Peer Lab
Precision Nanomedicine

TEL AVIV UNIVERSITY
אוניברסיטת תל אביב

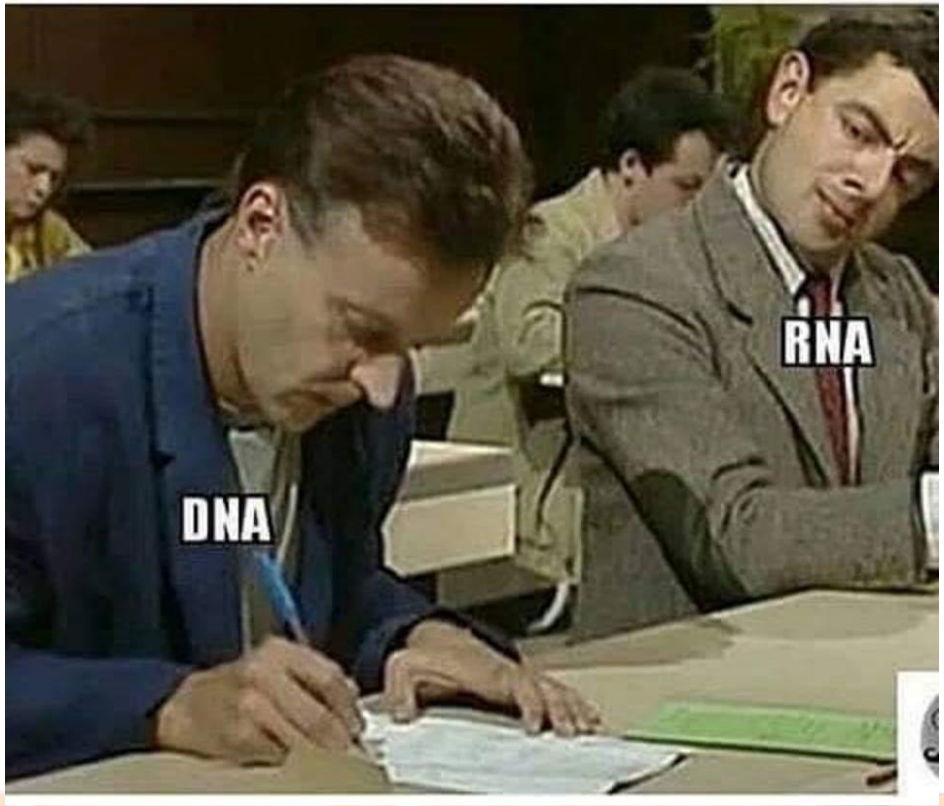
Therapeutic Genome Editing in Cancer via Targeted Lipid Nanoparticles Entrapping RNAs

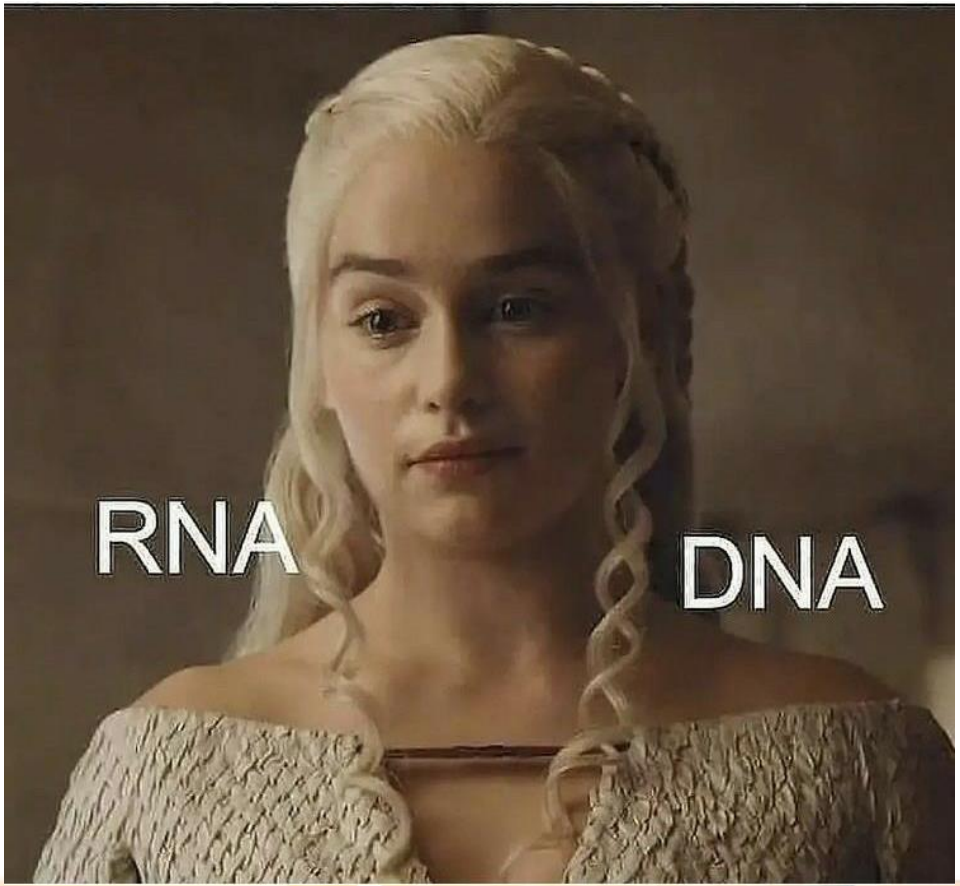
Dan Peer

CRS 2025 ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
JULY 13-18, 2025

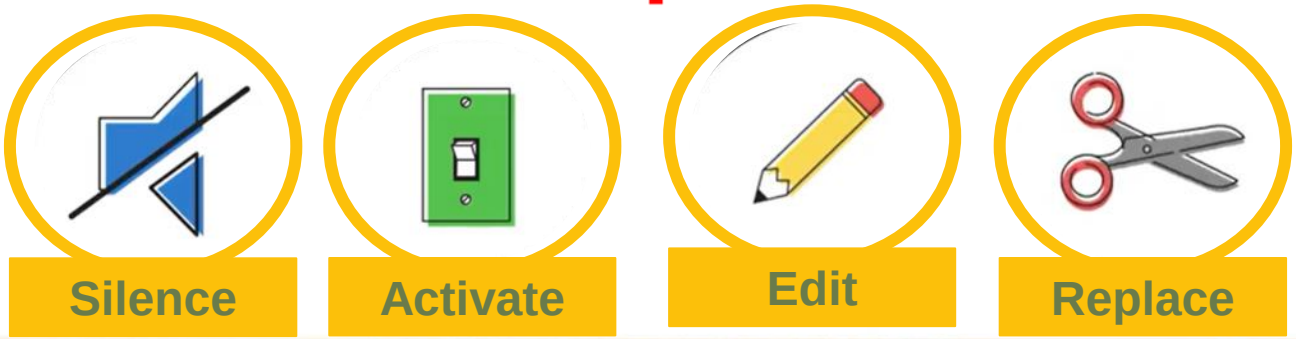
NEXT-GENERATION DELIVERY INNOVATIONS

The future is already here




RNA
DNA

Therapeutic approaches



RNA based therapeutics

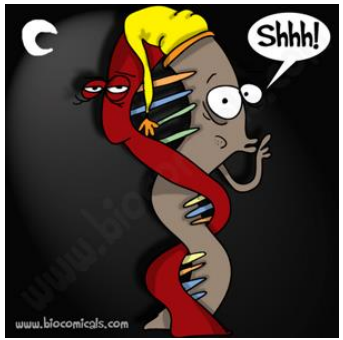
Silence

Activate

Edit

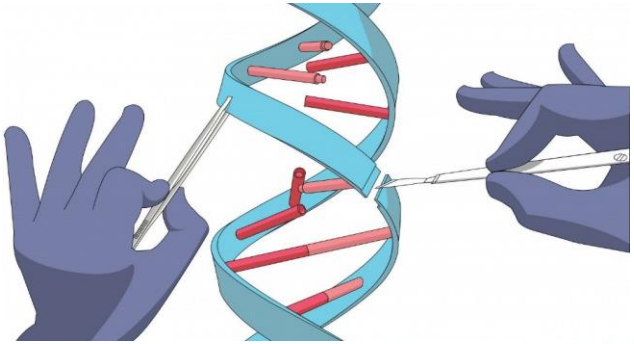
Replace

siRNA



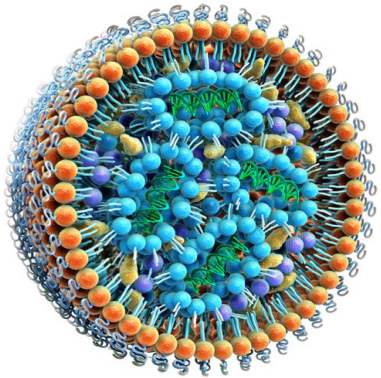
mRNA

Genome editing (e.g. CRISPR/Cas9)



The challenge in RNA therapeutics

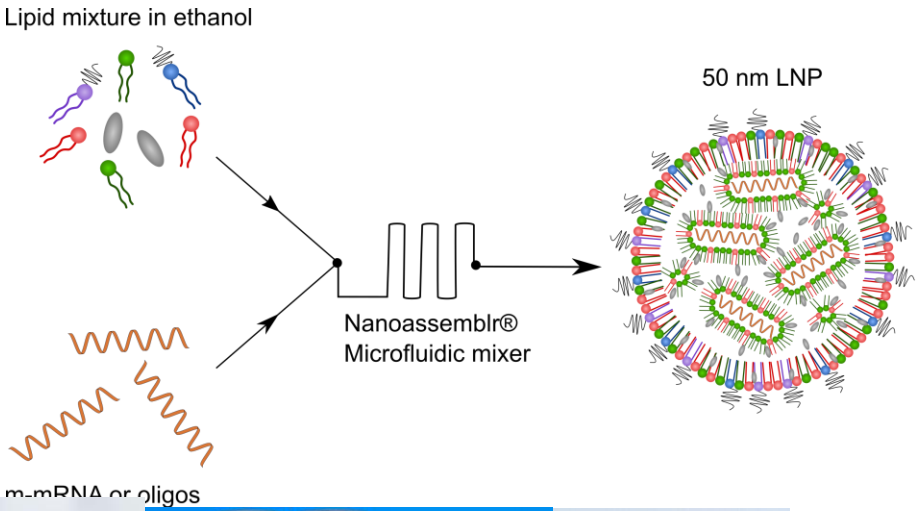
An appropriate delivery system.
Requirements:
Efficient RNA encapsulation
Evading clearance mechanism
Avoid toxicity and immune activation
Carrier internalization
RNA release
Specificity

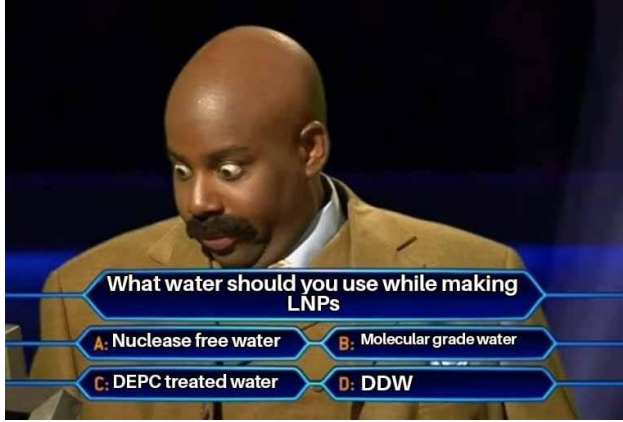


Lipid nanoparticles (LNPs)

Components:

- Cholesterol
- DSPC
- PEG-DMG
- Ionizable lipid



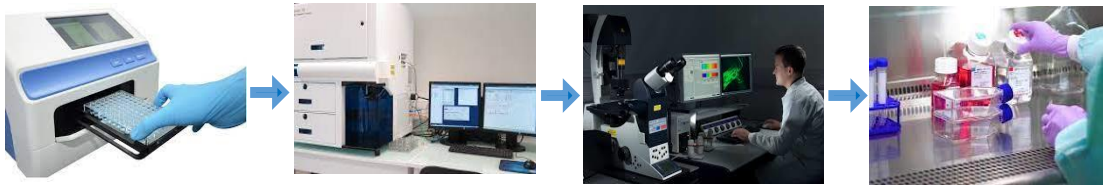


LNPs advantages:

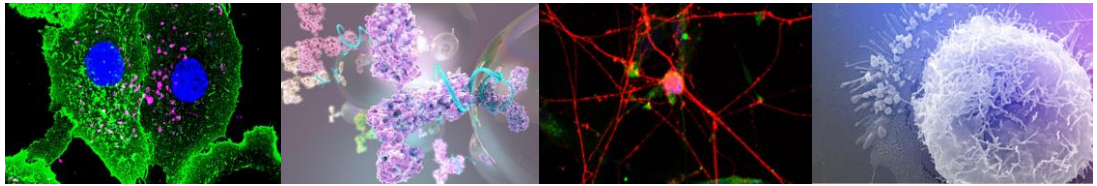
- Biocompatible
- Biodegradable
- Nucleic acid protection
- Increase stability in circulation
- Increase therapeutic effect
- Clinically approved

Laboratory of Precision NanoMedicine

Exome / Transcriptome analysis
Target Discovery & Validation



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

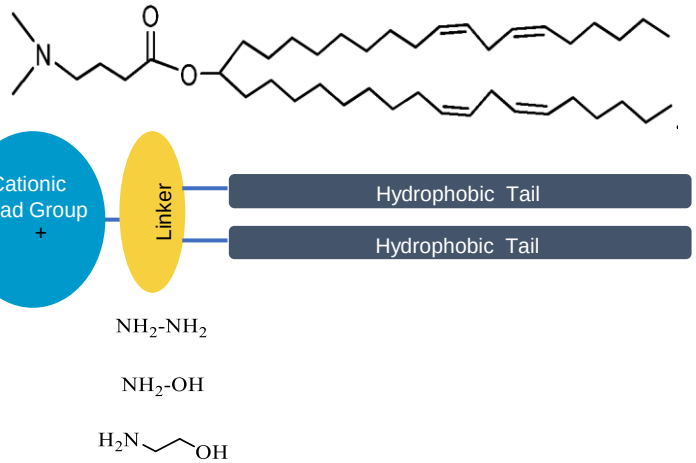


Tailor-made the appropriate nano-vehicle, selective targeting agent and specific therapeutic payload

Structural Design of Ionizable Lipids

Dr. Srinivas Ramishetti

Dlin-MC3-DMA



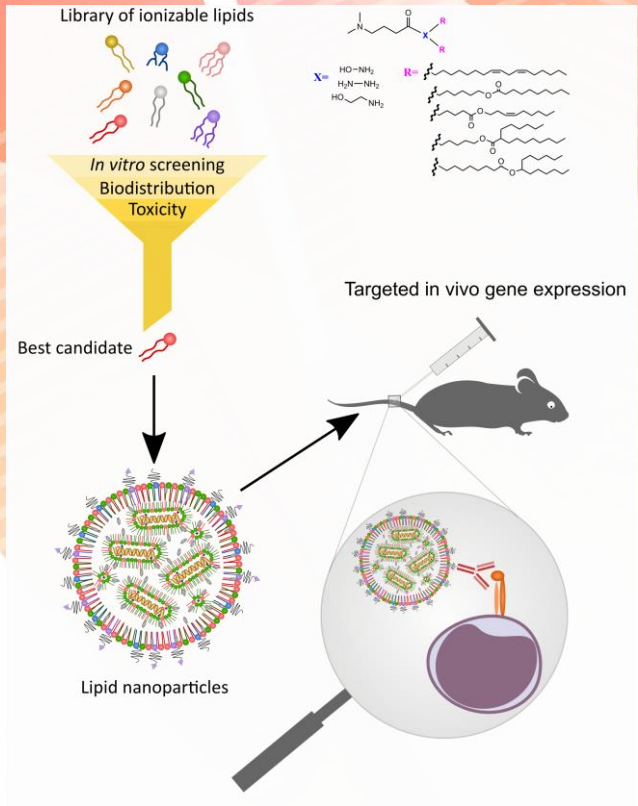
Based on the gold standard we have synthesized more than 100,000 different lipids from 2012.

