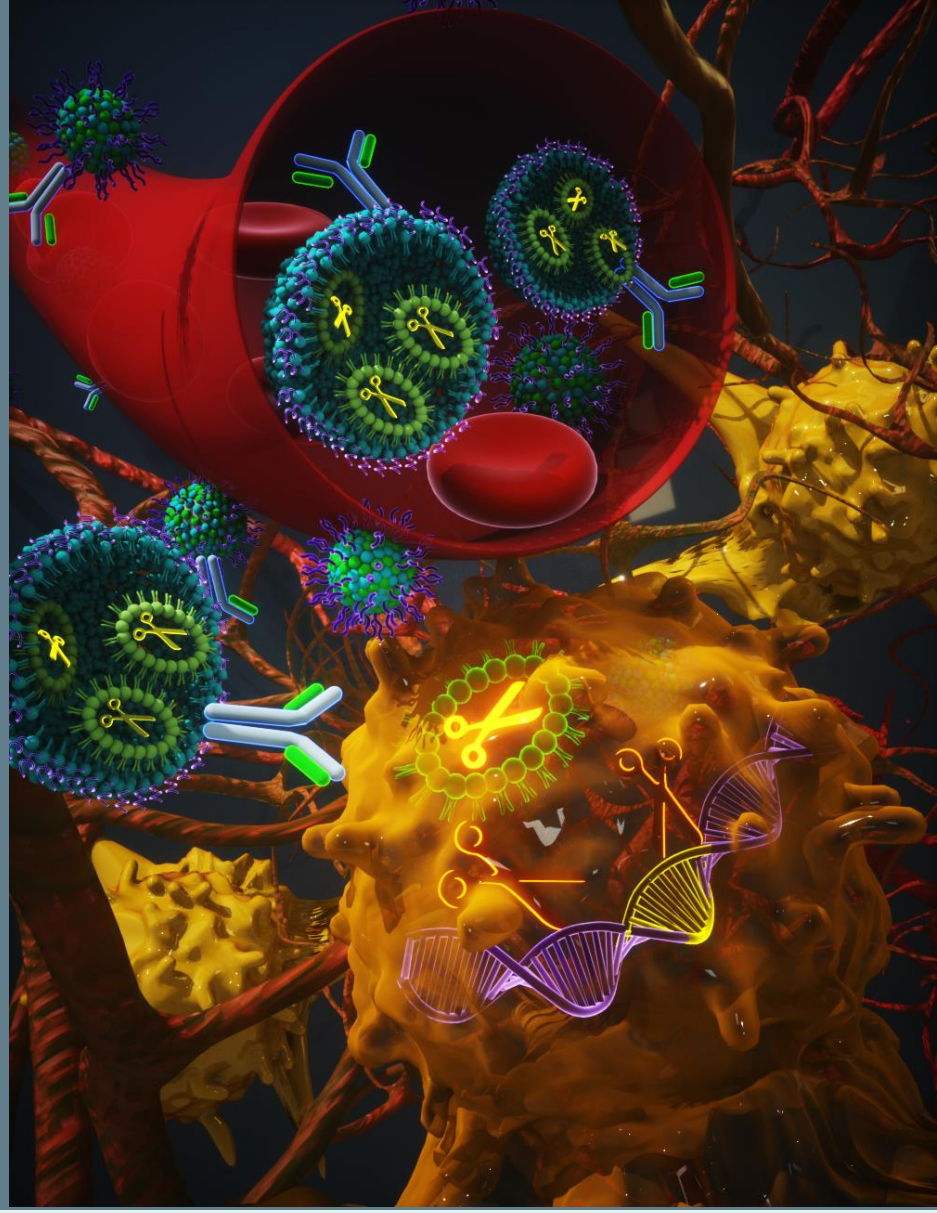
Cell Specific Targeting: Technological and Biological Considerations

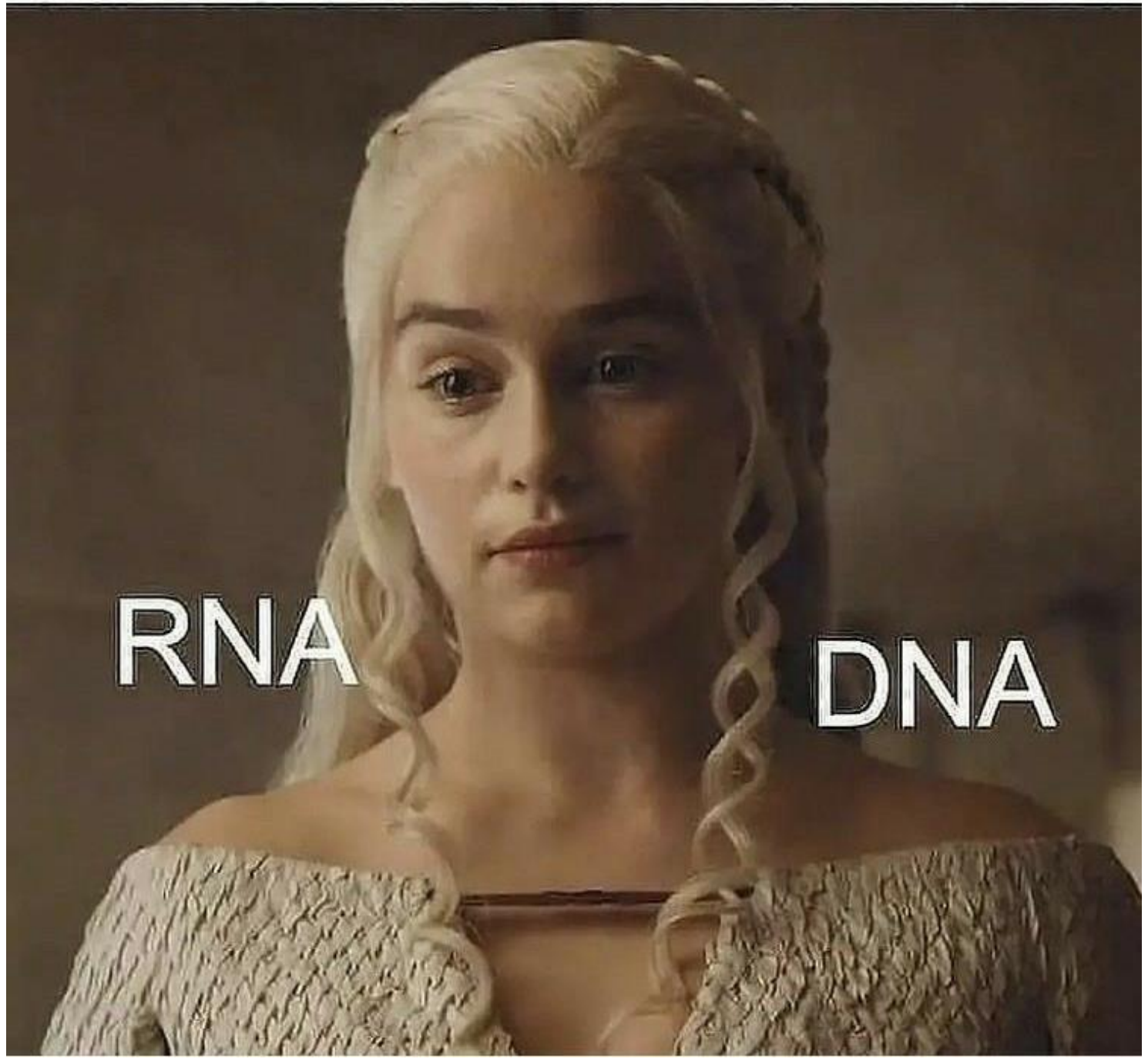


CRS annual meeting
Lisbon, Portugal
6-9th of July 2026

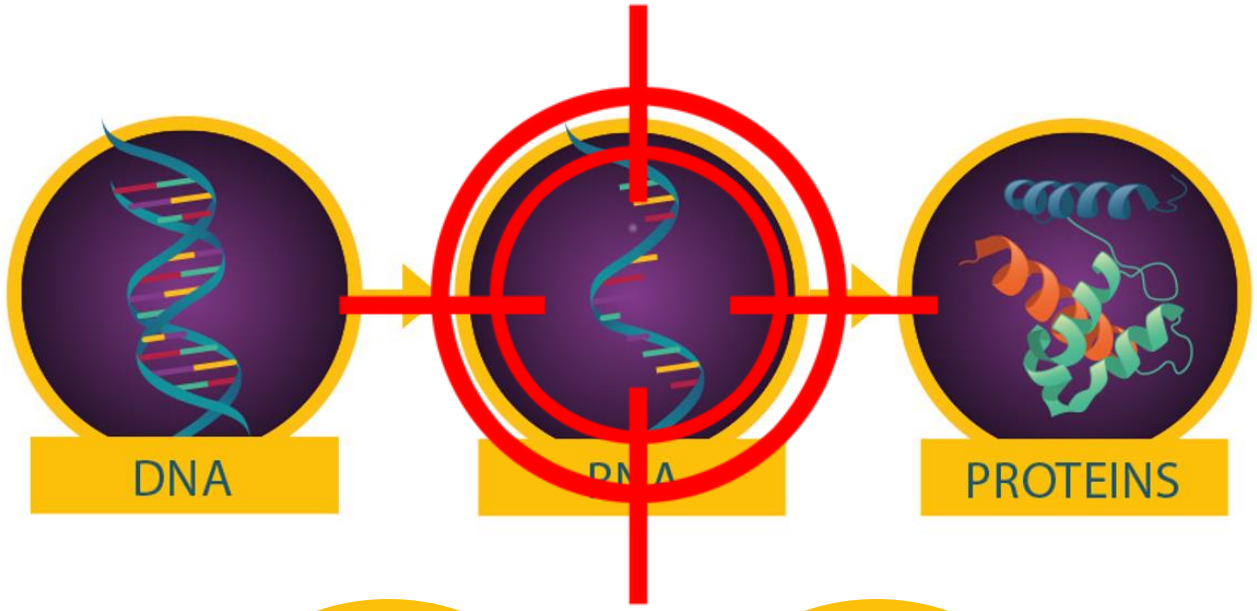
Dan Peer

Peer Lab
Precision Nanomedicine

TEL AVIV UNIVERSITY
אוניברסיטת תל אביב


RNA
DNA

Therapeutic approaches



Silence

Activate

Edit

Replace

RNA based therapeutics

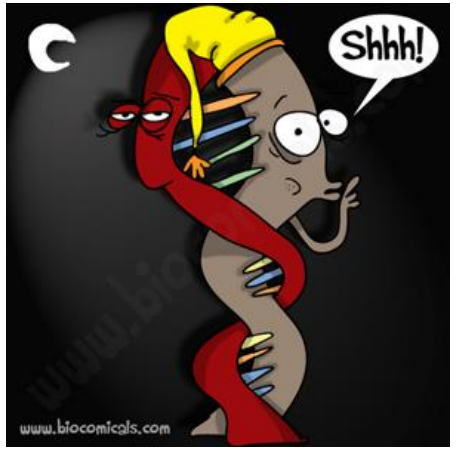
Silence

Activate

Edit

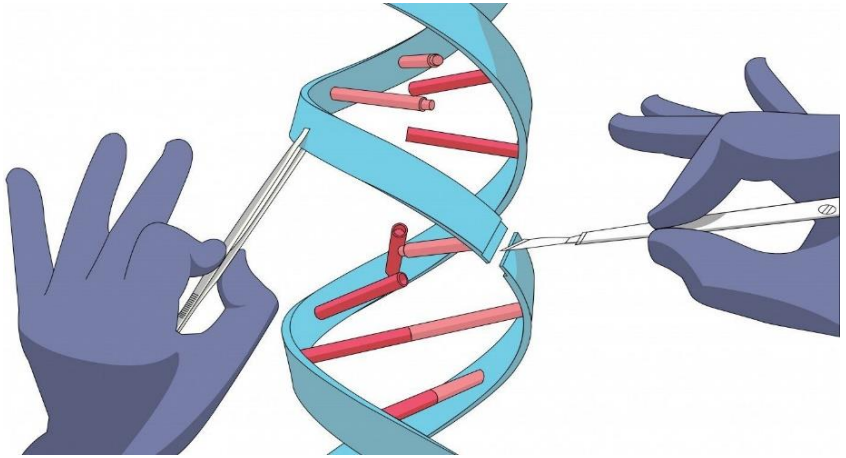
Replace

siRNA



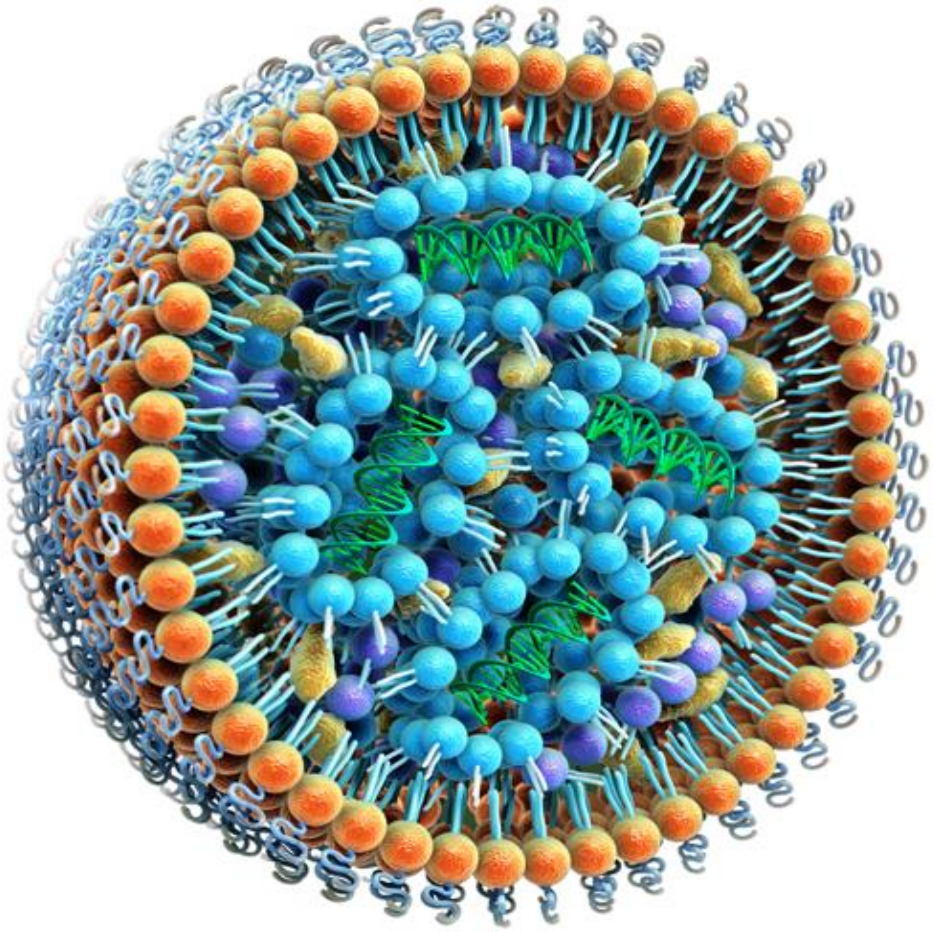
mRNA

Genome editing (e.g. CRISPR/Cas9)



The challenge in RNA therapeutics

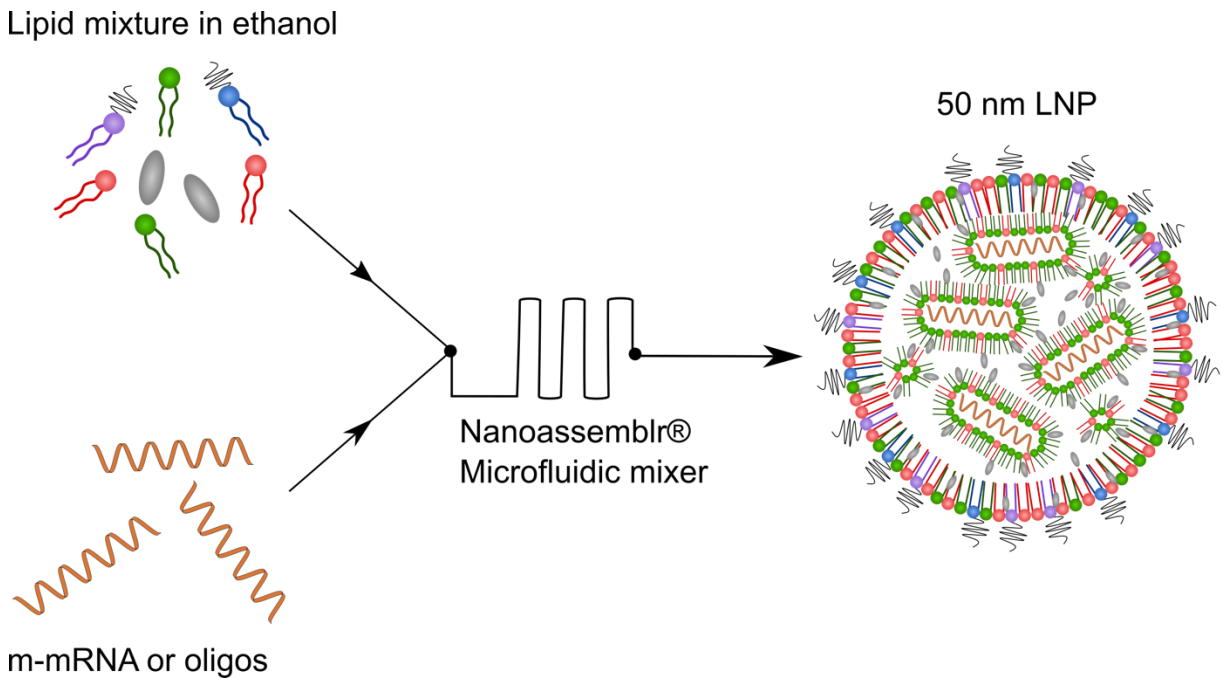
- An appropriate delivery system.
- Requirements:
  - Efficient RNA encapsulation
  - Evading clearance mechanism
  - Avoid toxicity and immune activation
  - Carrier internalization
  - RNA release
  - Specificity

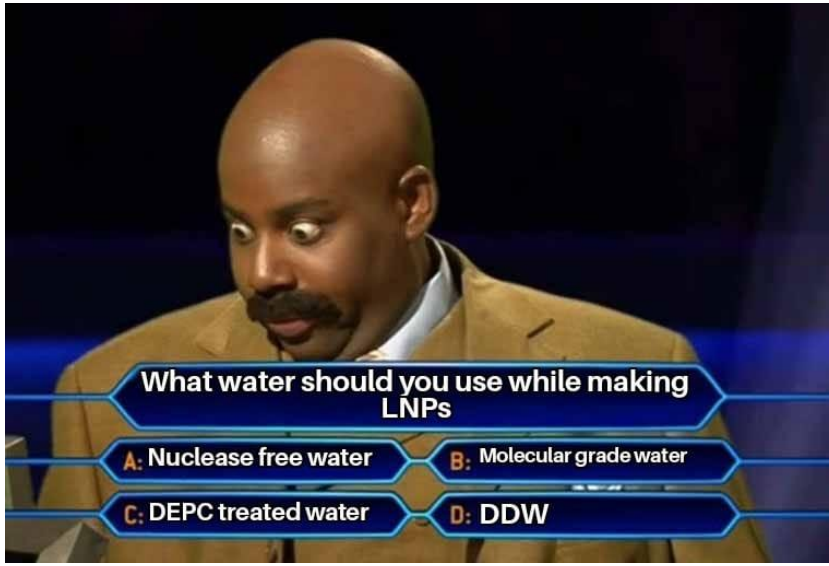


Lipid nanoparticles (LNPs)

Components:

- Cholesterol
- DSPC
- PEG-DMG
- Ionizable lipid



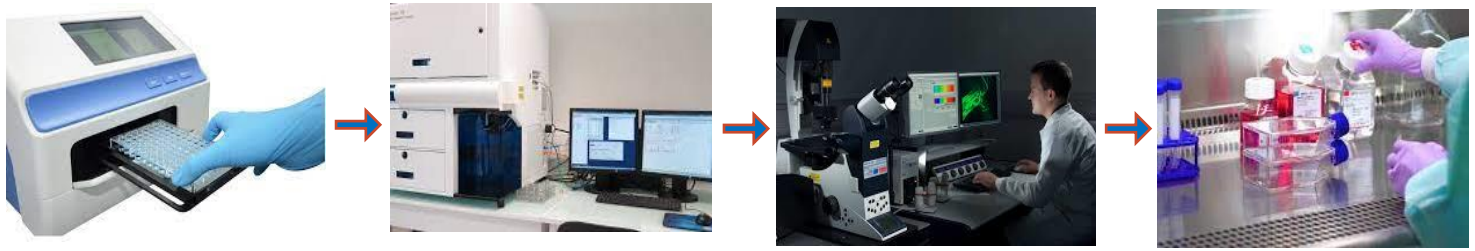


LNPs advantages:

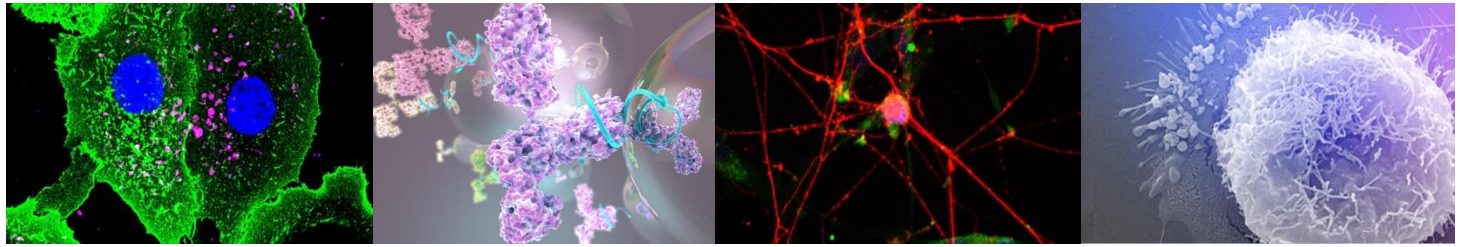
- Biocompatible
- Biodegradable
- Nucleic acid protection
- Increase stability in circulation
- Increase therapeutic effect
- Clinically approved

Laboratory of Precision NanoMedicine

Exome / Transcriptome analysis
Target Discovery & Validation



RNA Delivery systems (from lipid synthesis) to
Generation of mAbs & Protein Engineering

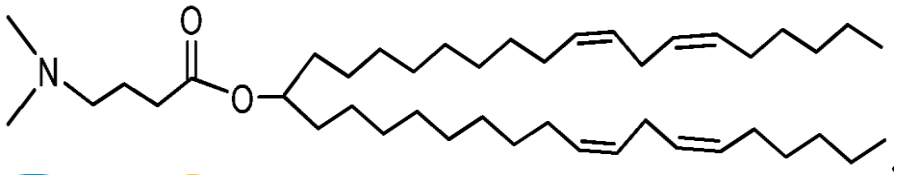


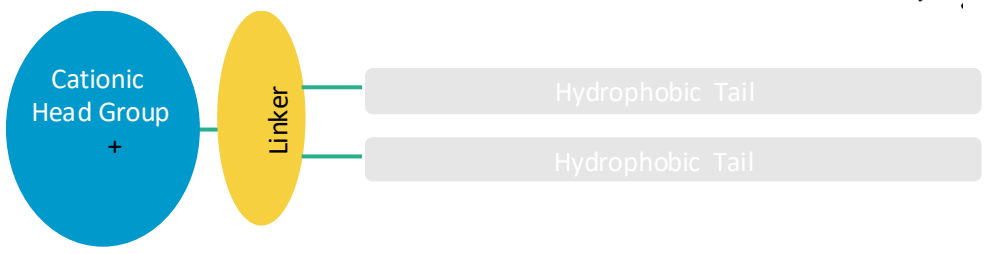
Tailor-made the appropriate nano-vehicle, selective targeting agent and specific therapeutic payload

Structural Design of Ionizable Lipids

Dr. Srinivas Ramishetti

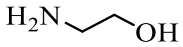
Dlin-MC3-DMA





NH_2-NH_2

NH_2-OH



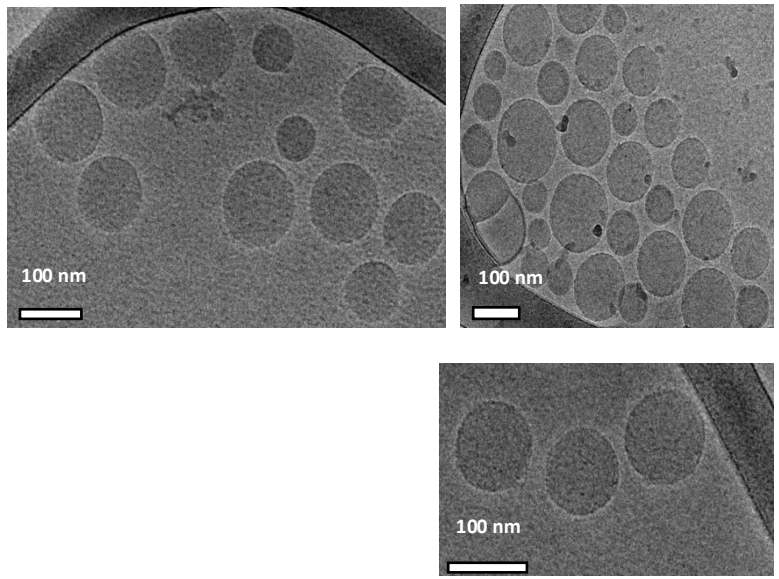
Based on the gold standard we have synthesized more than 50,000 lipids

Ramishetti S. et al. Advanced Materials 2020

LNPs Characterization

Formulation	Size (d.nm)	PDI	Zeta-potential (mV)
Dlin-MC3-DMA	44.5	0.18	-1
HZ-35	45.1	0.14	18.5
HZ-25	56.3	0.06	13.9
HZM-45	50.5	0.08	18.5
HZM-50	108.5	0.10	20.4
HZM-55	52.5	0.11	10.1
HZM-Im	46.0	0.15	-10.8
HZM-Pip	67.7	0.16	-2.8
HZM-Gly	52.8	0.07	-1.5
EA2	59.0	0.08	6.7
EA2-Pip	97.0	0.17	-1.28
EA-406	138.6	0.06	-1.82
HyAm-Gly	75.1	0.04	-13.6
HyAm-2	44.3	0.09	-3.6

