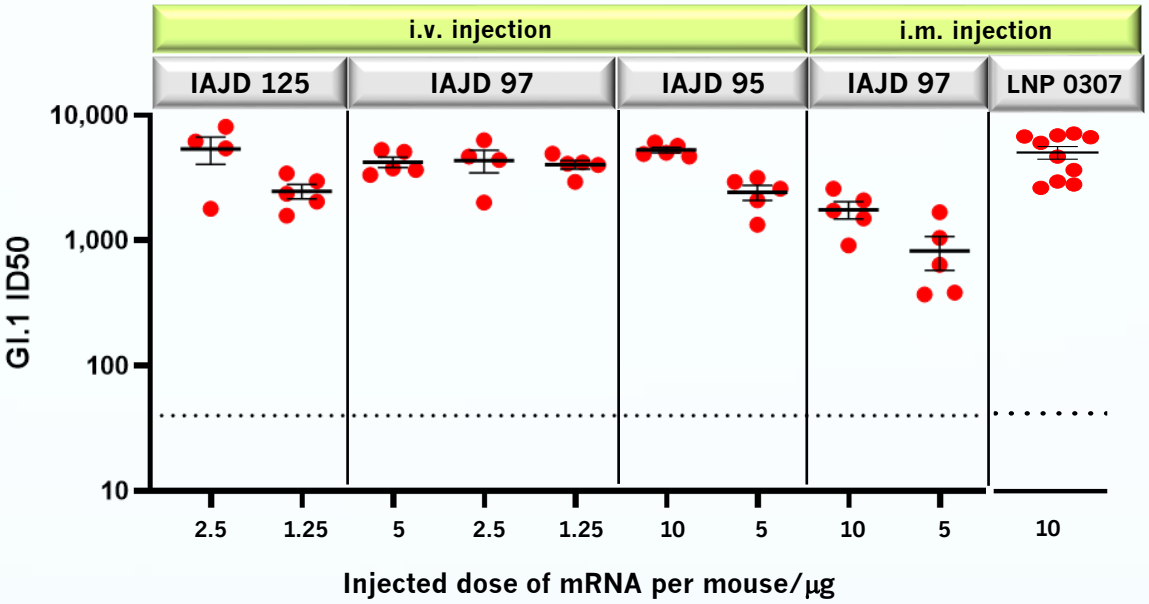


D. Zhang, E. N. Atochina-Vasserman, D. S. Maurya, N. Huang, Q. Xiao, N. Ona, M. Liu, H. Shahnawaz, H. Mi, K. Kim, M. M. Billingsley, D. J. Pochan, M. J. Mitchellk, D. Weissman, V. Percec. "One-Component Multifunctional Sequence-Defined Ionizable Amphiphilic Janus Dendrimer Delivery Systems for mRNA." J. Am. Chem. Soc. 143, 12315-12327(2021). D. Zhang, E.N. Atochina-Vasserman, D.S. Maurya, M. Liu, Q. Xiao, J. Lu, G. Lauri, N. Ona, E.K. Reagan, H. Ni, D. Weissman, V. Percec "Targeted Delivery of mRNA with One-Component Ionizable Amphiphilic Janus Dendrimers." J. Am. Chem. Soc. 143,17975-17982, (2021). D. Zhang, E.N. Atochina-Vasserman, J. Lu, D.S. Maurya, Q. Xiao, M. Liu, J. Adamson, N. Ona, E.K. Reagan, H. Ni, D. Weissman, V. Percec. "The Unexpected Importance of the Primary Structure of the Hydrophobic Part of One-Component Ionizable Amphiphilic Janus Dendrimers in Targeted mRNA Delivery Activity." J. Am. Chem. Soc. 11, 4746–4753 (2022)

One-Component IAJDs Exhibit a Variety of Specific Tissue Tropism



Vaccination with DNPs Co-Assembled from IAJDs & mRNA Encoding Norovirus Capsid Protein Induced High Titers of Neutralization Antibody



(A) Mice were injected by i.v. with indicated IAJDs formulated with modified mRNA luciferase. Four hours post injection, mice and their organs were analyzed on IVIS machine for luminescence intensity. **(B)** Serum was collected at 4 weeks after boost and analyzed for ability to block the interaction of VLP with binding ligand in a surrogate neutralization assay. n=5 per each group.

Acknowledgements

Katalin Kariko

Weissman Lab

- **Hamideh Parhiz**
- **Norbert Pardi**
- **Istvan Tombacz**
- **Qin Li**
- **Houping Ni**
- **Erin Reagan**
- **Mohamad-Gabriel Alameh**
- **Elena Vasserman**
- **Jaishree Beedsay**
- **Goutham Boija**
- **Wendy Bonilla-Acosta**
- **Nicholas Cerda**
- **Benjamin Davis**
- **Angela Desmond**
- **Garima Dwivedi**
- **Majed Ghattas**
- **Edward Kreider**
- **Jilian Melamed**
- **Xiomara Lopez**
- **Nathan Ona**
- **Tyler Papp**
- **Matthew Pine**
- **Bryn Queeley**
- **Ruiqi Shi**
- **Julie Taylor**
- **Amir Yadegari**
- **Jibin Zhou**

Hensley Lab

- **Kaela Parkhouse**

Acuitas Therapeutics

- **Ying Tam**
- **Chris Barbosa**

Percec Lab

- Dapeng Zhang
- Jasper Adamson
- Maurya, Devendra
- Juncheng Lu

Funding: Center For HIV/AIDS Vaccine Immunology-Immunogen Discovery grant (CHAVI-ID; grant UM1 AI100645), CHAVD, Takeda Pharmaceuticals, New Frontier Science, NIAID/NIH R01-AI050484 and RO1-AI084860, Gates Foundation CAVD 0PP1033102, NIH IPCAVD x 2, DARPA P3, BioNTech SRA for vaccine development, BioNTech SRA for targeted delivery of mRNA.