Ramishetti S. et al. Advanced Materials 2020

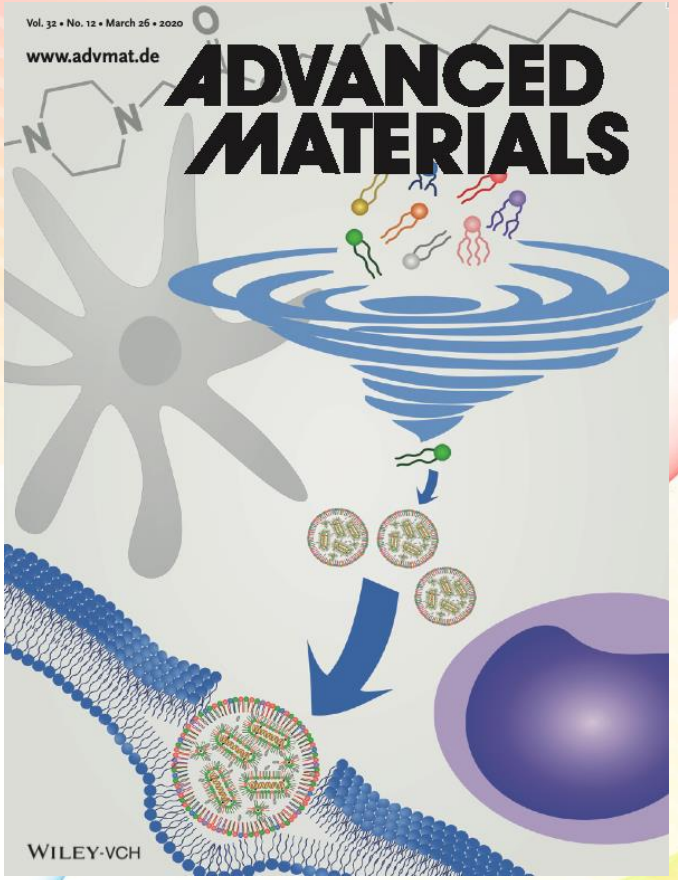
Dr. Inbal Hazan-Halevi

Dr. Srinivas Ramishetti

Dr. Somu Gonna Naidu



Ramishetti S. et al. Advanced Materials 2020



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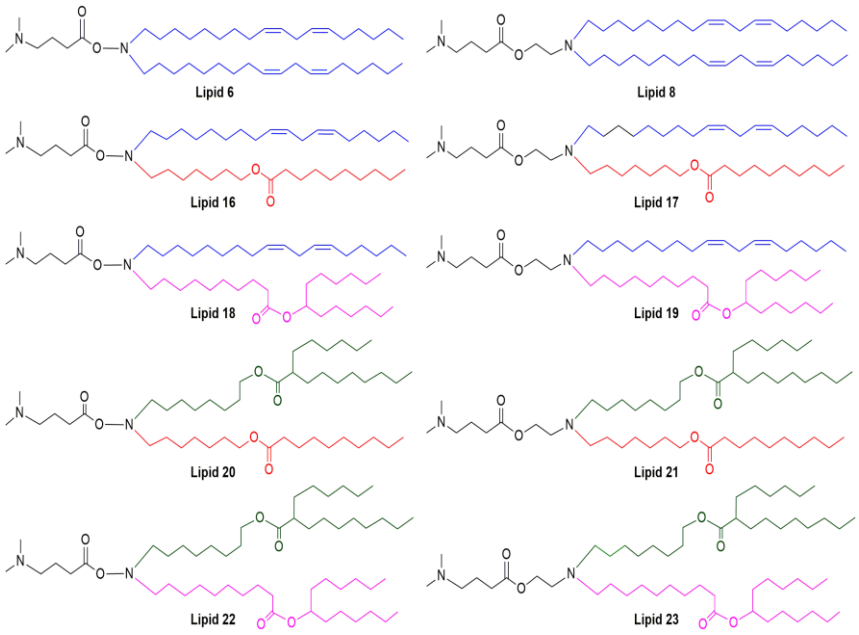
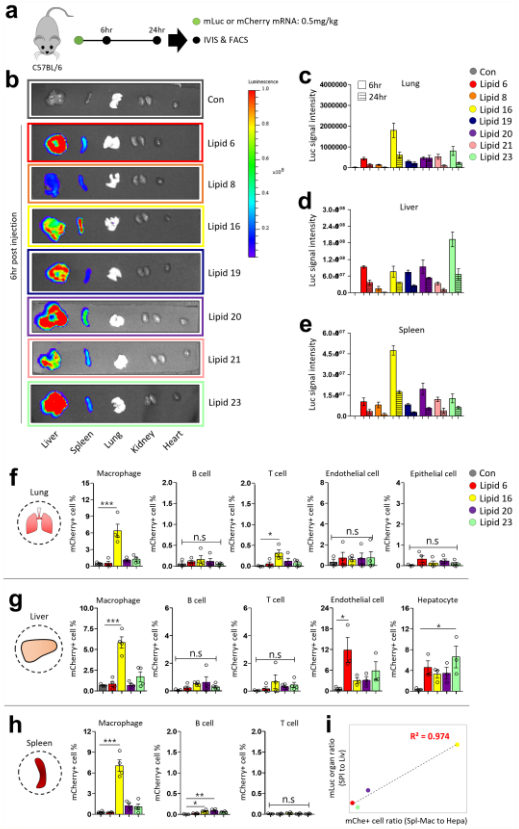
NEXT-GENERATION DELIVERY INNOVATIONS

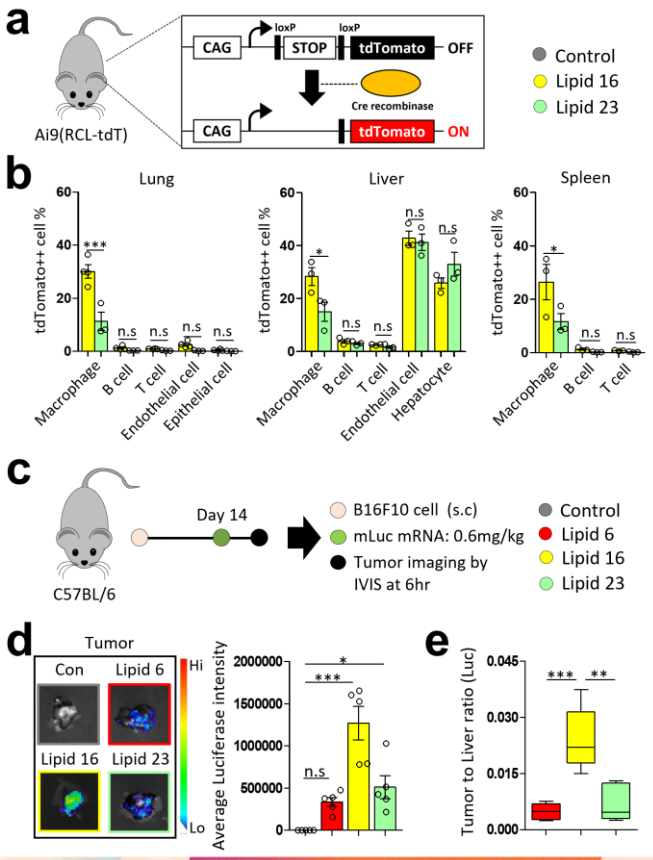
Dr. Somu Gonna Naidu



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

Organ specific delivery or cell specific ?





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

Ionizable lipids with optimized linkers enable lung-specific, lipid nanoparticles-mediated mRNA delivery for treatment of metastatic lung tumors

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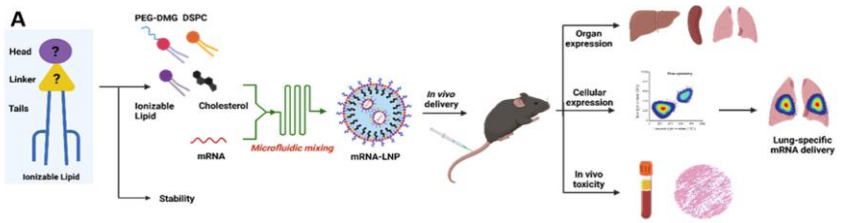
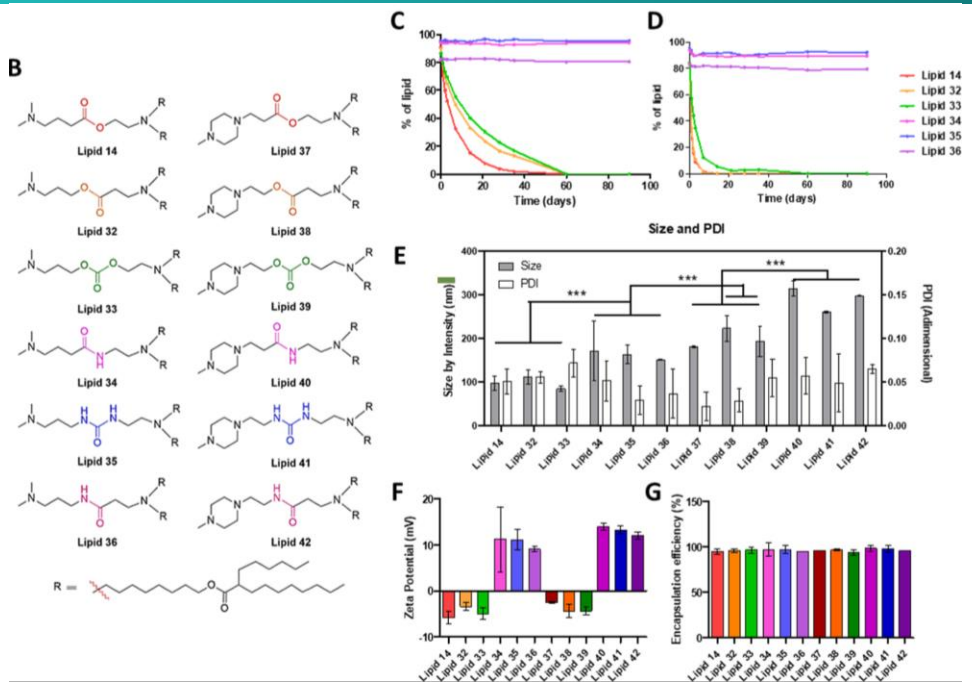
NEXT-GENERATION DELIVERY INNOVATIONS

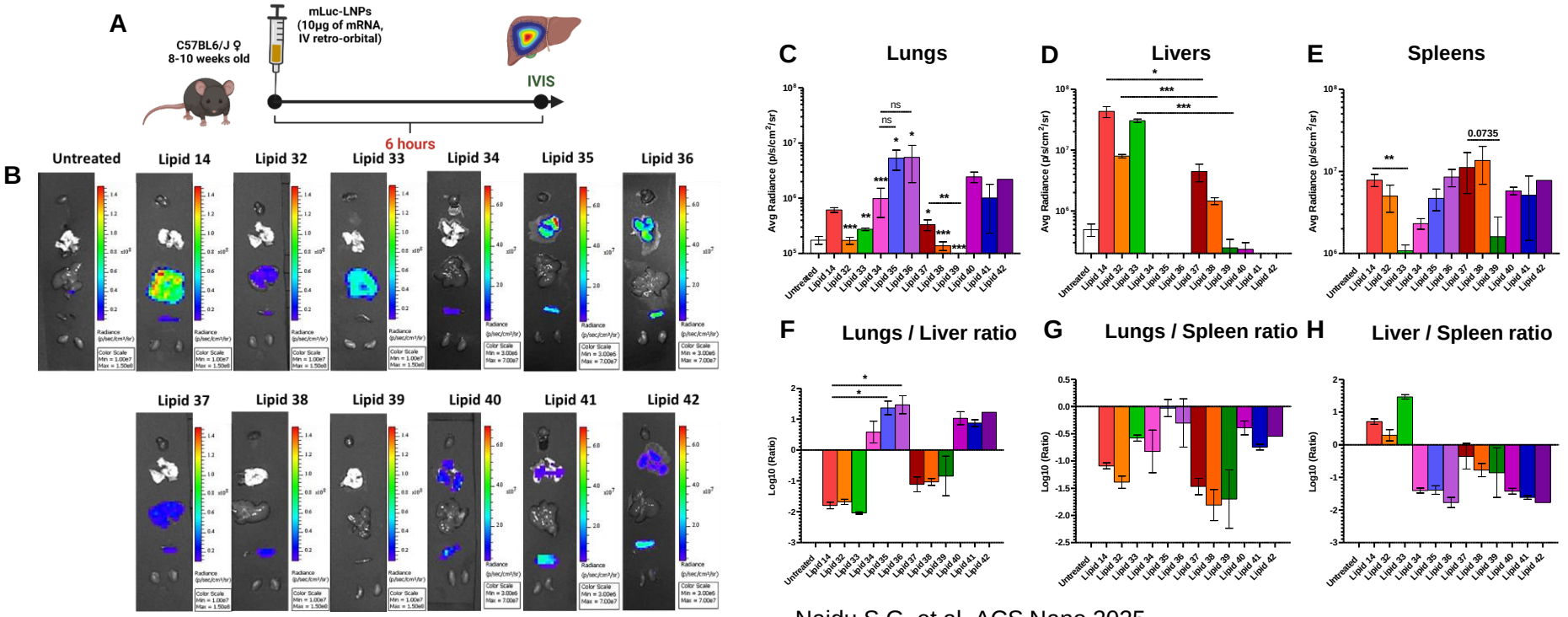
Ionizable lipids structure and LNPs characterization





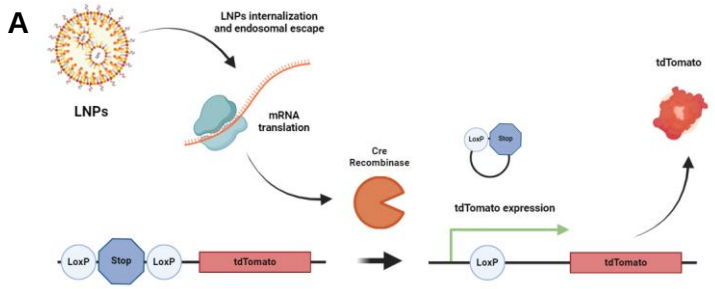
Naidu S.G. et al. ACS Nano 2025

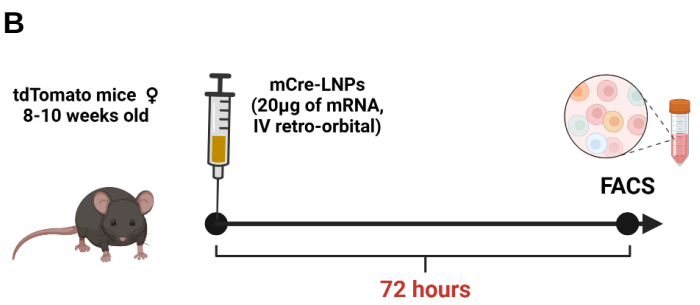
Luciferase mRNA expression in vivo

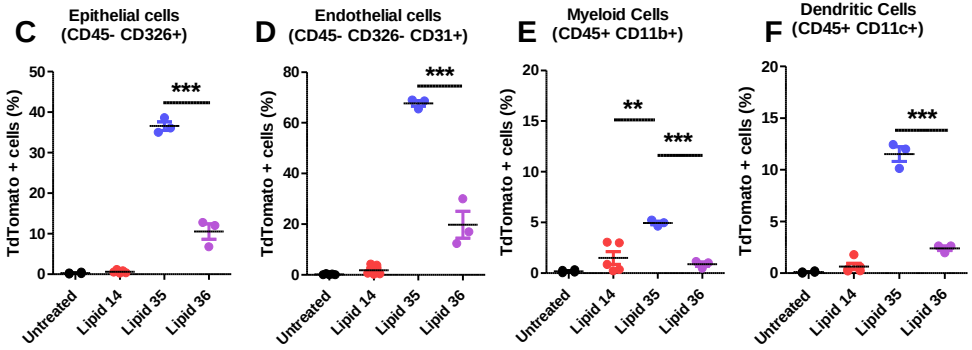


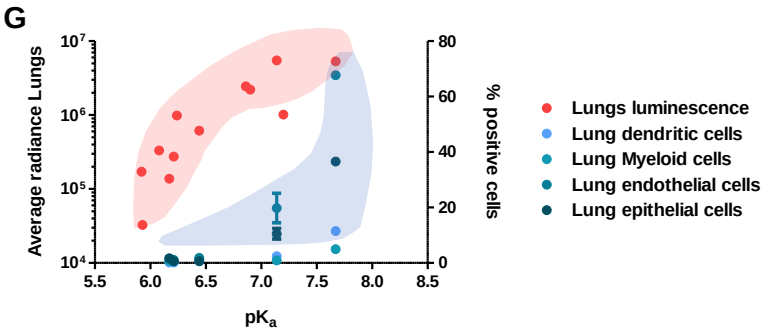
Naidu S.G. et al. ACS Nano 2025

LNPs targets endothelial and epithelial lung cells



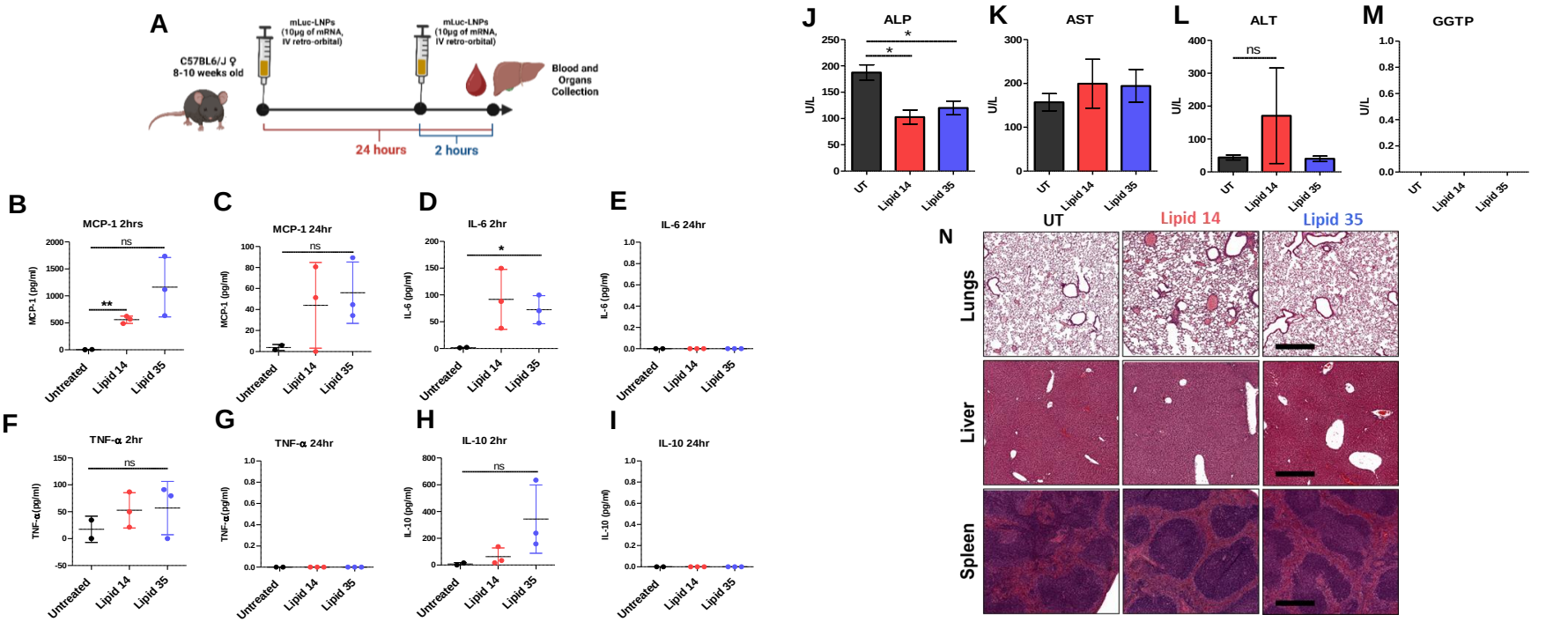






Naidu S.G. et al. ACS Nano 2025

LNPs biocompatibility



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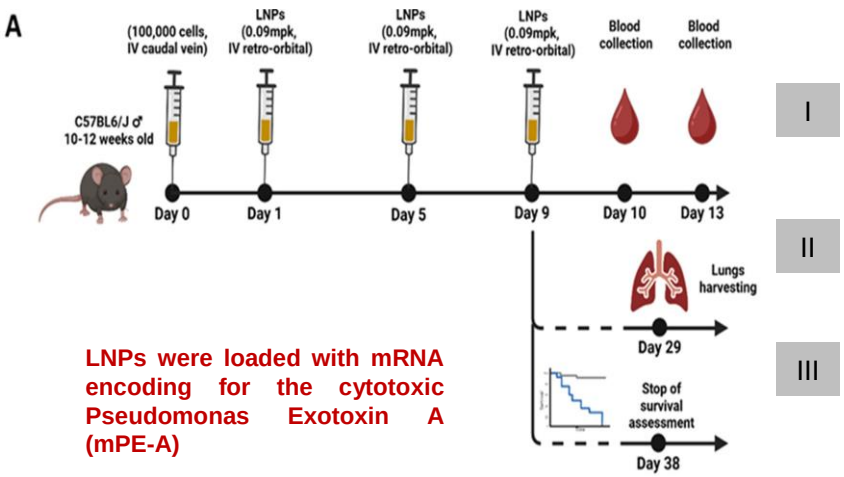
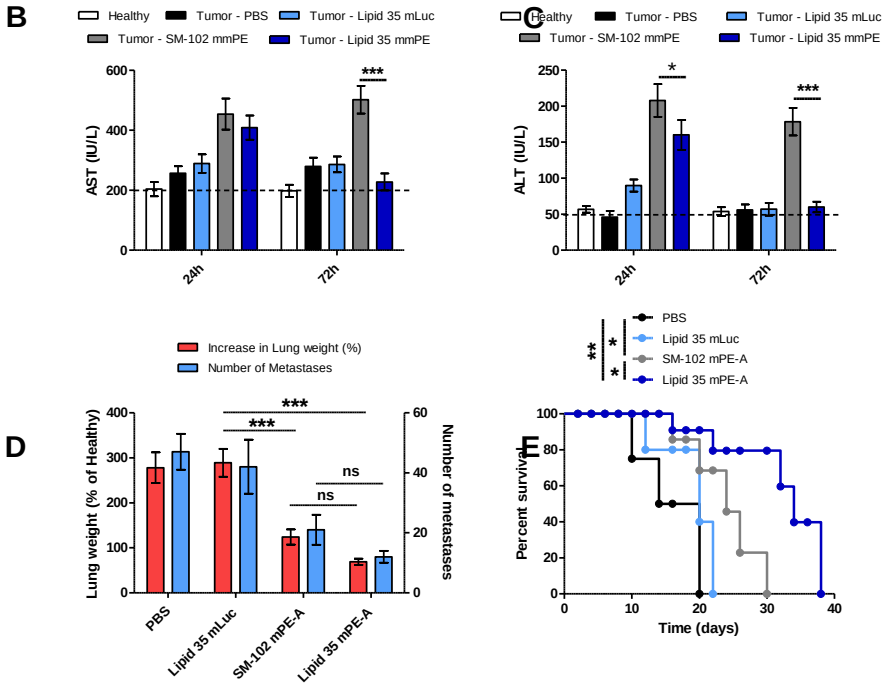
LNPs therapeutic efficacy against lung tumor metastases