Structural characterization of siRNA-EA2-LNPs by cryoTEM

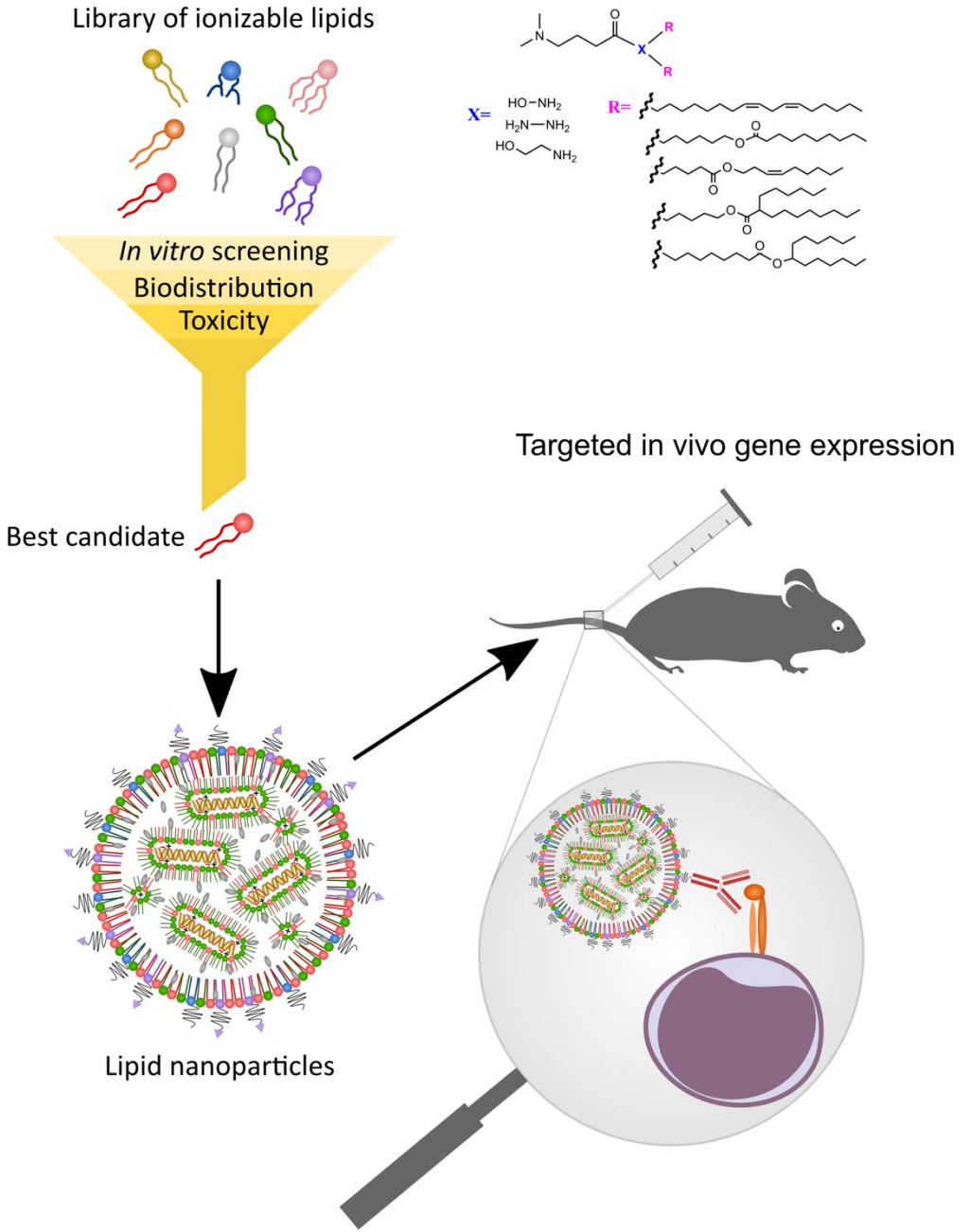


Ramishetti S. et al. Advanced Materials 2020

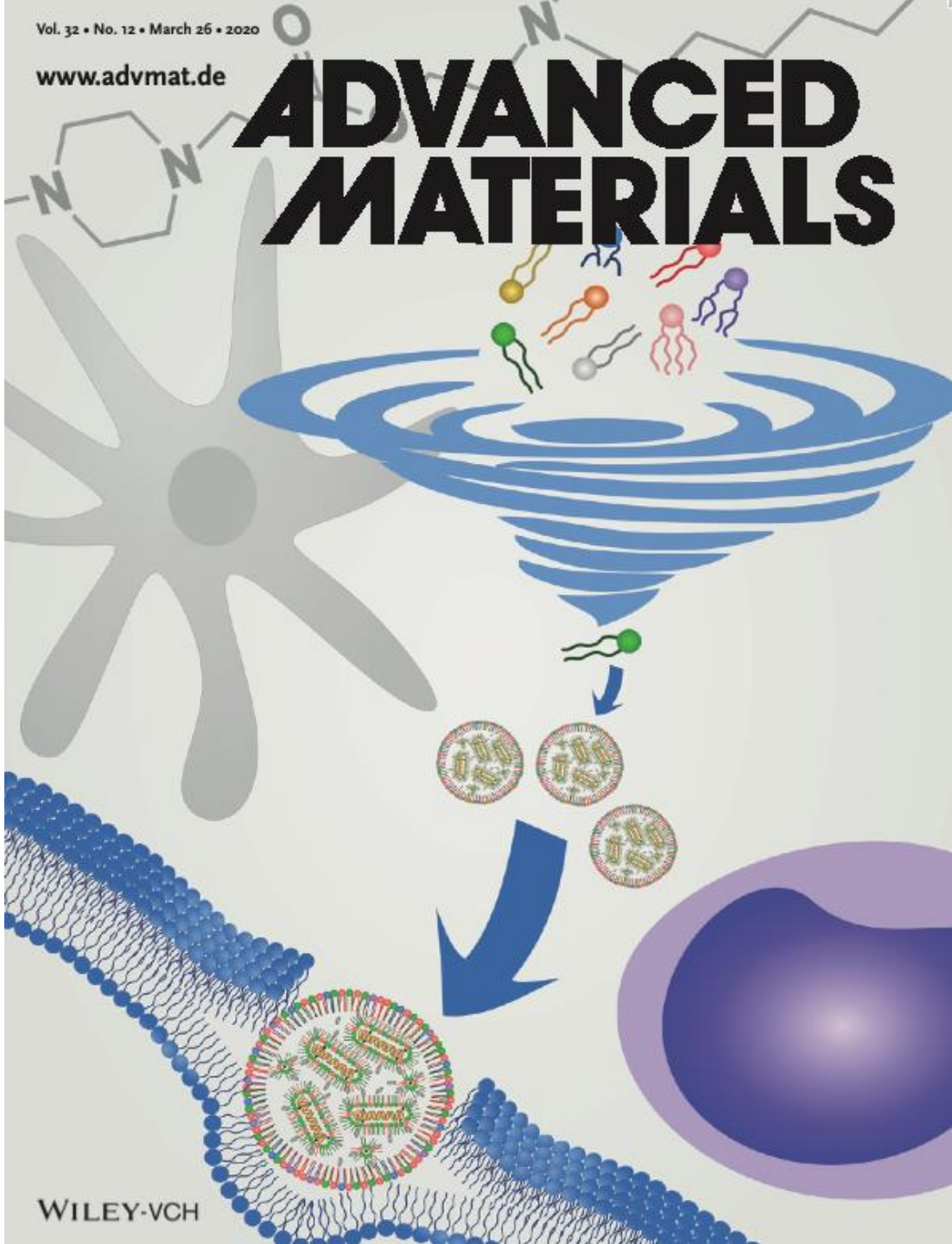
Dr. Inbal Hazan-Halevi

Dr. Srinivas Ramishetti

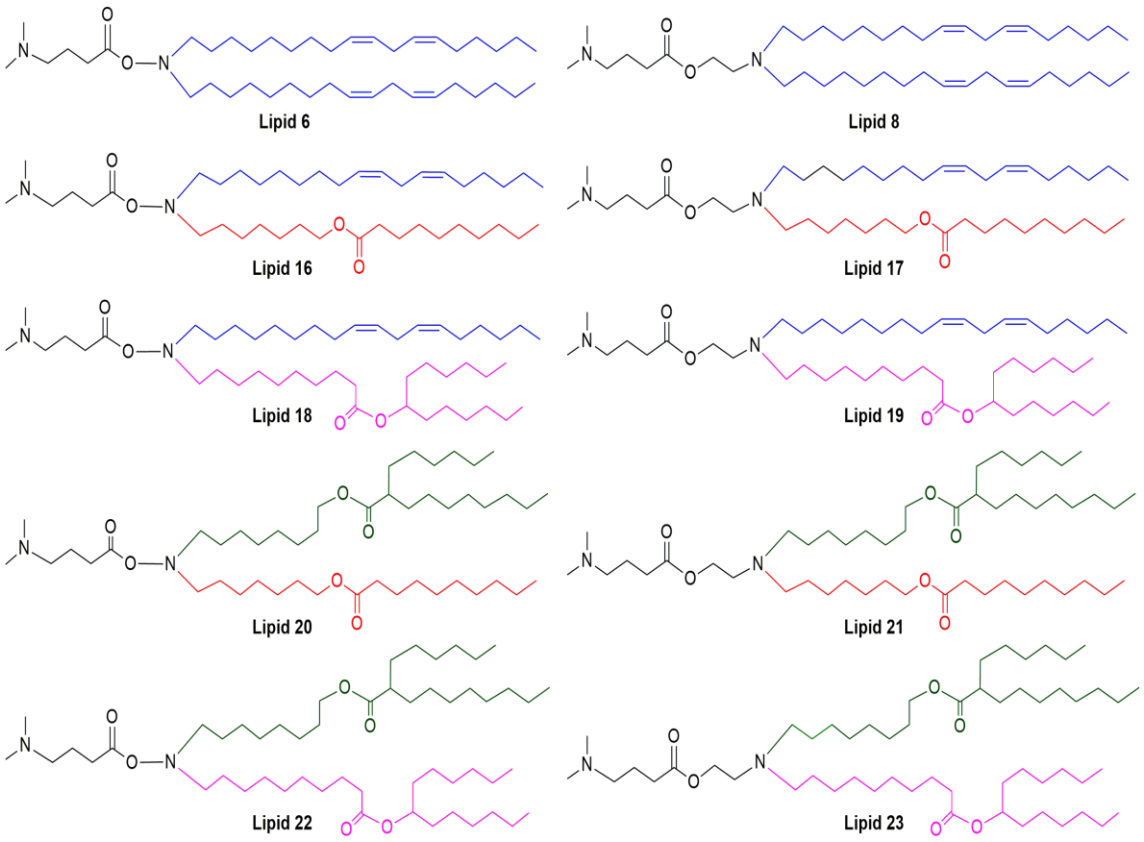
Dr. Somu Gonna Naidu



Ramishetti S. et al. Advanced Materials 2020

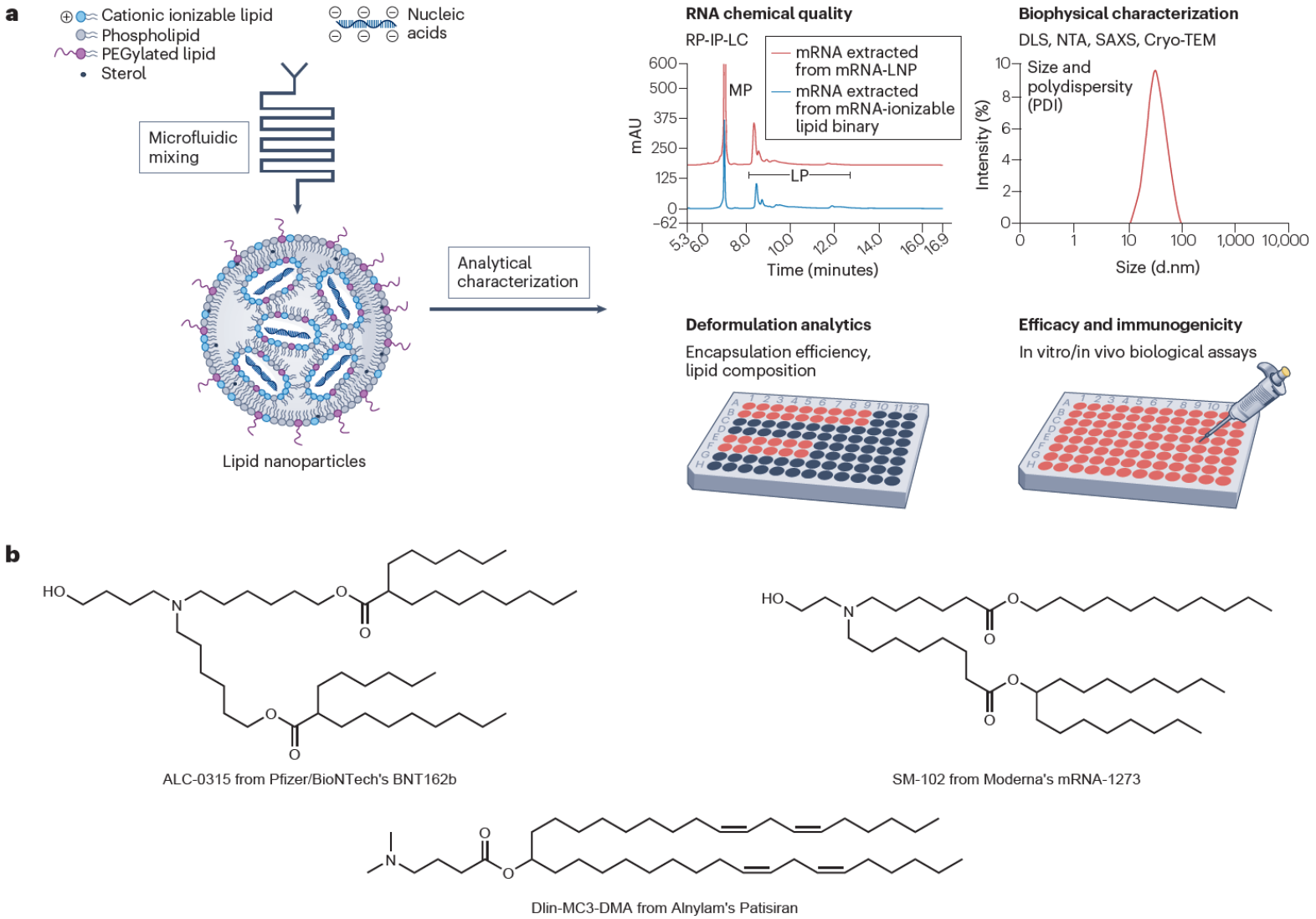


Dr. Somu Gonna Naidu

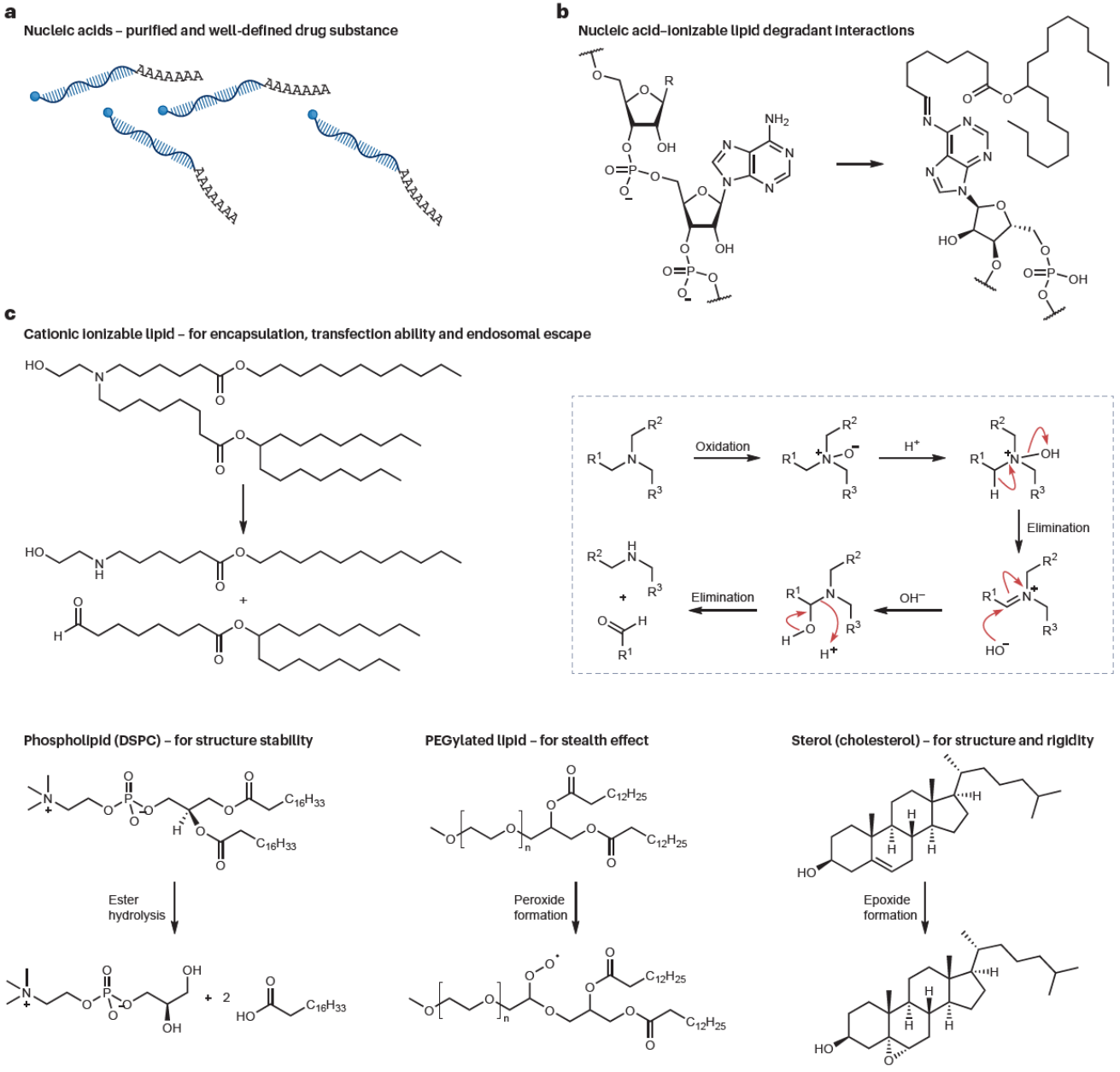


Naidu GS*, Yong S-B* et al. Advanced Science 2023

Analytics – the promise of improved formulations



Kovačič T. et al. Nature Reviews Chemistry 2025

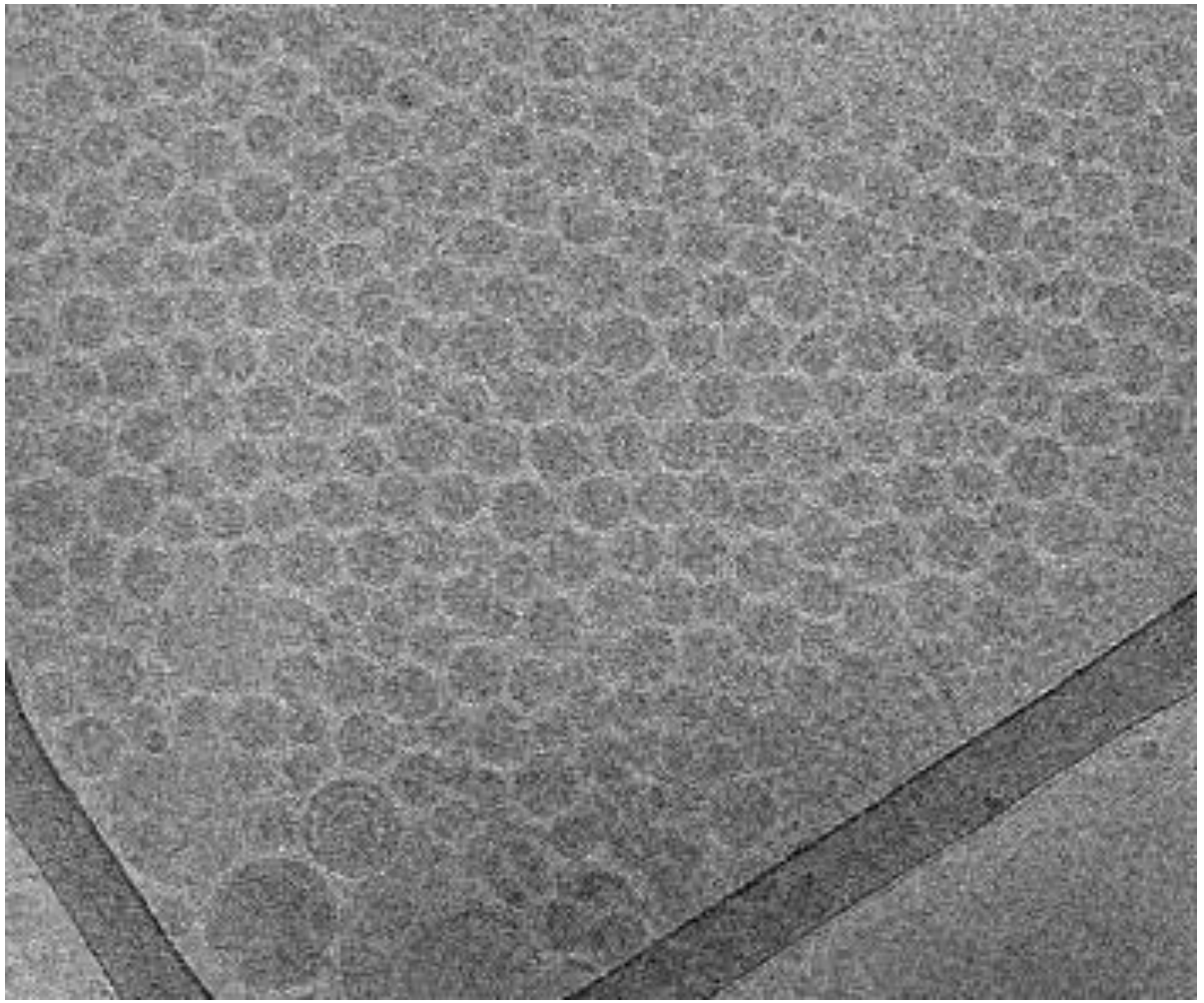


Kovačič T. et al. Nature Reviews Chemistry 2025


MRNA
VACCINE
MRNA
VACCINE

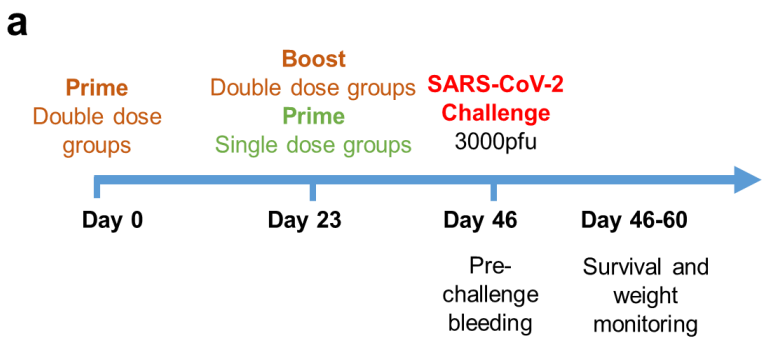
mRNA Vaccines

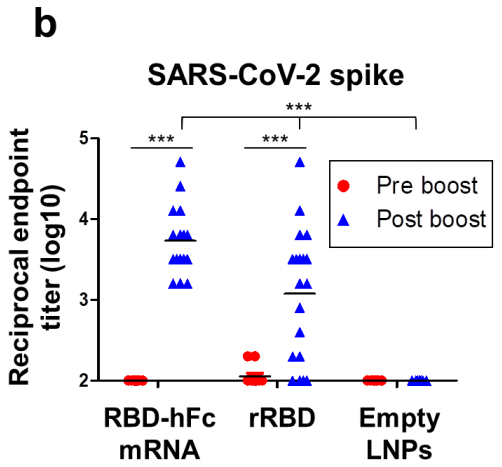
LNP RBD-hFc mRNA vaccine protects hACE2 trangenic mice against a lethal SARS-CoV-2 challenge .



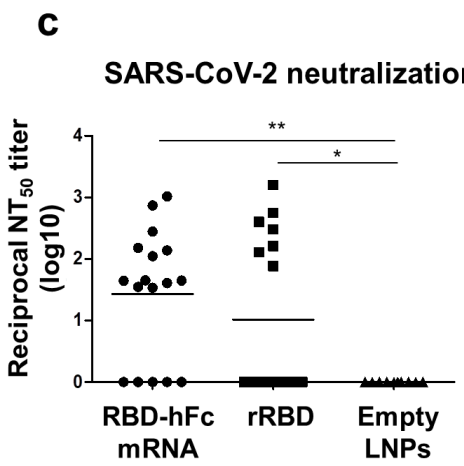
LNP RBD-hFc mRNA vaccine protects hACE2 trangenic mice against a lethal SARS-CoV-2 challenge

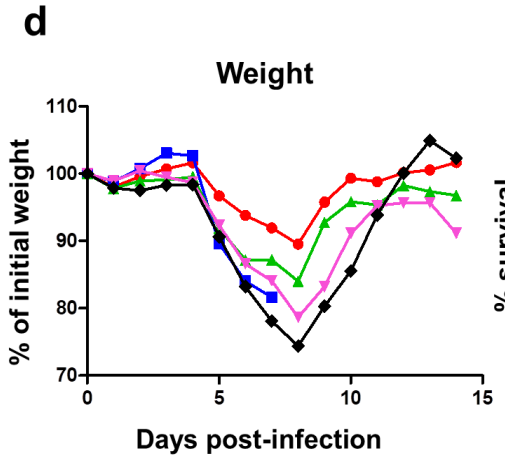
Dr. Uri Elia

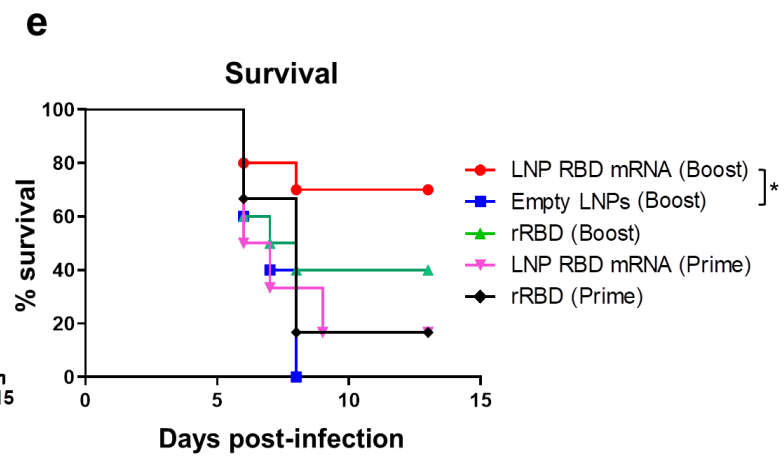




Collaboration – Dr. Ofer Cohen







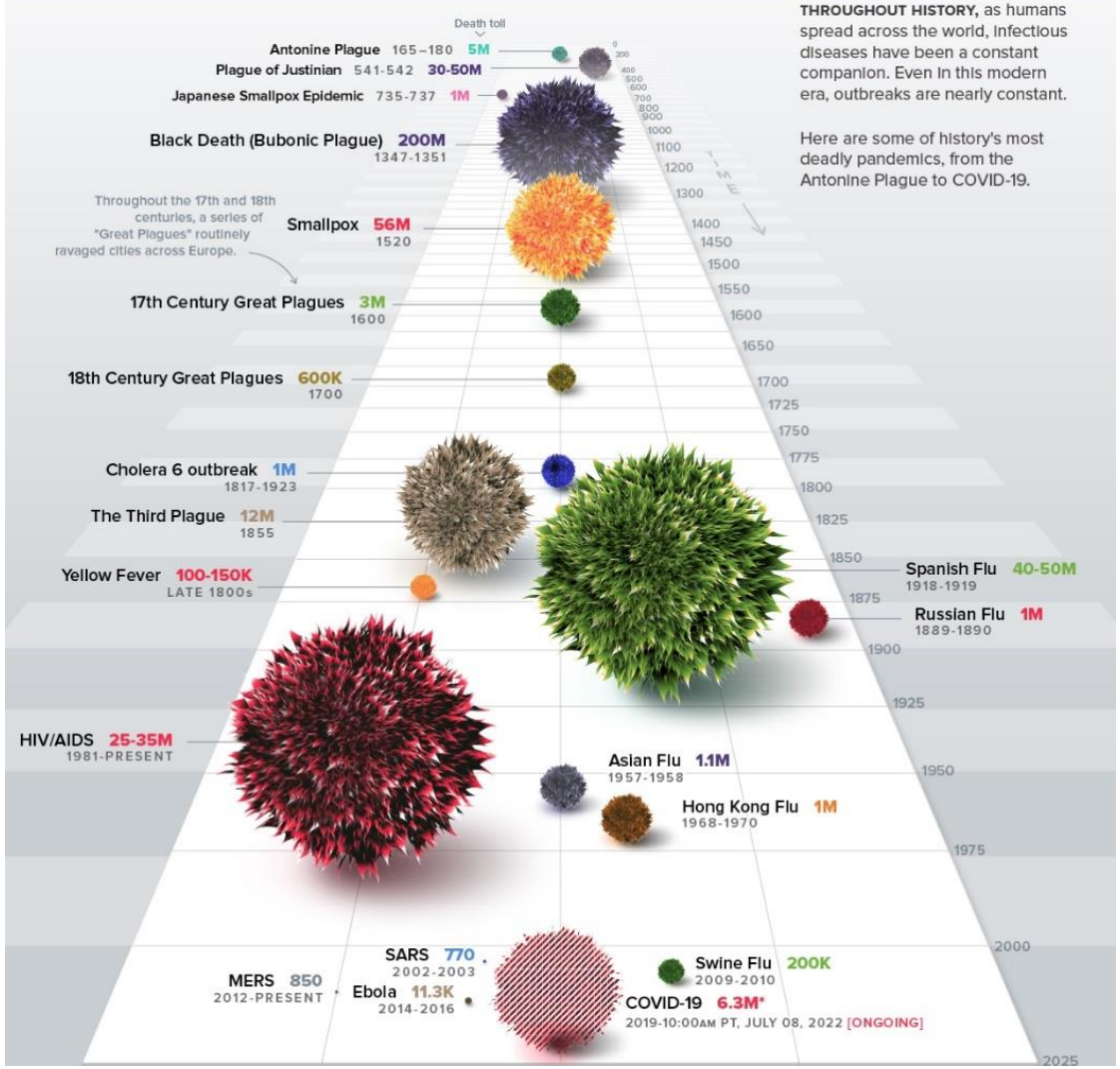
Elia U et al. ACS Nano 2021
Elia U. et al. Nano Letters 2021

HISTORY OF **PANDEMICS**

PAN·DEM·IC (of a disease) prevalent over a whole country or the world.

THROUGHOUT HISTORY, as humans spread across the world, infectious diseases have been a constant companion. Even in this modern era, outbreaks are nearly constant.

Here are some of history's most deadly pandemics, from the Antonine Plague to COVID-19.

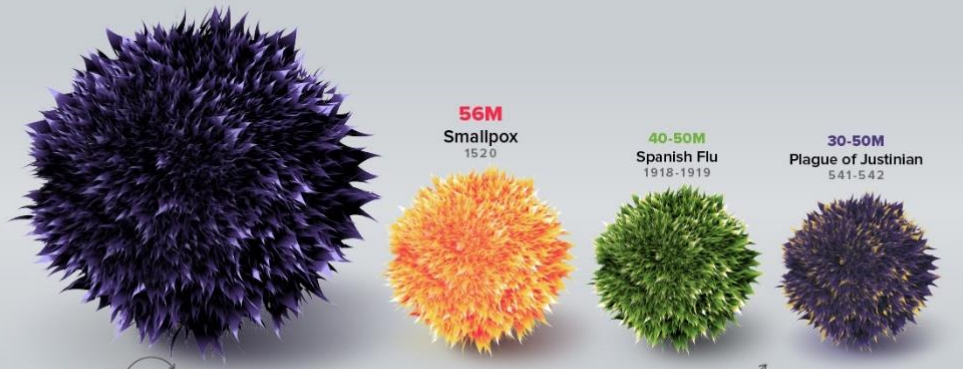


WHO officially declared COVID-19 a pandemic on Mar 11, 2020.

It is hard to calculate and forecast the impact of COVID-19 because the disease is new to medicine, and data is still coming in.

*Johns Hopkins University estimates

DEATH TOLL
[HIGHEST TO LOWEST]



The plague originated in rats and spread to humans via infected fleas.

The outbreak wiped out 30-50% of Europe's population. It took more than 200 years for the continent's population to recover.

Smallpox killed an estimated 90% of Native Americans. In Europe during the 1800s, an estimated 400,000 people were being killed by smallpox annually. The first ever vaccine was created to ward off smallpox.

The death toll of this plague is still under debate as new evidence is uncovered, but many think it may have helped hasten the fall of the Roman Empire.

25-35M HIV/AIDS 1981-PRESENT
12M The Third Plague 1855
6.3M* COVID-19 2019-10:00AM PT, JULY 08, 2022 [ONGOING]
5M Antonine Plague 165-180
3M 17th Century Great Plagues 1600
1.1M Asian Flu 1957-1958
1M Russian Flu 1889-1890
1M Hong Kong Flu 1968-1970

1M Cholera 6 outbreak 1817-1923
1M Japanese Smallpox Epidemic 735-737
600K 18th Century Great Plagues 1700
200K Swine Flu 2009-2010
100-150K Yellow Fever LATE 1800s
11.3K Ebola 2014-2016
850 MERS 2012-PRESENT
770 SARS 2002-2003

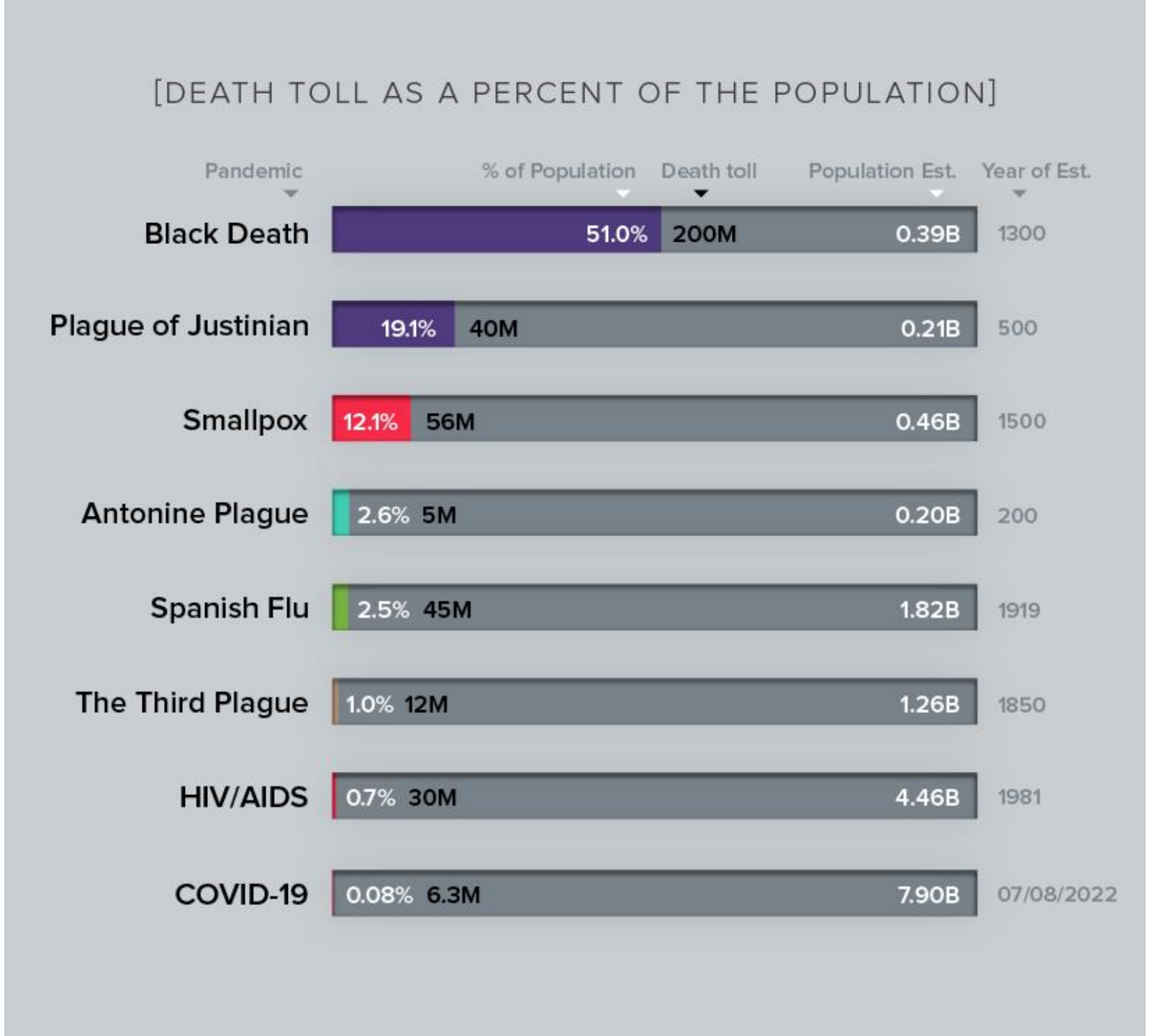
*Johns Hopkins University estimates

VISUAL CAPITALIST

Sources:
CDC, WHO, BBC, Wikipedia, Historical records, Encyclopedia Britannica Johns Hopkins University

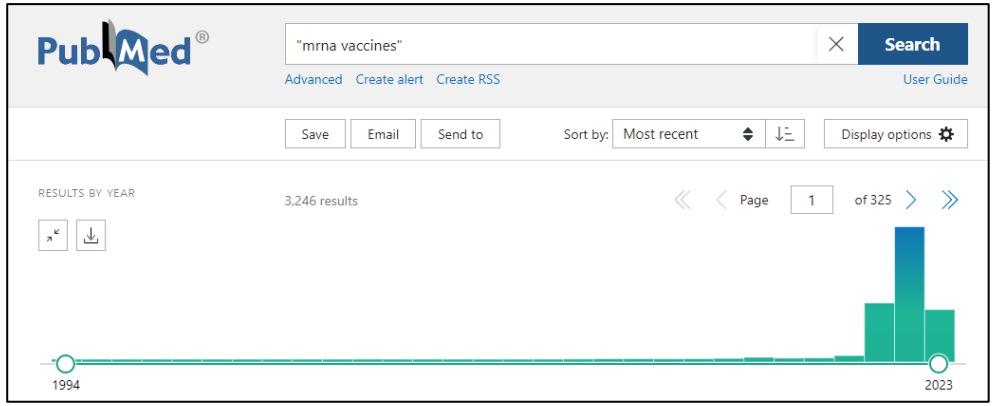
/visualcapitalist @visualcap visualcapitalist.com