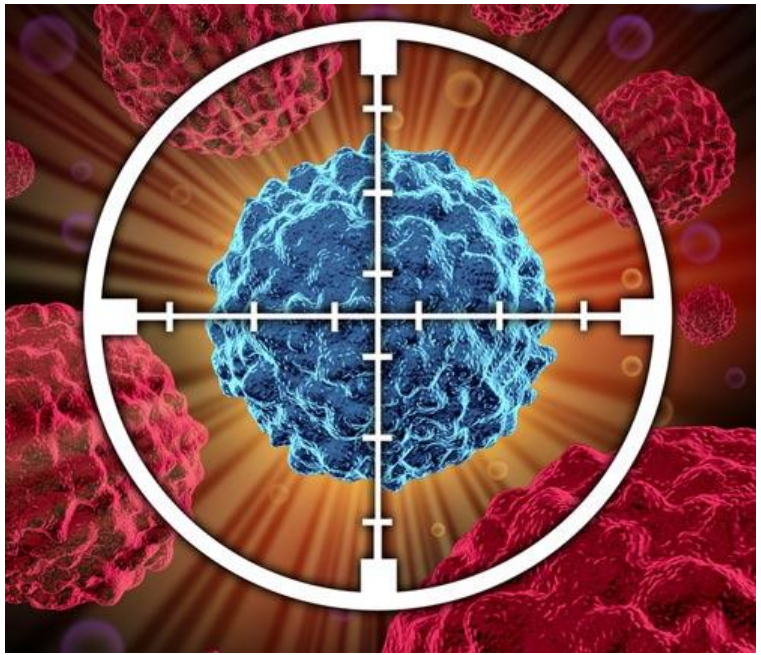
LNPs were loaded with mRNA encoding for the cytotoxic Pseudomonas Exotoxin A (mPE-A)



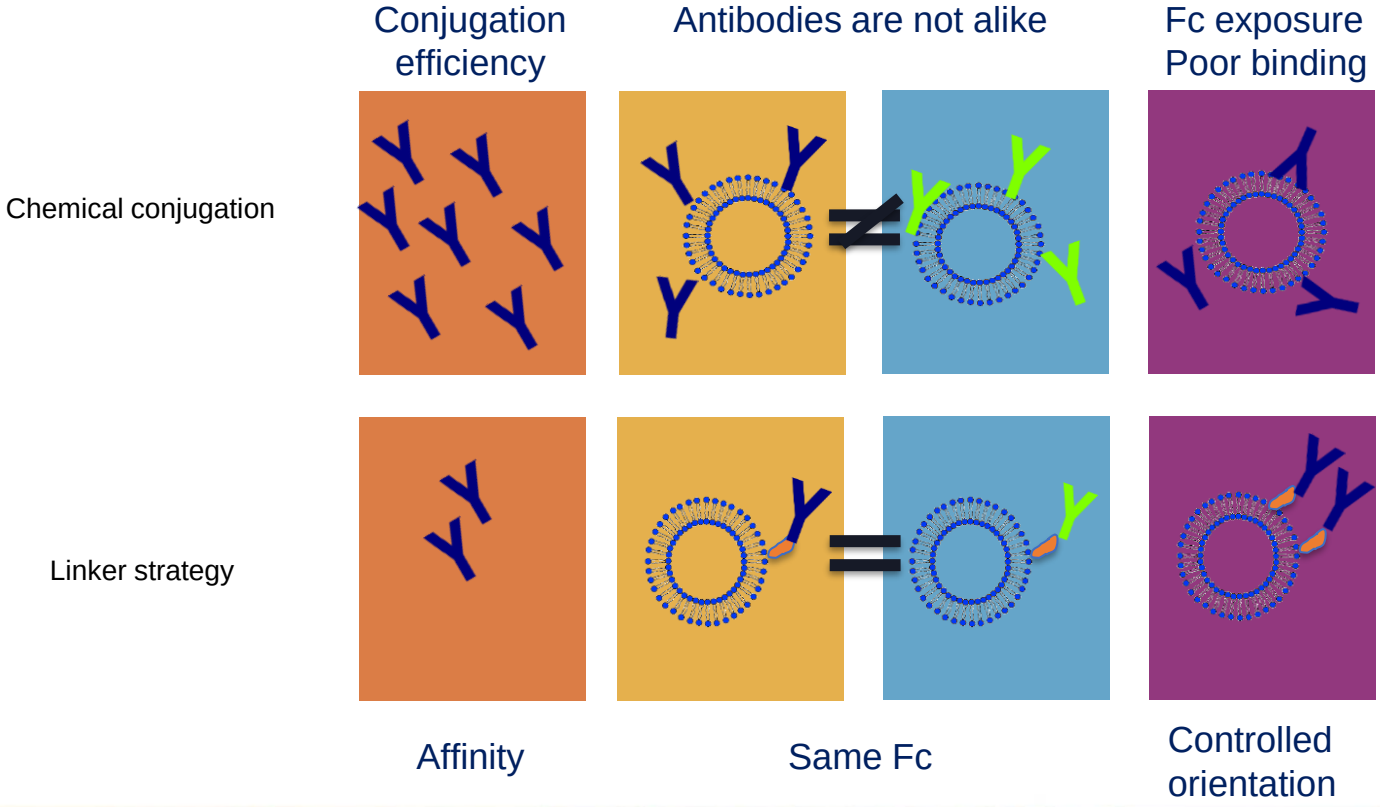
Naidu S.G. et al. ACS Nano 2025

Targeted delivery platform

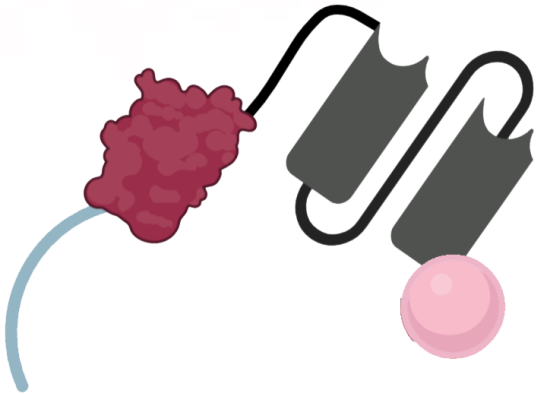
Targeting moiety-
Antibodies
Peptides
Ligands
Aptamers



Antibody targeted LNPs construction



Anchored Secondary scFv Enabling Targeting – ASSET



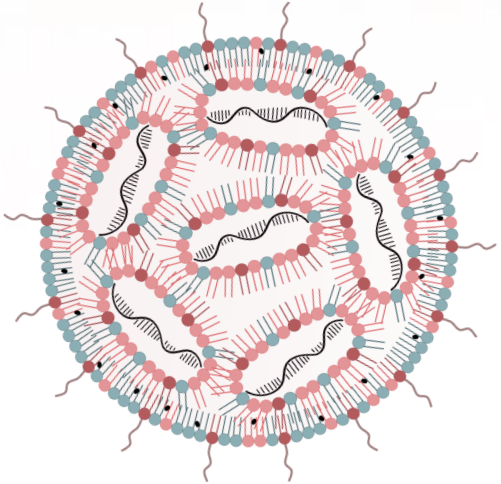
Dr. Ranit Kedmi

Dr. Nuphar Veiga

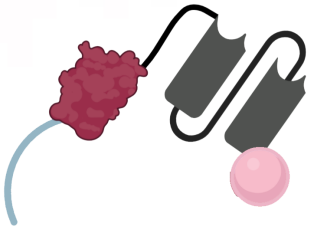
Kedmi, R. *et al. Nat. Nanotechnol.* **13**, 214–219 (2018).