COLLABORATORS RESEARCH + WRITING Nick LePan, Nick Routley | DESIGN Harrison Schell | ART DIRECTION Harrison Schell

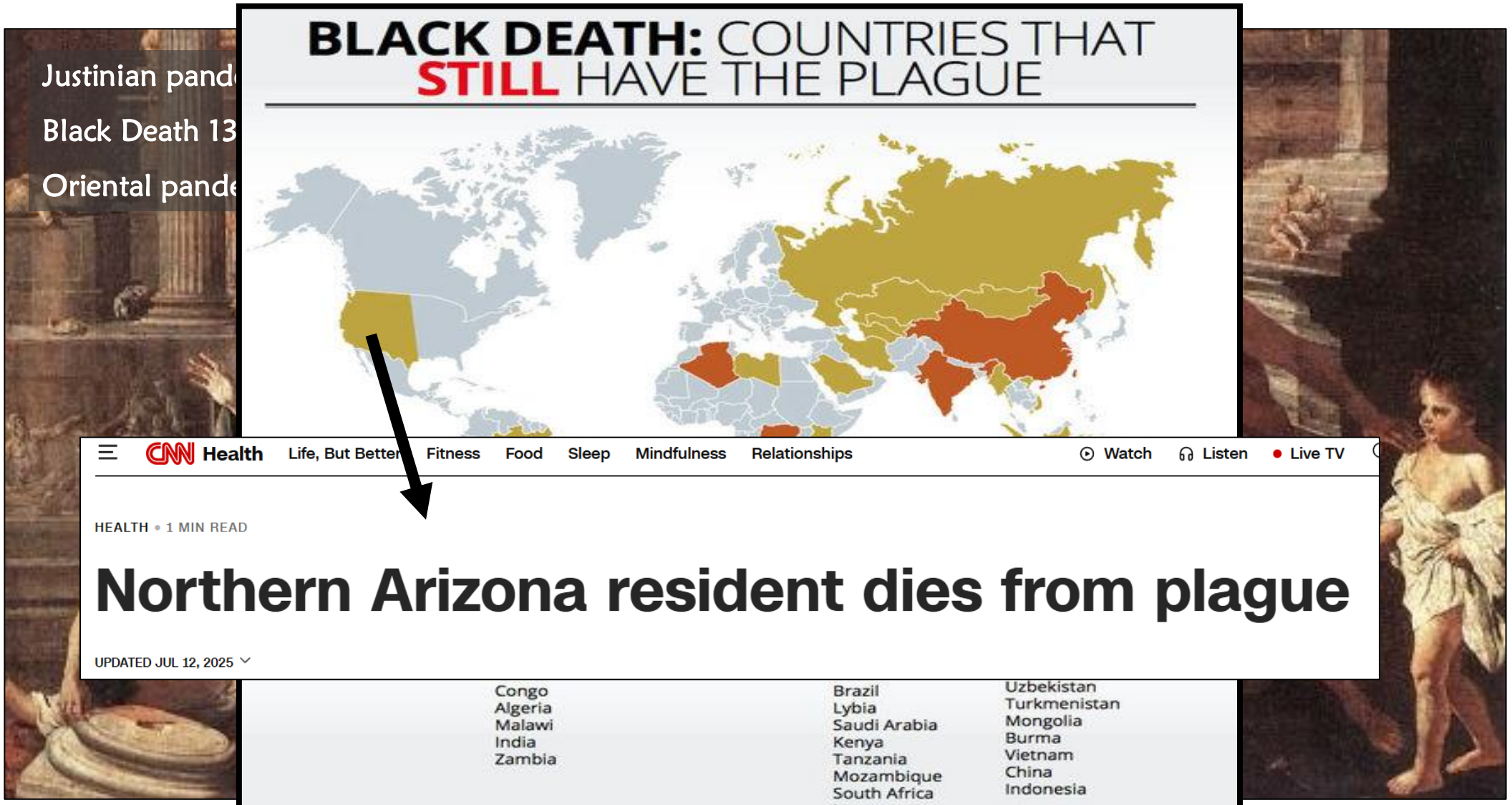

[DEATH TOLL AS A PERCENT OF THE POPULATION]
Pandemic
% of Population
Death toll
Population Est.
Year of Est.
Black Death
51.0%
200M
0.39B
1300
Plague of Justinian
19.1%
40M
0.21B
500
Smallpox
12.1%
56M
0.46B
1500
Antonine Plague
2.6%
5M
0.20B
200
Spanish Flu
2.5%
45M
1.82B
1919
The Third Plague
1.0%
12M
1.26B
1850
HIV/AIDS
0.7%
30M
4.46B
1981
COVID-19
0.08%
6.3M
7.90B
07/08/2022

mRNA vaccines against bacterial pathogens

- “Gold rush” for mRNA vaccines
- Mostly against viral pathogens and cancer
- Very limited data on the platform’s applicability against bacteria
- Antibiotic resistance necessitates alternative countermeasures



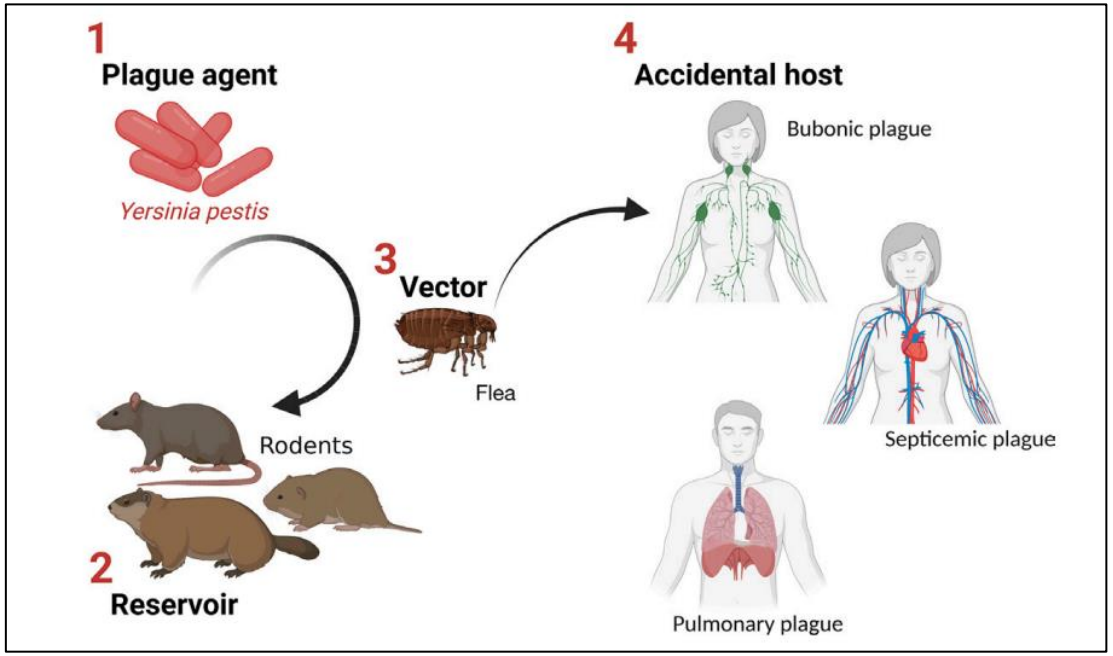
Plague Outbreaks

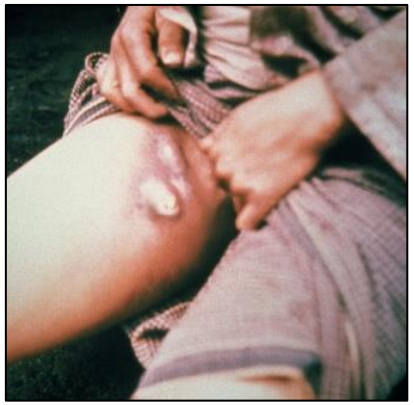


The Plague in Ashdod, Nicolas Poussin. Musée du Louvre, Paris, France.

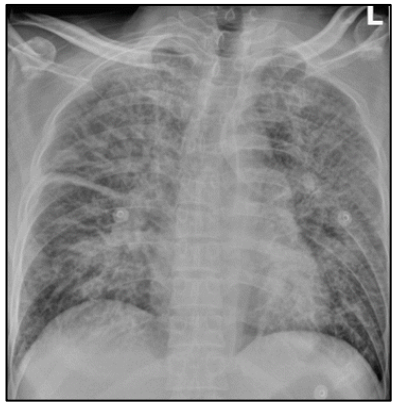
Yersinia pestis

- The etiological agent of plague
- Gram-negative bacterium
- **Highly infectious and lethal**
- Millions of deaths throughout history, several hundreds annually
- **No approved vaccine**
- Can be treated with antibiotics
- **Antibiotic-resistant strains**
- **Potential bioweapon (Tier 1 select agent)**
- **Extensively studied at the IIBR**

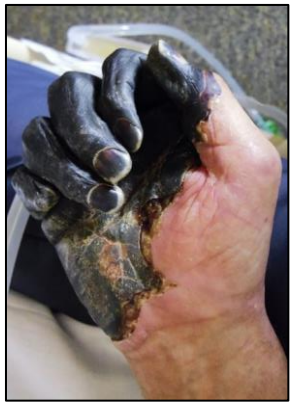




Bubonic



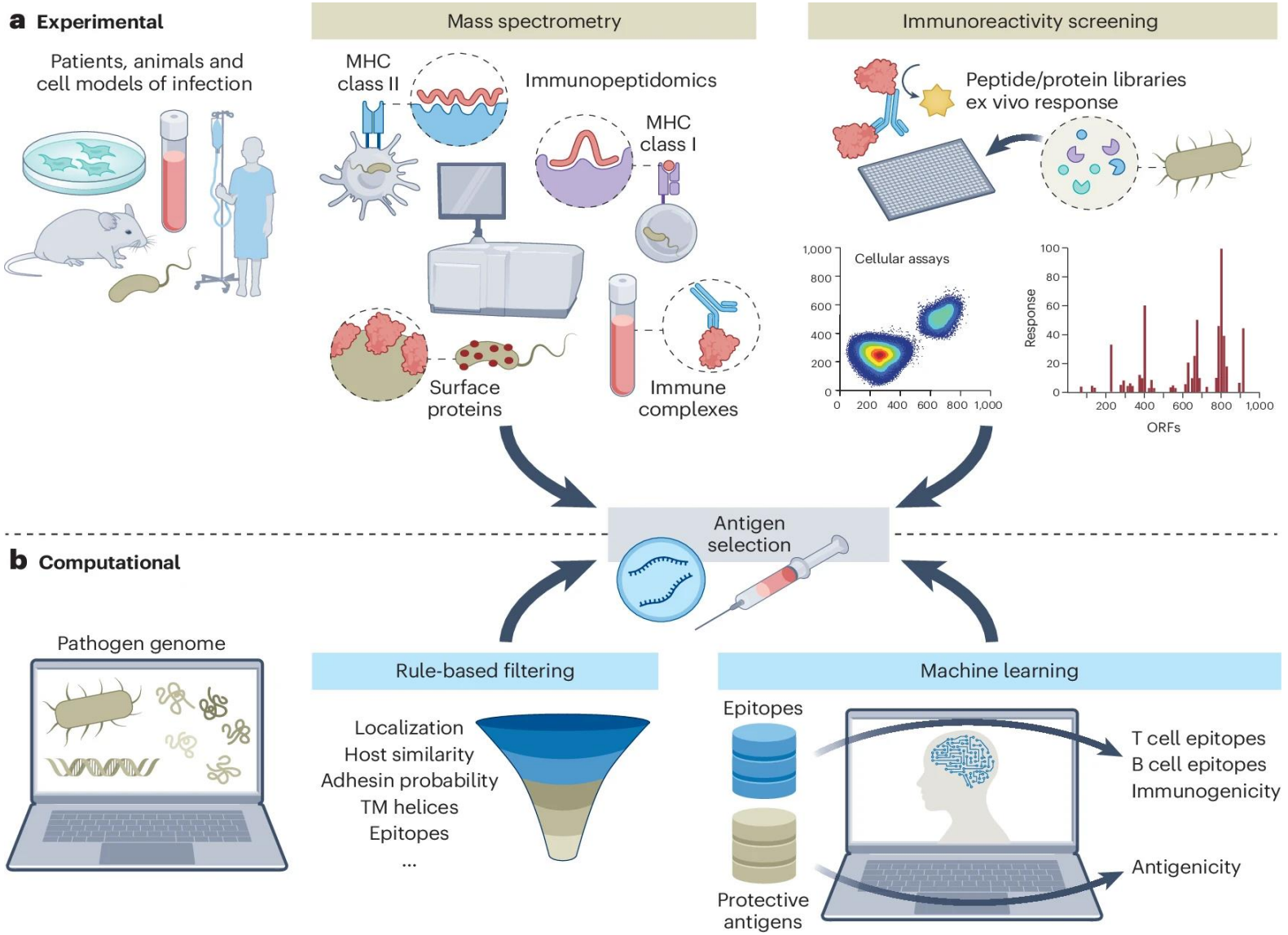
Pneumonic



Septicemic

Guillem Mas Fiol et al. The Biochemist, 2023, 45 (2), pp.1-5. ff10.1042/bio_2023_109ff. ffpasteur-04087871f

Antigen discovery methods in bacterial vaccine development



Aernout I et al. Nature Microbiology 2025

Could mRNA Vaccines Do the Job?

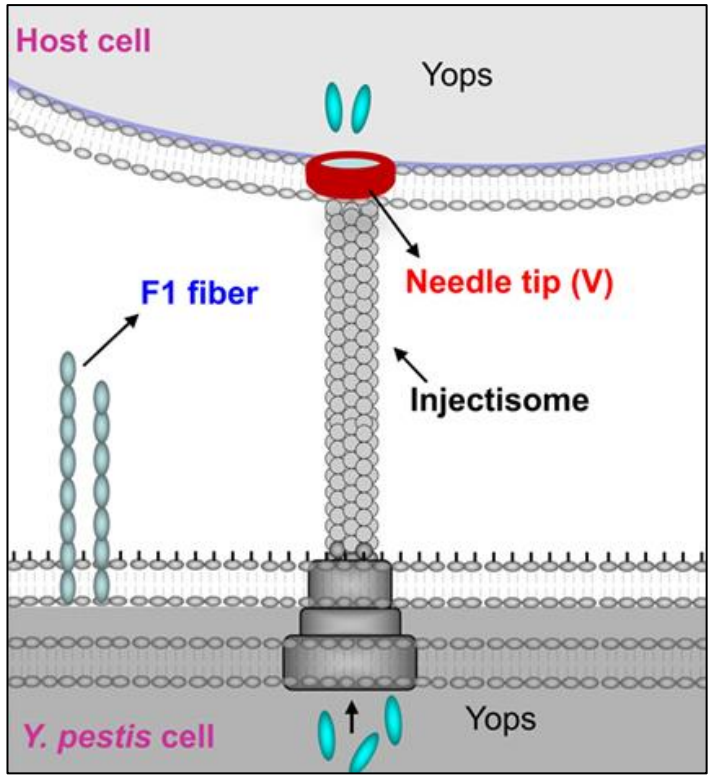
- Non-infectious
- Non-integrating
- Induction of both humoral and cellular immune responses
- Cell- and animal-free, rapid production
- Potentially cheap(er)

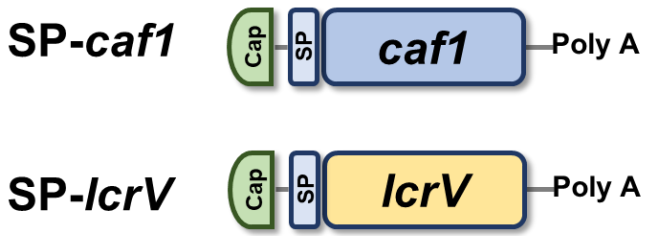
Treating a 14th century pathogen with 21st century technology

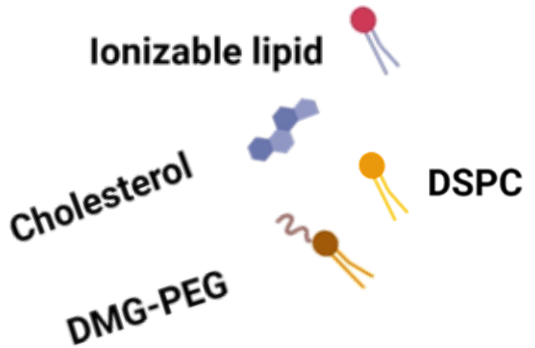
mRNA Design and LNP Formulation

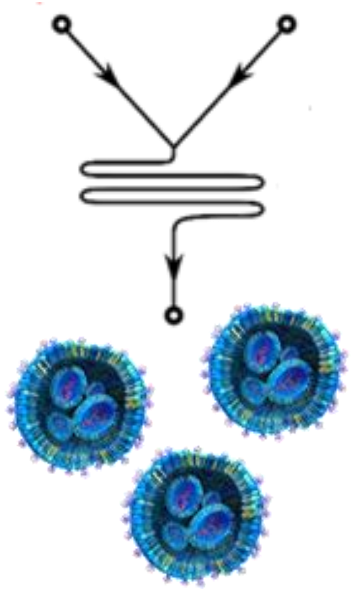
F1 (*caf1*) and LcrV antigens were chosen for vaccination study

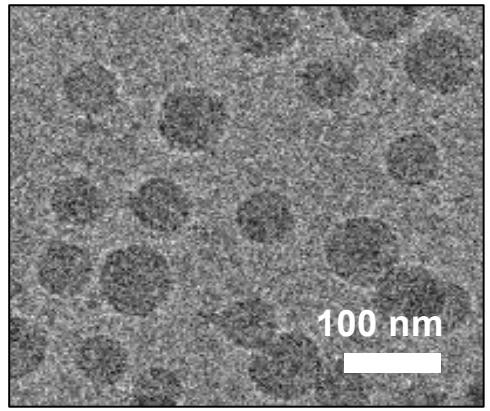
Signal peptide (SP) added for secretion





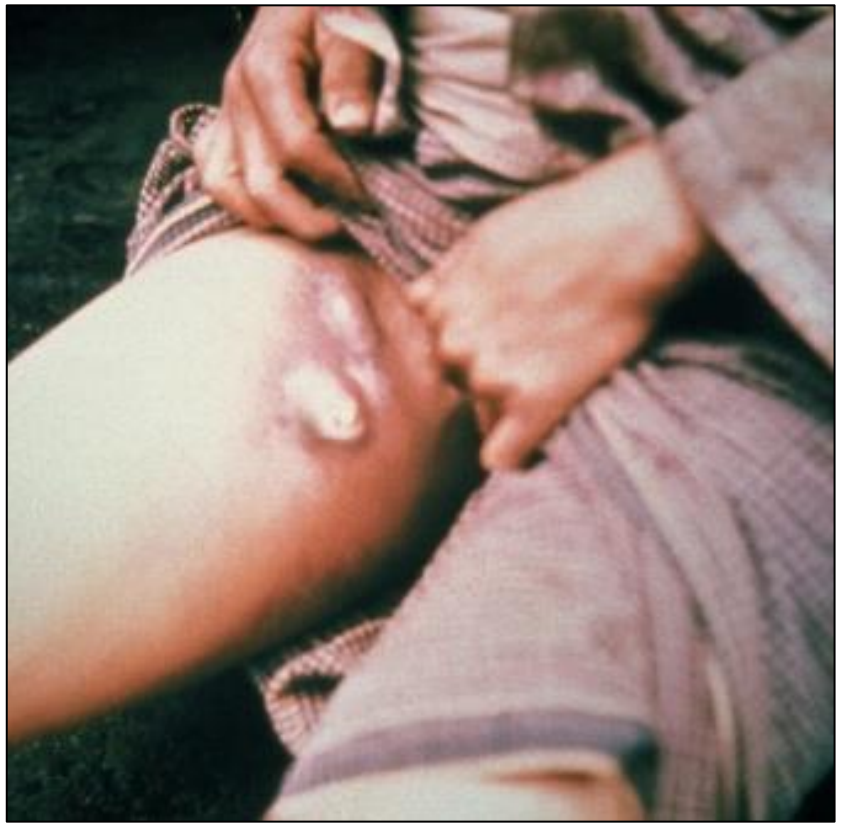






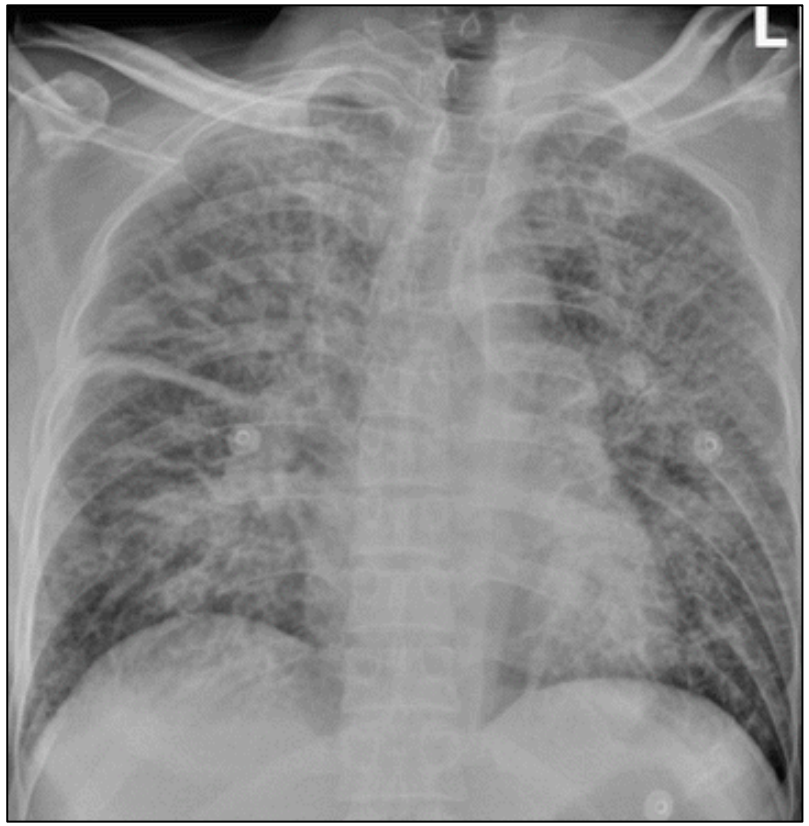
Kon et al., Science Advances (2023)

Animal Models



Bubonic Plague

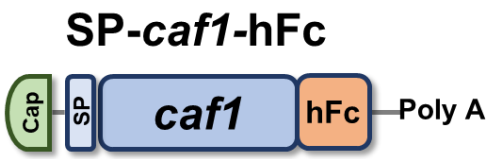
Subcutaneous injection

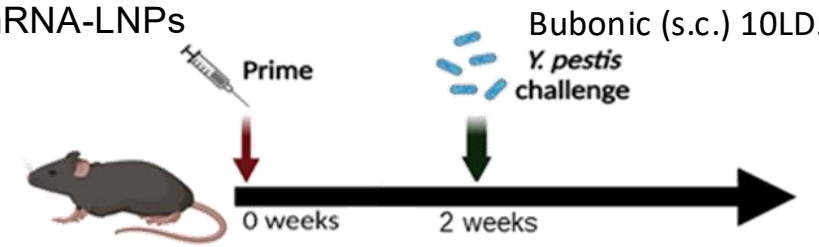


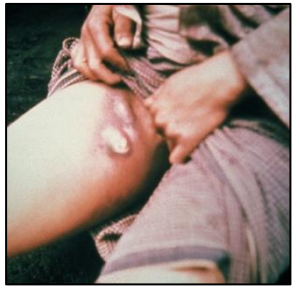
Pneumonic Plague

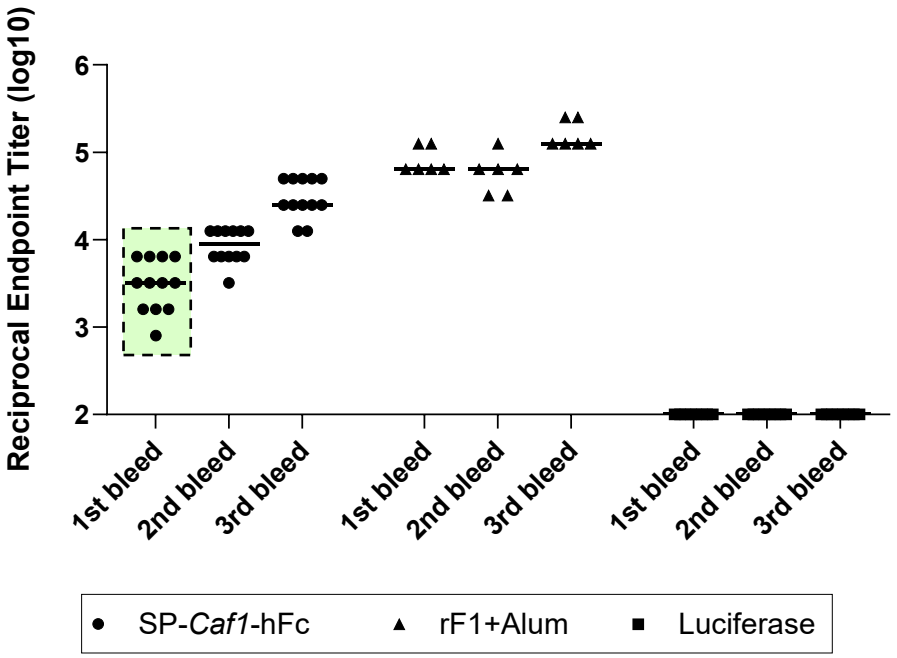
Intranasal administration

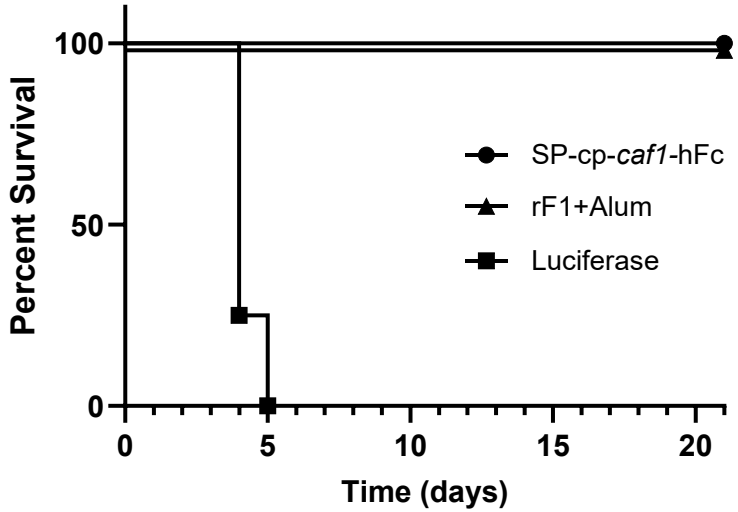
Y. pestis LNP-mRNA Vaccine – Bubonic Plague
Full protection after a single dose







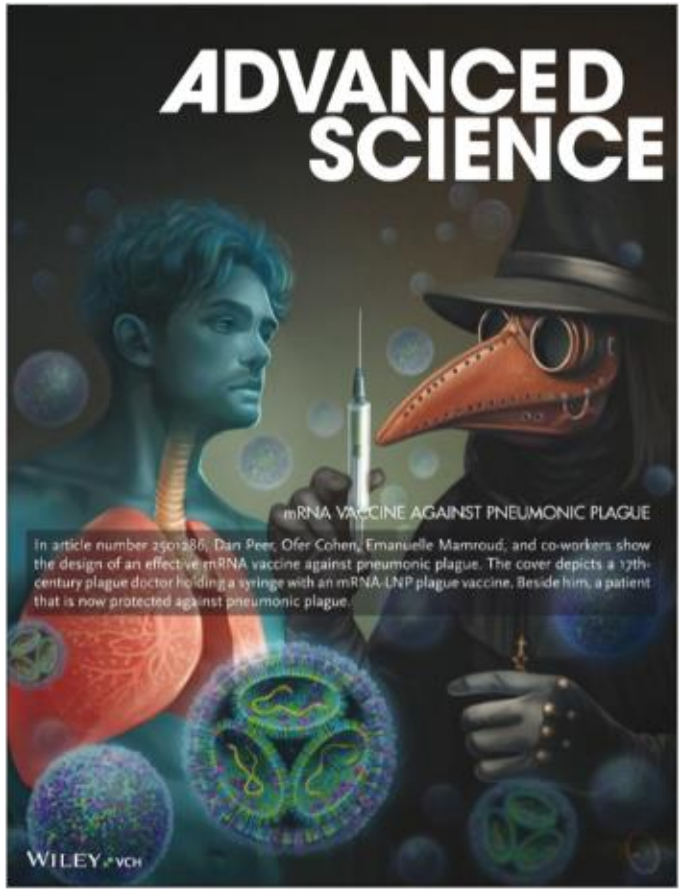


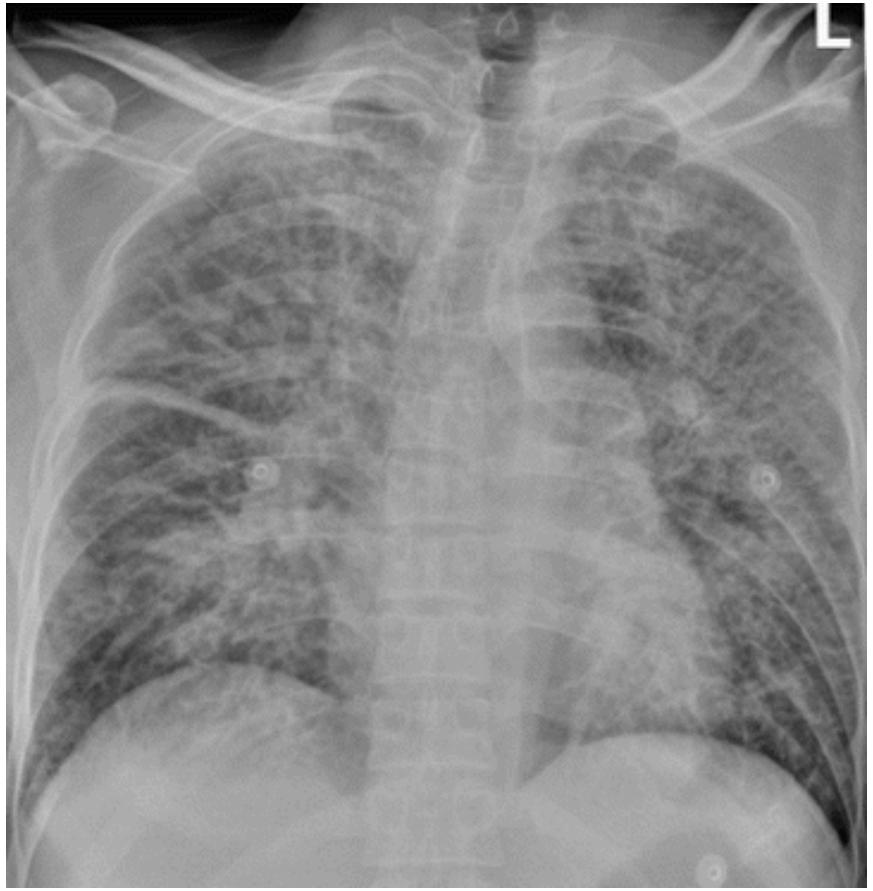


Ab titers correlate well with likelihood of survival
Full protection after single dose of F1-Fc LNP-mRNA

Kon, Levy , Elia *et al., Science Advances* (2023)