ASSET as a Targeting Platform

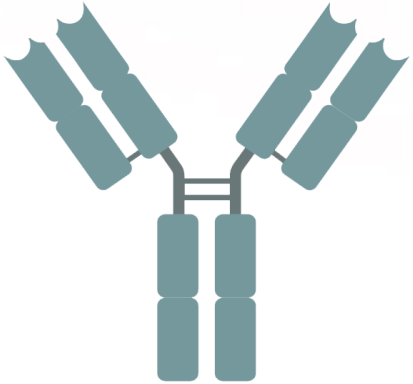
LNPs



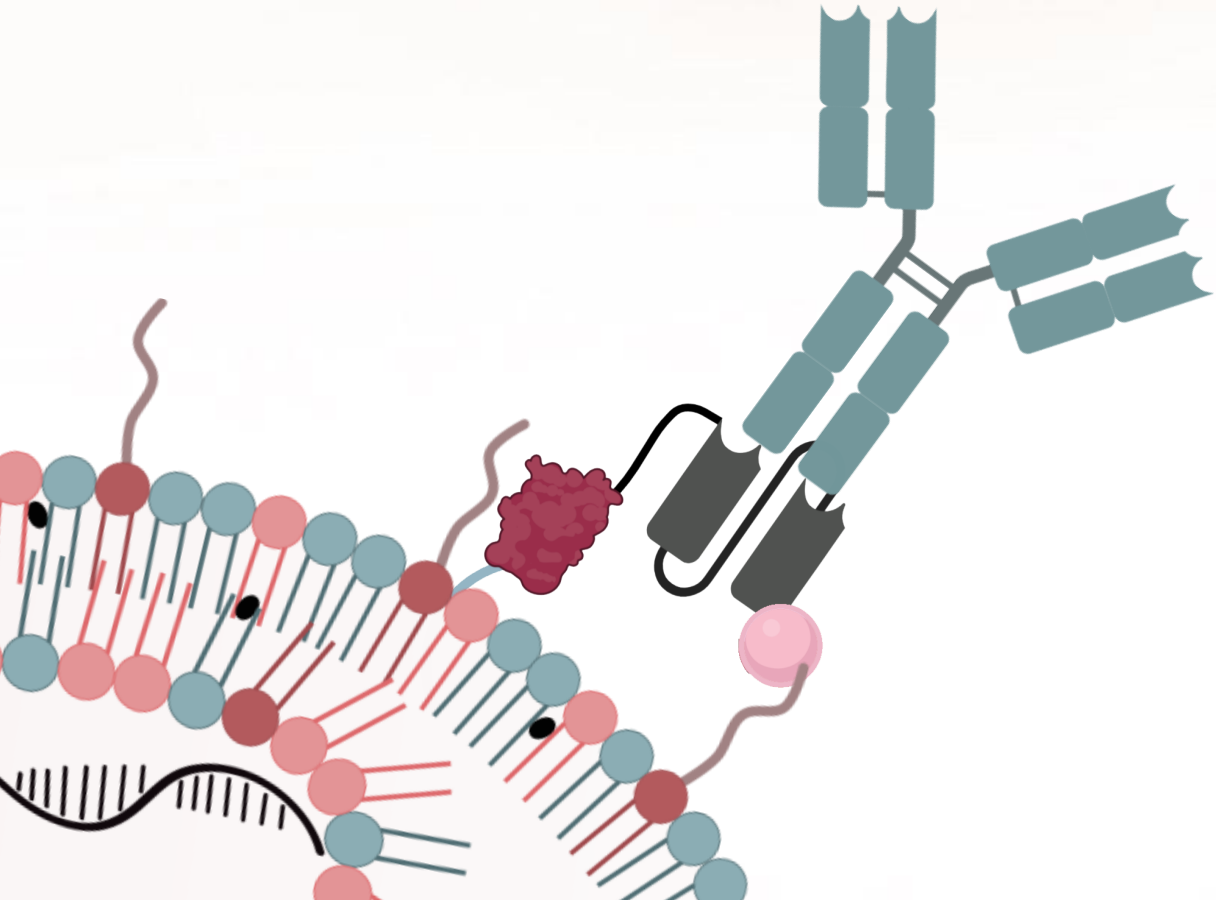
ASSET



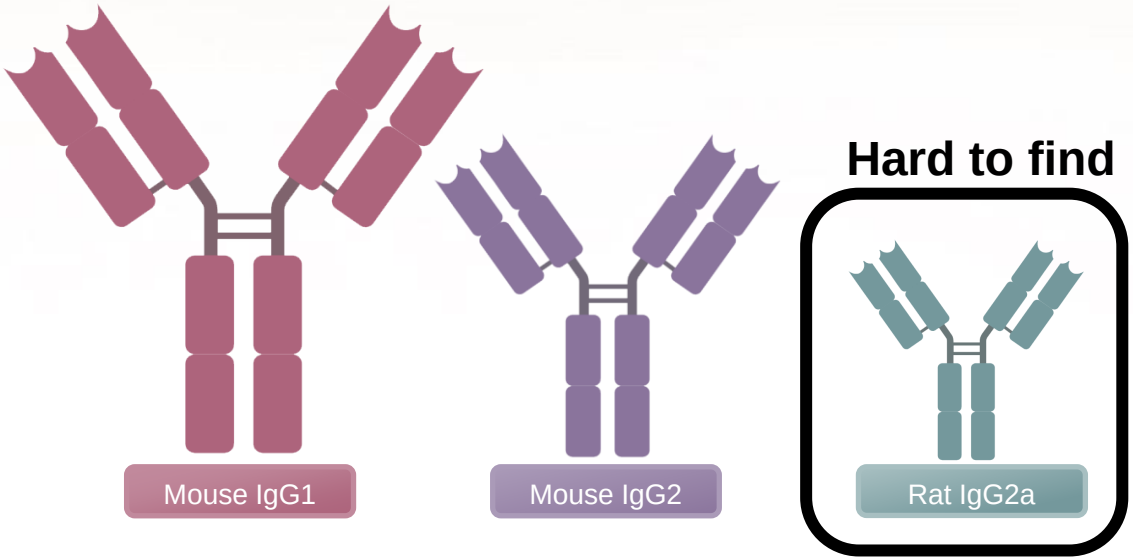
Rat IgG2a antibody



ASSET as a Targeting Platform

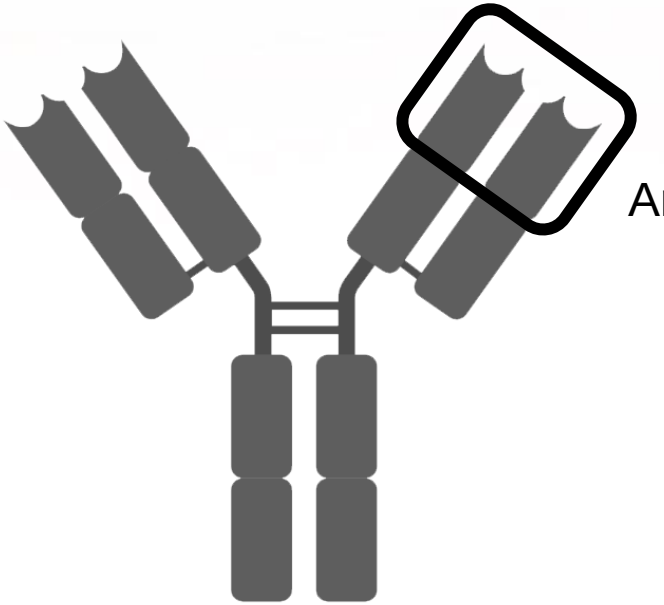


Antibodies used with human cells



Lipidated Antibody Nanoparticle Delivery – LAND

Utilizing the ASSET platform to construct a direct targeting moiety



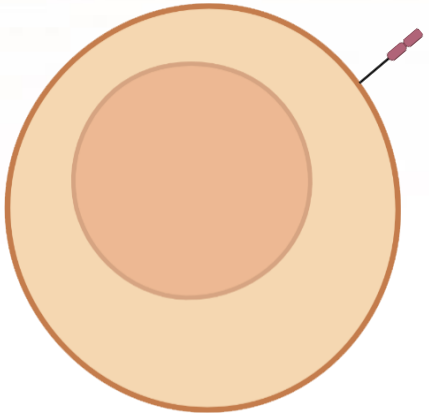
Anti XXX scFv

Anti XXX antibody

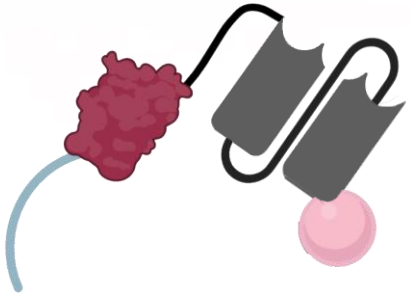
LAND Direct Binding to Target Cells

Target Cell

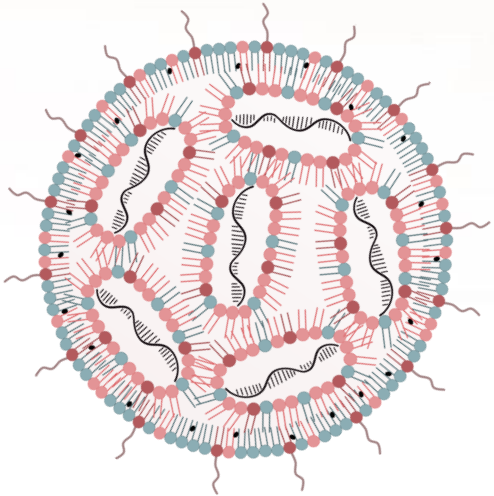
Target Receptor



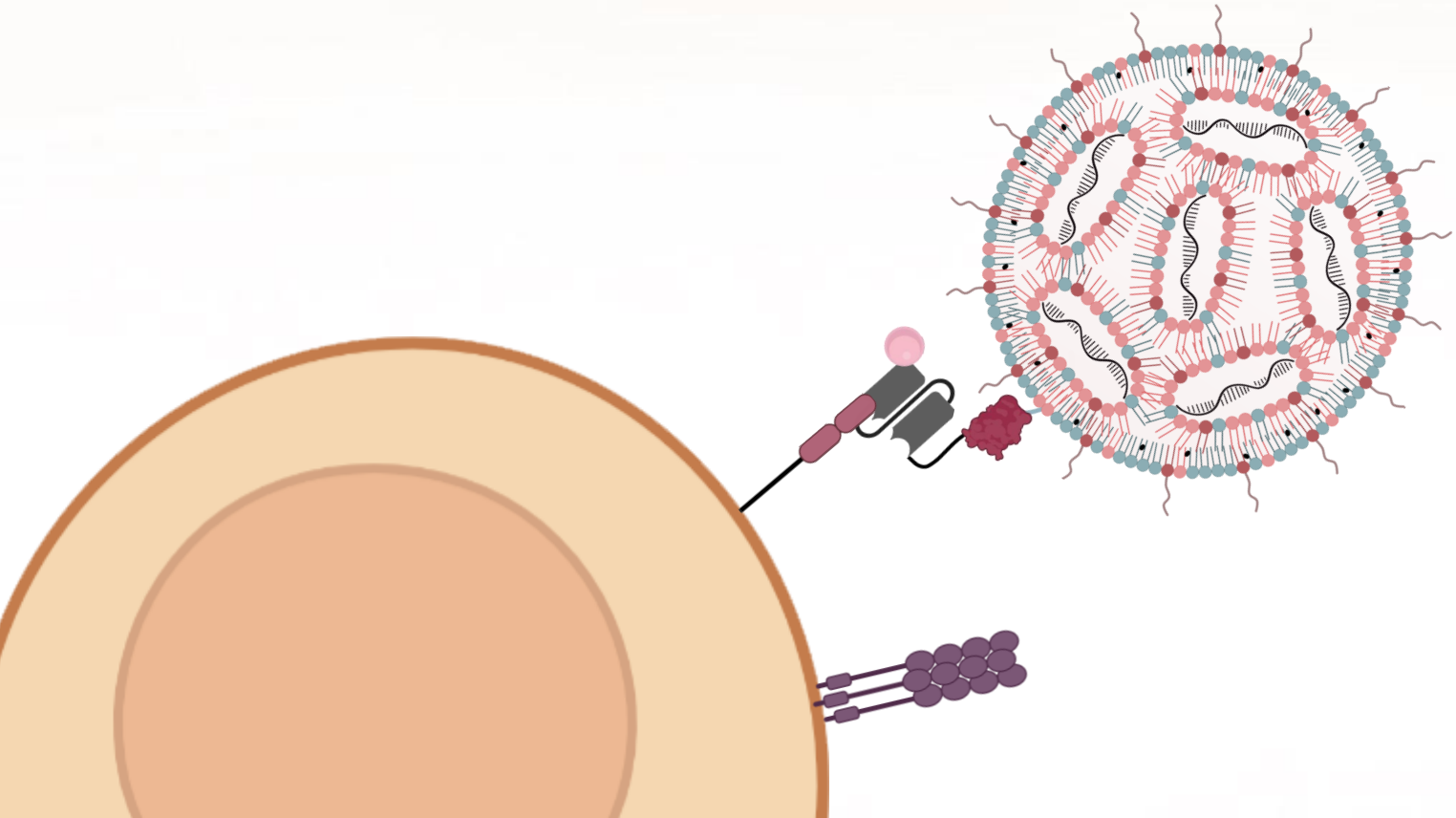
Anti-Target receptor LAND



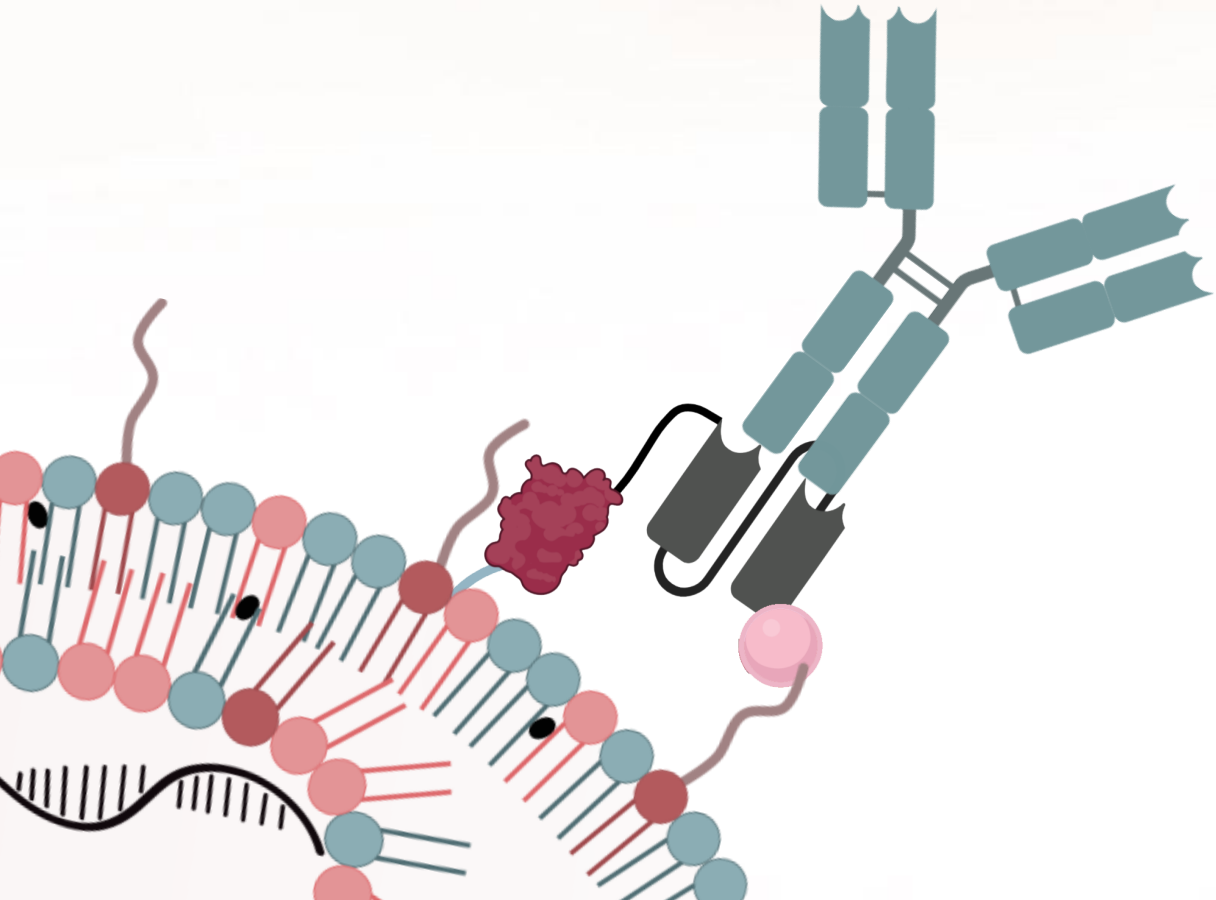
LNPs



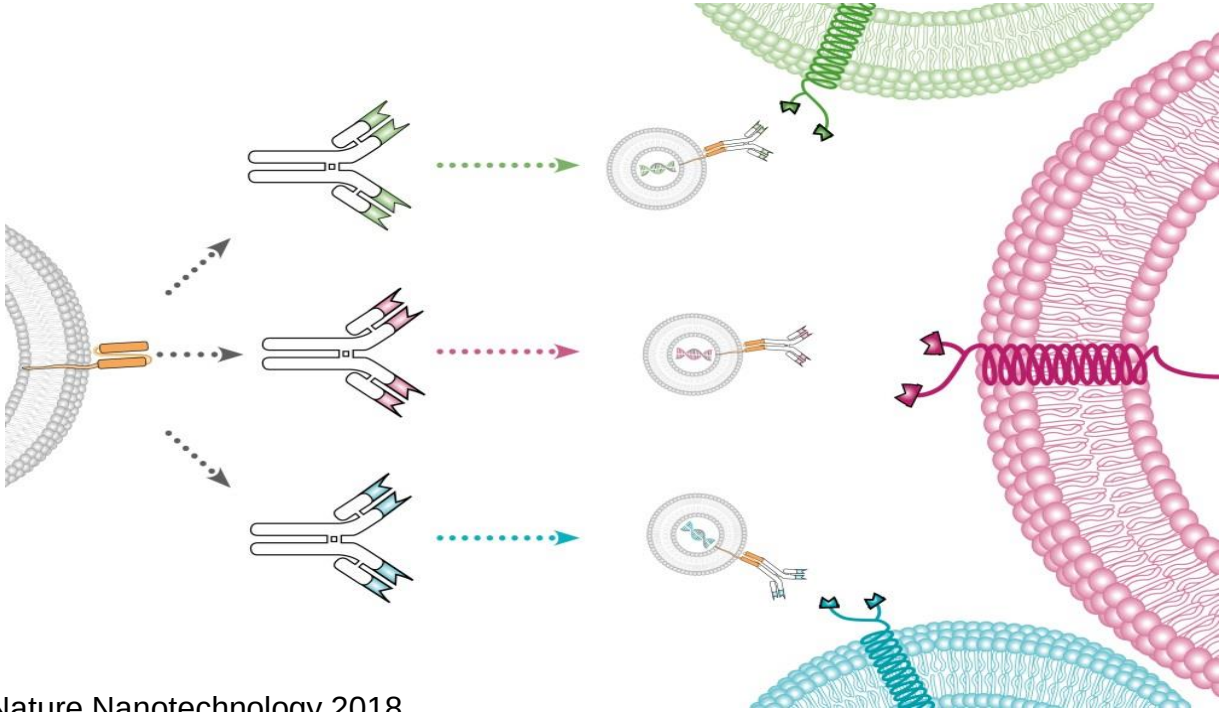
LAND Direct Binding to Target Cells



ASSET as a Targeting Platform

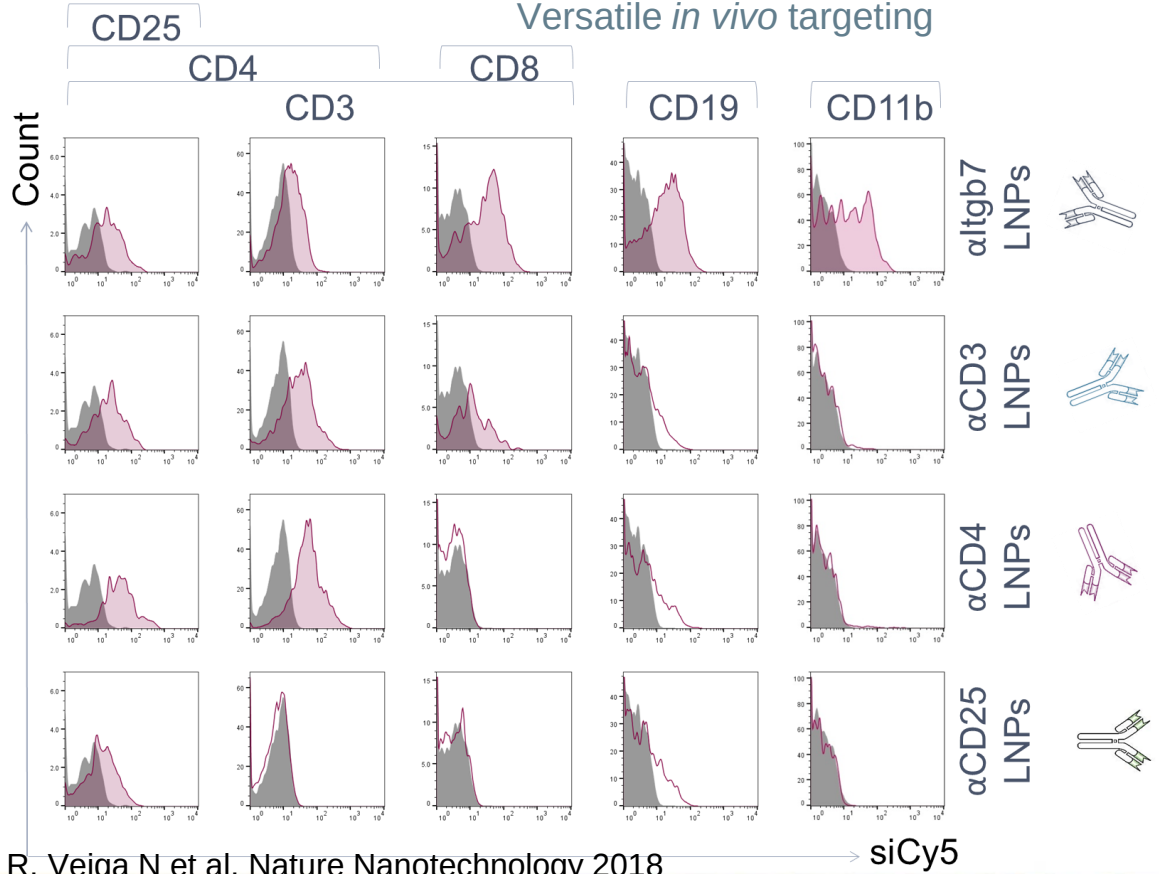


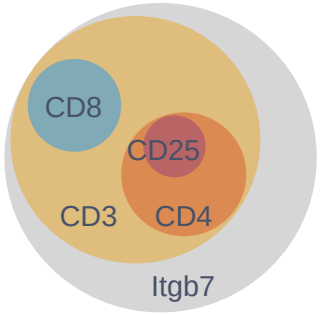
Modular platform



Kedmi R, Veiga N et al. Nature Nanotechnology 2018

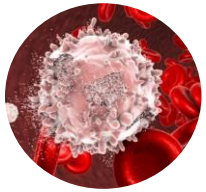
Versatile *in vivo* targeting





Kedmi R, Veiga N et al. Nature Nanotechnology 2018

Therapeutic applications



Hematological malignancies

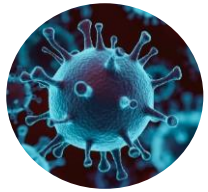
- Myeloma
- Leukemia
  - CML
  - AML
  - CLL
  - ALL
- Lymphoma
  - Hodgkin’s
  - NHL: MCL

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

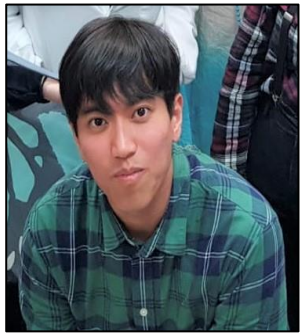
Genetic disorders

- Duchenne Muscular Dystrophy
- CF
- XBP-1

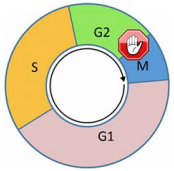
Therapeutic approach: Silencing

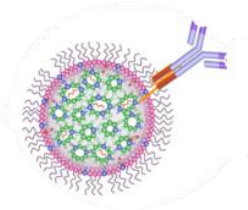
Target gene: HO1

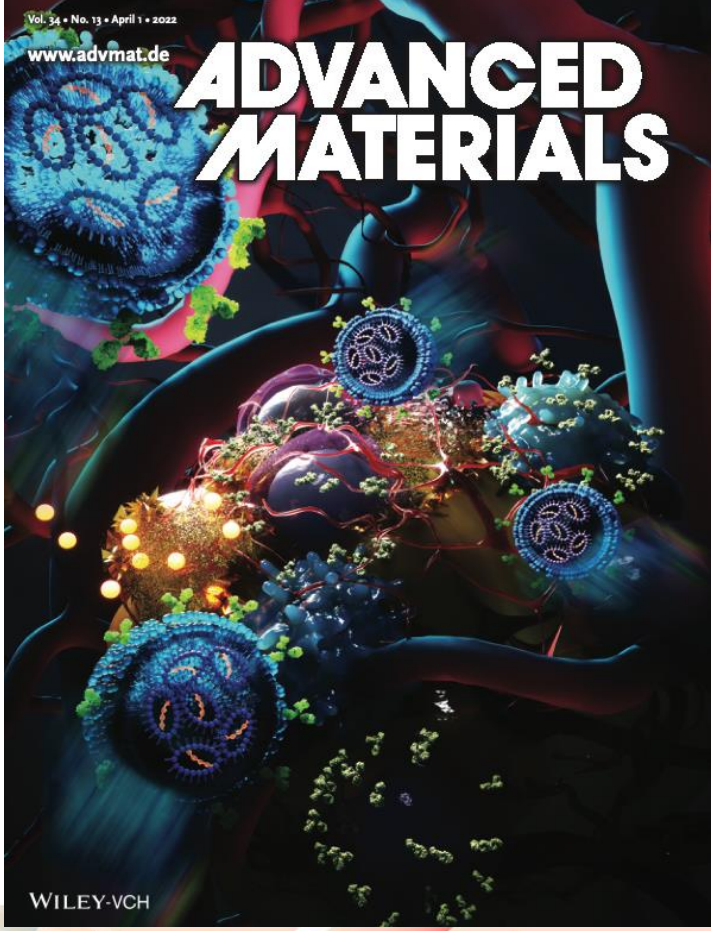
Target Receptor: PDL-1



Seok-Beom Yong, Ph.D





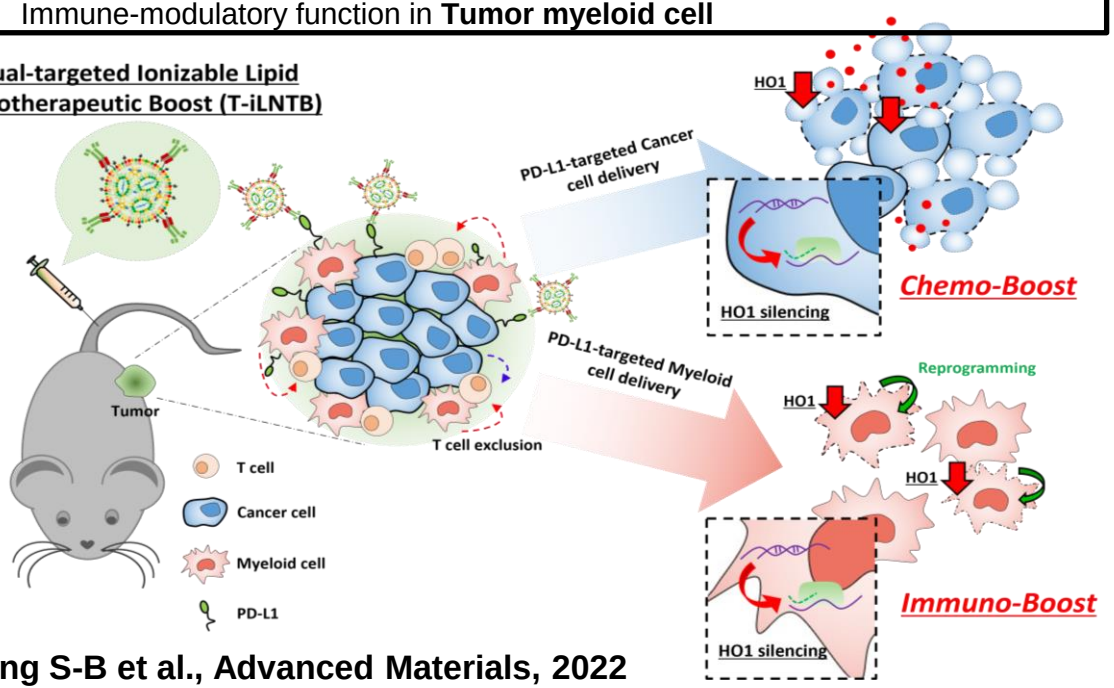


Single gene (HO1)-targeted lipid nanoparticle for chemo-immunotherapeutic boost in cancer

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

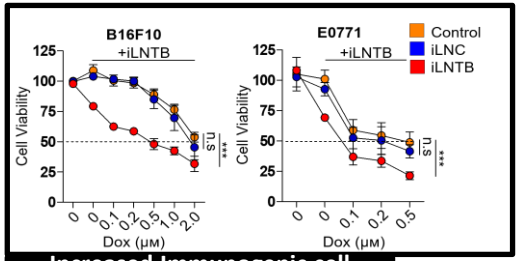
1. Chemo-resistance function in **Cancer cell**
2. Immune-modulatory function in **Tumor myeloid cell**

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

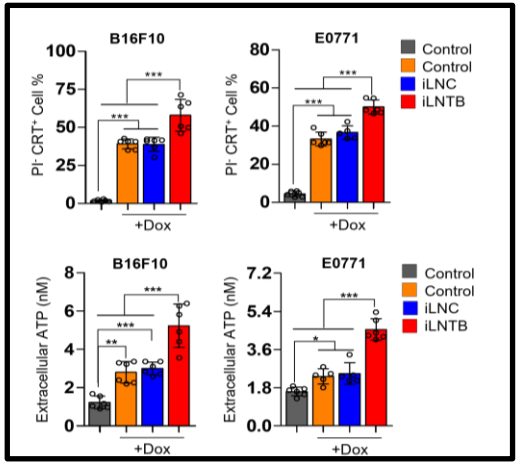


Yong S-B et al., Advanced Materials, 2022

Chemo-sensitization effect

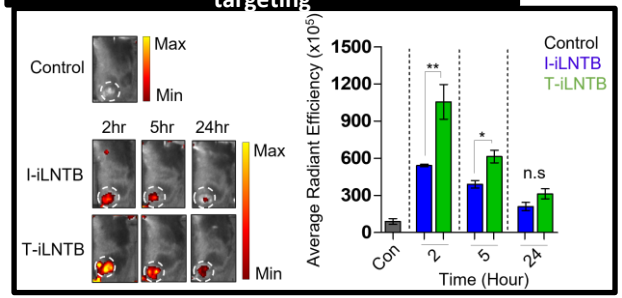


Increased Immunogenic cell death

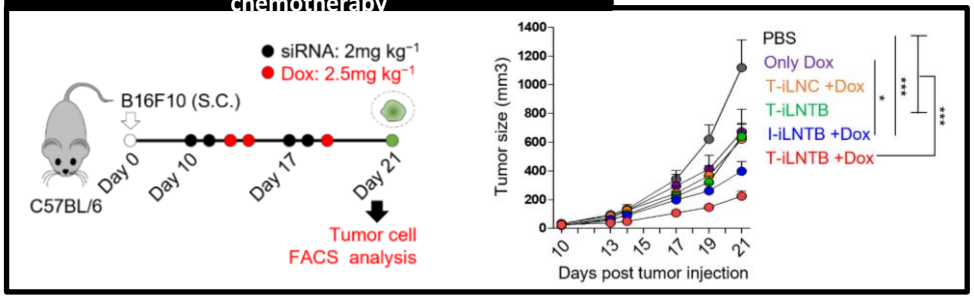


In vivo anti-tumor effect in combination with chemotherapy (Dox) & immune reprogramming effect

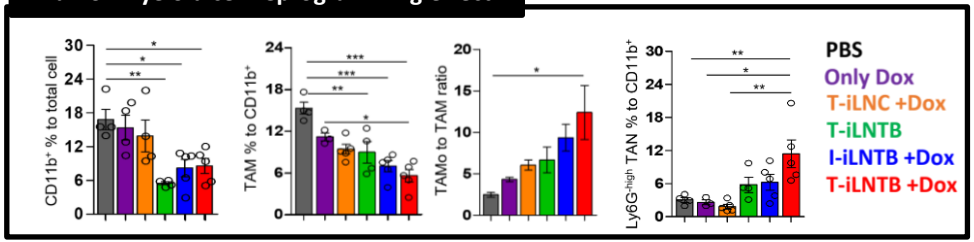
Increased tumor delivery by PD-L1-targeting



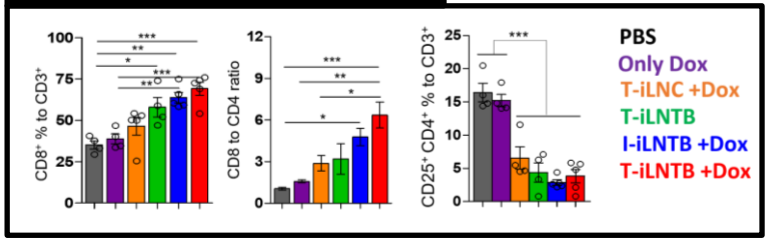
Anti-tumor effect in combination with chemotherapy



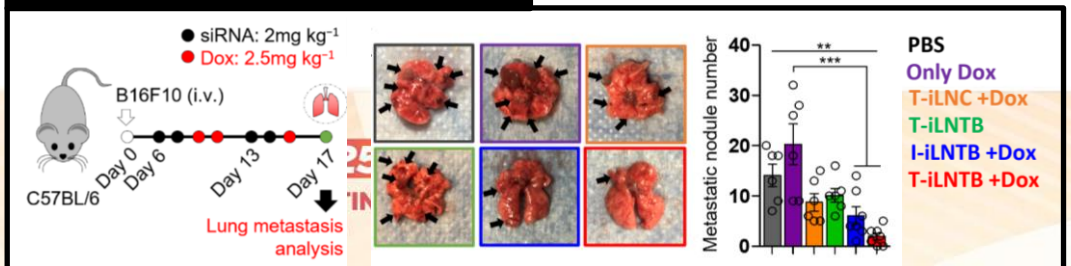
Tumor myeloid cell reprogramming effect



Tumor T cell reprogramming effect

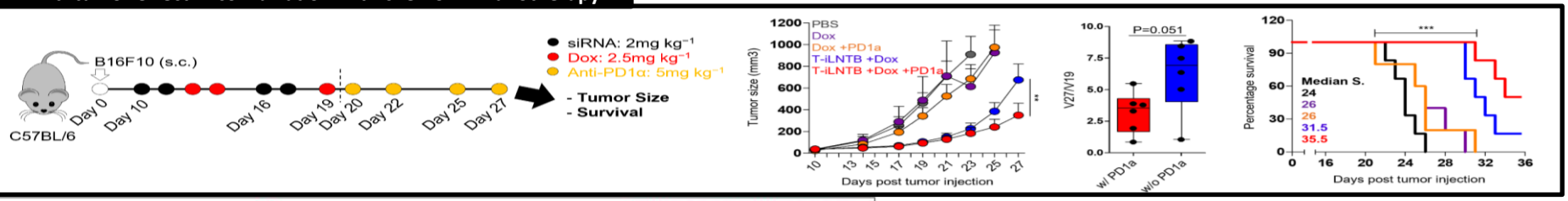


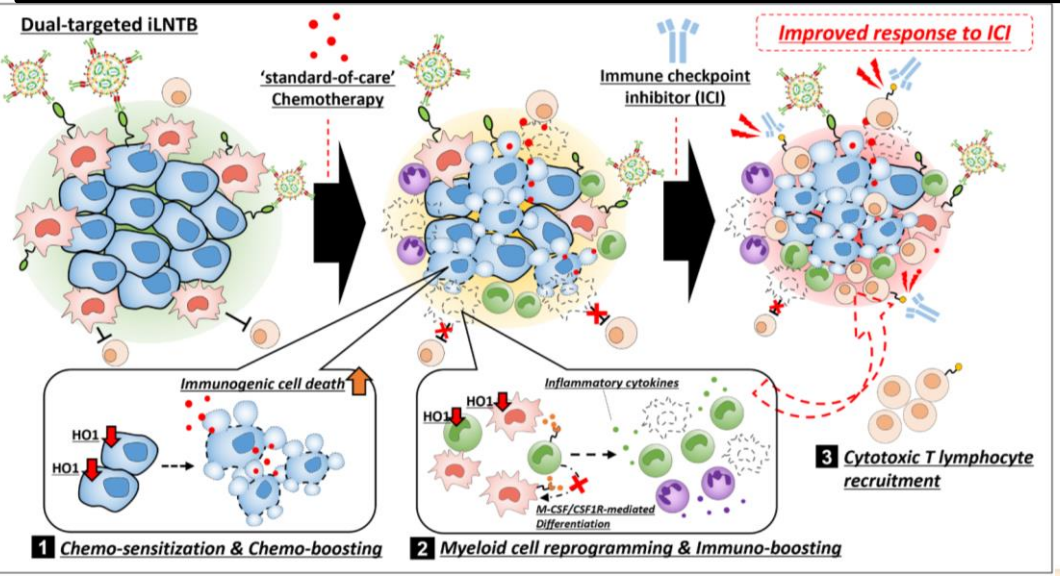
Anti-tumor effect in metastatic tumor model



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

Anti-tumor effect in combination with chemo-immunotherapy





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

RNA based therapeutics

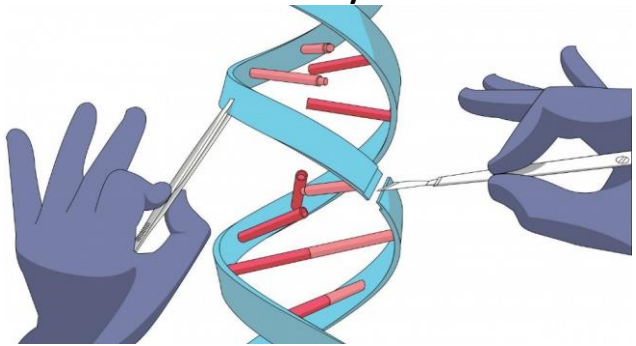
Silence

Activate

Edit

Replace

CRISPR/Cas9



Targeted CRISPR/Cas9 LNPs for therapeutic genome editing in cancer

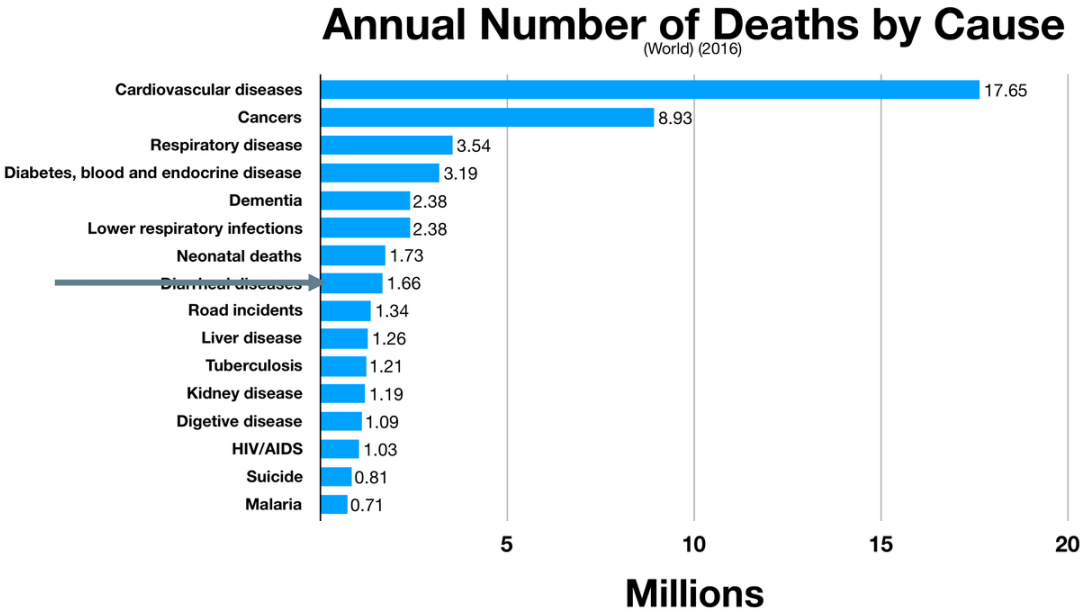
Dr. Daniel Rosenblum

Dr. Anna Gutkin

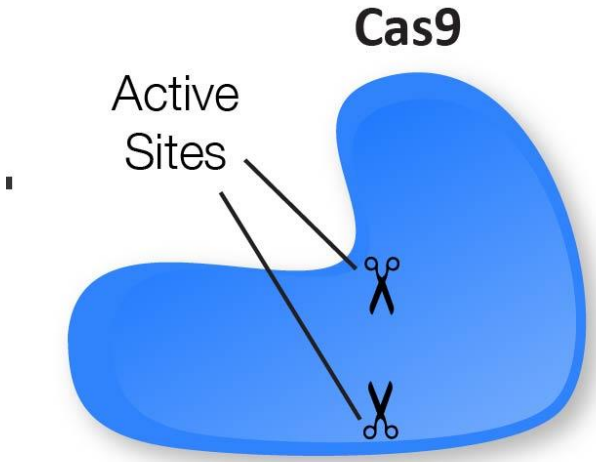
Rosenblum D.*, Gutkin A* et. al. Science Advances 2020
Gutkin A**,** Rosenblum D.*, Peer D. Nature Biotechnology 2021
Elia U.*, Kon E*, Peer D. Nature Biotechnology 2023.

Why cancer?

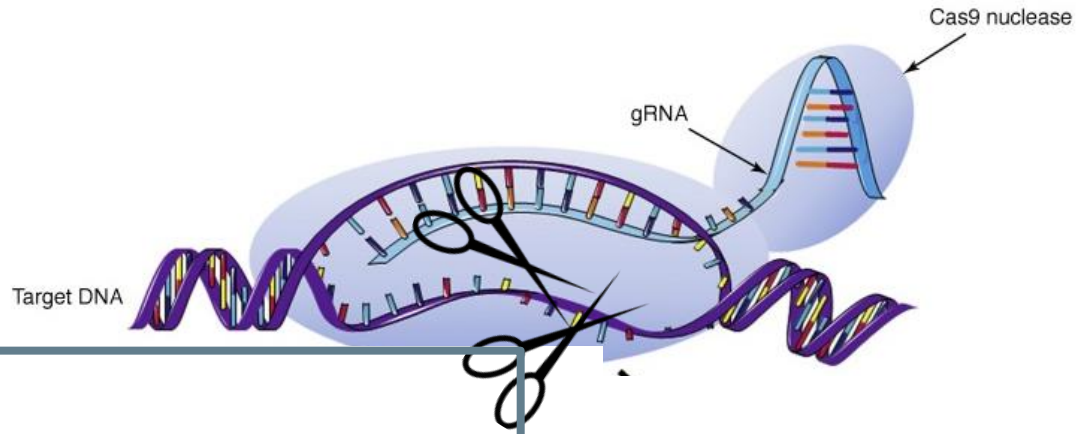
Cancer remains the second major cause of mortality worldwide.