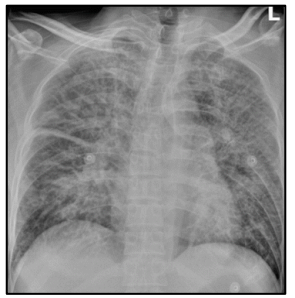
Pneumonic Plague





Y. pestis LNP-mRNA Vaccine – Pneumonic Plague

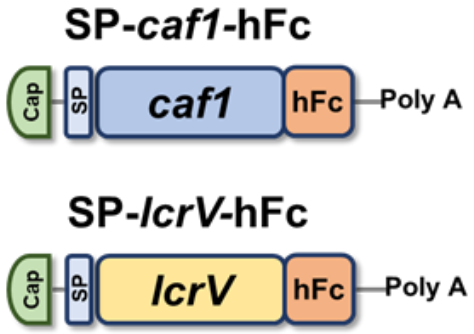
Challenges in vaccine development

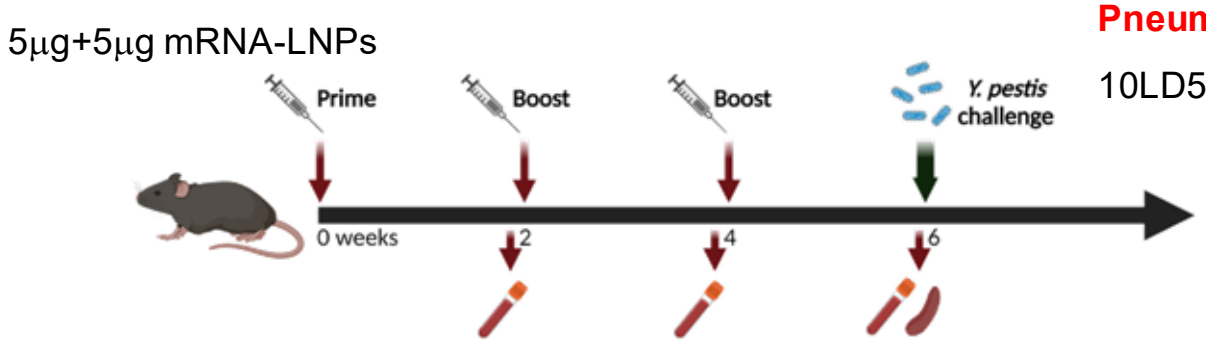


- Severe disease, harder to protect
- A combination of antigens is required for protection
- Vaccine must be effective against F1-devoid strains

Y. pestis LNP-mRNA Vaccine - Pneumonic Plague

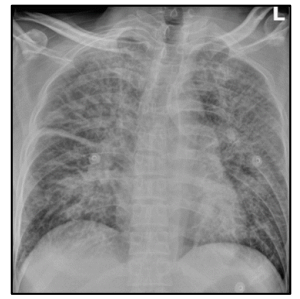
A bivalent F1+LcrV mRNA vaccine

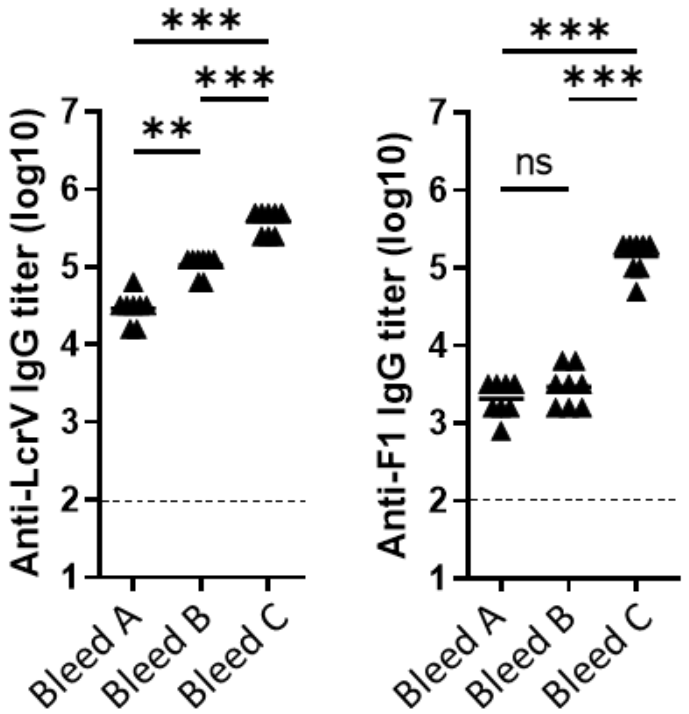


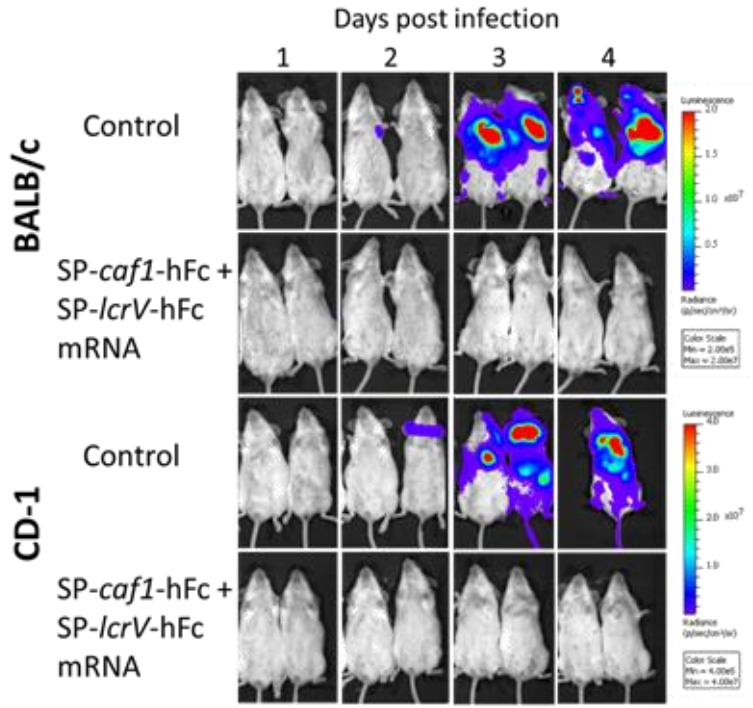


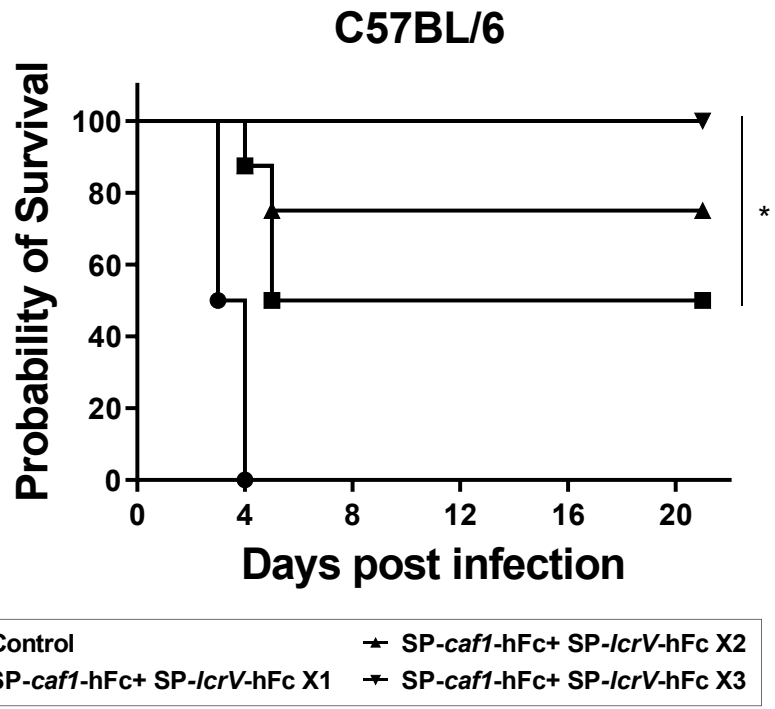
Pneumonic (i.n.)

10LD50





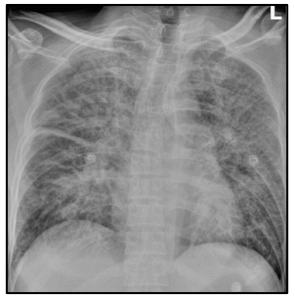


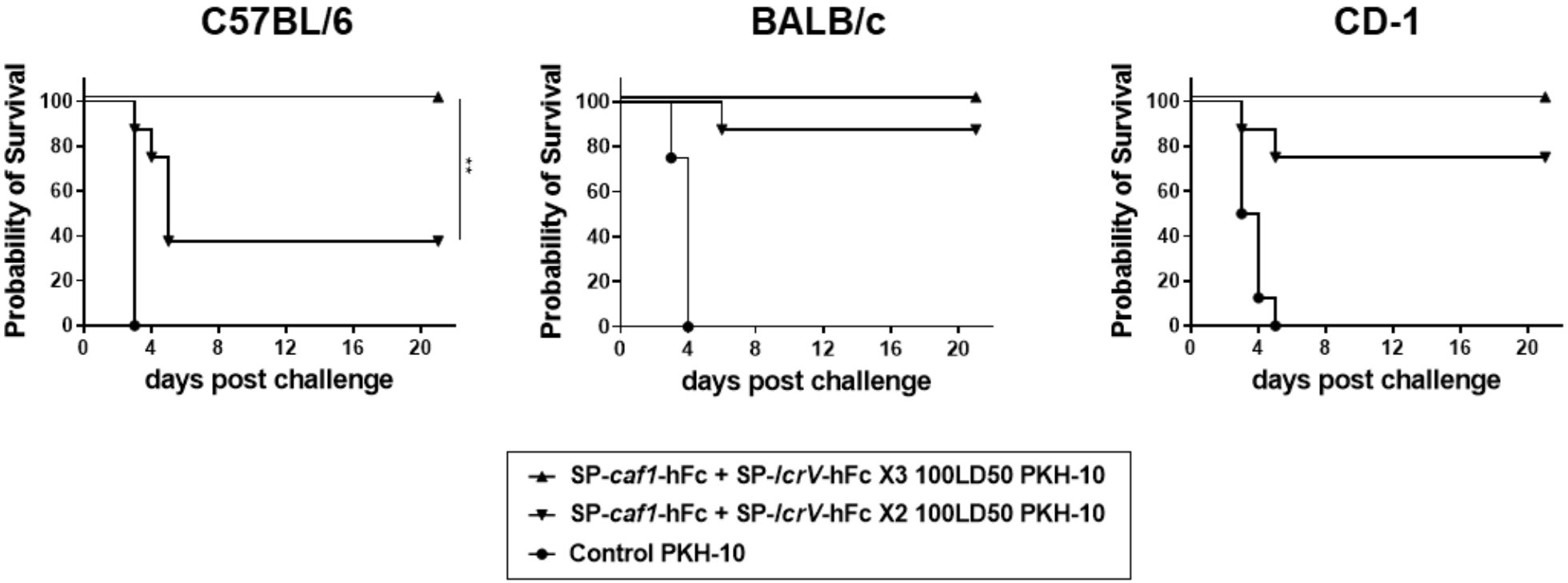


Elia, Levy et al., Advanced Science (2025)

Y. pestis LNP-mRNA Vaccine - Pneumonic Plague

Protection against high dose (100LD50) challenge





Robust protection against 100LD50 (~100,000 cfu) infection across 3 mouse strains

Elia, Levy et al., Advanced Science (2025)

Next Generation LNP Platform

for Precision RNA Delivery

NeoVac

NeoVac's Modular Lipid Architecture Creates Superior LNPs

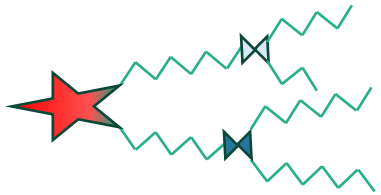
NeoVac

NeoVac's lipid library reaches beyond standard lipid structures, supporting improved LNP tailoring for diverse uses

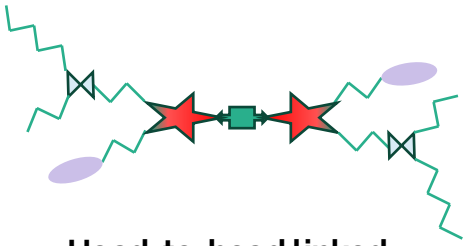
Building Blocks

- Ionizable center
- Hydrophobic tail
- Hydrophilic moiety
- Scaffold
- Cleavable Element

Novel Lipid Structures

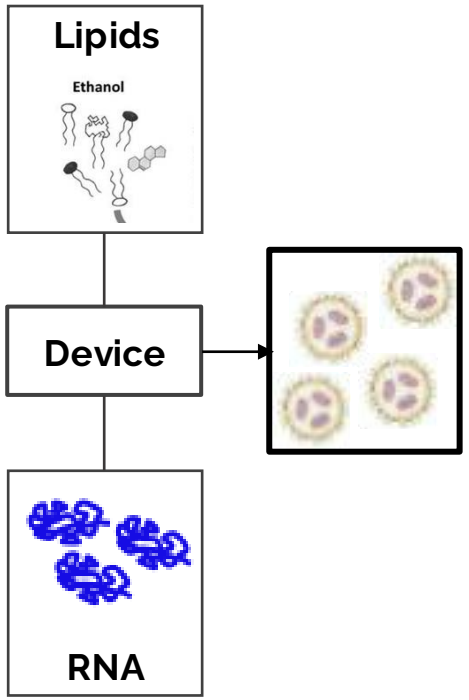


Double branched, staggered cleavibility

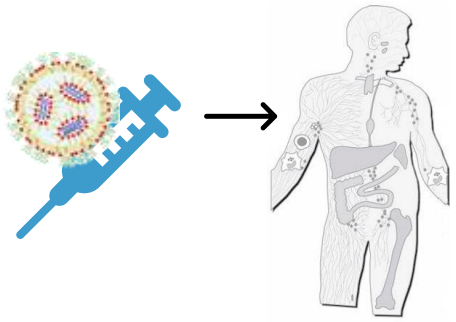


Head-to-head linked dendrimer inspired

Dedicated LNP Technologies



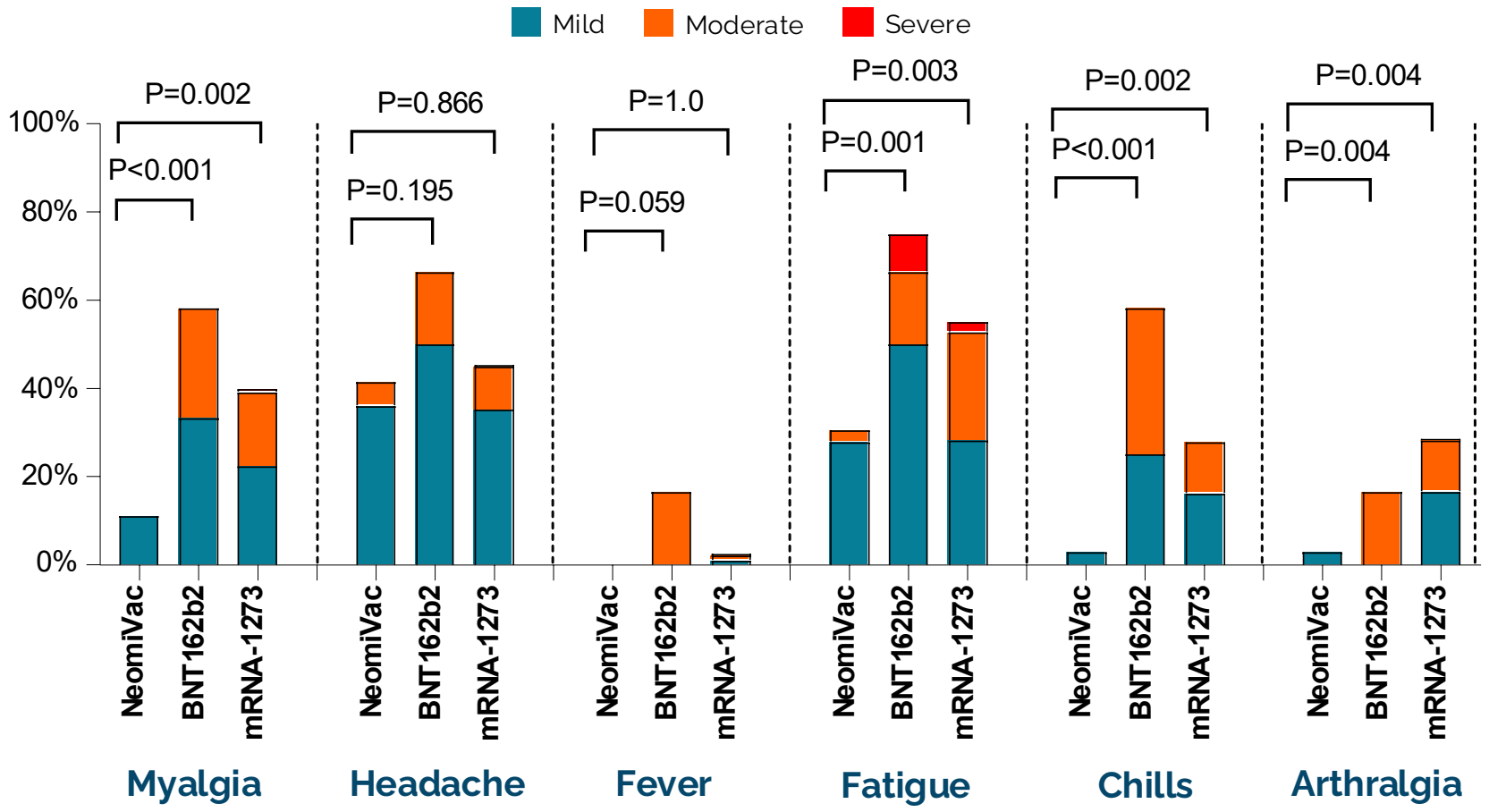
Improved mRNA Pharmaceuticals



- Superior Safety
- Tunable Immunogenicity
- Organ-specific Targeting
- Controlled Biodegradability

Superior Safety – Human Clinical Data

Phase I / II clinical study showed both numerically fewer and less severe side effects compared to marketed products



Fewer

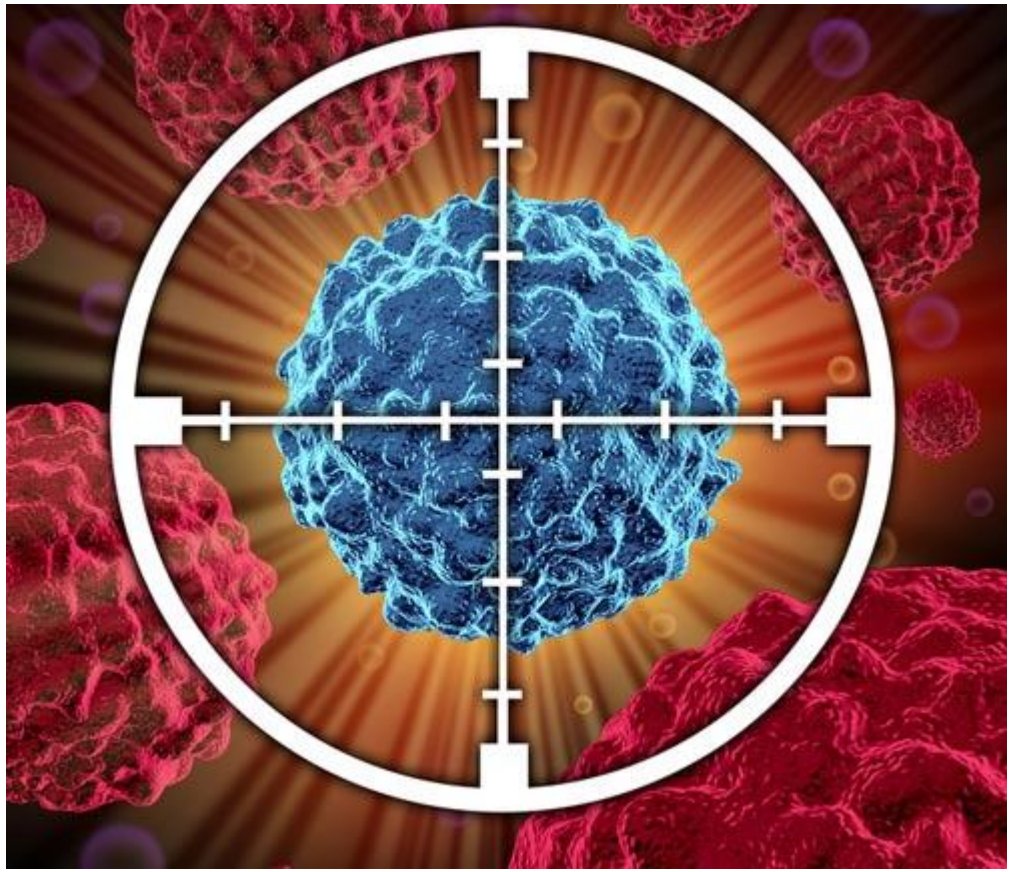
NeomiVac shows significantly lower number of adverse events compared to other mRNA vaccines

Less Severe

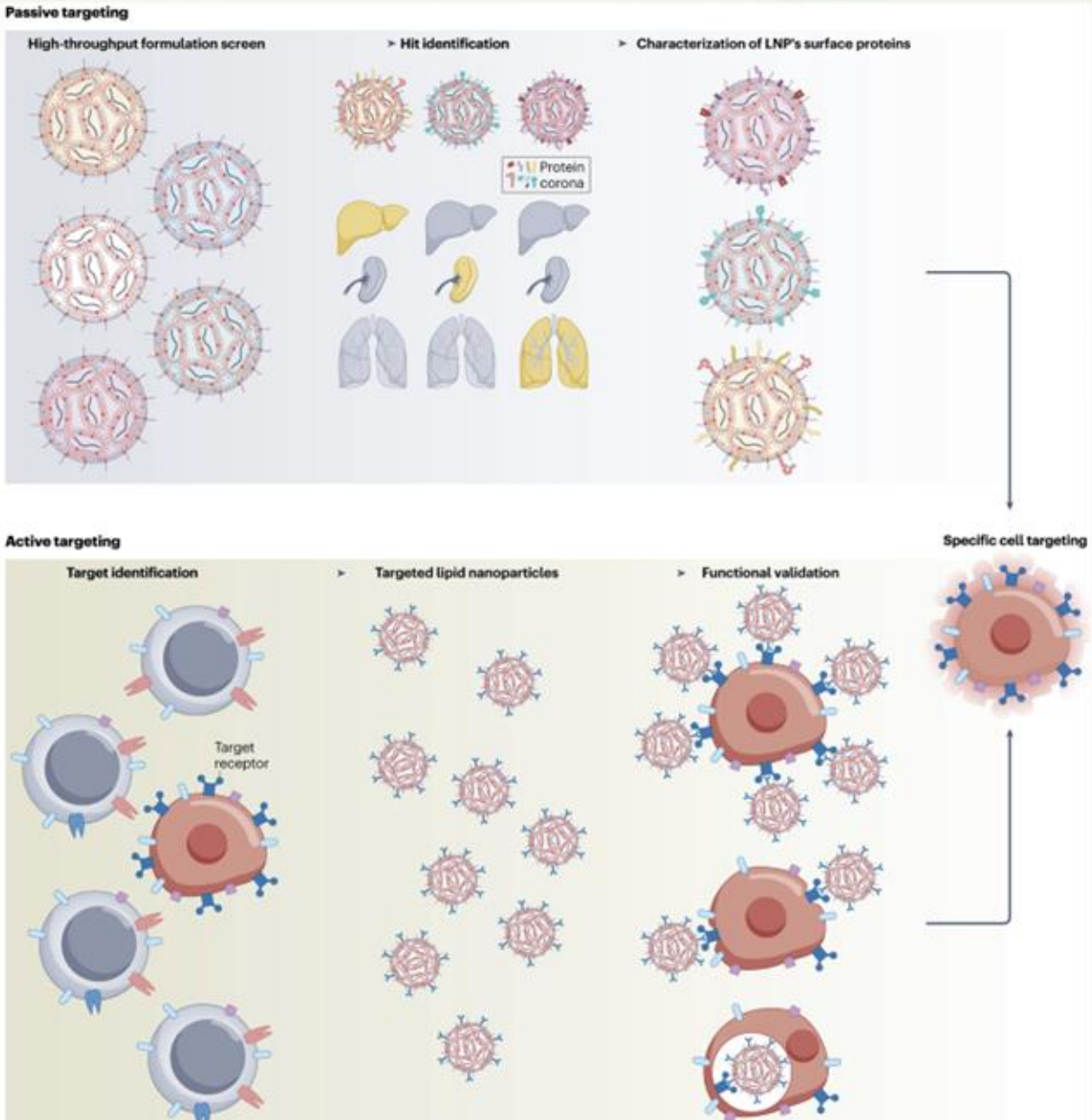
NeomiVac shows mostly mild adverse events, with no severe cases observed

Targeted delivery platform

- Targeting moiety-
  - Antibodies
  - Peptides
  - Ligands
  - Aptamers

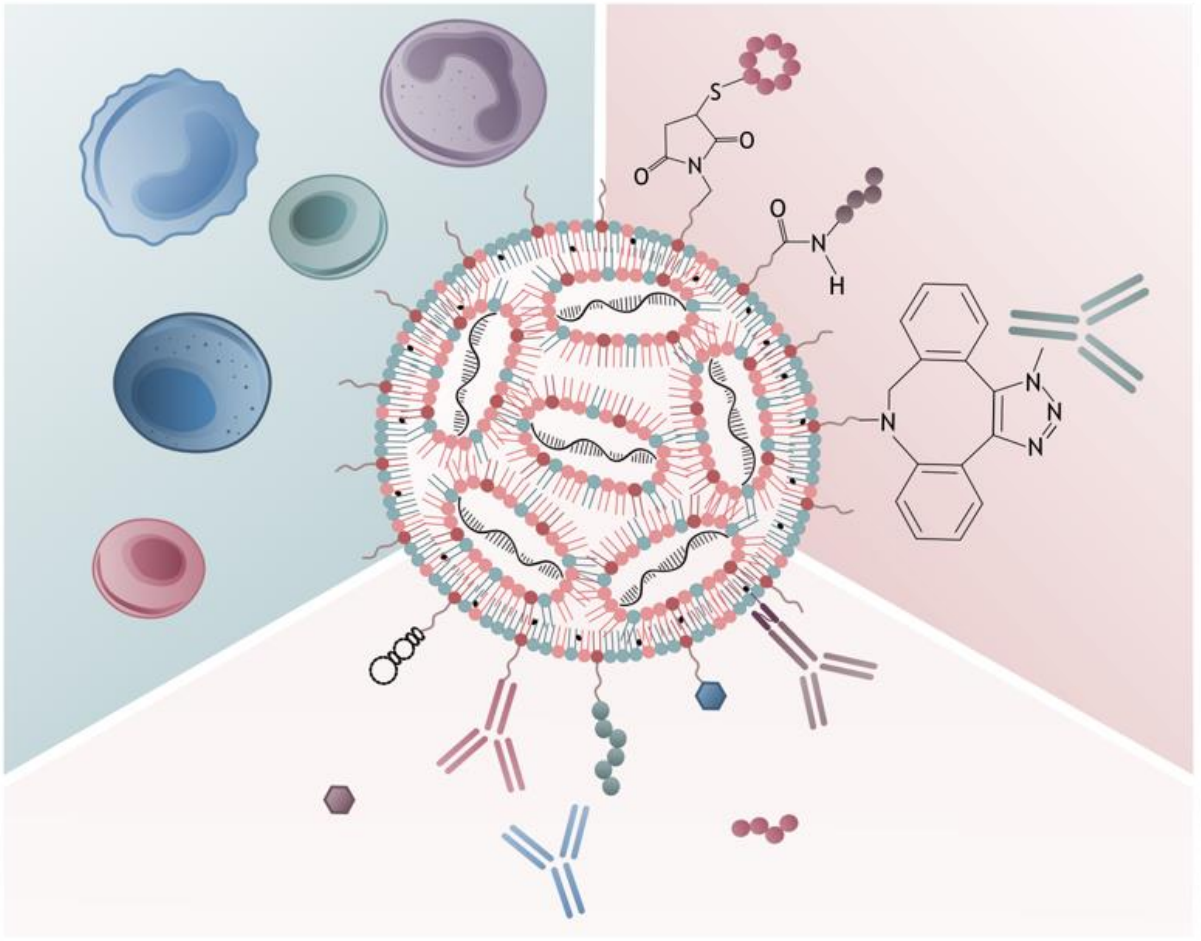


Reaching the Target Organ and Tissue

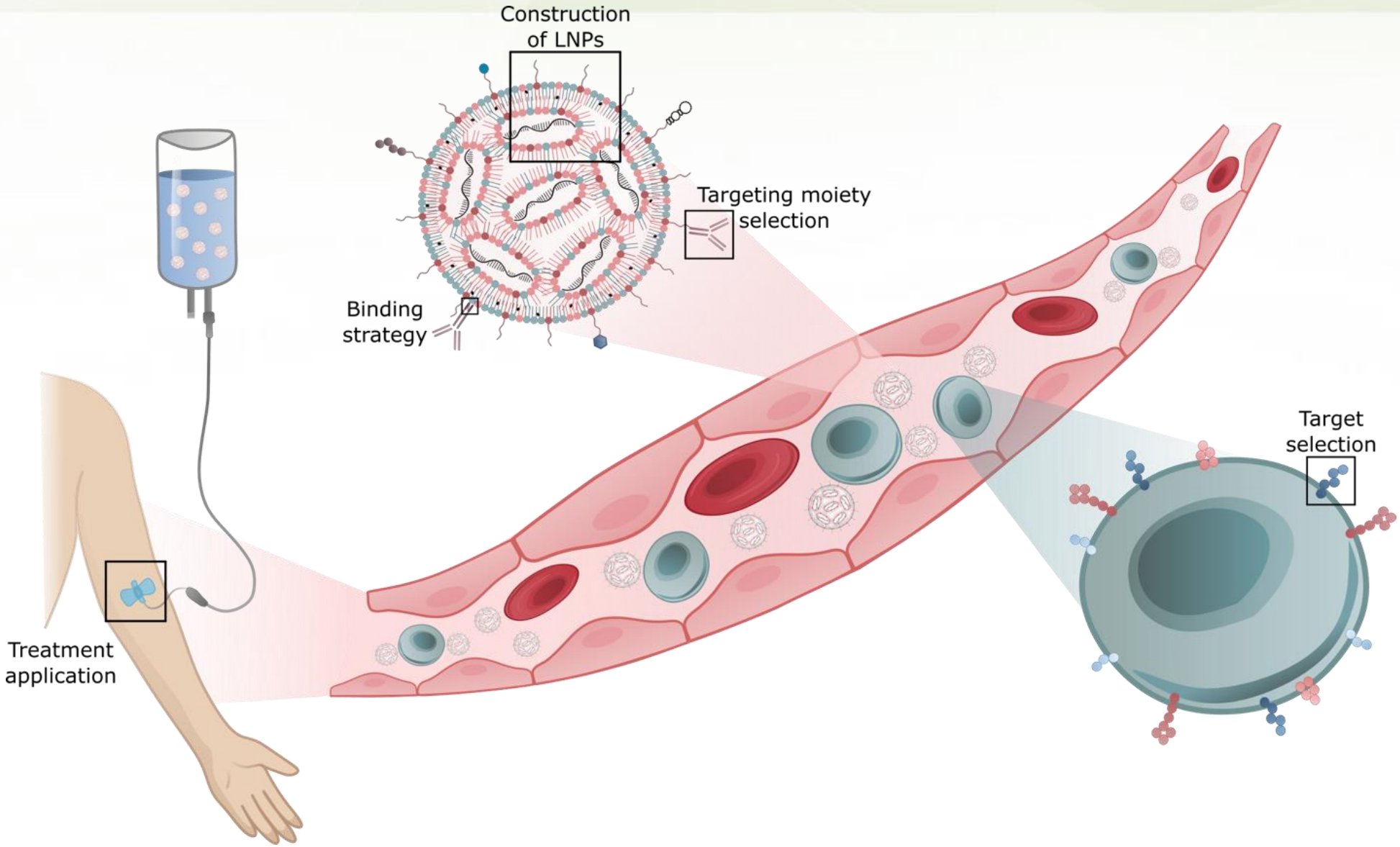


Kon, E., et al. *Nature Rev. Clin. Oncol.* 2023.