CRISPR/Cas9 mediated genome editing



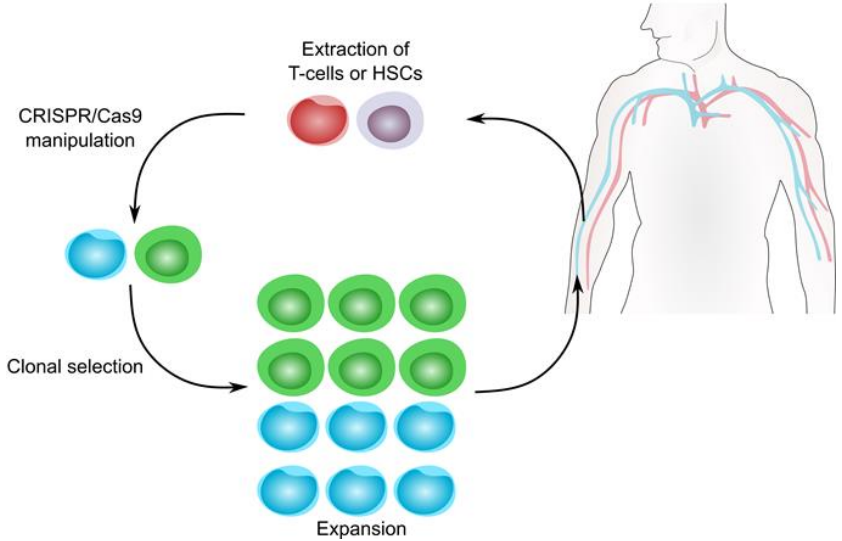
CRISPR/Cas9 mediated genome editing



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

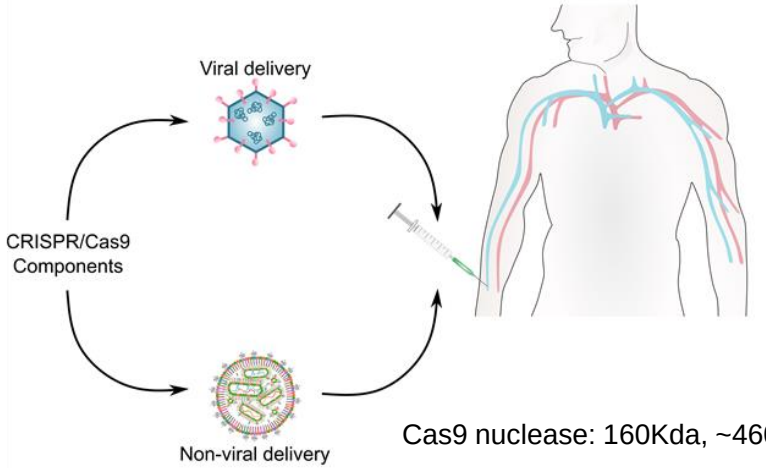
Clinical applications of CRISPR/Cas9

Ex vivo editing

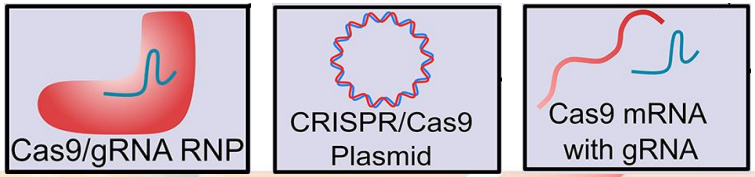


Rosenblum D., Gutkin A. et. al. ADDR (2020)

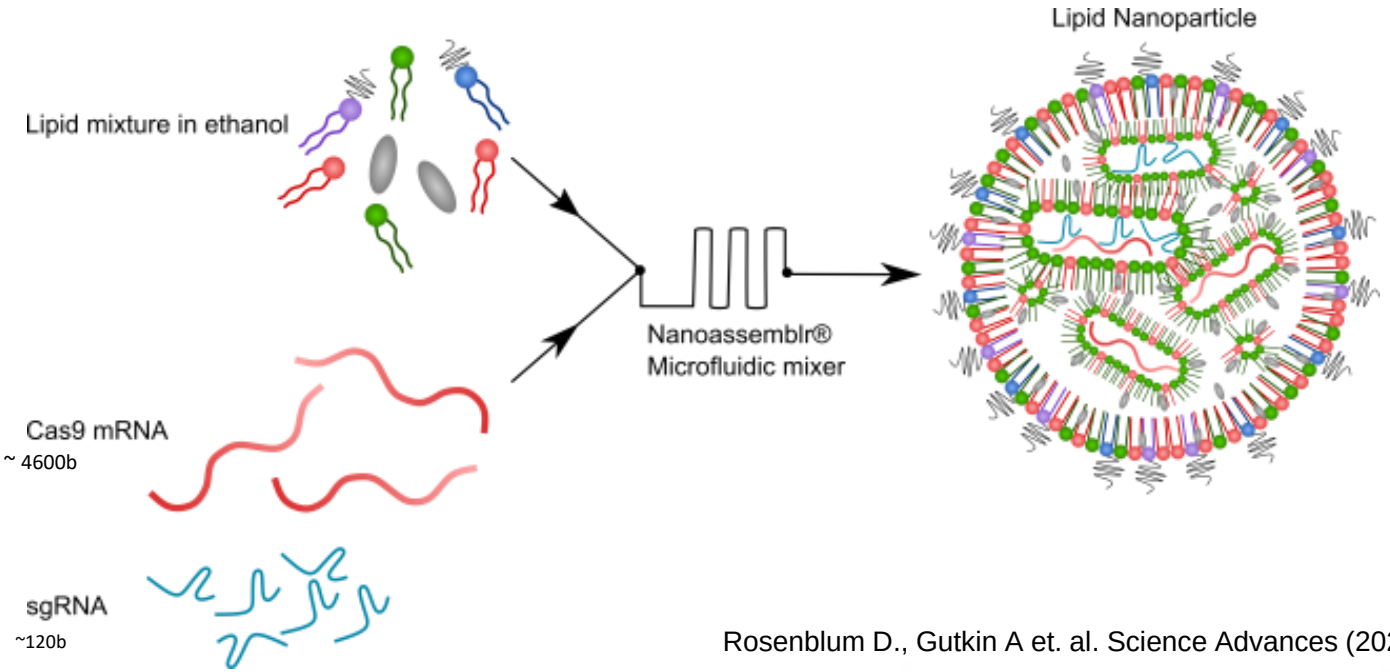
In vivo editing



Cas9 nuclease: 160Kda, ~4600b

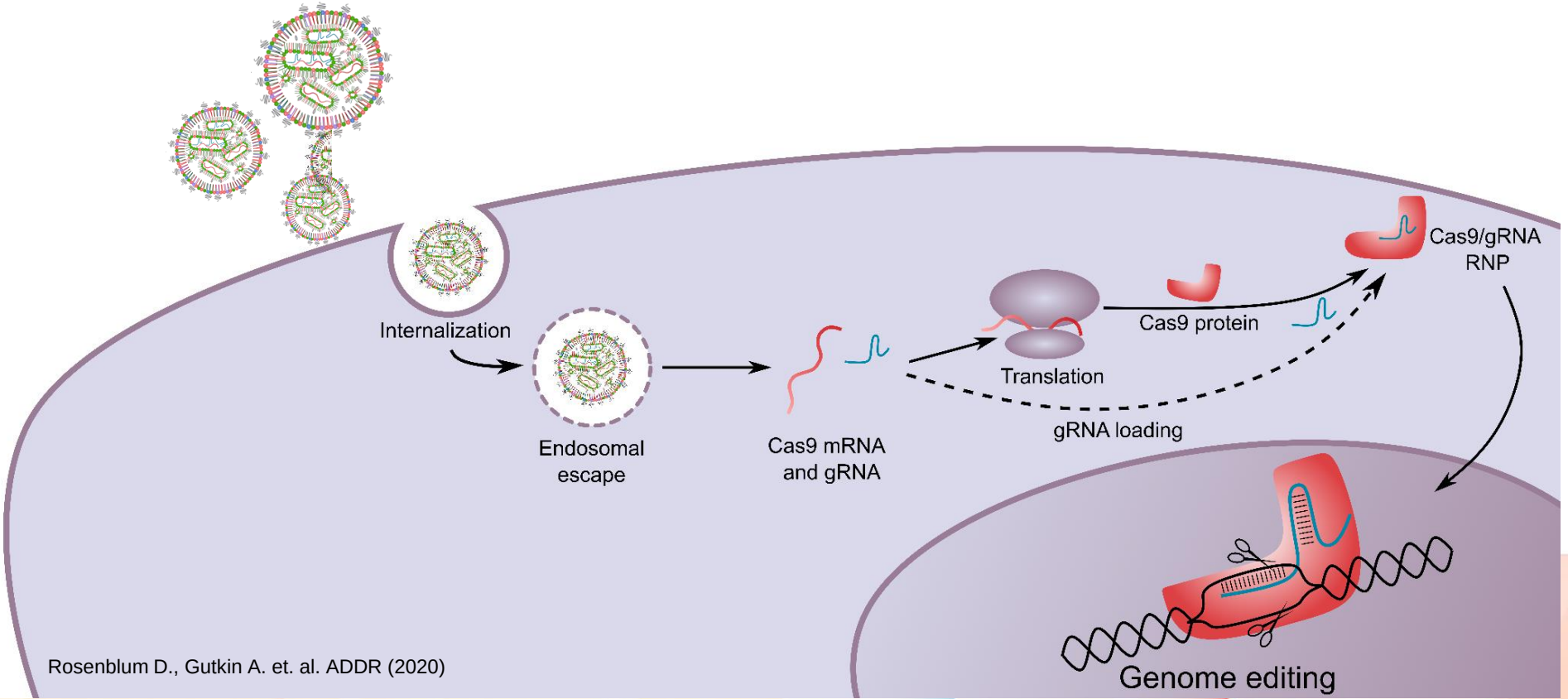


CRISPR/Cas9 Lipid nanoparticles (cLNPs)



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

cLNP's mechanism of action



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

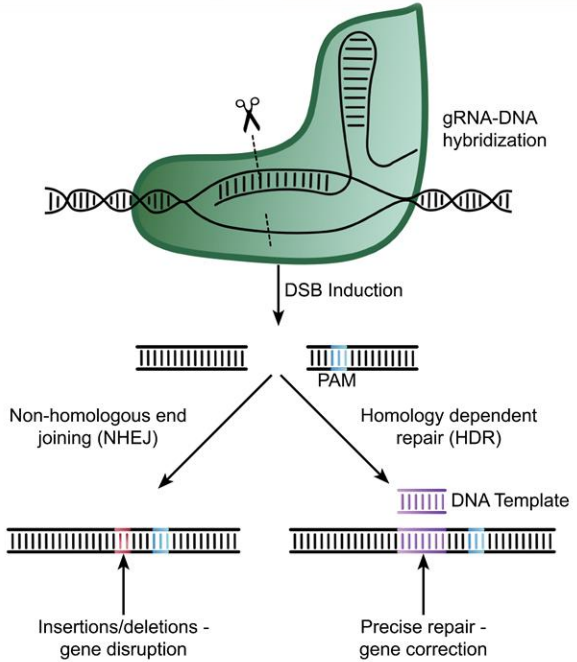
Challenges in Gene Editing

Specificity issues:

- On target
- Off target

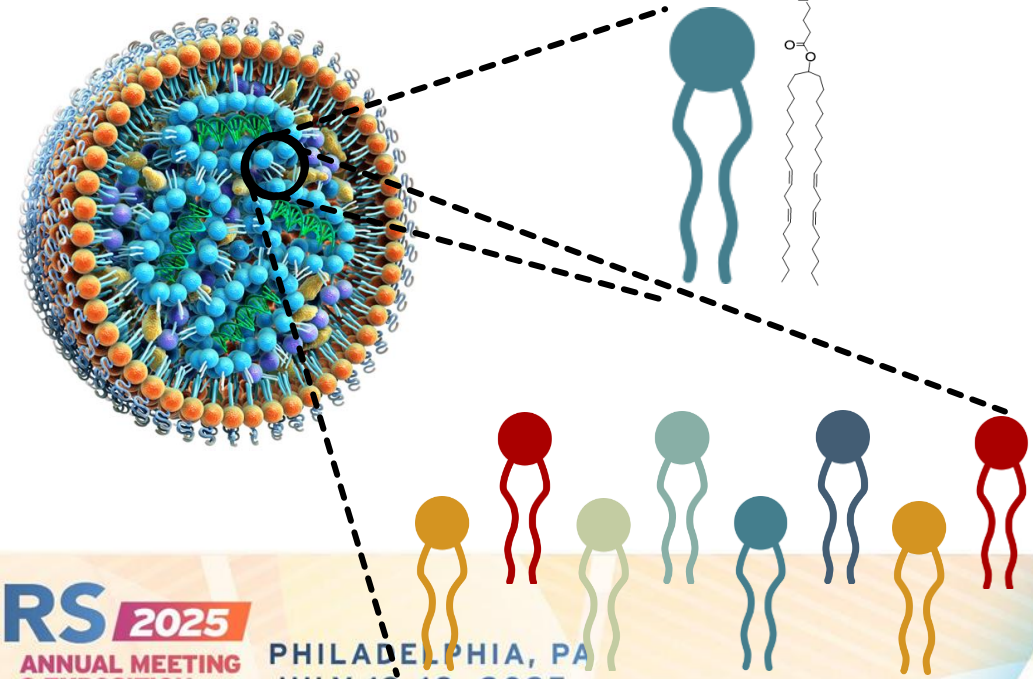
Cargo-related issues:

- mRNA size and instability
- Kinetics of mRNA expression and gRNA presence
- HDR has low efficiency compared with the NHEJ – relevant in cases of DNA template integration



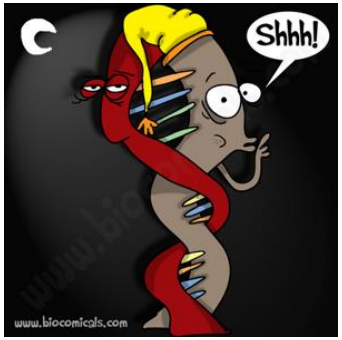
1. Breier, D. & Peer, D. *Bioact. Mater* 2022.
2. Kon, E., et al. . *Nature. Rev. Clin. Oncol.* 2023.
3. Masarwy, R., et al. *Adv. Drug Deliv. Rev.* 2024.

Design and construction of cLNPs



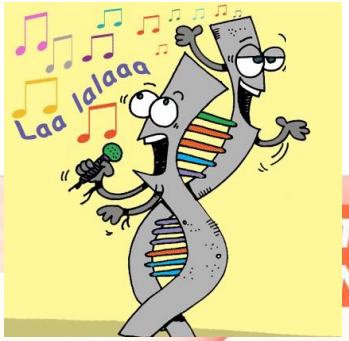
Dlin-MC3-DMA

siRNA



~20-24b

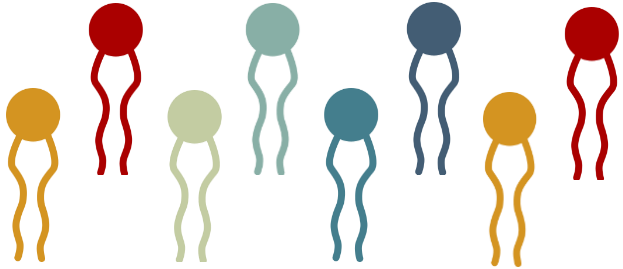
mRNA



~4600b

Design and construction of cLNPs

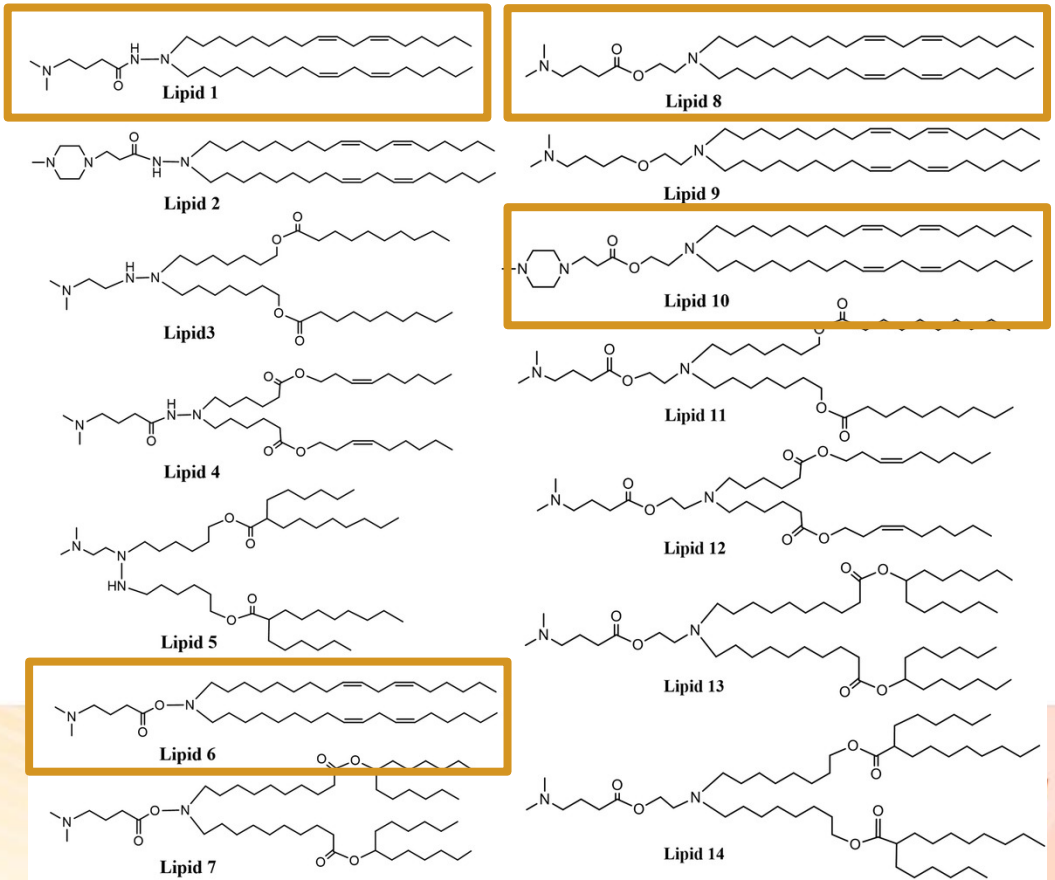
Ramishetti S, Hazan-Halevy I, Palakuri R et. al. Adv Mater (2020)