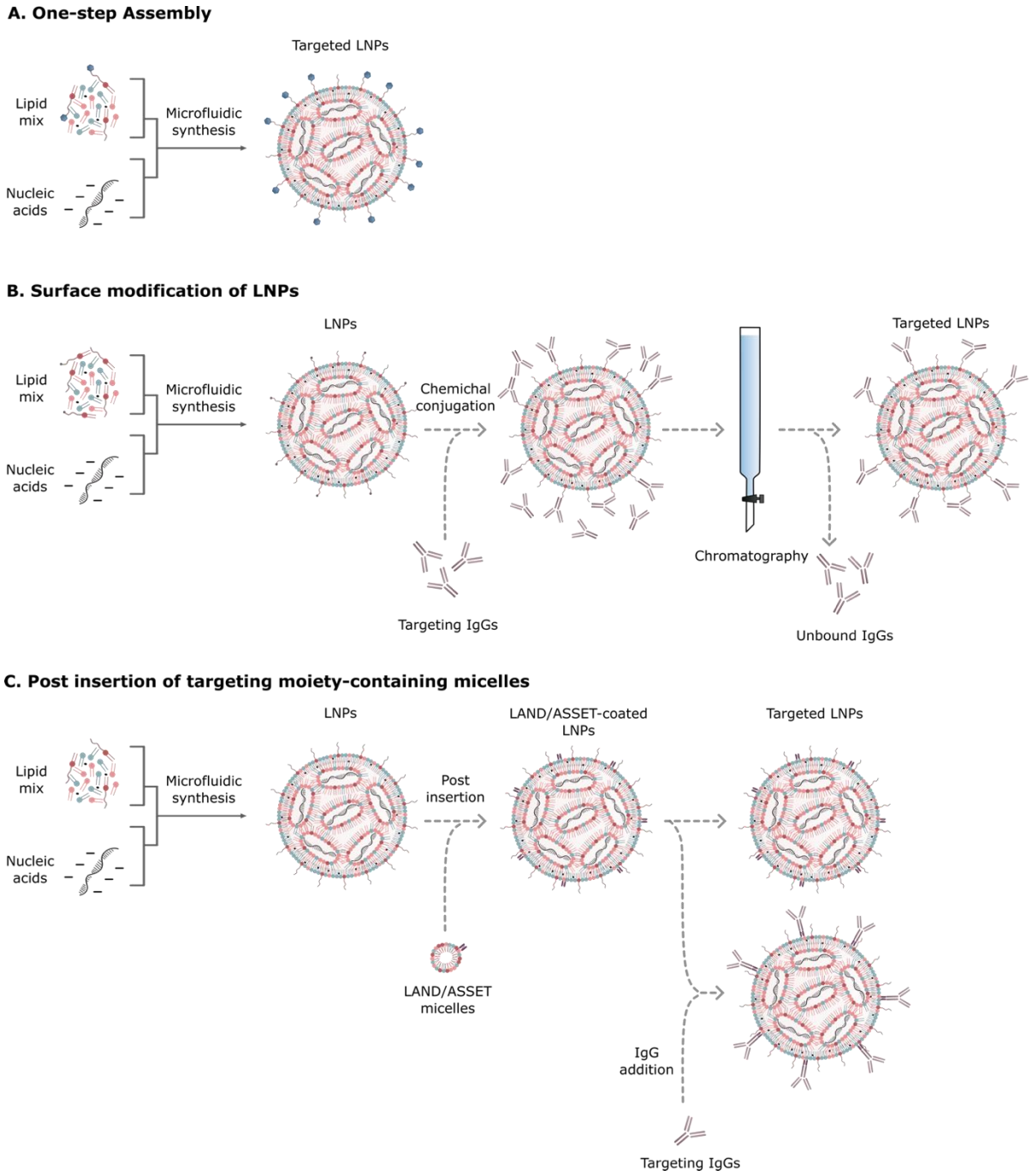
Many ways to conjugate ligands and targeting agents on LNPs surface.



Cell-Specific Targeting Methods

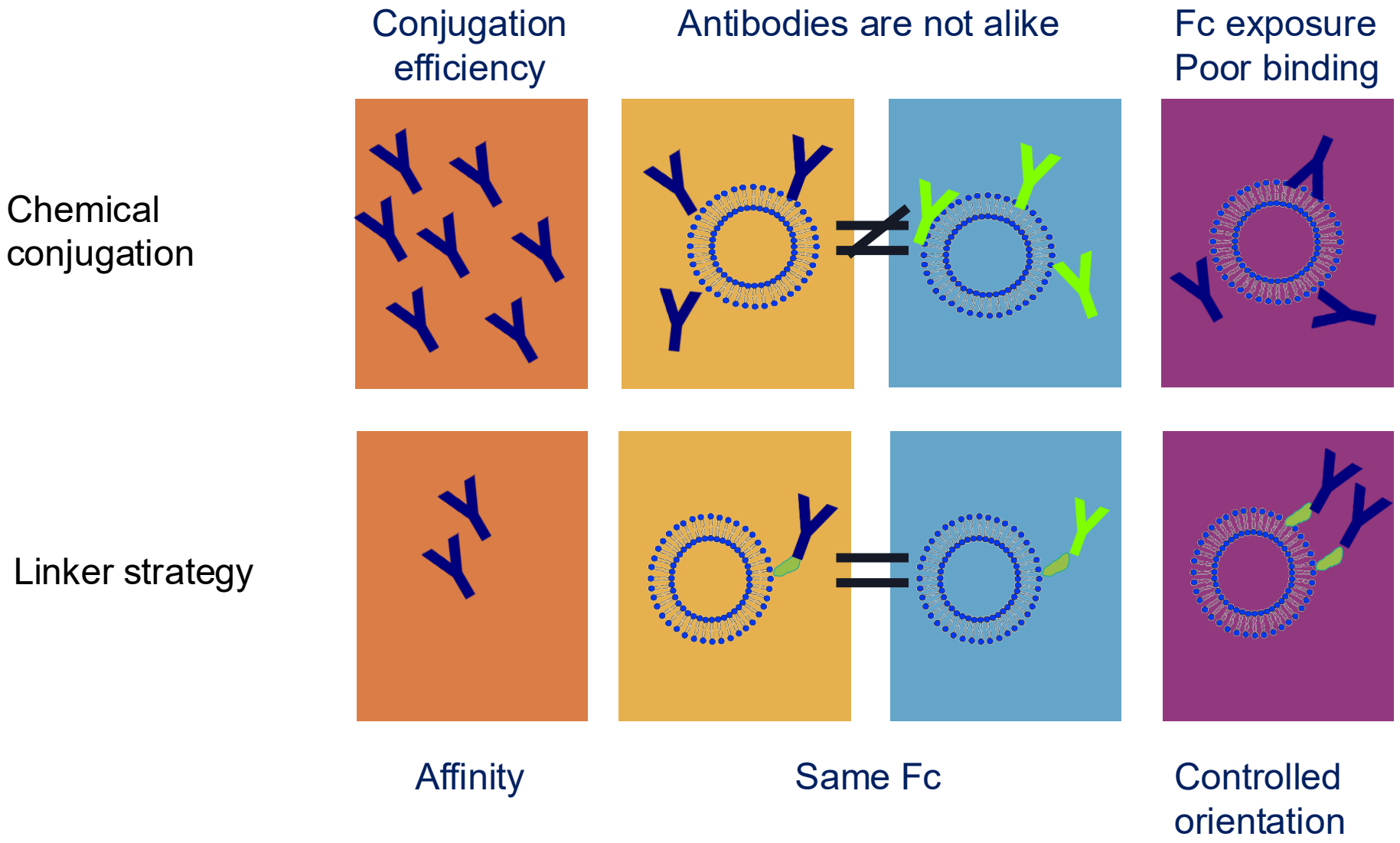


Tarab – Ravski D. et al. *JCR.* 2024



Tarab – Ravski D. et al. *JCR.* 2024

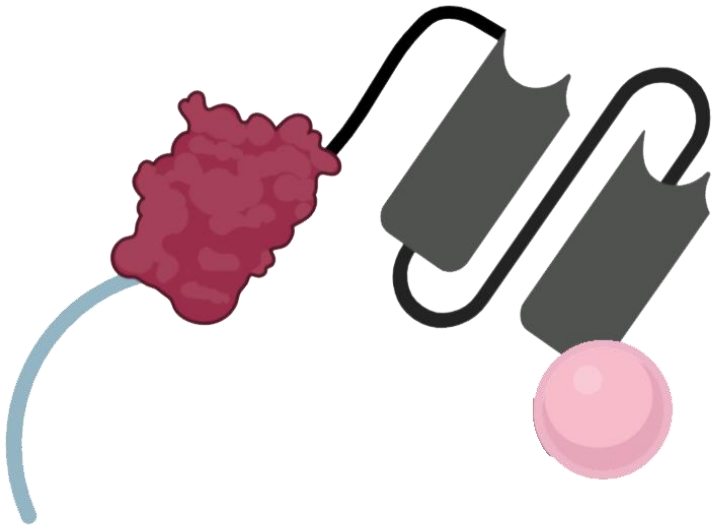
Antibody targeted LNPs construction



Anchored Secondary scFv Enabling Targeting – ASSET

Dr. Ranit Kedmi

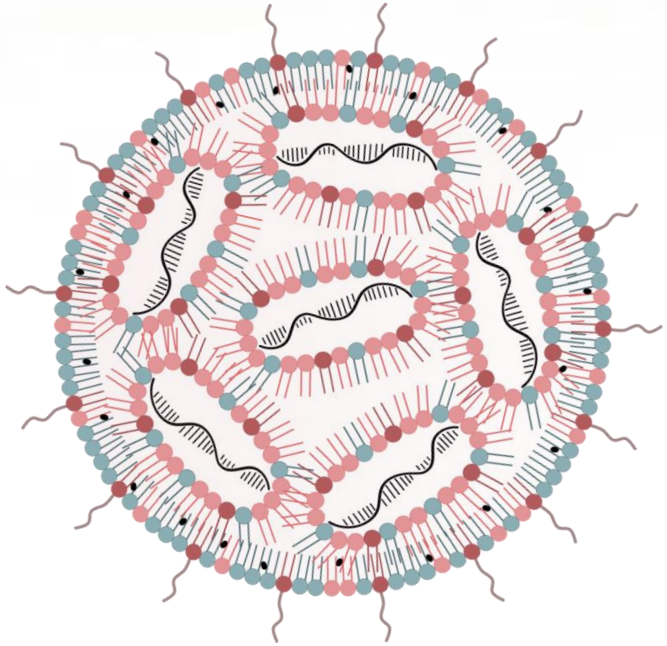
Dr. Nuphar Veiga



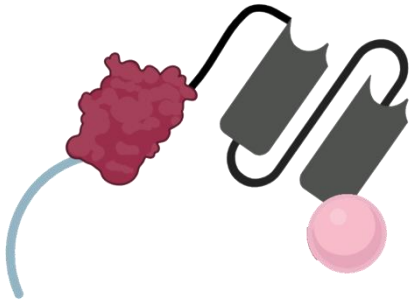
Kedmi, R. *et al. Nat. Nanotechnol.* **13**, 214–219 (2018).

ASSET as a Targeting Platform

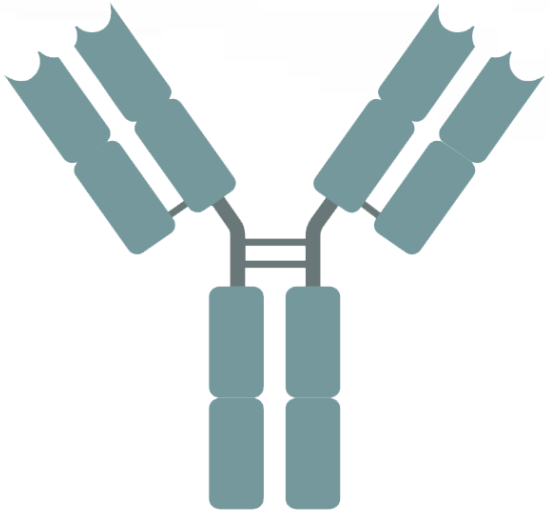
LNPs



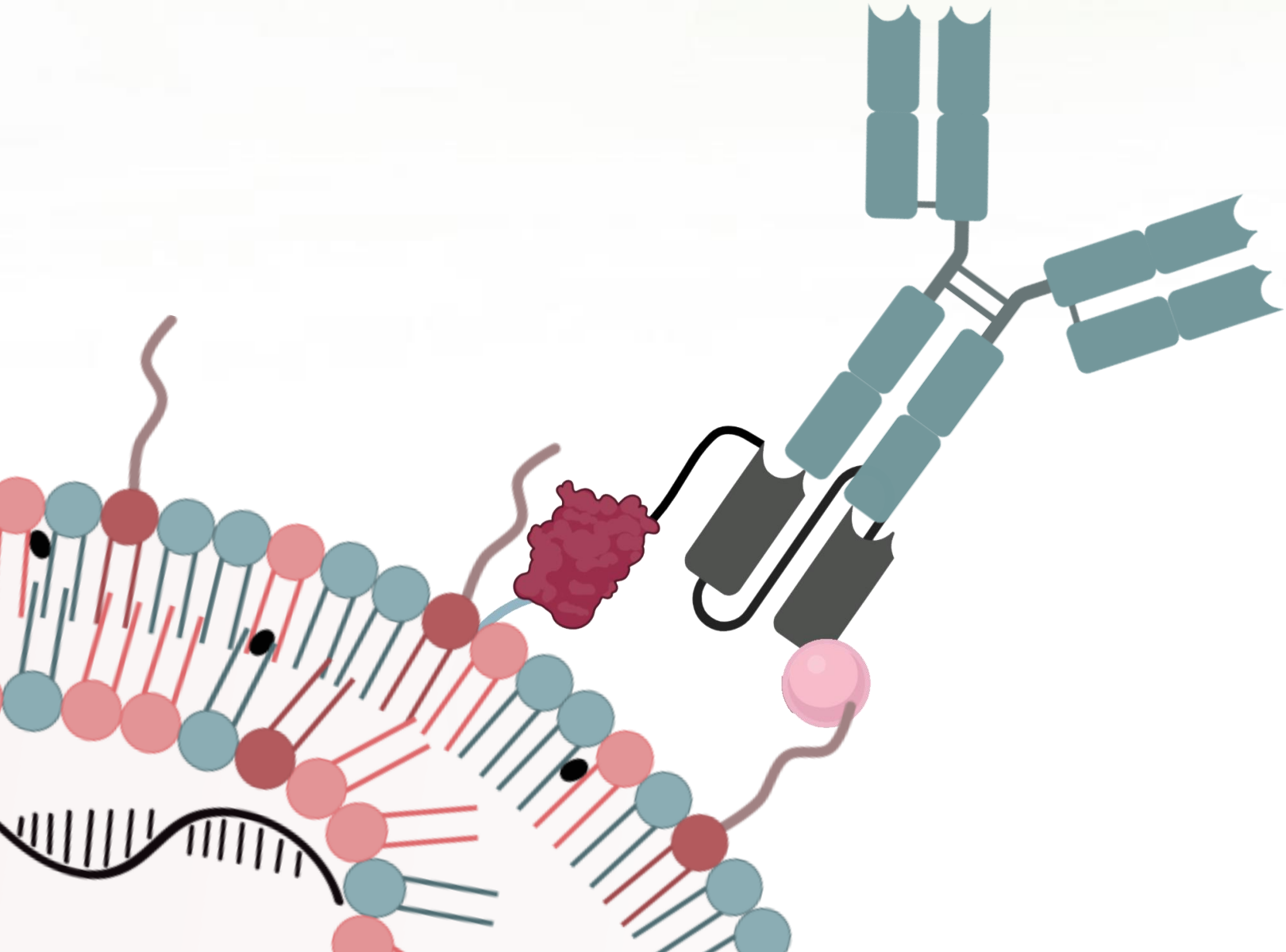
ASSET



Rat IgG2a antibody

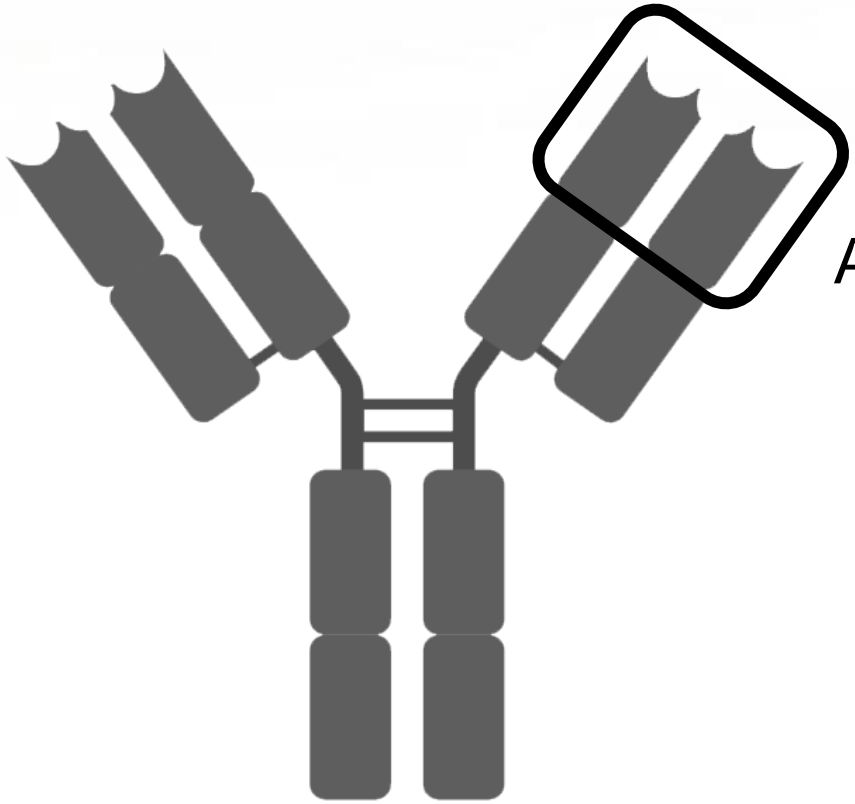


ASSET as a Targeting Platform



Lipidated Antibody Nanoparticle Delivery – LAND

Utilizing the ASSET platform to construct a direct targeting moiety

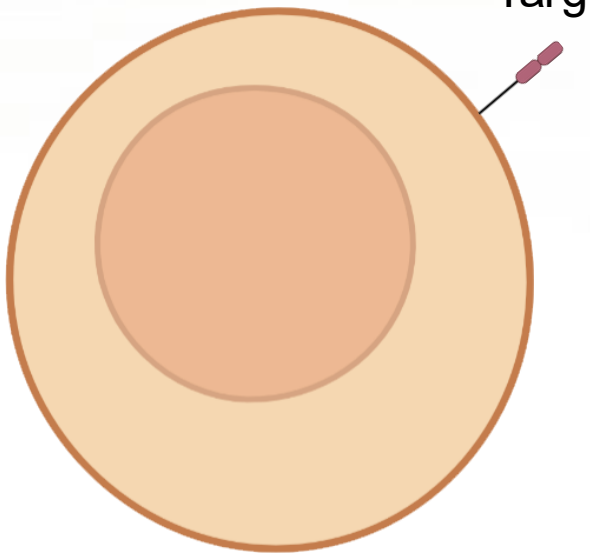


Anti XXX antibody

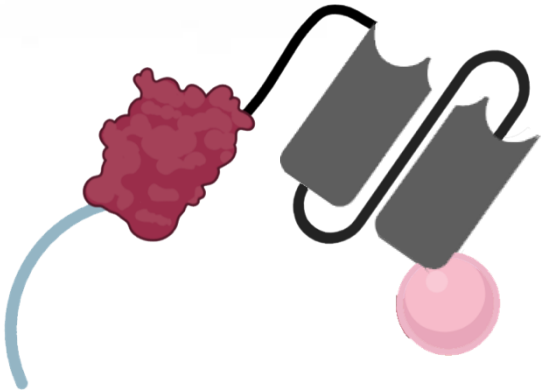
LAND Direct Binding to Target Cells

Target cell

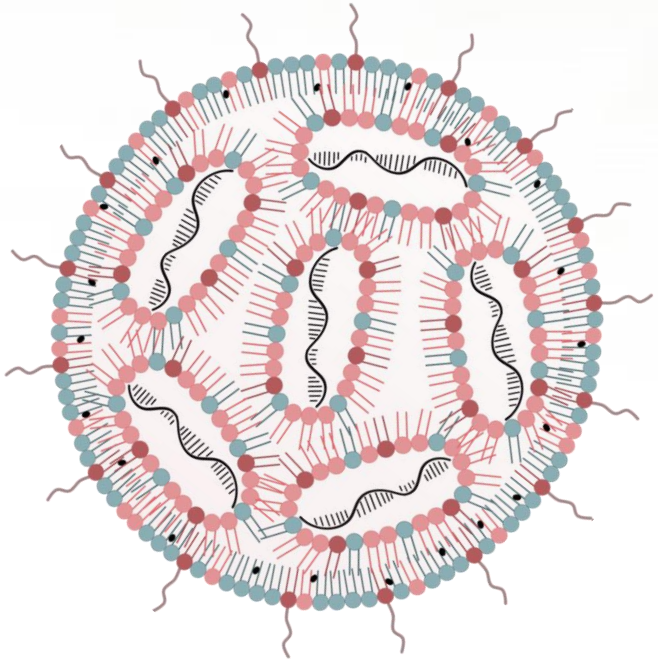
Target Receptor



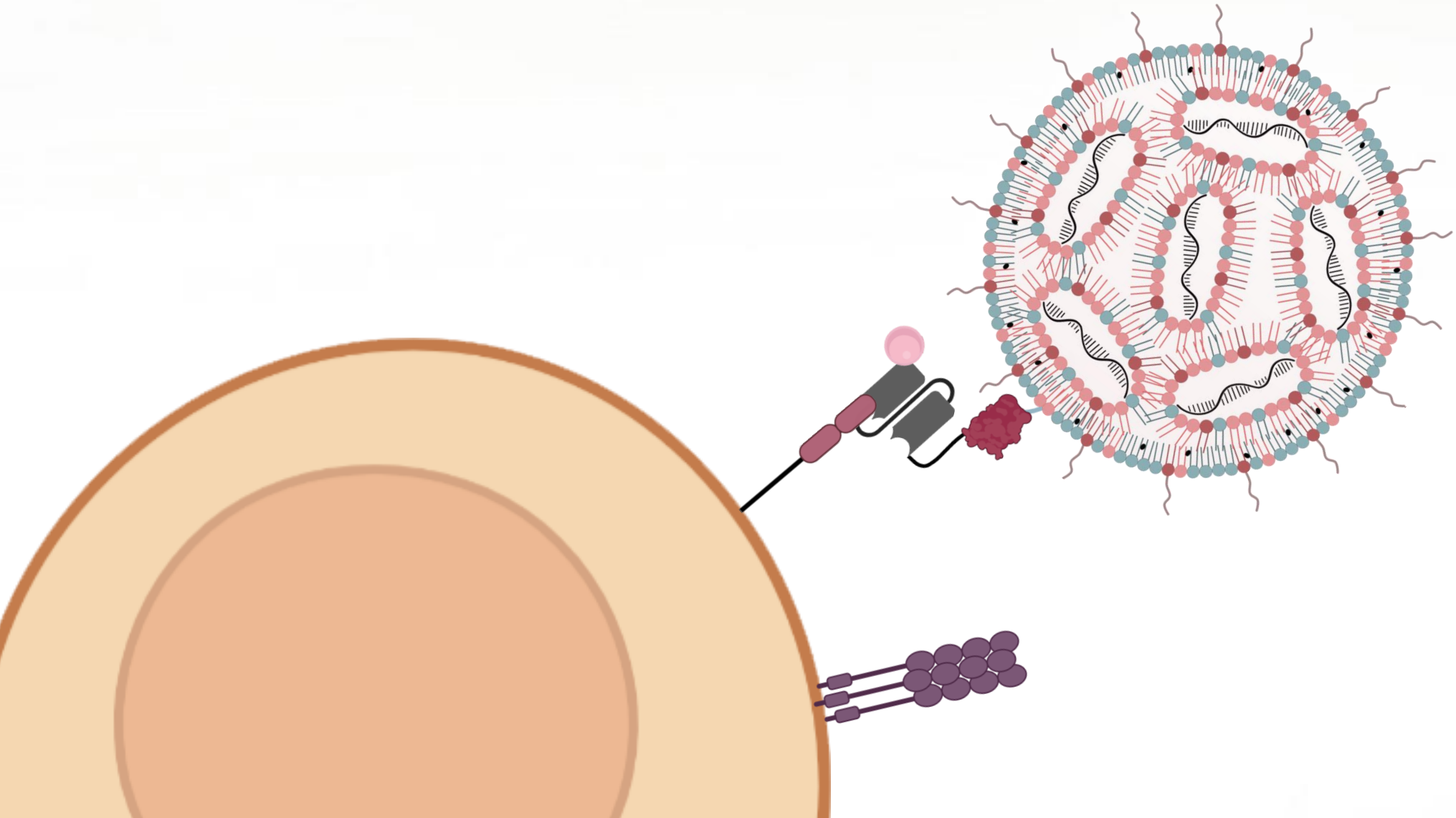
Anti-Target receptor LAND



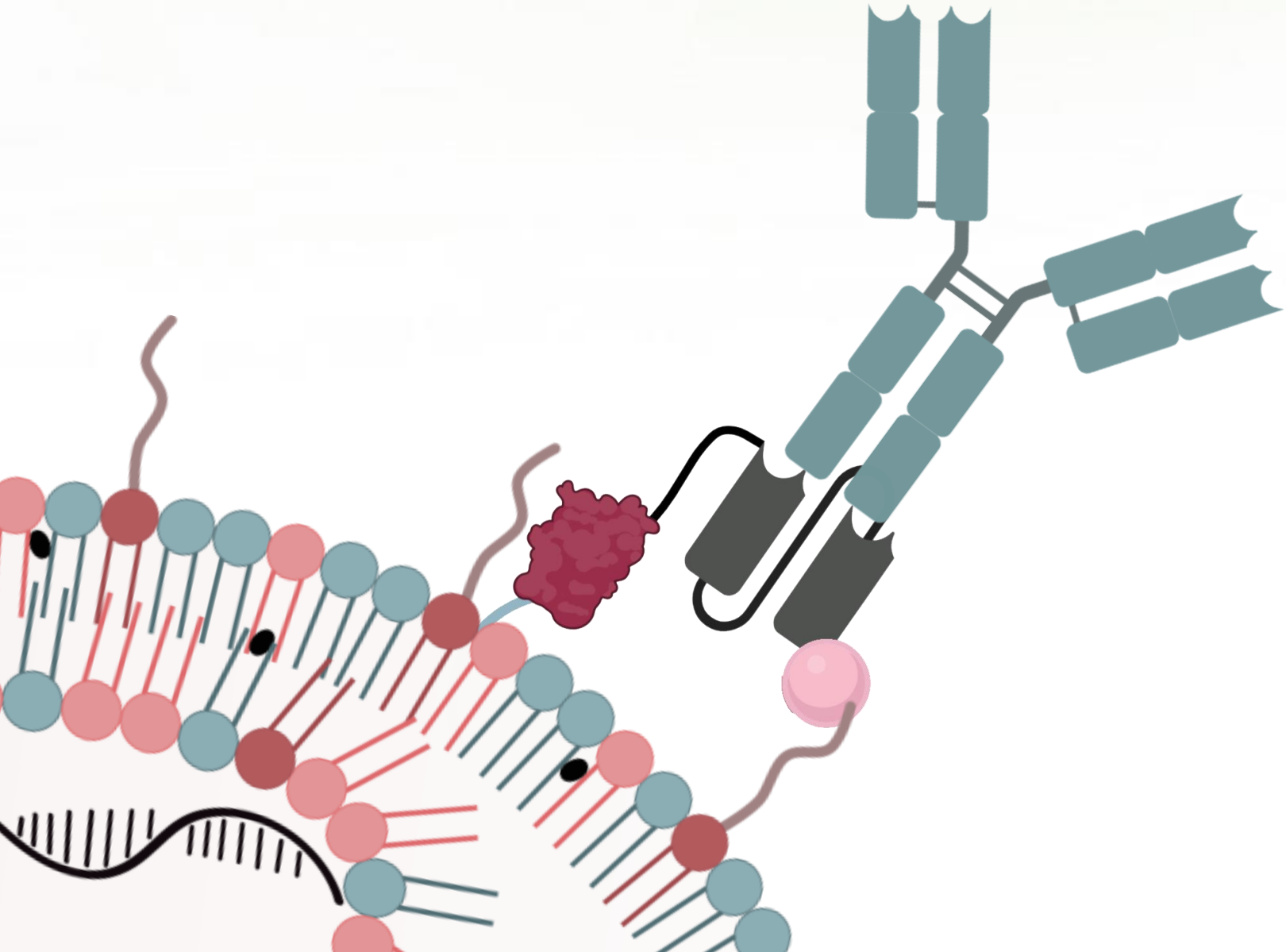
LNPs



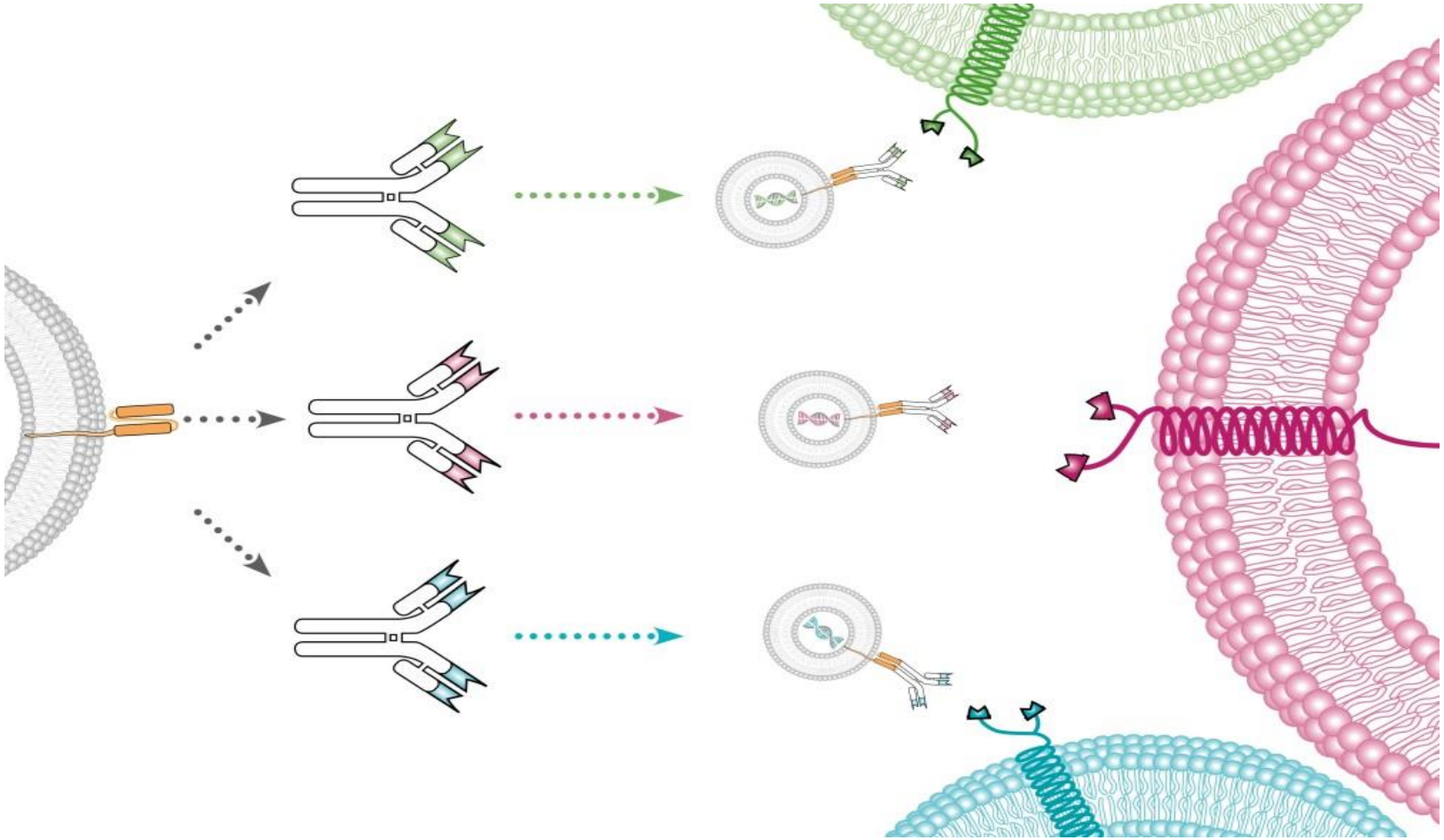
LAND Direct Binding to Target Cells



ASSET as a Targeting Platform

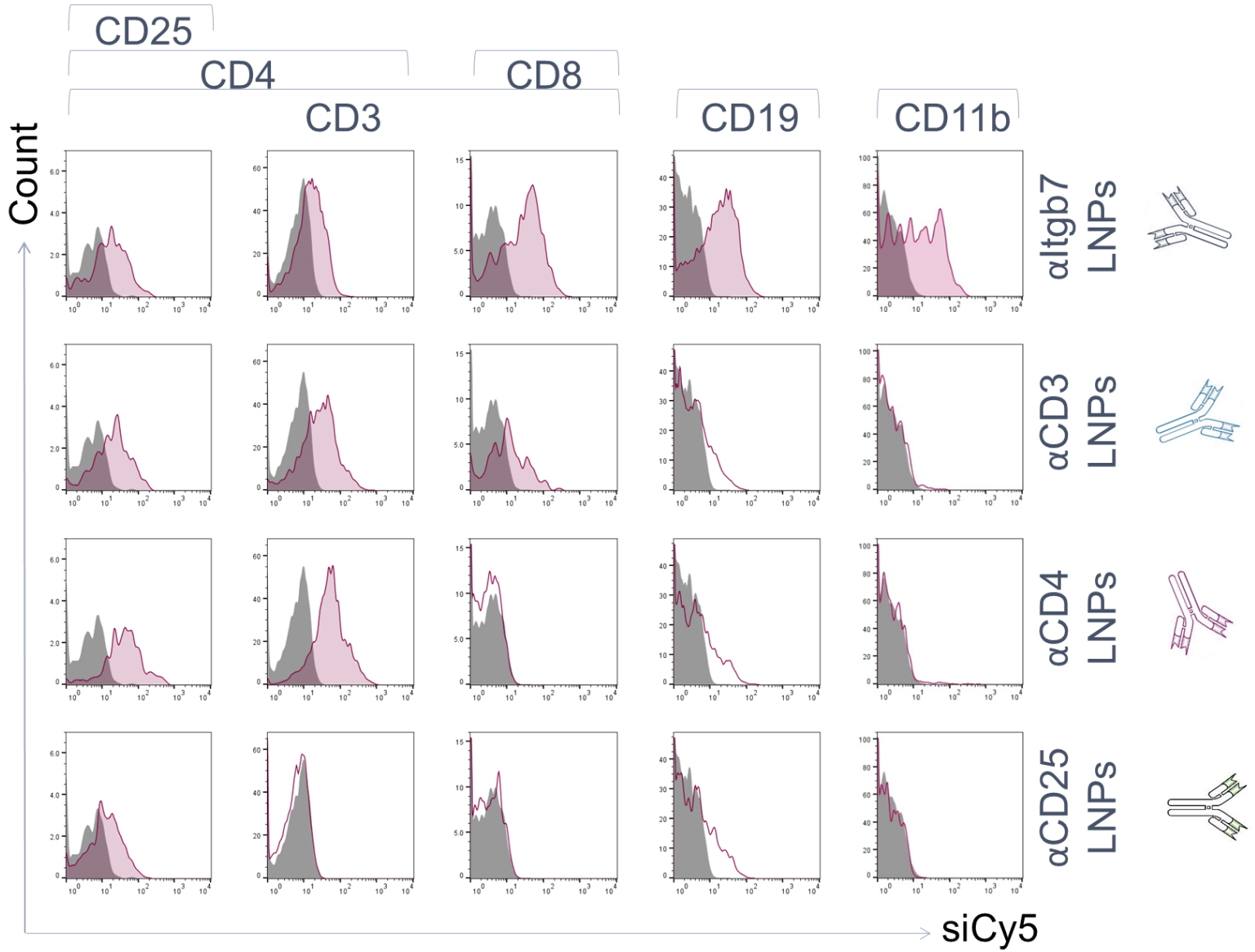


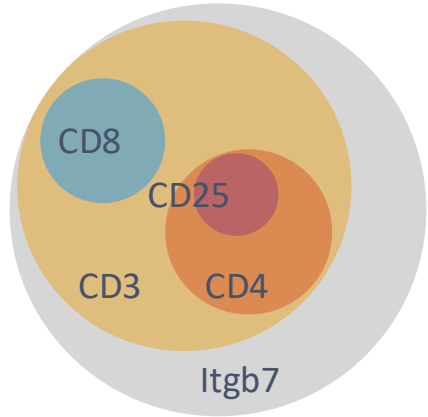
Modular platform



Kedmi R, Veiga N et al. Nature Nanotechnology 2018

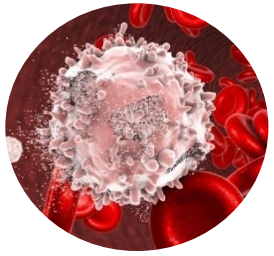
Versatile *in vivo* targeting





Kedmi R, Veiga N et al. Nature Nanotechnology 2018

Therapeutic applications



Hematological malignancies

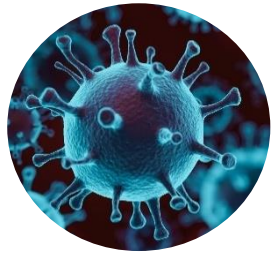
- Myeloma
- Leukemia
  - CML
  - AML
  - CLL
  - ALL
- Lymphoma
  - Hodgkin's
  - NHL: MCL

Solid Tumors

- Ovarian cancer
- GBM
- Head and neck

Inflammation

- Autoimmune diseases
  - Colitis
  - Crohn's
  - RA
  - Psoriasis
  - Lupus/SLE
  - MS
  - Allergy/Asthma
  - GVHD
  - IR injury



Viral infection

- HIV
- Ebola
- COVID - 19

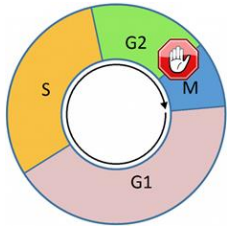
Genetic disorders

- Duchenne Muscular Dystrophy
- CF
- XBP-1

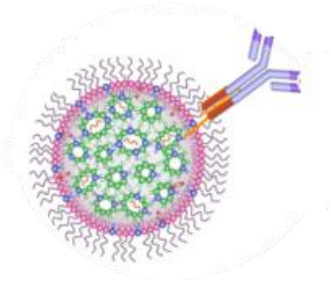
Cancer

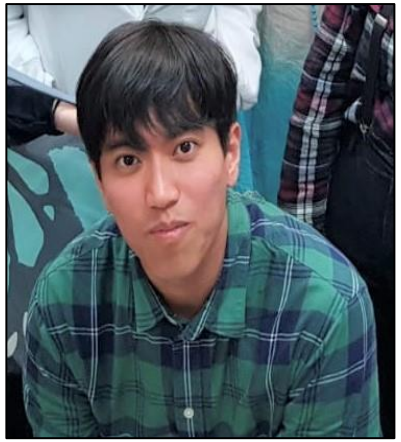
- Therapeutic approach: Silencing

- Target gene: HO1

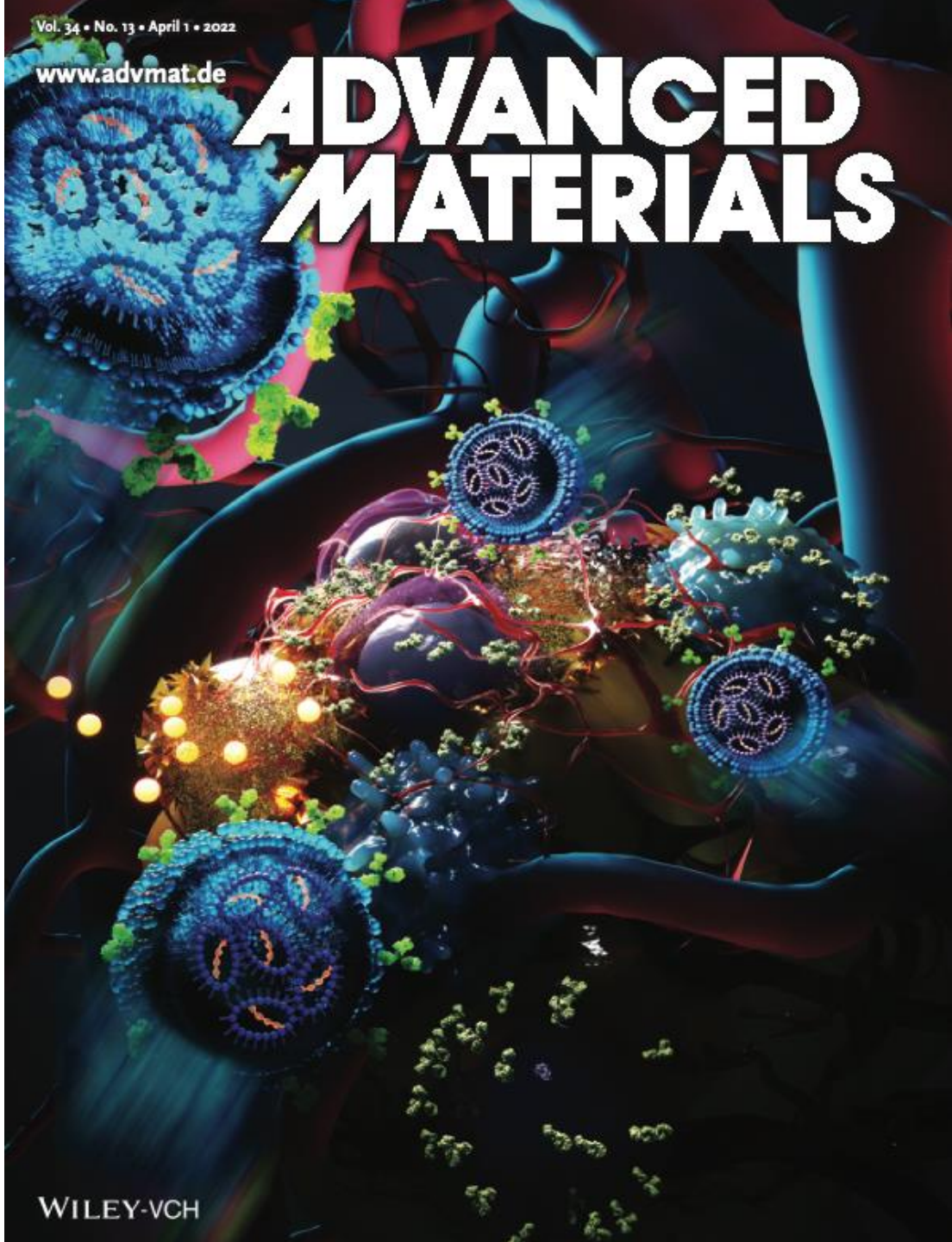


- Target Receptor: PDL-1





Seok-Beom Yong, Ph.D

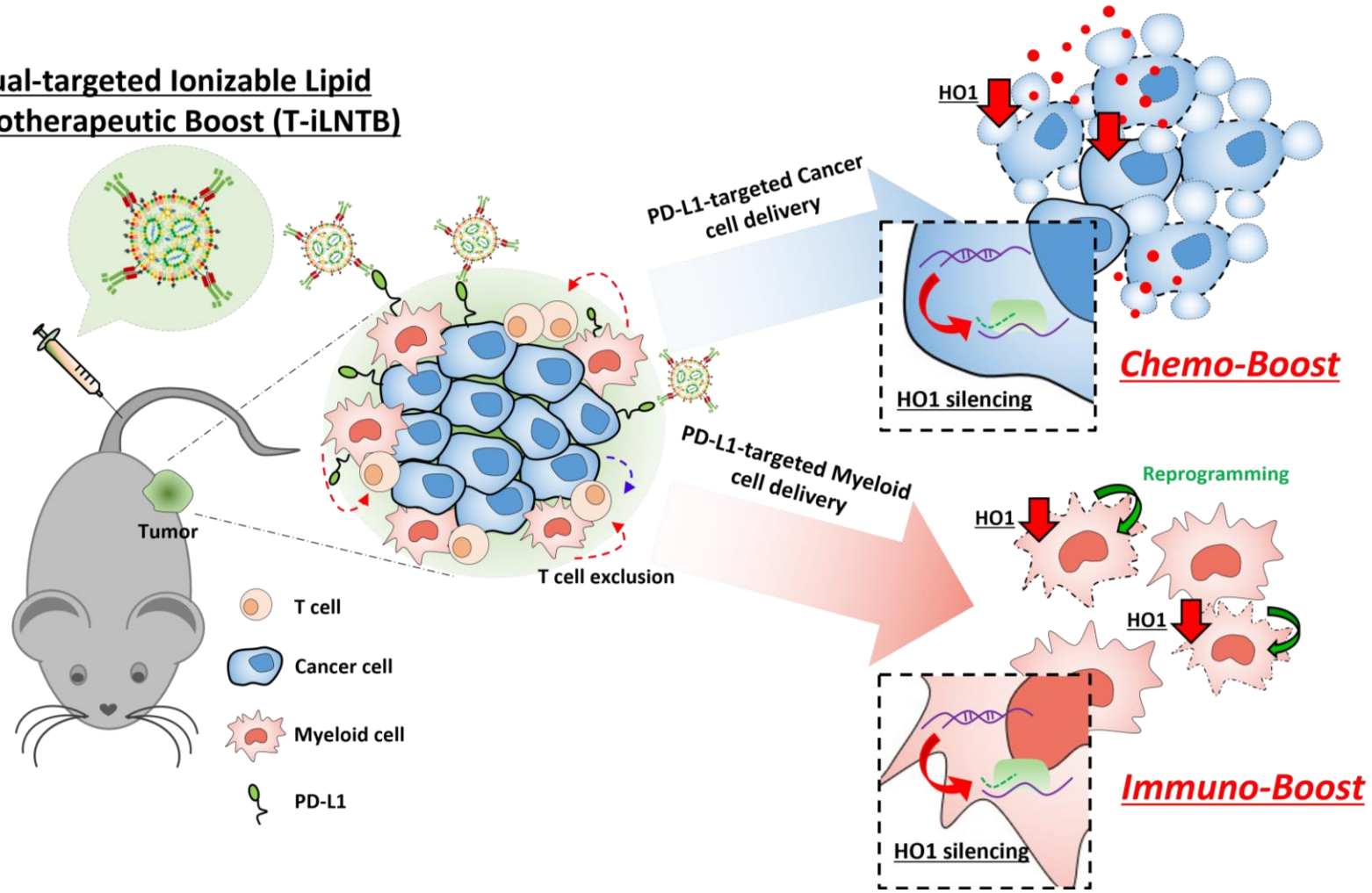


Single gene (HO1)-targeted lipid nanoparticle for chemo-immunotherapeutic boost in cancer

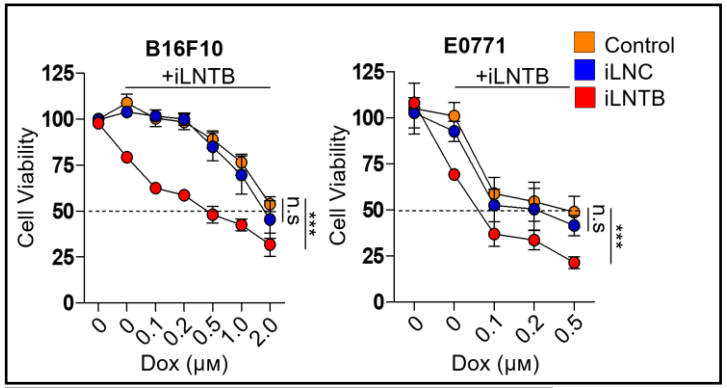
***Dual function of HO1 gene in tumor**

1. Chemo-resistance function in **Cancer cell**
2. Immune-modulatory function in **Tumor myeloid cell**

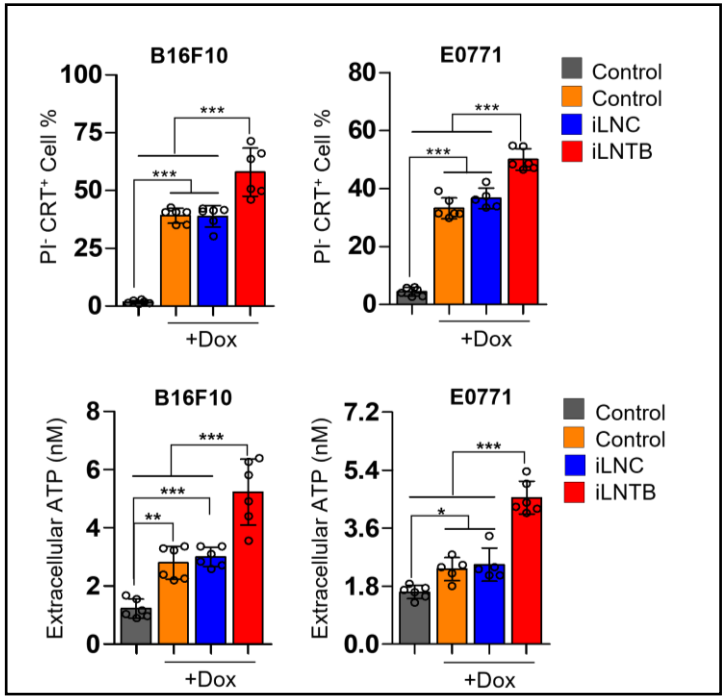
Dual-targeted Ionizable Lipid Nanotherapeutic Boost (T-iLNTB)



Chemo-sensitization effect



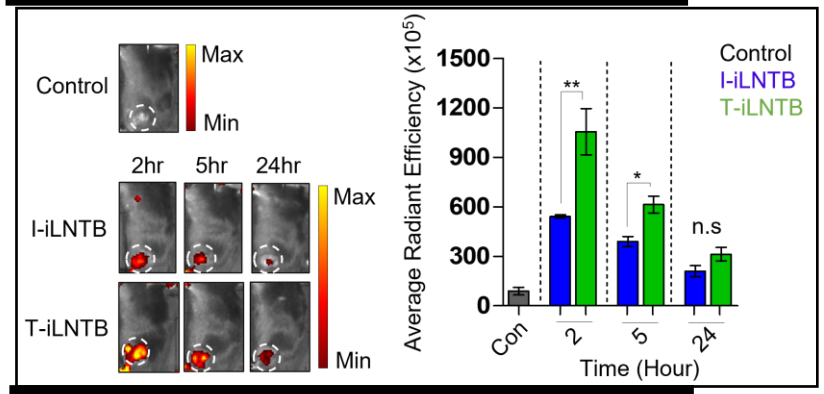
Increased Immunogenic cell death



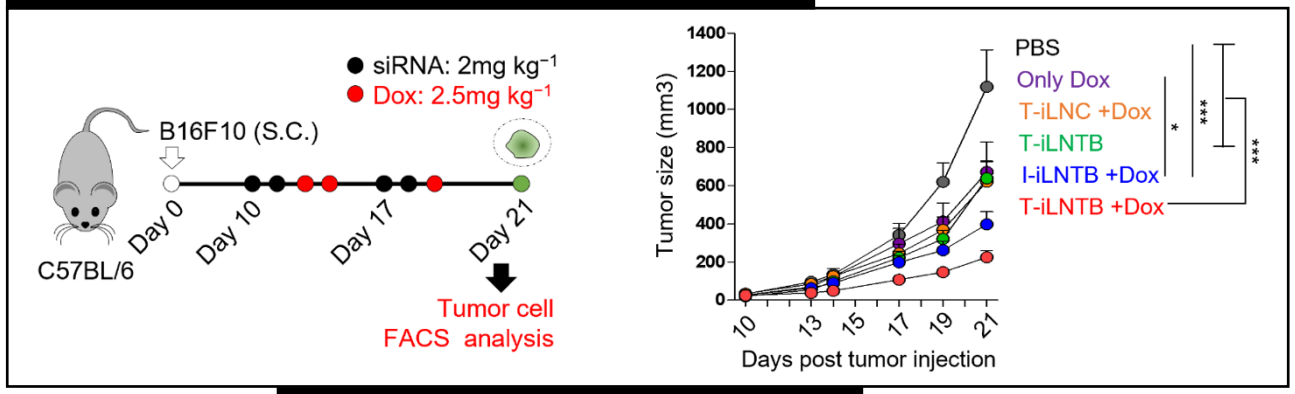
Yong S-B et al., Advanced Materials, 2022

In vivo anti-tumor effect in combination with chemotherapy (Dox) & immune reprogramming effect

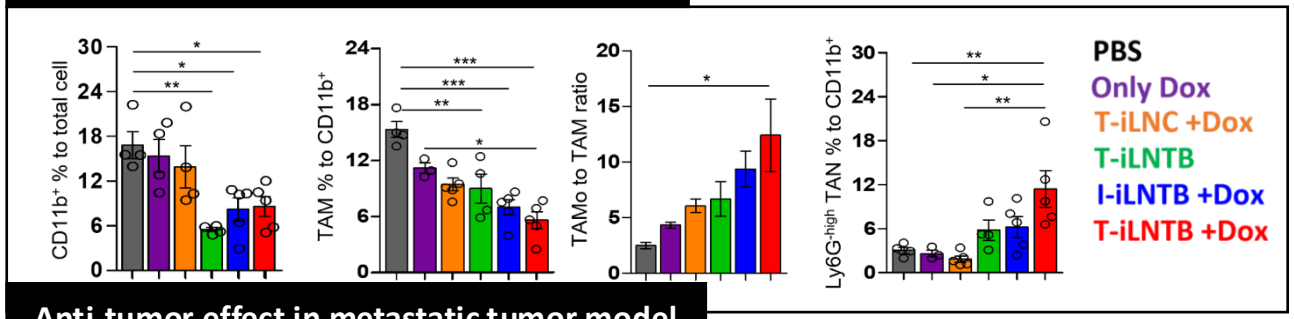
Increased tumor delivery by PD-L1-targeting



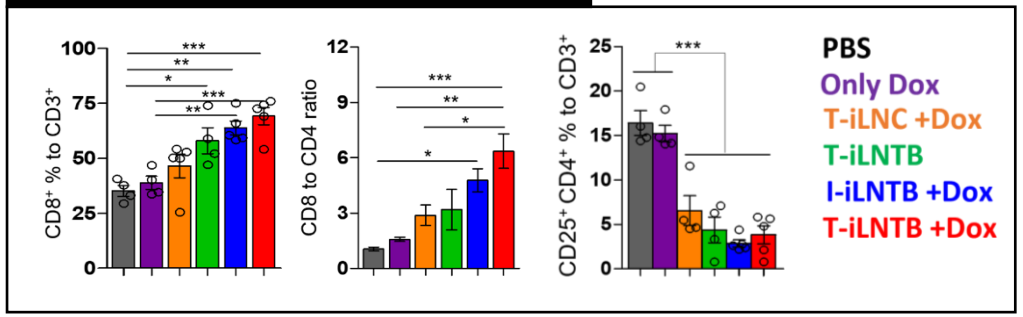
Anti-tumor effect in combination with chemotherapy



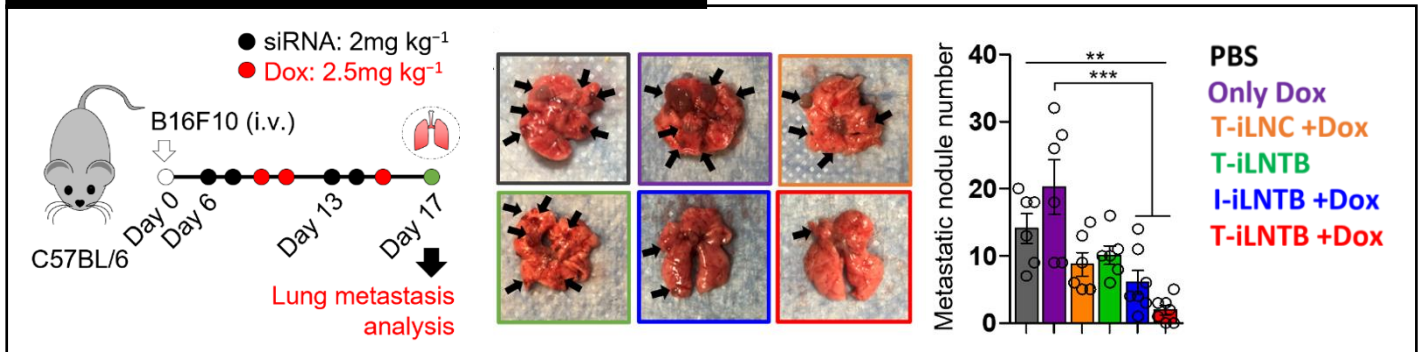
Tumor myeloid cell reprogramming effect



Tumor T cell reprogramming effect

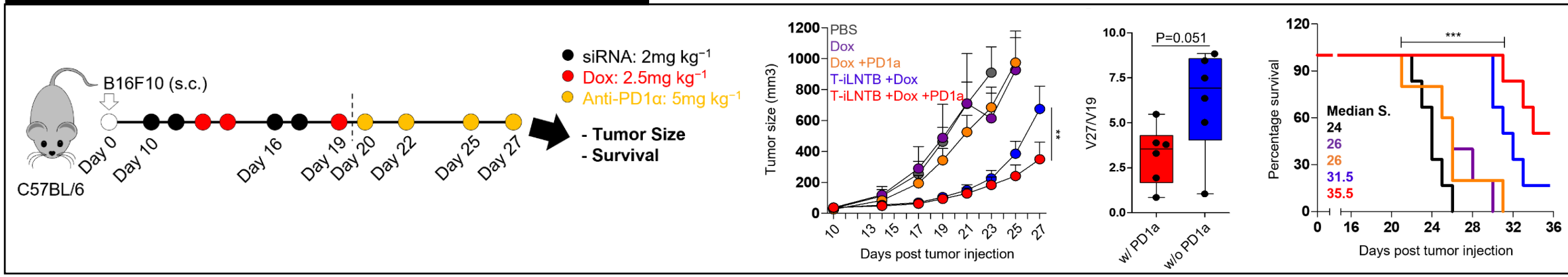


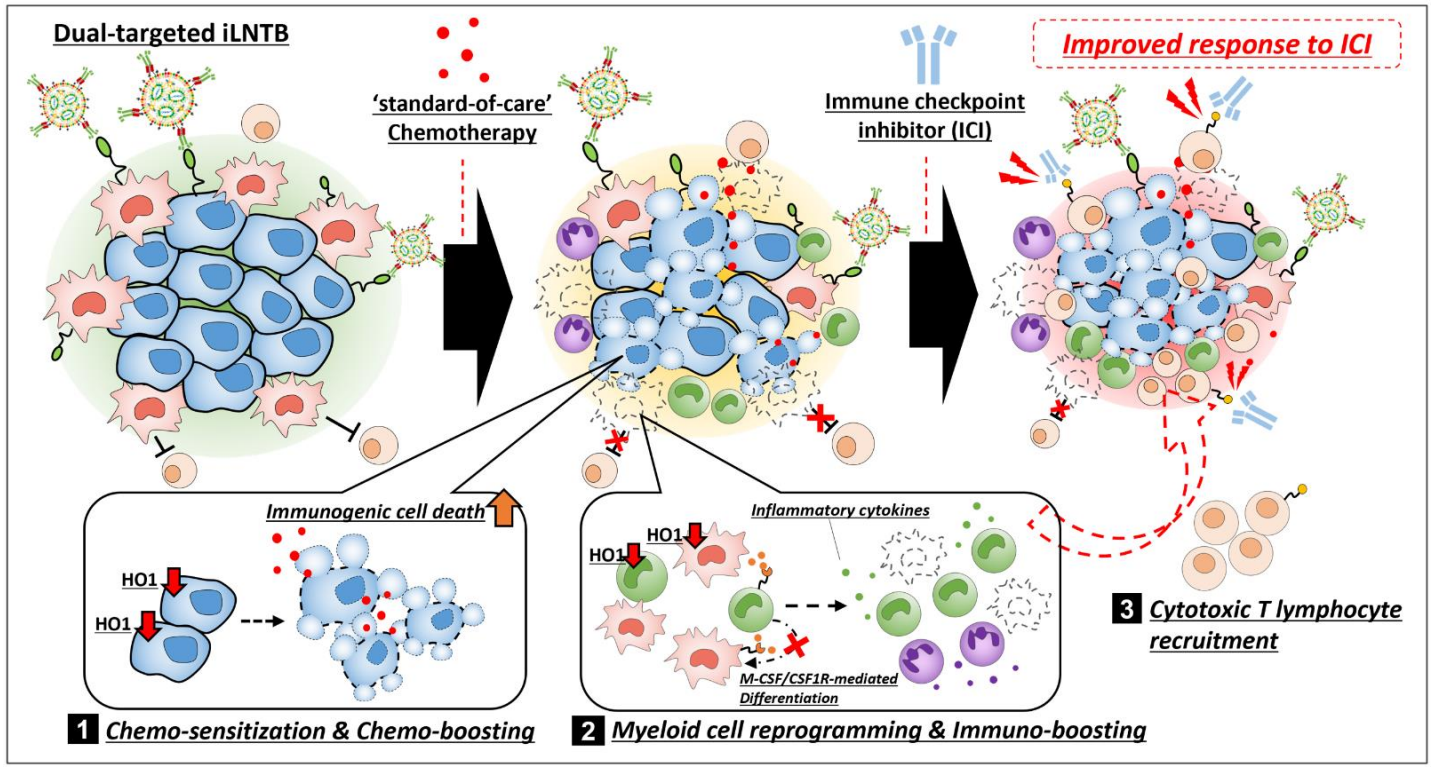
Anti-tumor effect in metastatic tumor model



Yong S-B et al., Advanced Materials, 2022

Anti-tumor effect in combination with chemo-immunotherapy





Yong S-B et al., Advanced Materials, 2022

Cancer

- Therapeutic approach: Silencing
- Target gene: HO1
- Target Receptor: PDL-1

RNA based therapeutics

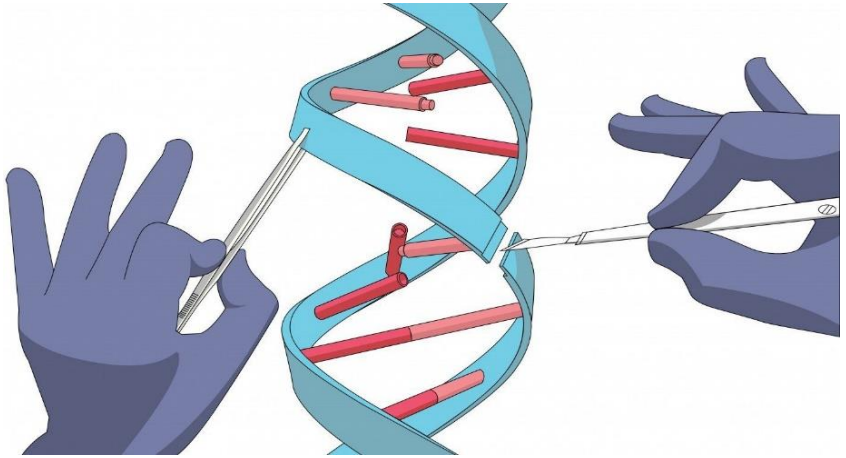
Silence

Activate

Edit

Replace

CRISPR/Cas9

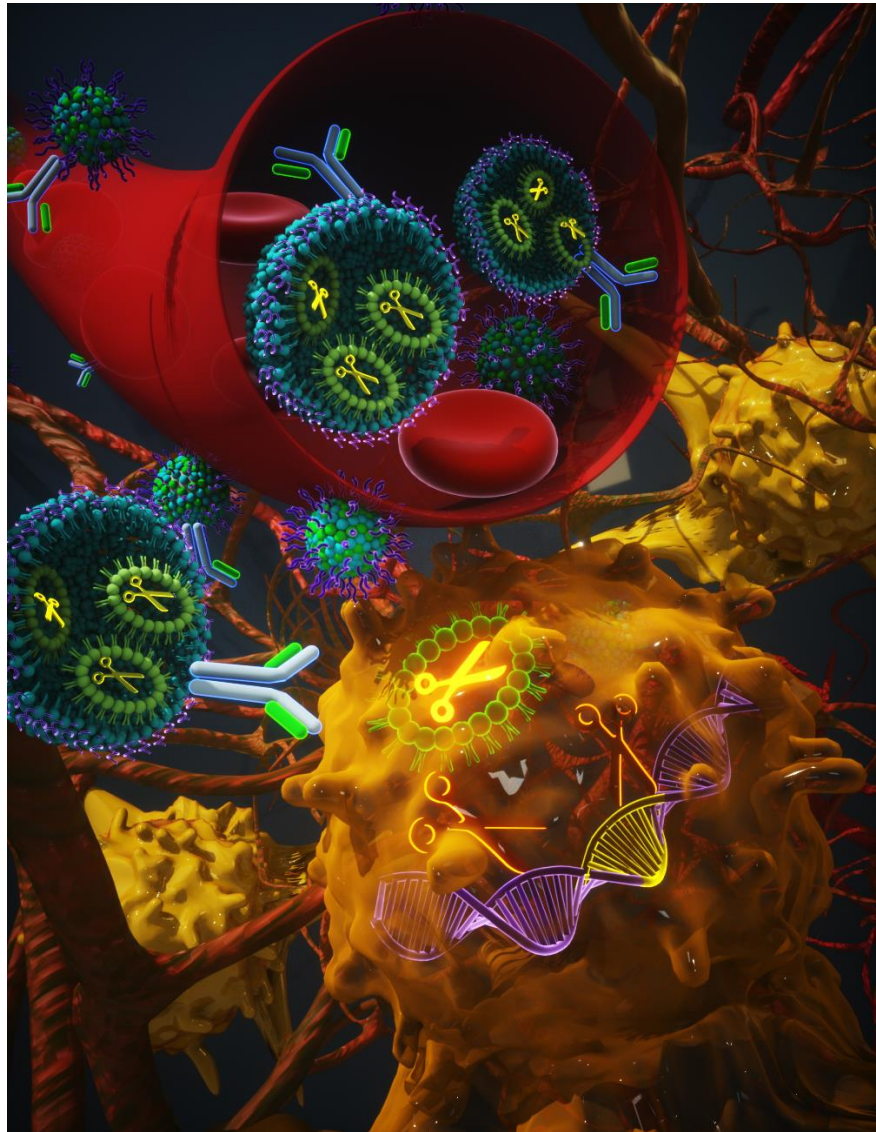


Targeted CRISPR/Cas9 LNPs for therapeutic genome editing in cancer

Dr. Daniel Rosenblum

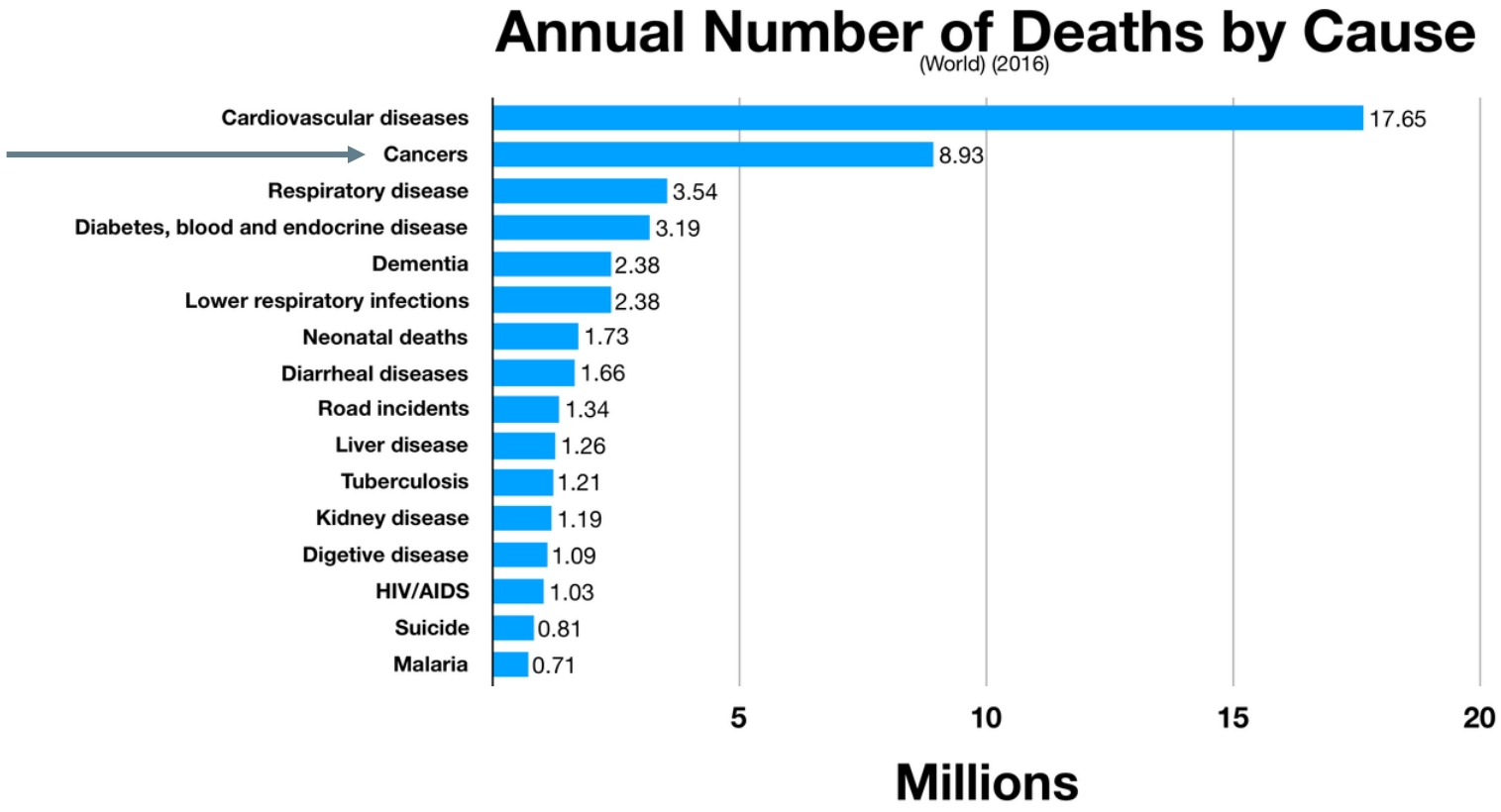
Dr. Anna Gutkin

Rosenblum D.*, Gutkin A* et. al. Science Advances 2020
Gutkin A*, Rosenblum D.*, Peer D. Nature Biotechnology 2021
Elia U.*, Kon E*, Peer D. Nature Biotechnology 2023.