Dr. Srinivas Ramishetti

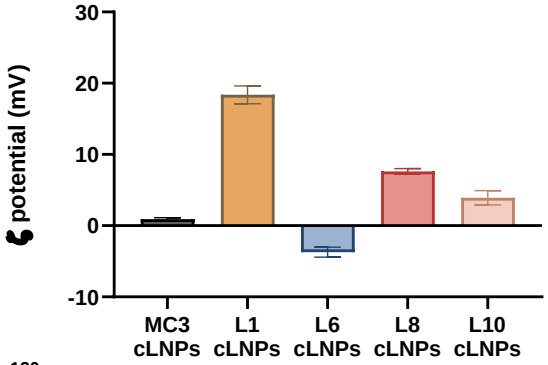
Dr. Inbal Hazan-Halevi

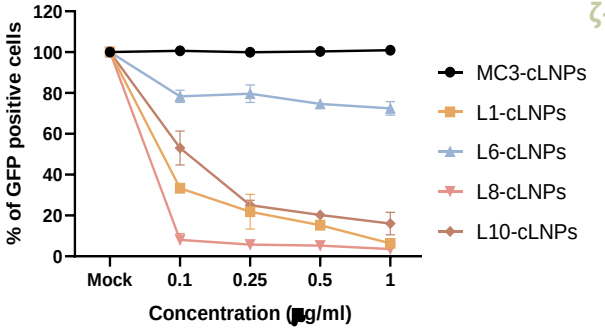
Dr. Ramesh Palakuri

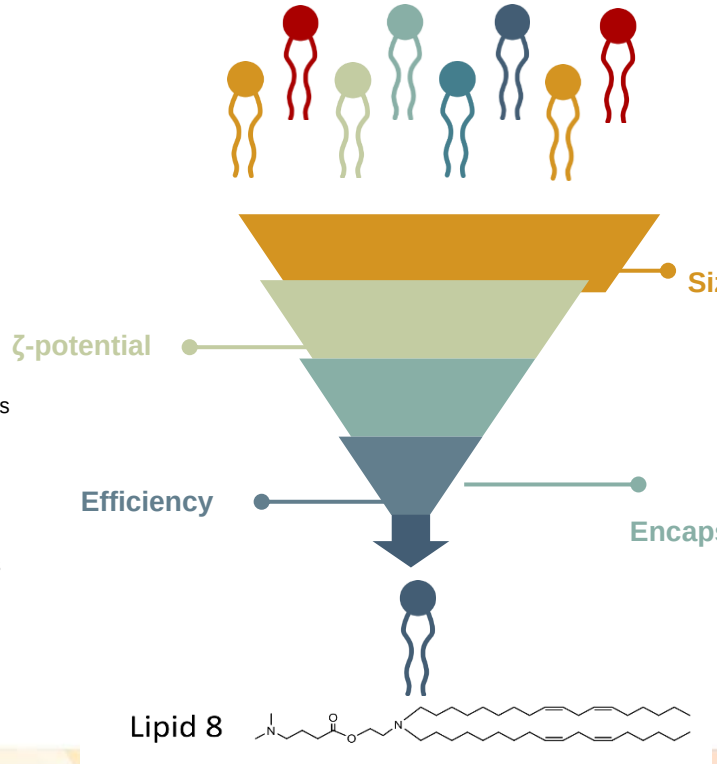


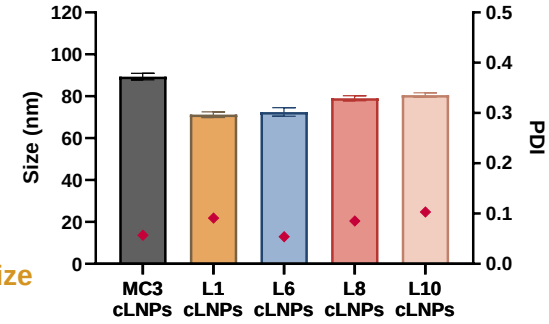
CRS 2025 ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
JULY 13-18, 2025

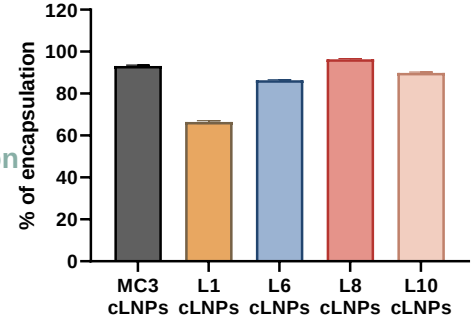
Design and construction of cLNPs





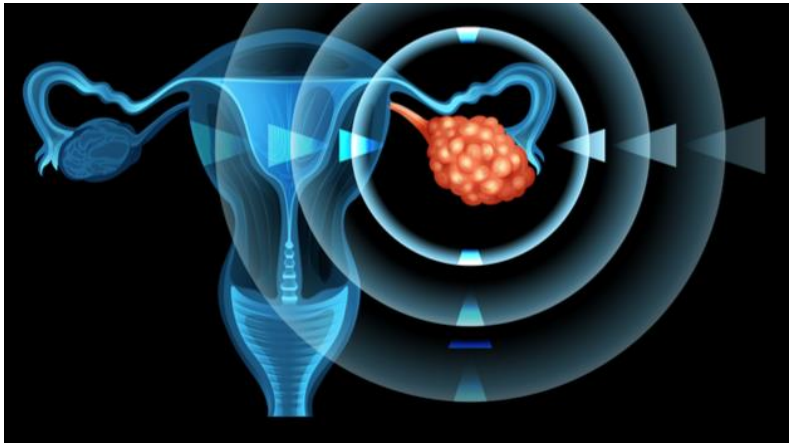






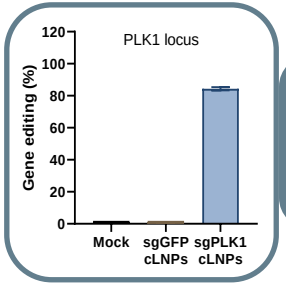
Tumor models

Murine orthotopic glioblastoma multiforme (GBM)
Human metastatic ovarian adenocarcinoma



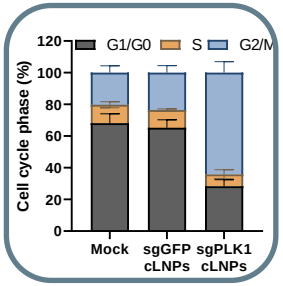
PLK1 gene disruption in two tumor models *in vitro*

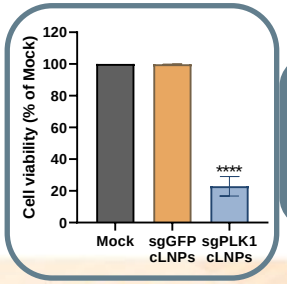
GBM



Gene editing- **85%**

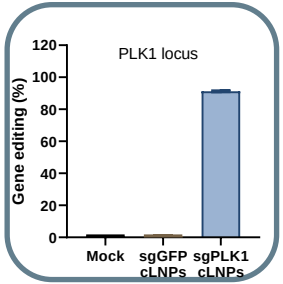
Cell cycle arrest- 3 fold





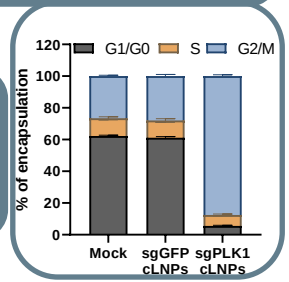
Cell death- 5 fold

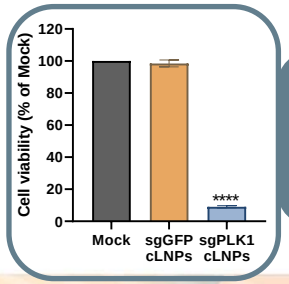
Ovarian cancer



Gene editing- **91%**

Cell cycle arrest- 4 fold

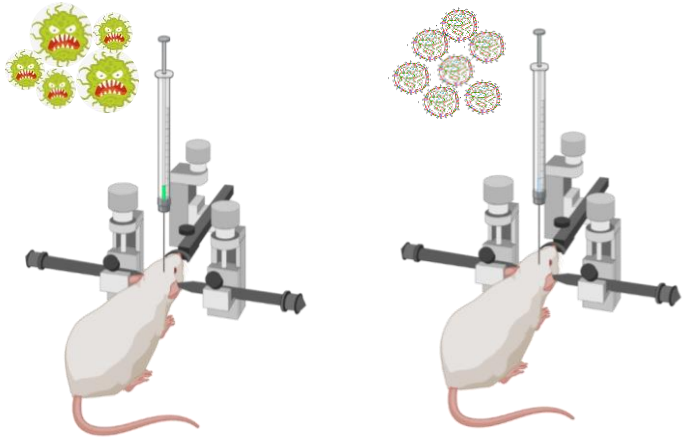


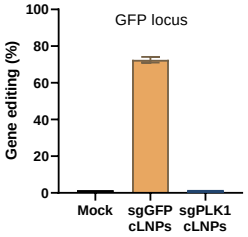


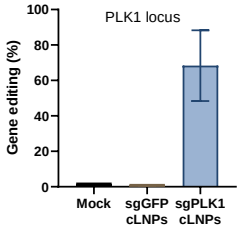
Cell death- 10 fold

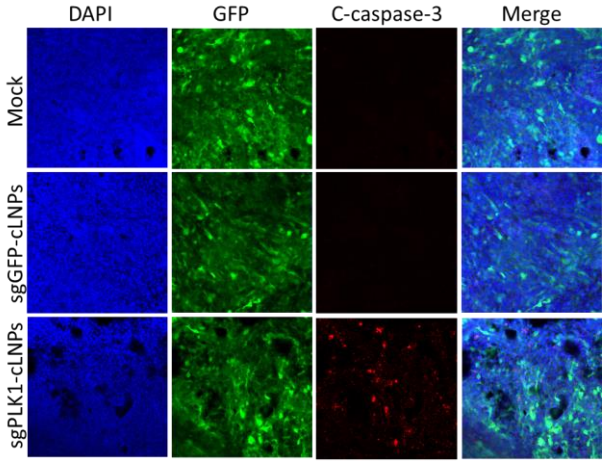
Local delivery of cLNPs to GBM

GBM cells injection → 10d → cLNPs injection → 2d → Gene disruption analysis → 1d → Apoptosis induction analysis



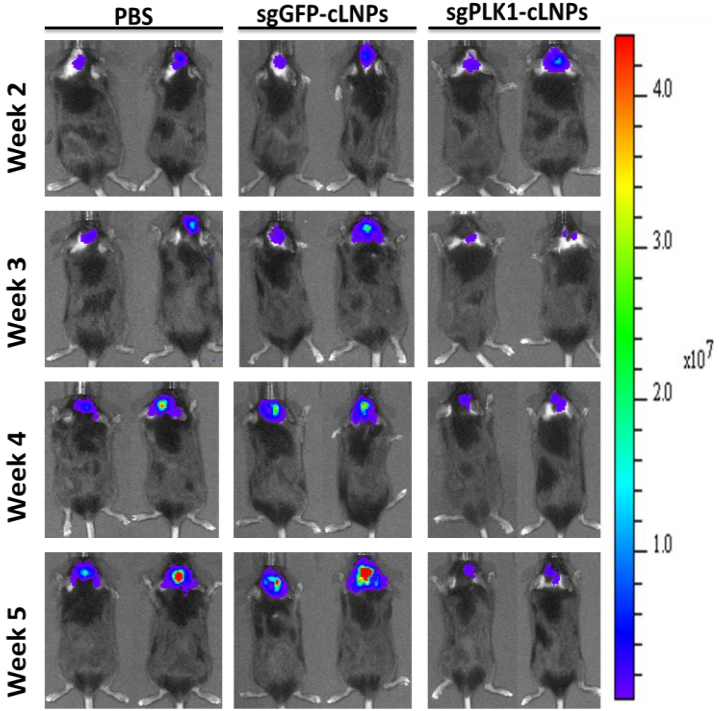


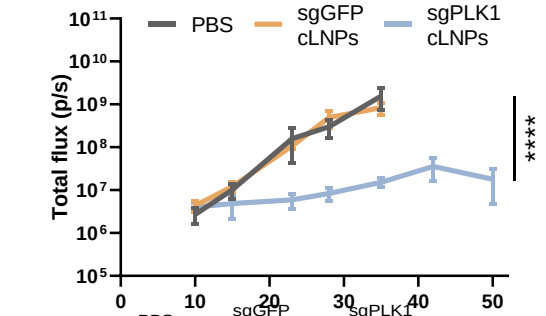


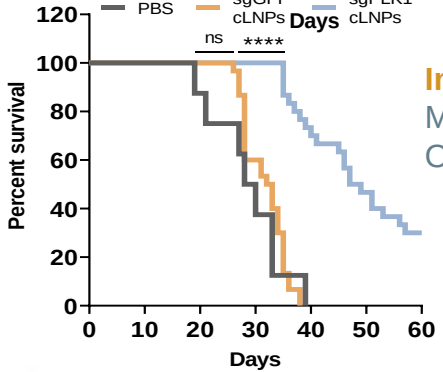


Rosenblum D., Gutkin A. et. al. Science Advances (2020)

Single administration of sgPLK1-cLNPs results in potent tumor growth inhibition and prolong survival of GBM bearing mice



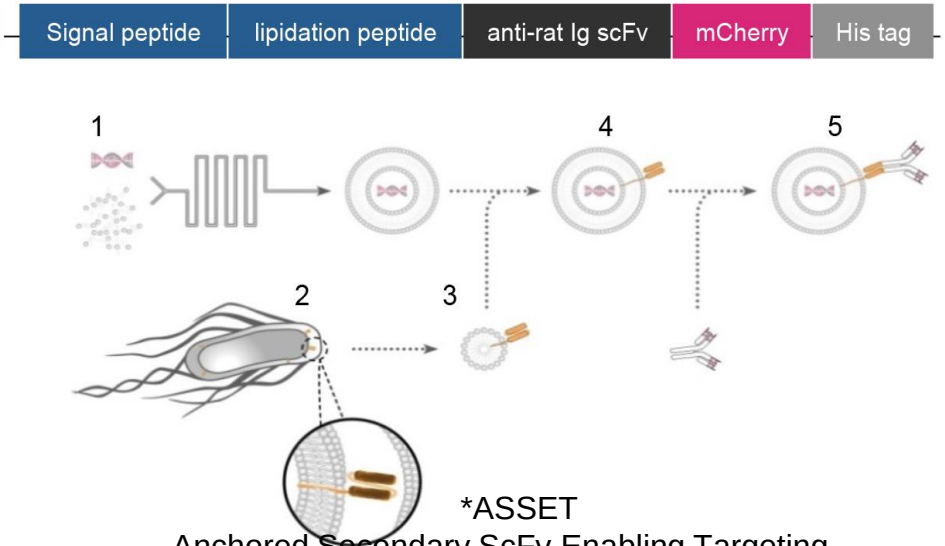




Increase:
Median survival 50%
Overall survival 30%

Systemic delivery to metastatic ovarian adenocarcinoma

ASSET* incorporation to LNPs.
Targeting the overexpressed epidermal growth factor receptor (EGFR).



*ASSET
Anchored Secondary ScFv Enabling Targeting

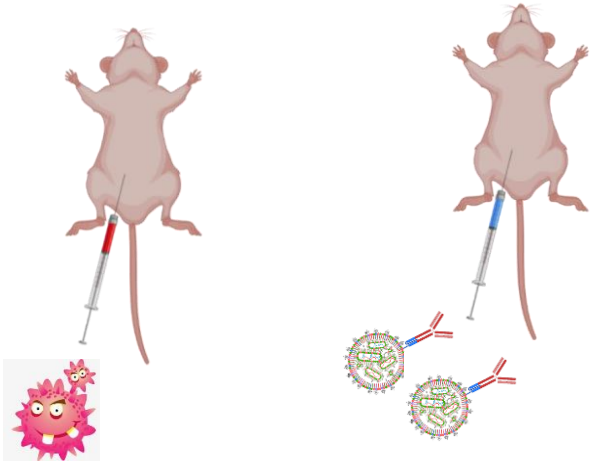
Dr. Ranit Kedmi

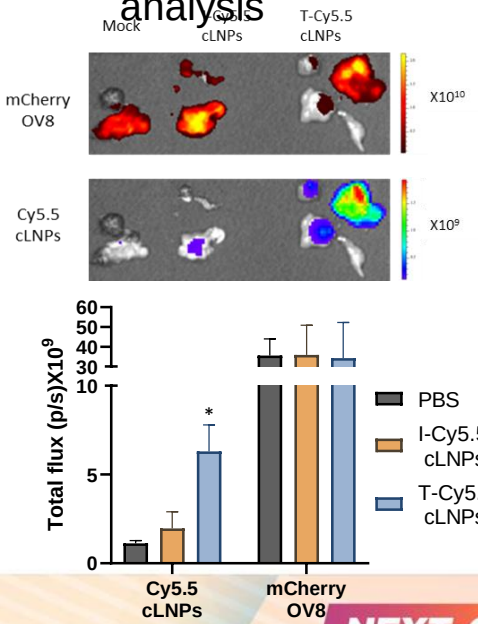
Dr. Nuphar Veiga

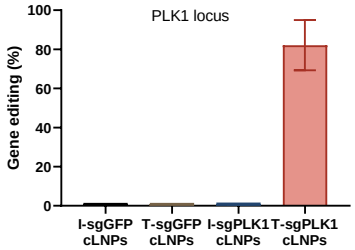
Kedmi R., Veiga N. et al. Nature Nanotechnology 2018