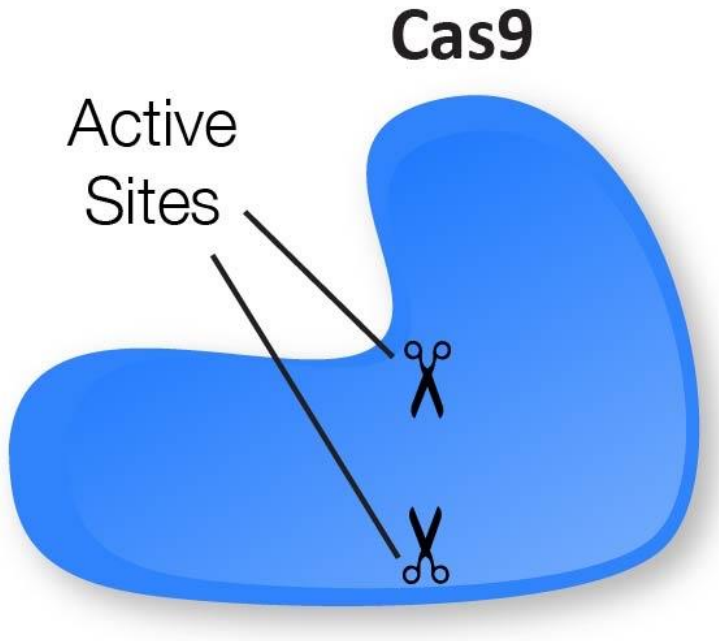


Why cancer?

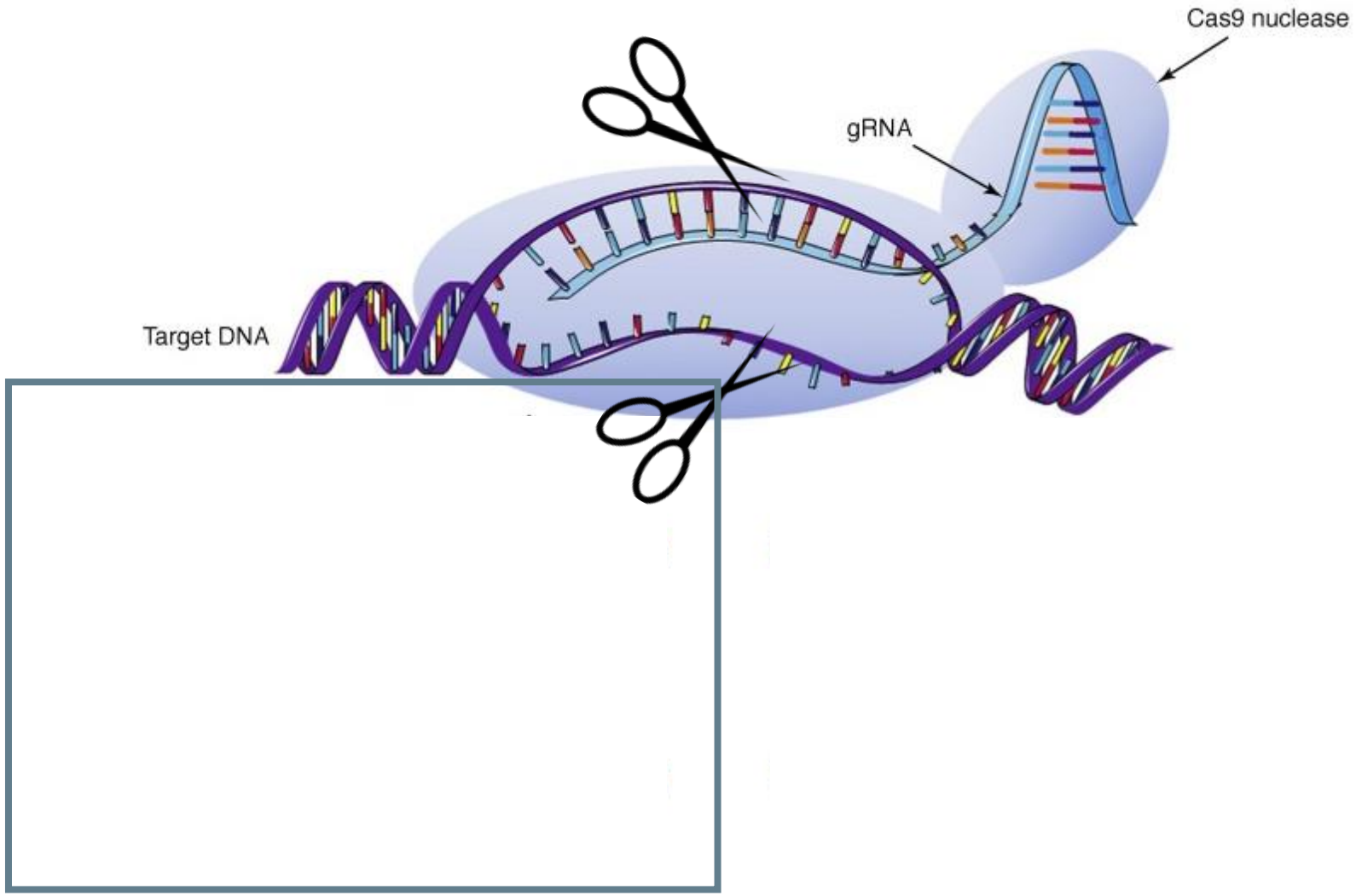
- Cancer remains the second major cause of mortality worldwide.



CRISPR/Cas9 mediated genome editing



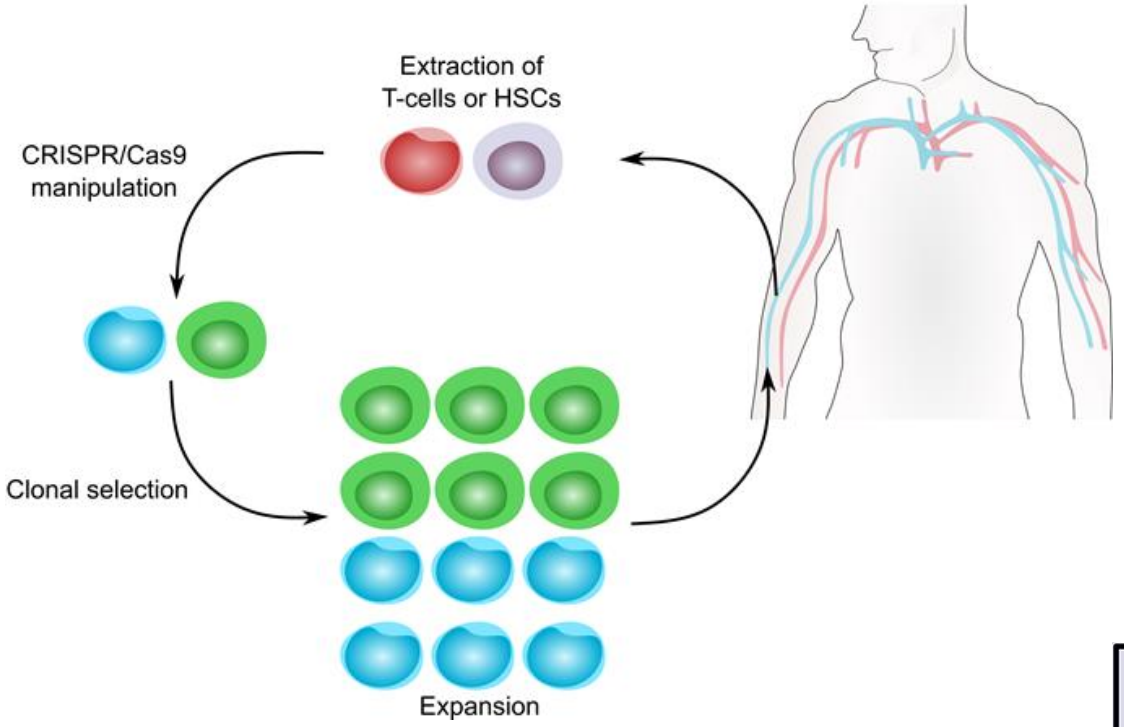
CRISPR/Cas9 mediated genome editing



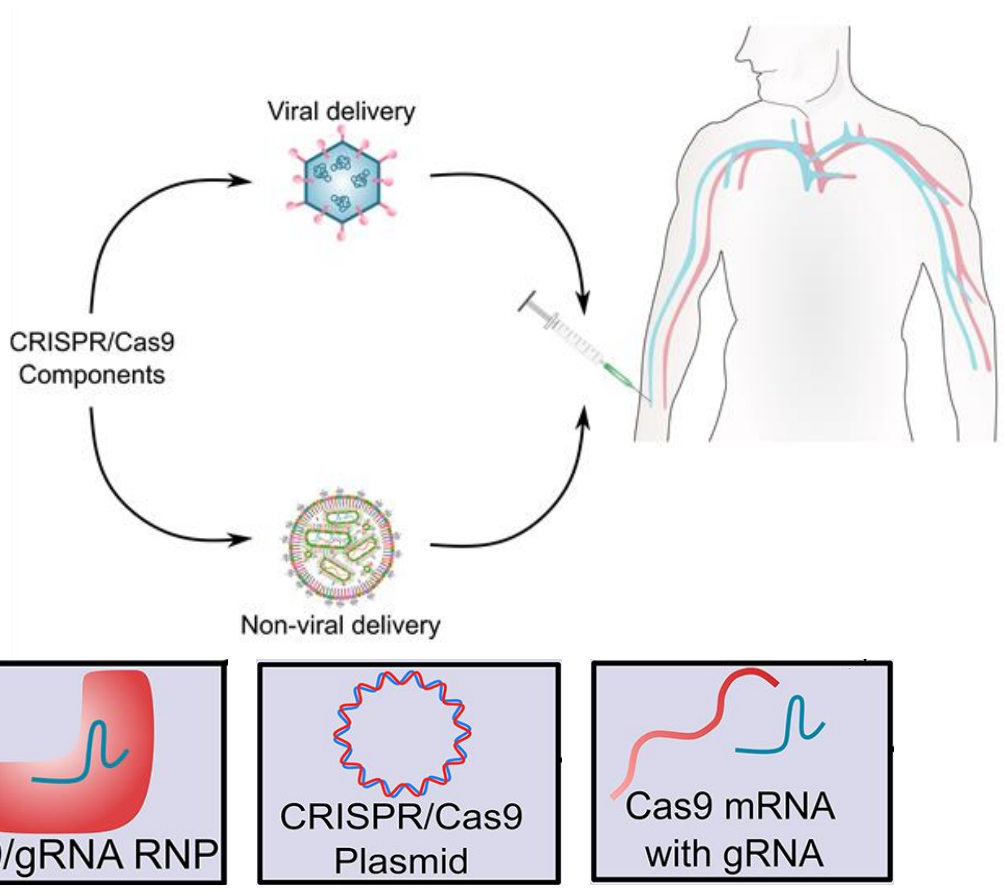
Bin L. et.al, Drug Discovery Today (2019)

Clinical applications of CRISPR/Cas9

Ex vivo editing



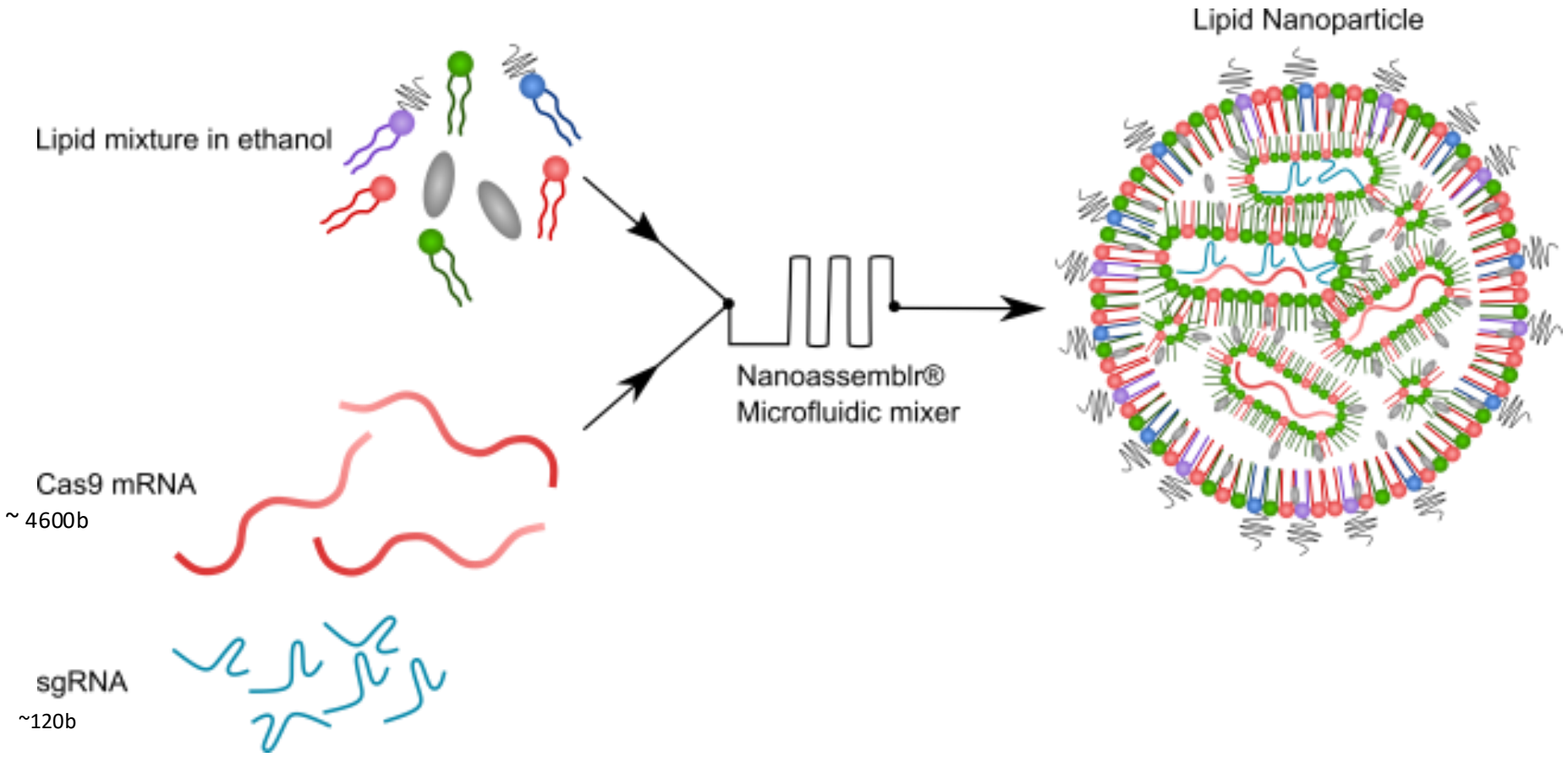
In vivo editing



Rosenblum D., Gutkin A. et. al. ADDR (2020)

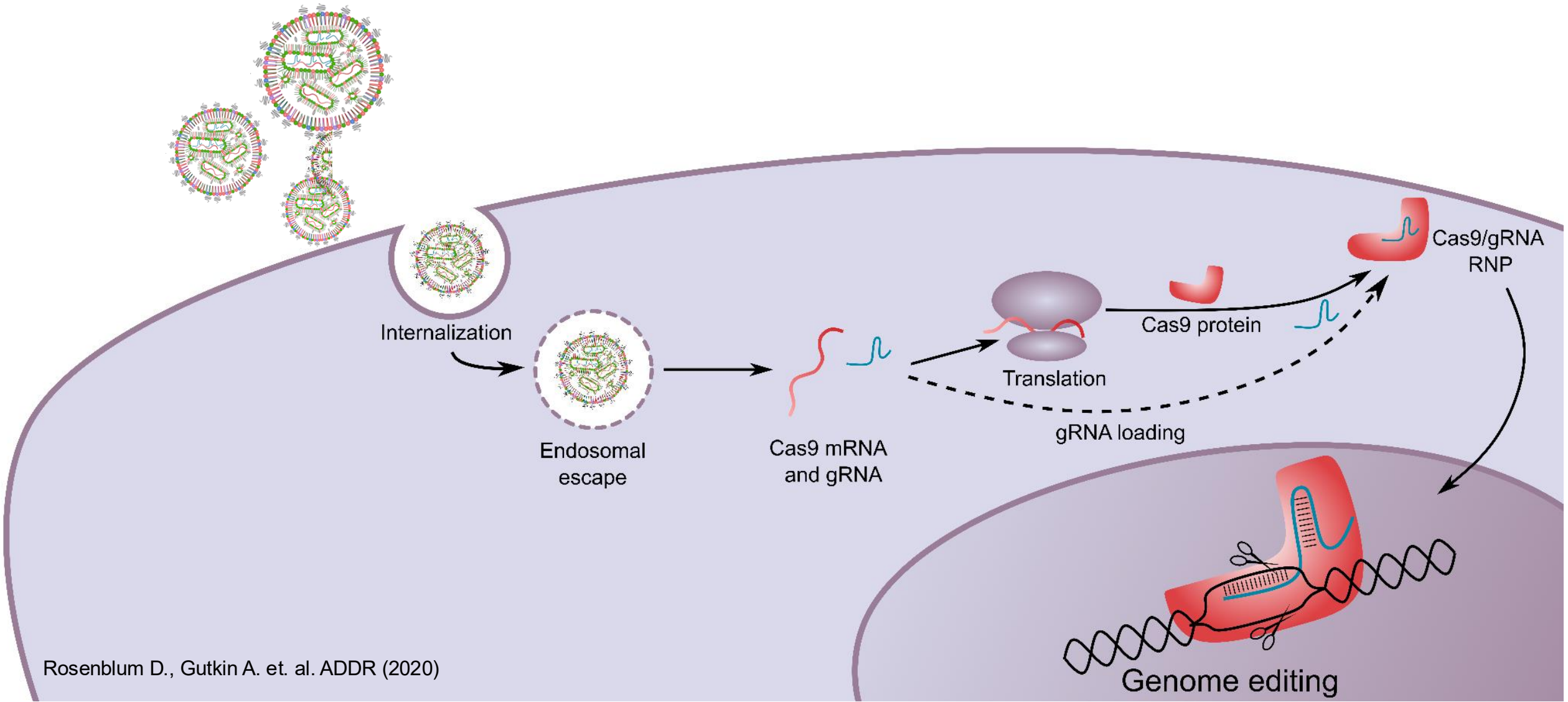
Cas9 nuclease: 160Kda, ~4600b

CRISPR/Cas9 Lipid nanoparticles (cLNPs)



Rosenblum D., Gutkin A et. al. Science Advances (2020)

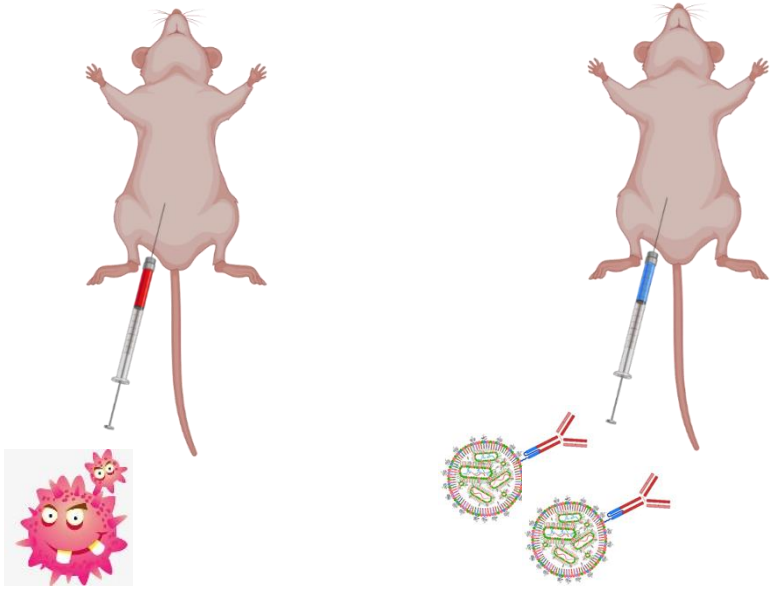
cLNPs mechanism of action

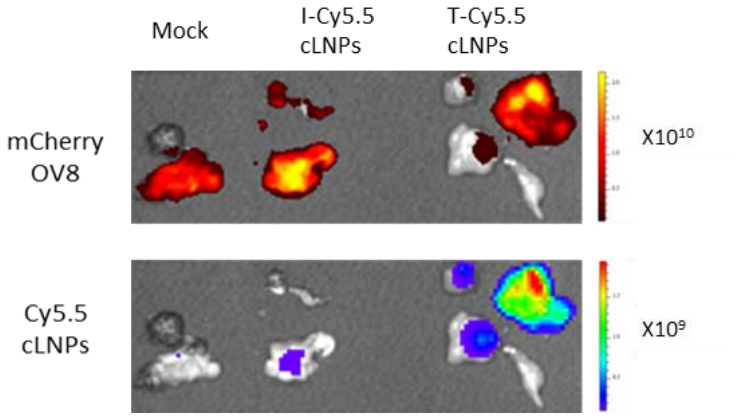


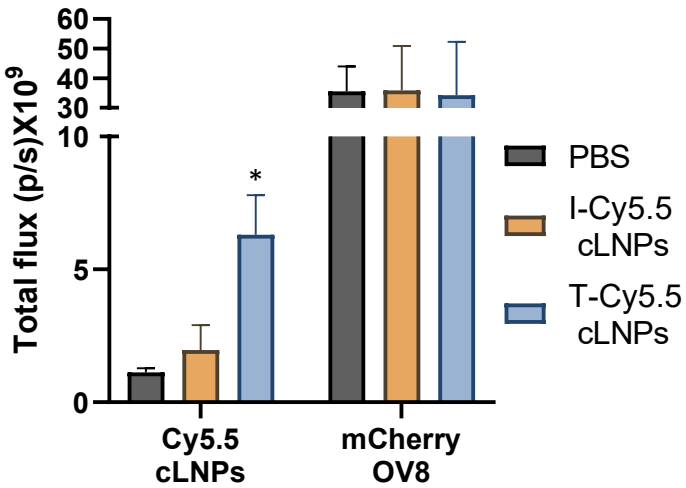
Rosenblum D., Gutkin A. et. al. ADDR (2020)

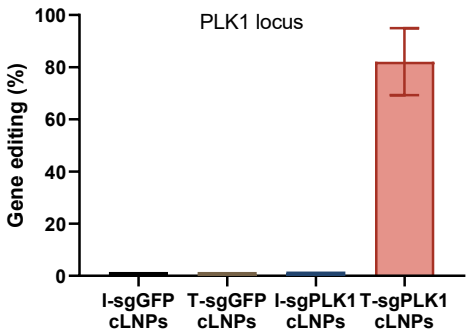
Systemic delivery of targeted cLNPs to metastatic ovarian adenocarcinoma

OV8 cells injection → 10d → cLNPs injection → 4h → Tumor targeting analysis → 3d → Gene disruption analysis







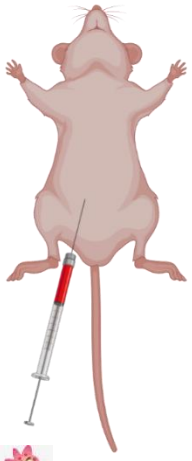


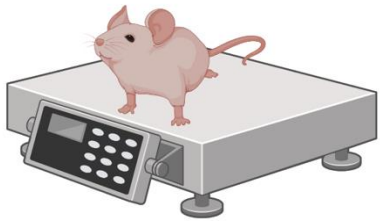
Gene editing at the PLK1 locus- 80%

Rosenblum D., Gutkin A. et. al. Science Advances (2020)

Schematic experimental design: Systemic delivery of targeted cLNPs to metastatic ovarian adenocarcinoma

OV8 cells injection → 10d → cLNPs injection → 60d follow up

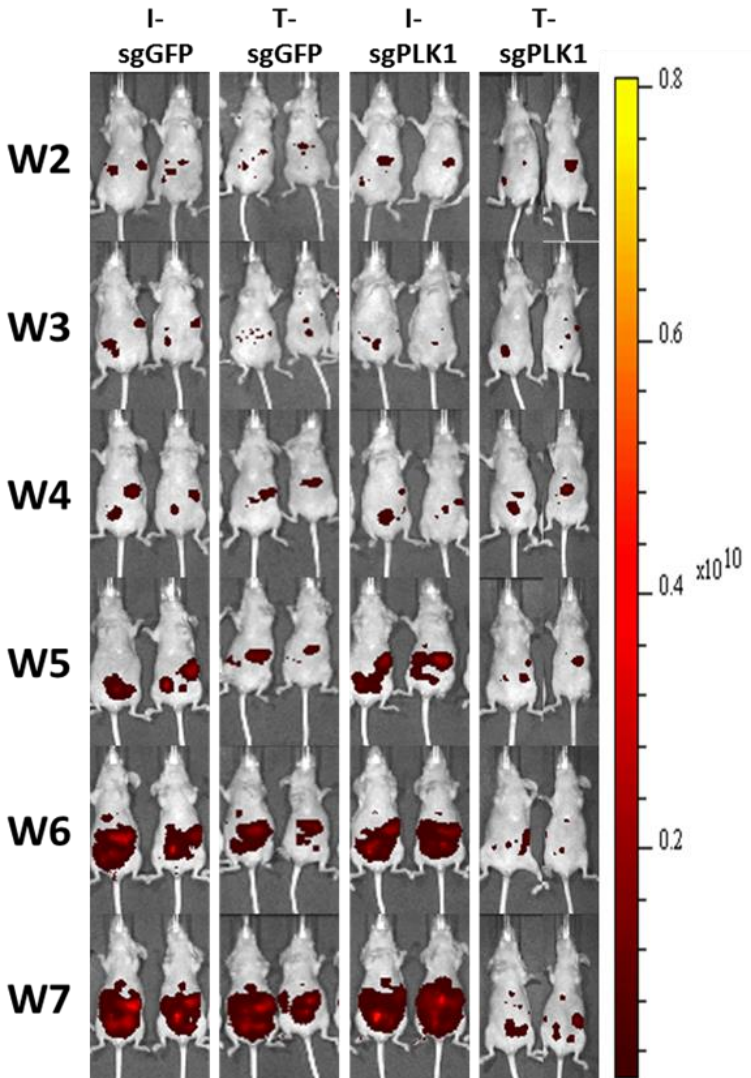


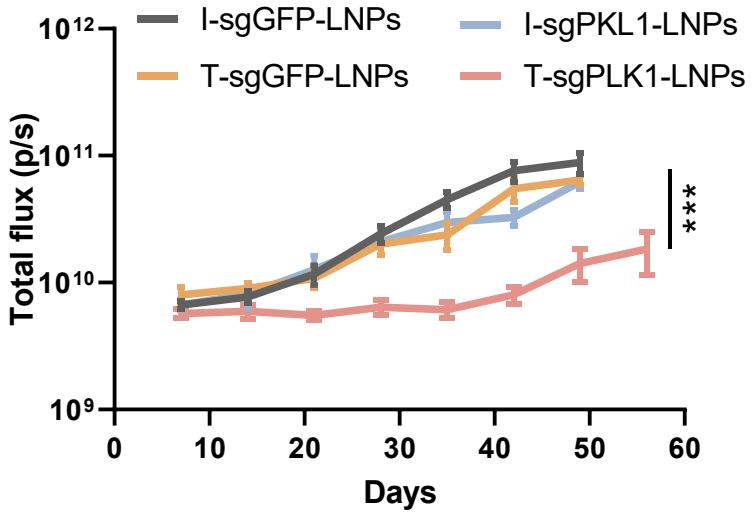


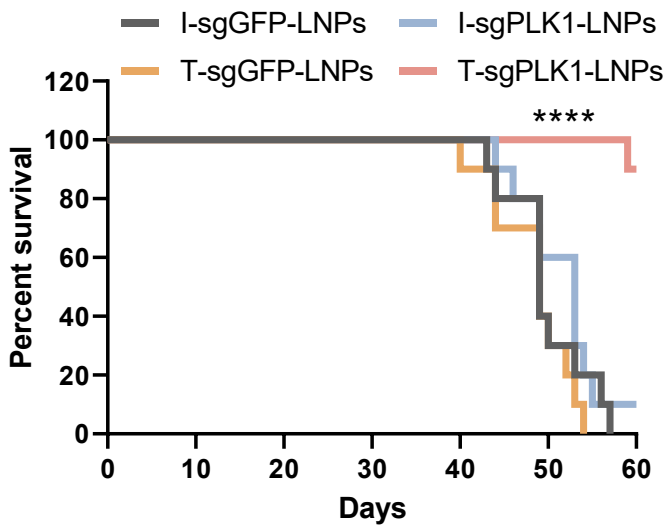
Treatments:

1. I-sgGFP-cLNPs
2. T-sgGFP- cLNPS
3. I-sgPLK1- cLNPS
4. T-sgPLK1-cLNPS

EGFR targeted sgPLK1-cLNPs potently inhibit tumor growth and increased overall survival in metastatic OV8 bearing mice





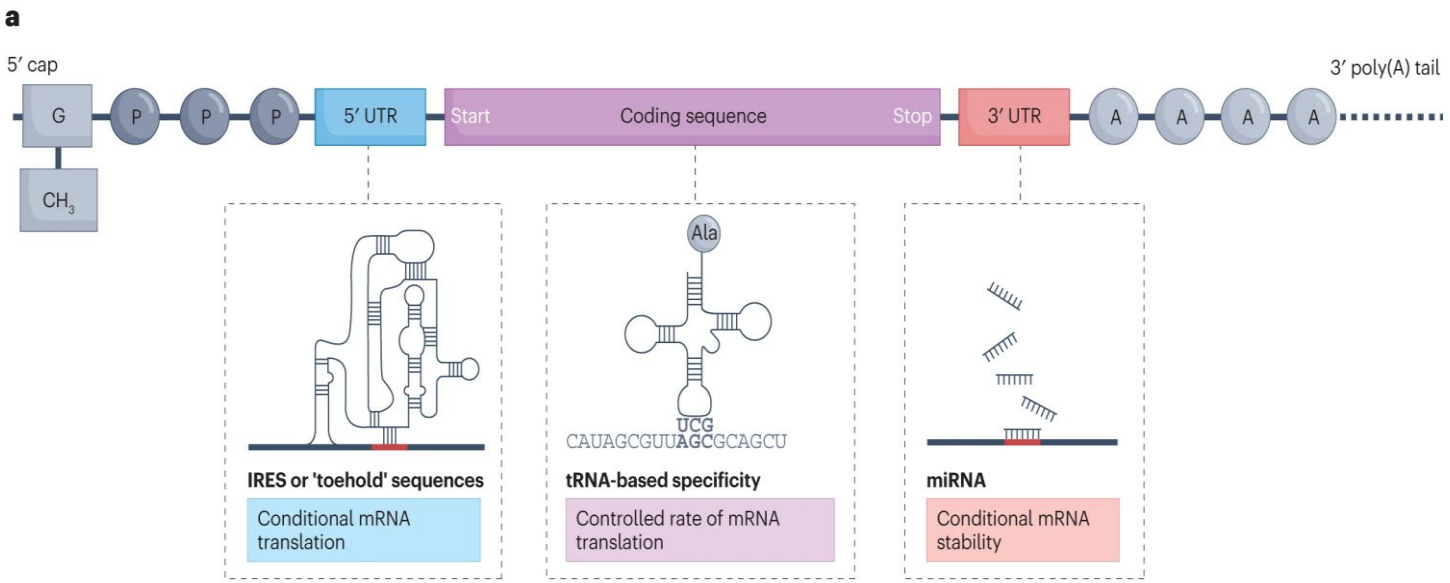


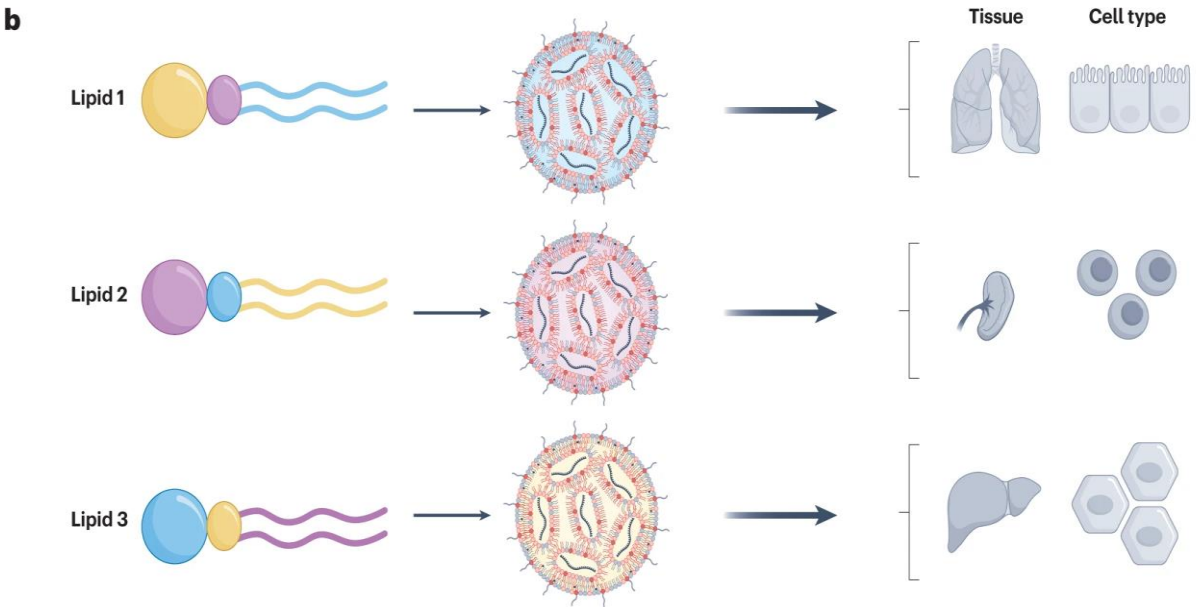
Increase:
Overall survival 90%

n=10 mice/group

Vision

Tailoring the expression of specific genes in diseased cells





Kon E. et al. Nature Reviews Clinical Oncology (2023)

Dr. Edo Kon

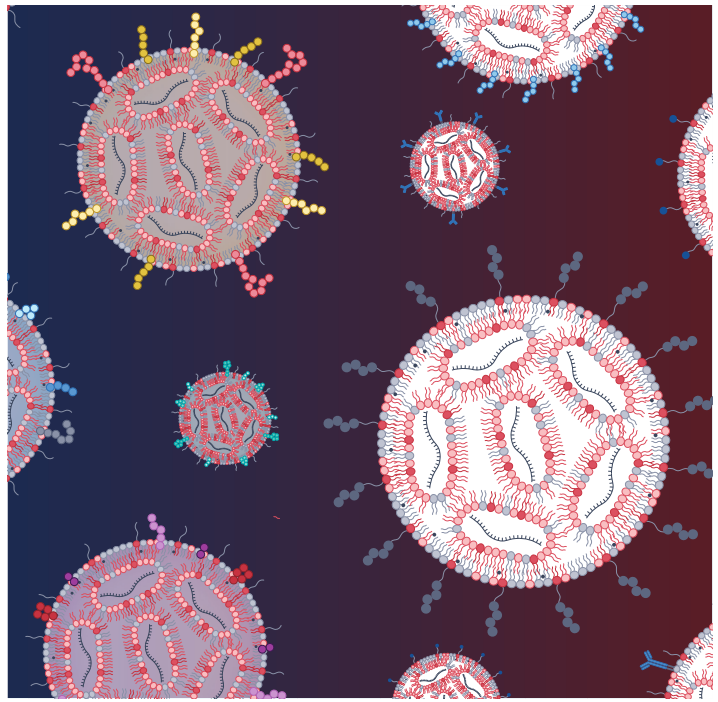
Dr. Inbal Hazan-Halevi

Lior Stostky-Oterin

Nitay Ad-El

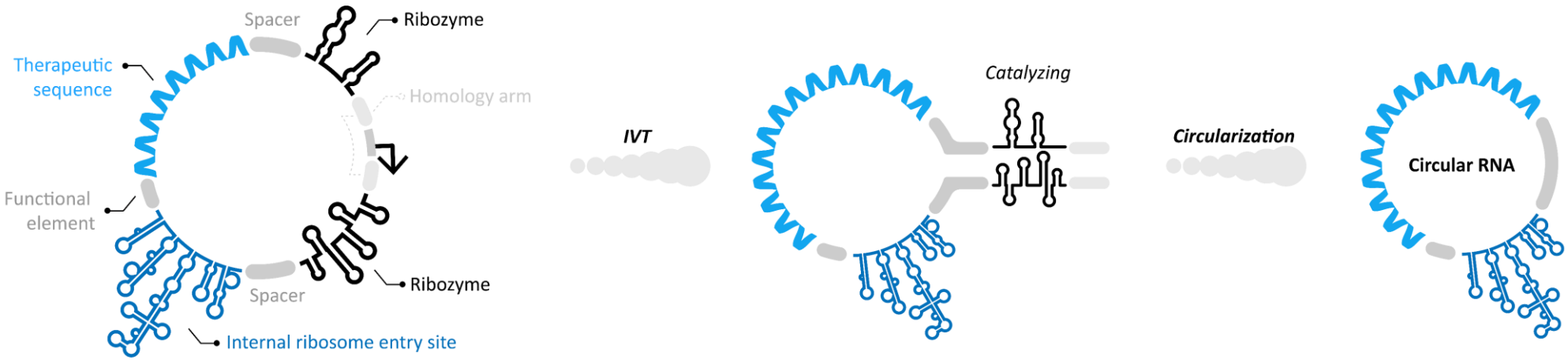
nature reviews clinical oncology

November 2023



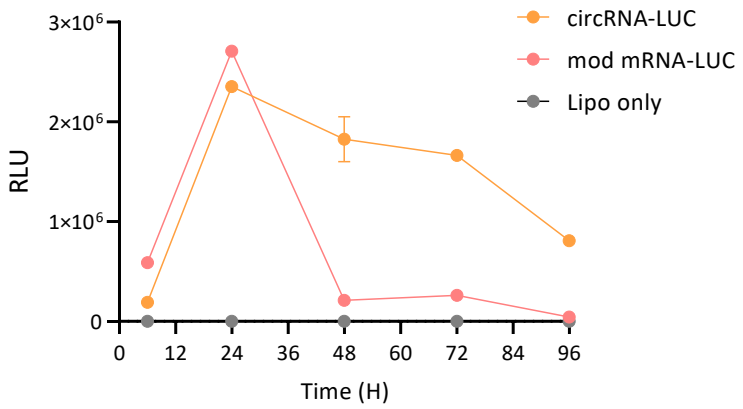
WHAT IS CIRCULAR RNA?

- Circular RNAs are covalently closed single-stranded RNA, naturally produced via back-splicing of pre-mRNA exon(s) in eukaryotic cells
- Broadly expressed in metazoans, but generally at low levels with cell/tissue-specific patterns
- Inherent stability with resistance to known exonucleases
- Can be engineered and produced *in vitro* via *in vitro* transcription and circularization

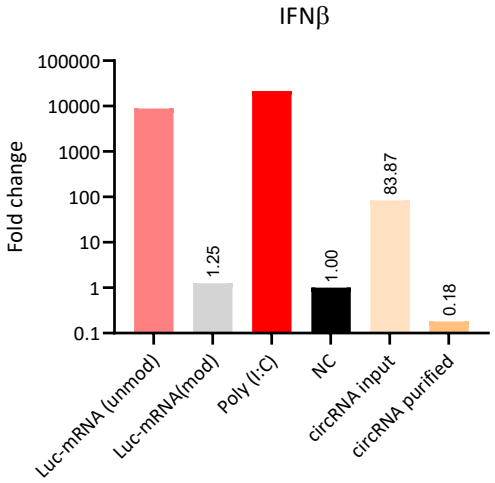


ENGINEERED CIRCULAR RNAS SHOW ADVANTAGES IN MANY ASPECTS

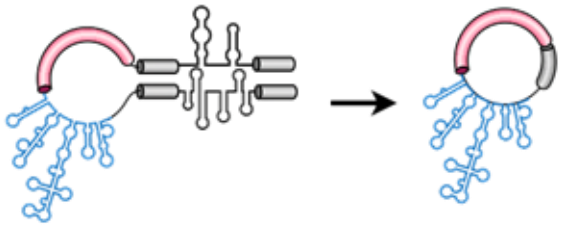
STABILITY AND TRANSLATIONAL EFFICIENCY



LOW IMMUNOGENICITY



MANUFACTURE FRIENDLY



No need for capping, polyadenylation

DIVERSE BIOMEDICAL APPLICATIONS

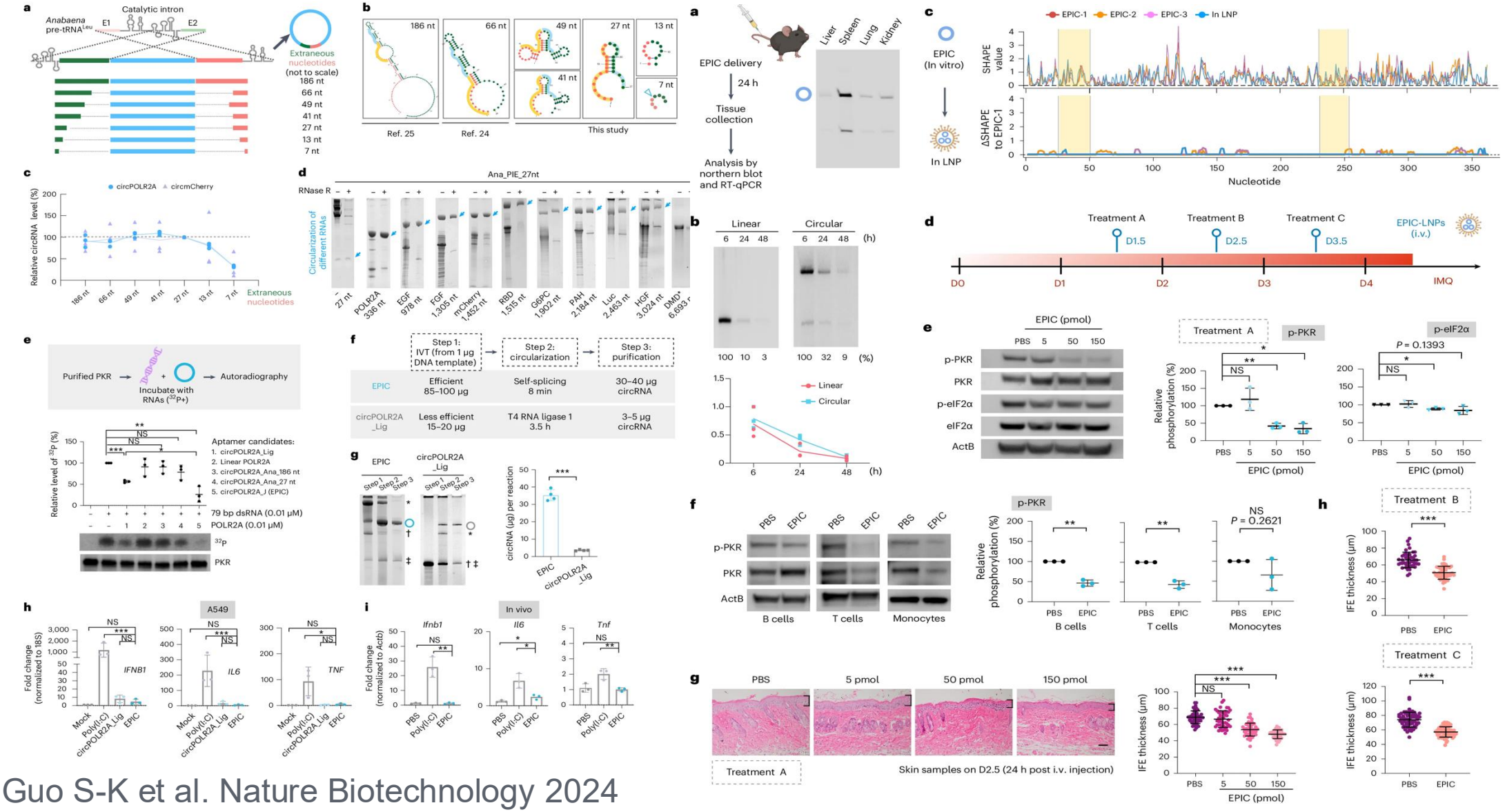
Express protein

miRNA/protein sponges

Inhibitors/boosters of innate immune responses

Protein scaffold

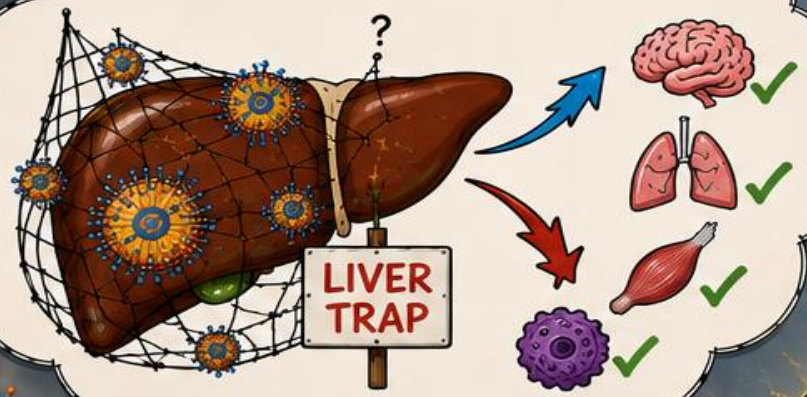
Therapeutic application of circular RNA aptamers in a mouse model of psoriasis



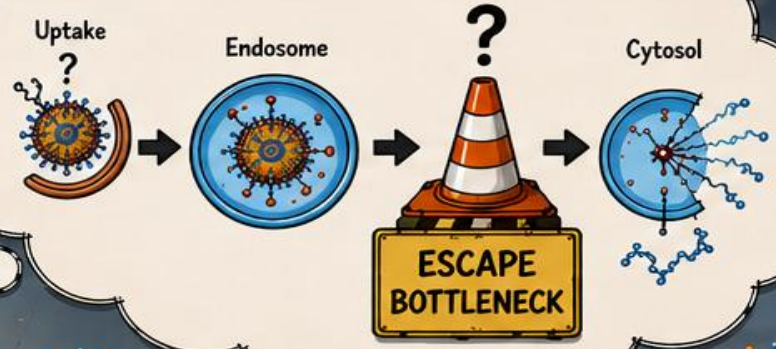
Guo S-K et al. Nature Biotechnology 2024

OPEN QUESTIONS

1 THE "LIVER TRAP" AND EXTRAHEPATIC TARGETING

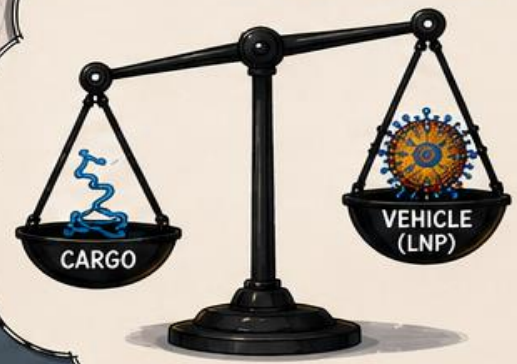


2 THE ENDOSOMAL ESCAPE BOTTLENECK



3 THE "PEG DILEMMA" AND REPEAT DOSING IMMUNOGENICITY (LIPIDS ?)

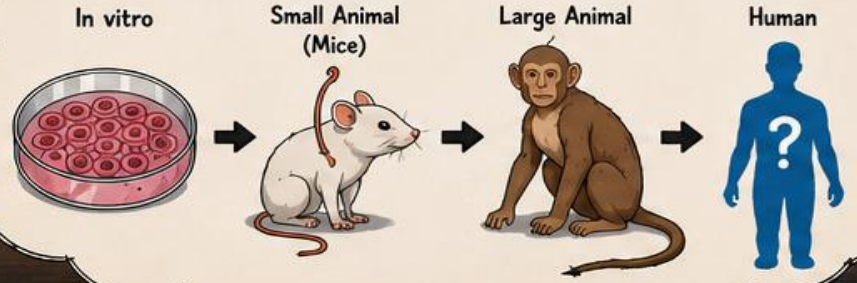
4 CARGO AND VEHICLE CO-DEPENDENCY

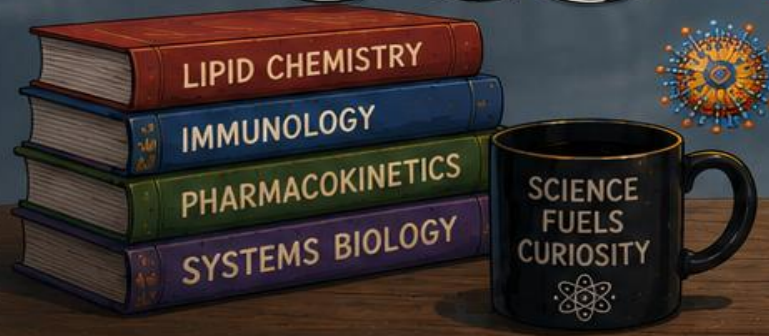


- ✓ Right cargo
- ✓ Right vehicle
- ✓ Right ratio
- ✓ Right route
- ✓ Right synergy

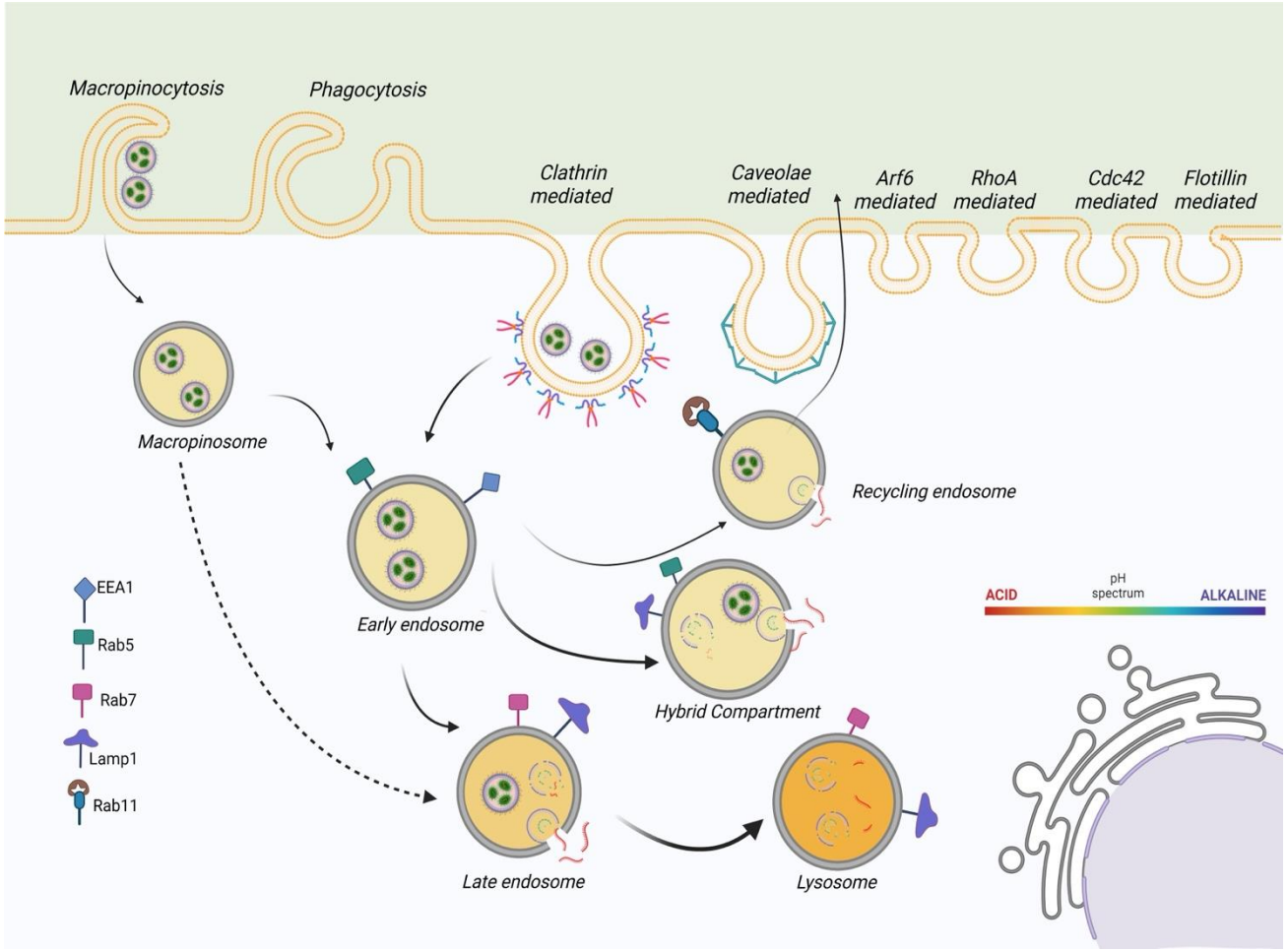
Or it all falls apart!

5 PRECLINICAL MODEL TRANSLATION



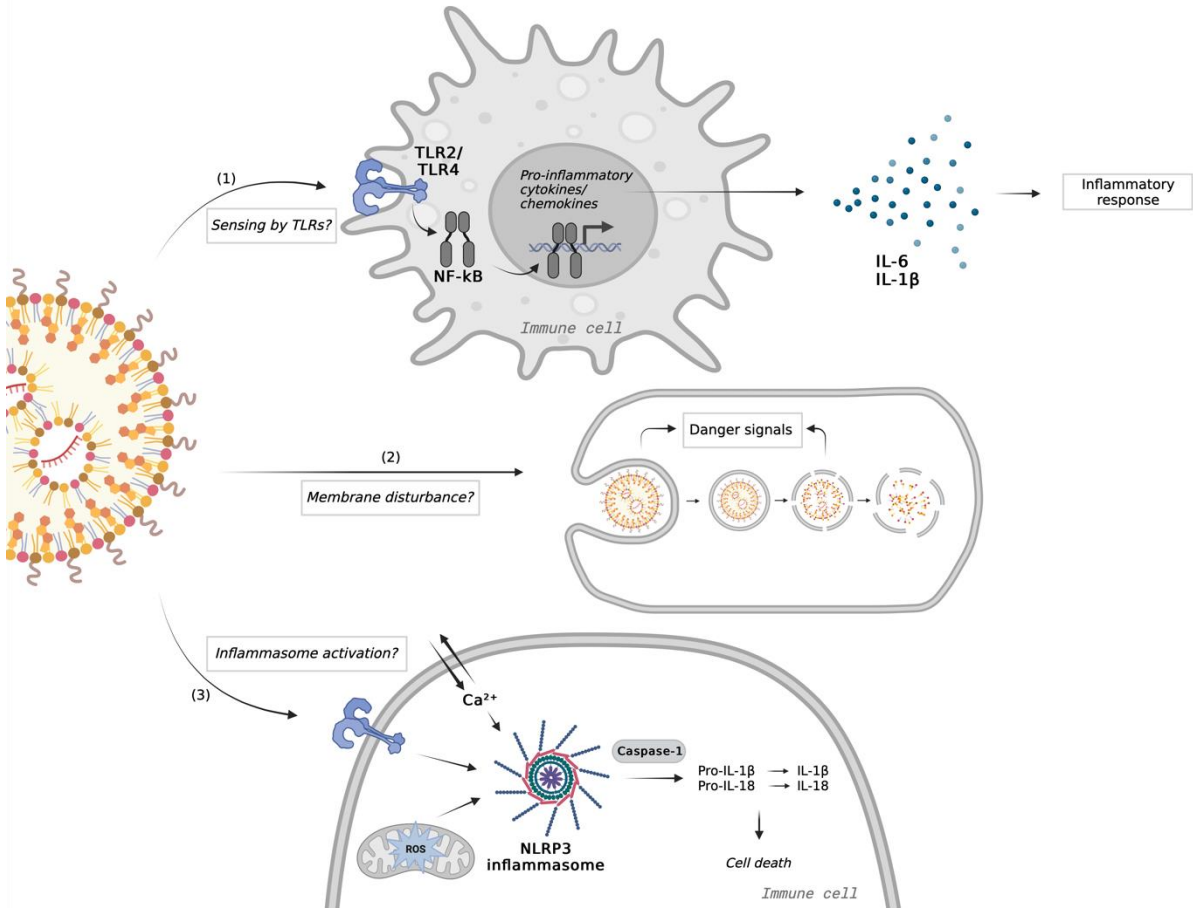


Endosomal Escape: A Bottleneck for LNP Mediated Therapeutics ?



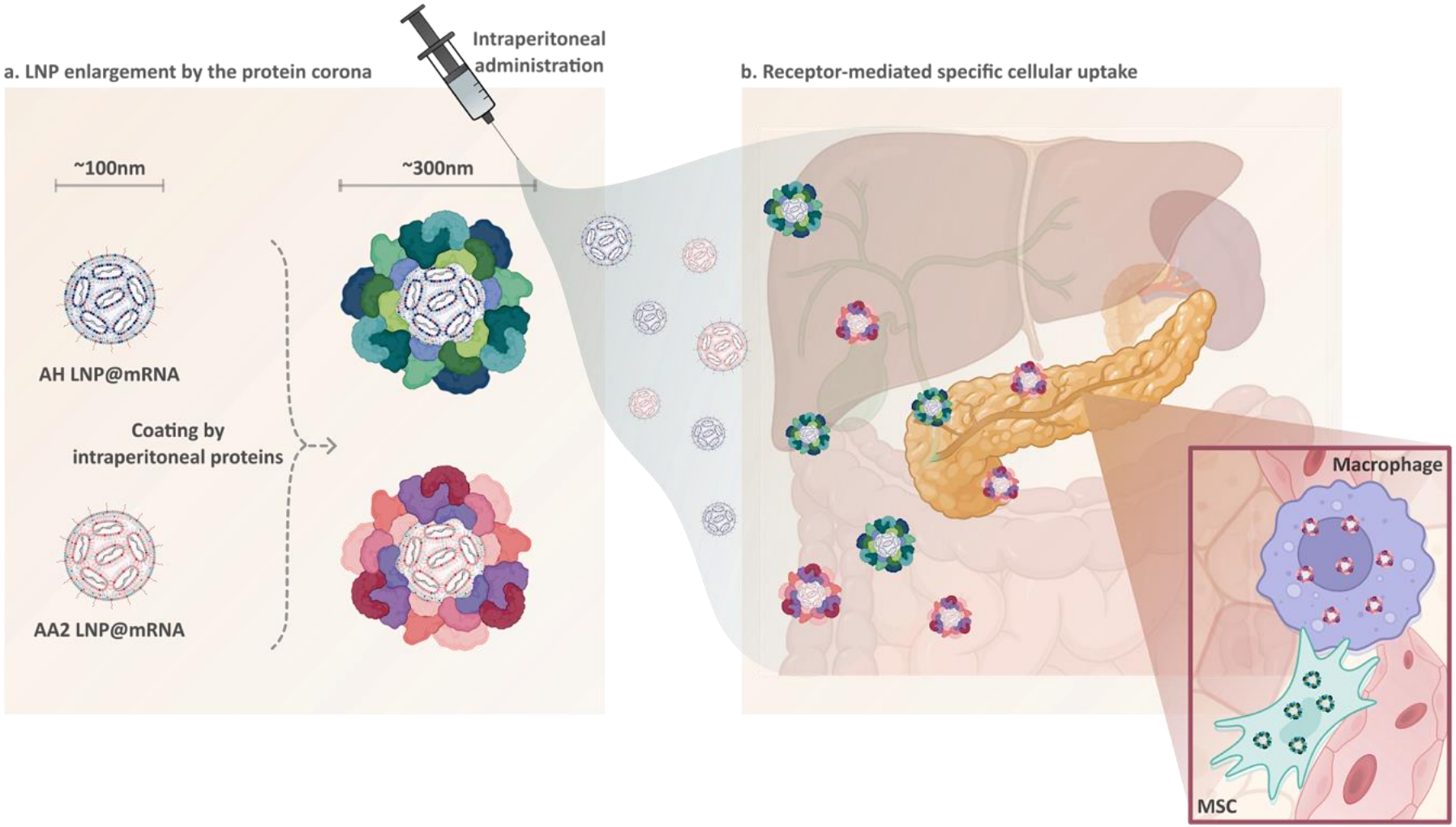
Chatterjee S. et al. PNAS (2024)

Understanding intracellular immune response of LNPs



Sharma P. et al. Nature Biomedical Engineering (2024)

Cell specific targeting in extrahepatic organs



Elisha A. and Peer D Nature (2026)

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המרכז לחקר המטאונקולוגי
על שם ורדה ובעז דותן

National Institute of Allergy and Infectious Diseases

National Cancer Institute

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LAD THERAPEUTICS

Roche

BIONTECH

MERCK

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RIDOX THERAPEUTICS

teva

ART BioScience
BRIDGING THE THERAPEUTIC GAP

Alnylam EXPERT