Systemic delivery of targeted cLNPs to metastatic ovarian adenocarcinoma

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

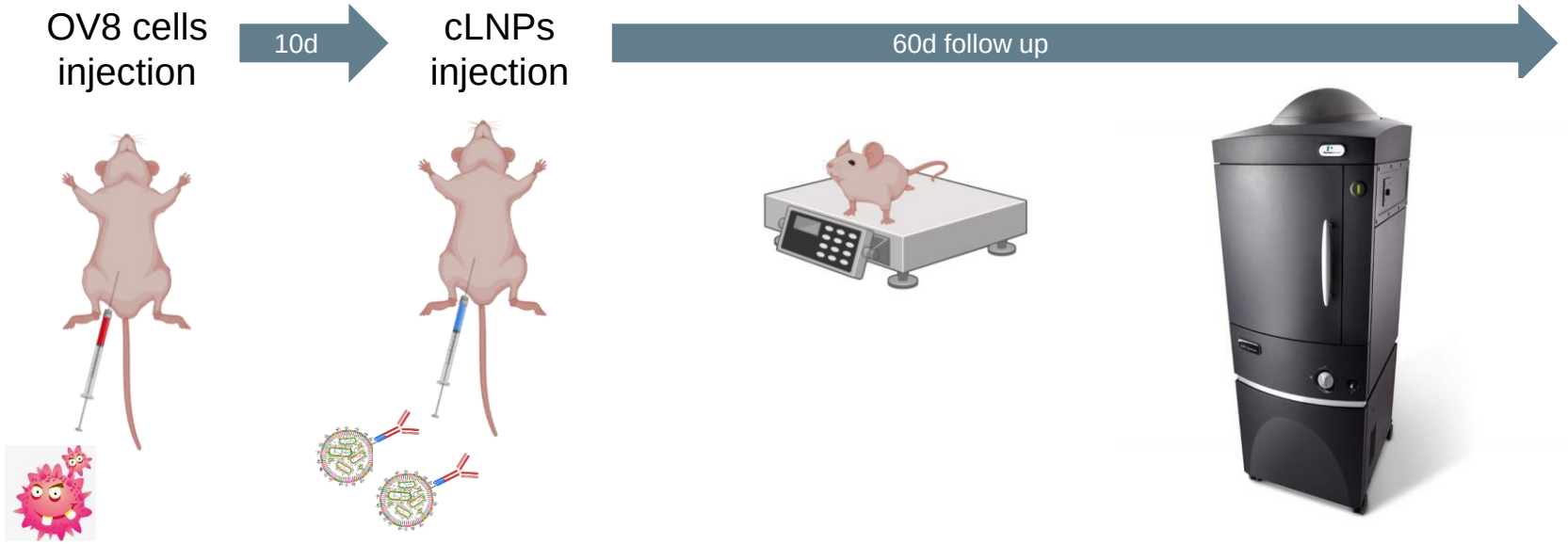




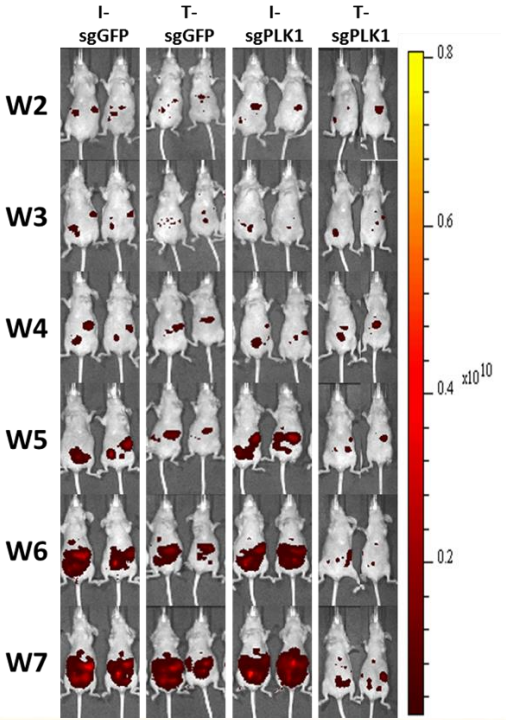


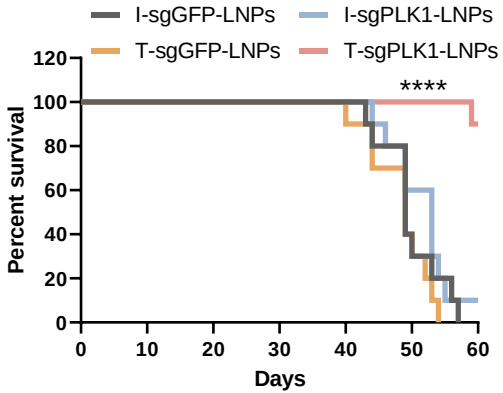
Rosenblum D., Gutkin A. et. al. Science Advances (2020)

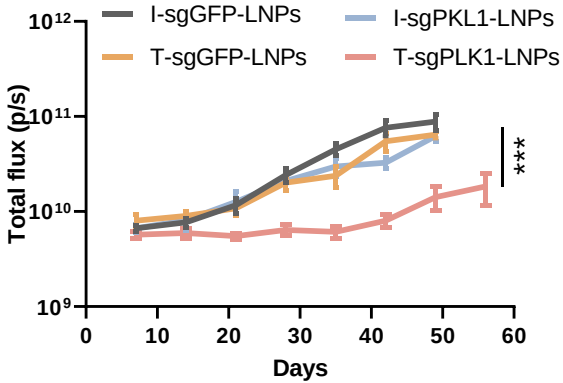
Schematic experimental design: Systemic delivery of targeted cLNPs to metastatic ovarian adenocarcinoma



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





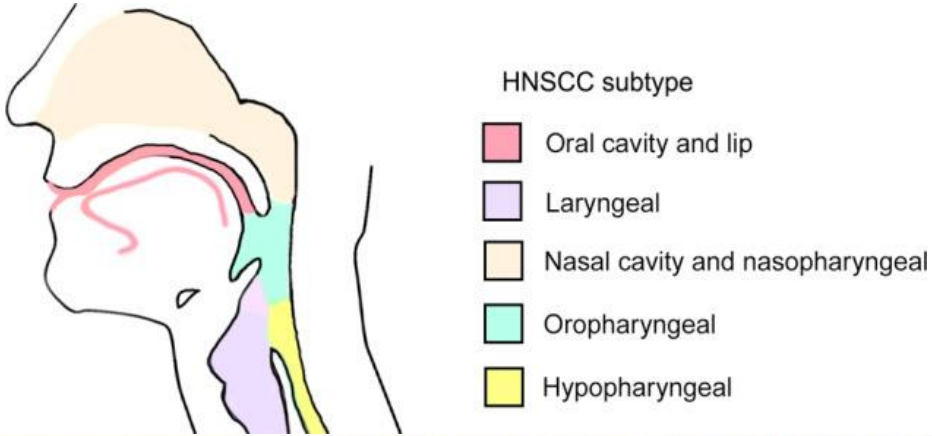


Increase:
Overall survival 90%

n=10 mice/group

Management of Locally Advanced - HNC

Fifth most common cancer worldwide.
>90% squamous cell carcinoma (SCC).
HNC: oral cavity, nasal cavity, pharynx, and larynx.



Prognosis

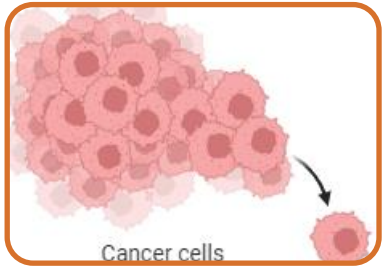
5-OS between 30 - 70%, depending on the stage and location of the tumor

40–50% of the patients experience disease recurrence

doi:10.1038/s12276-020-00492-9

Challenges

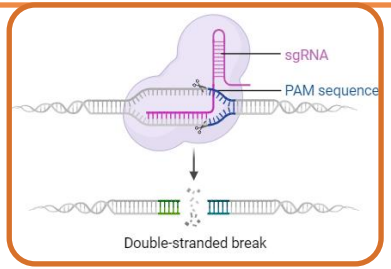
Tumor-related



1. Limitless replicative potential (Editing a large number of cells **)**

2. Dense and rigid microenvironment serves as a barrier in solid tumors (restrict access of LNPs**)**

Editing-related

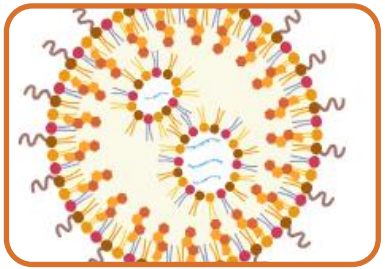


1. On-target effects→ chromosomal aberrations which may cause another cancer

2. Off-target effects which affects→ unwanted gene disruption, cellular damage, or cancerous transformation

3. Low editing efficiency

Delivery-related

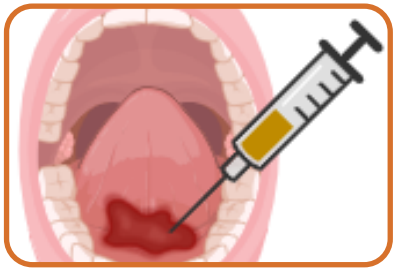


1. Lack of tissue-specific delivery system

2. Lack of tumor cell-specific delivery system

Proposed solutions

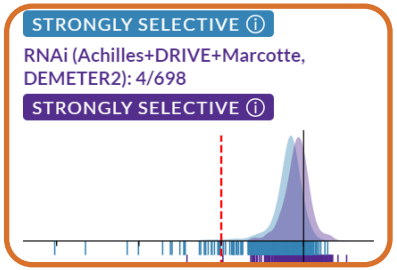
IT injection



1. Visible, palpable, or detectable → relevant to intratumoral therapy

2. Minimize the off-target toxicities

Careful selection of the Target-gene



Exclusively expressed in cancerous cells

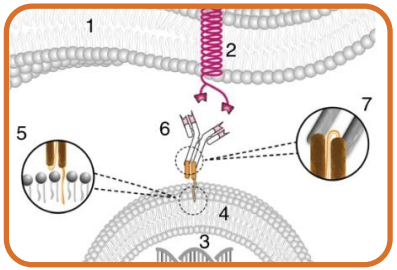
+

Critical for their survival

=

Reduce the dangers of off-target activity.

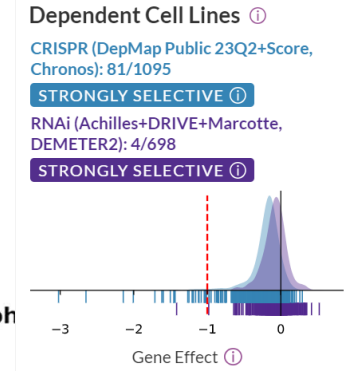
Targeted Platform

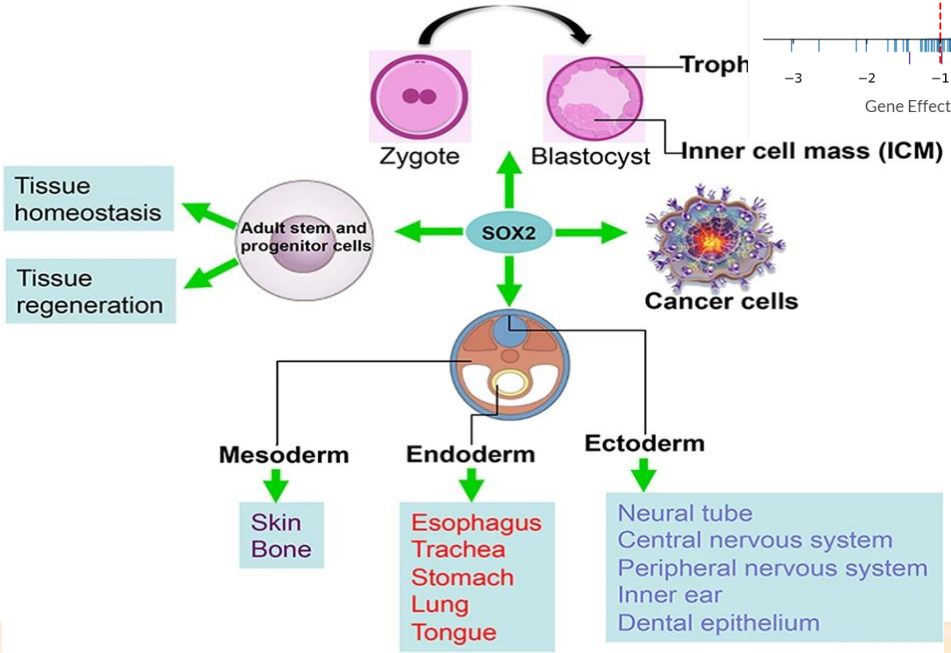


Organ and tumor cells-specific delivery system

Specific and efficient delivery

SRY-Related HMG box 2 (Sox2)





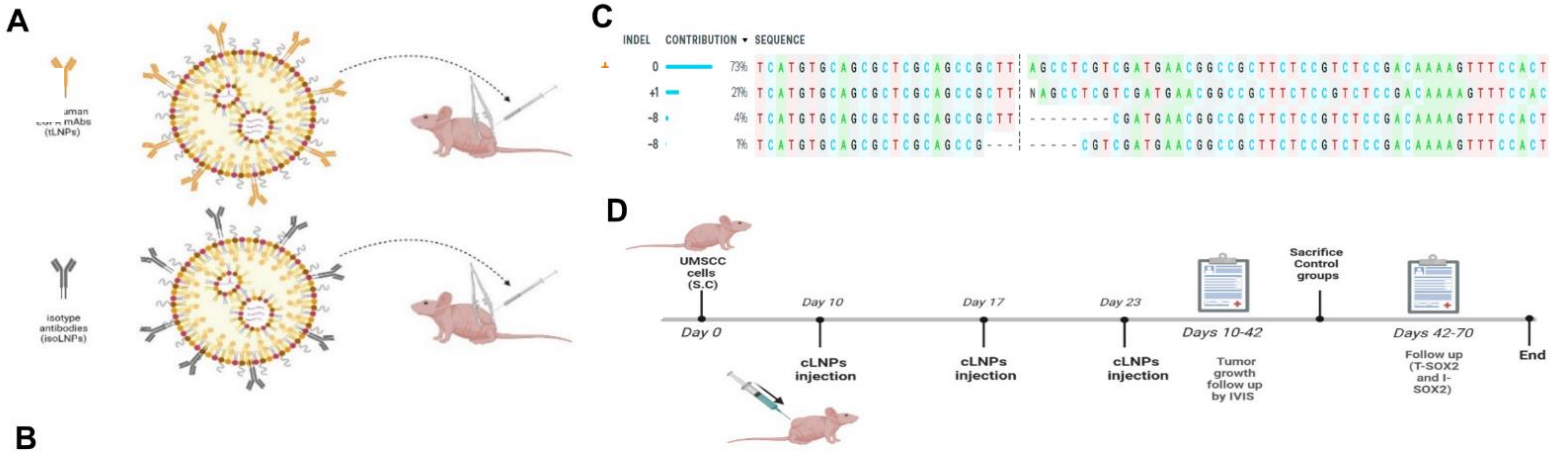
Embryonic stem cells (ESCs) transcription factor-required for embryogenesis and regulates expression of genes during development.

In normal adult tissues only detected in central nervous system (preserve stem-cell maintenance)

Head and neck cancer.
GI cancers: Hepatic, colorectal, esophageal, pancreatic, and gastric cancer
GU cancers: ovarian, cervical, prostate

Others-
Lung carcinoma
Breast carcinoma
Melanoma
Glioblastoma
Osteosarcoma

EGFR targeted sgSOX2-cLNPs Mediates Therapeutic Genome Editing in a Head and Neck Tumor post IT administration administration



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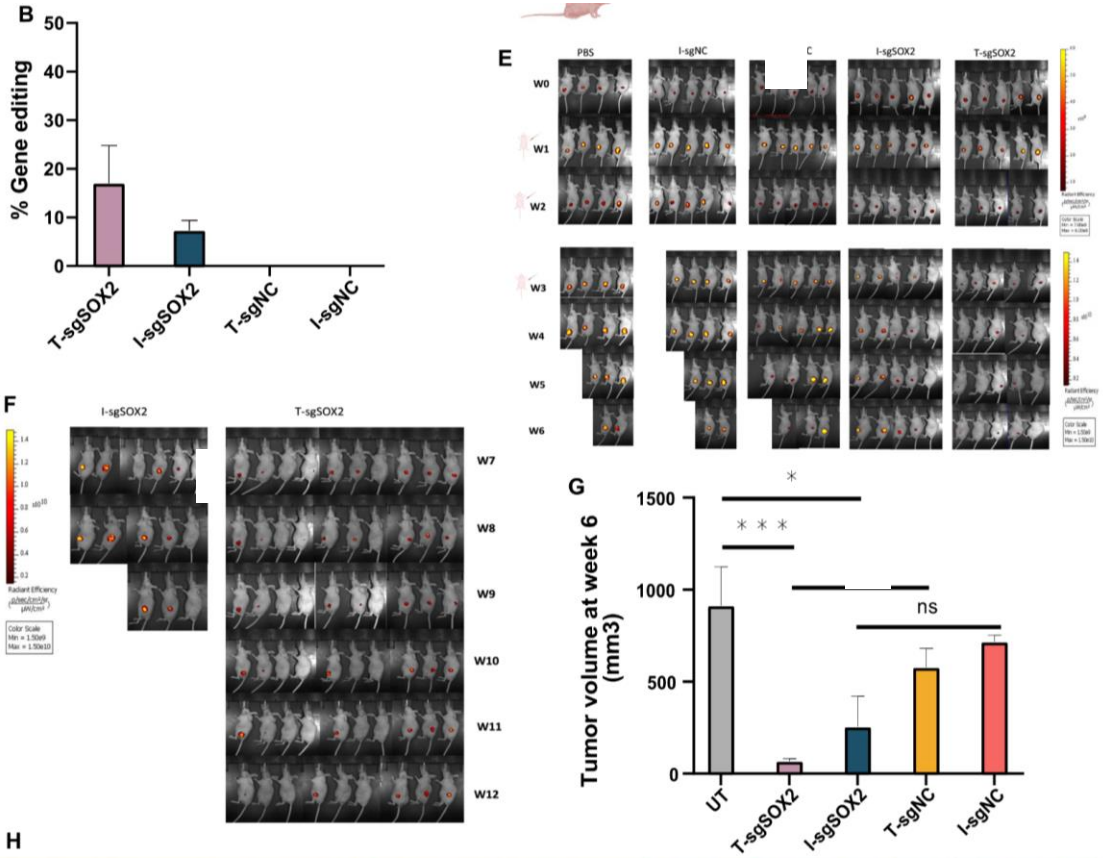

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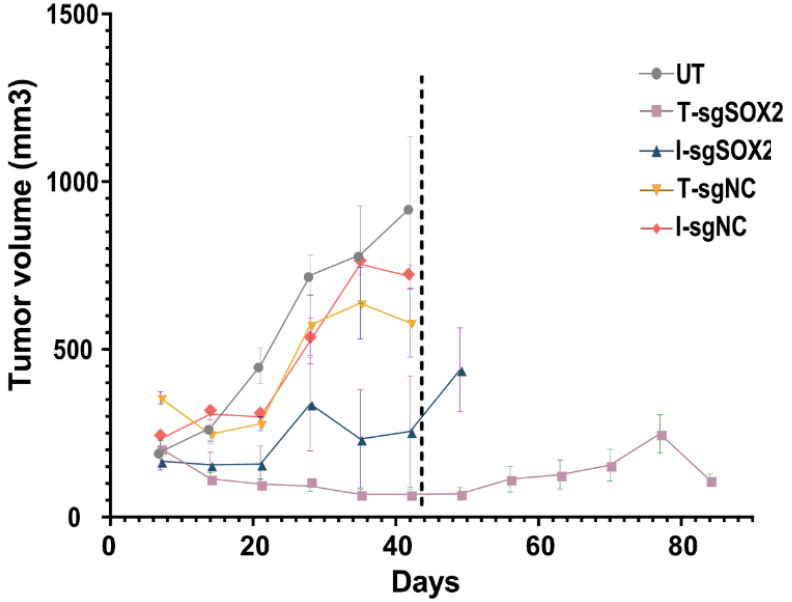
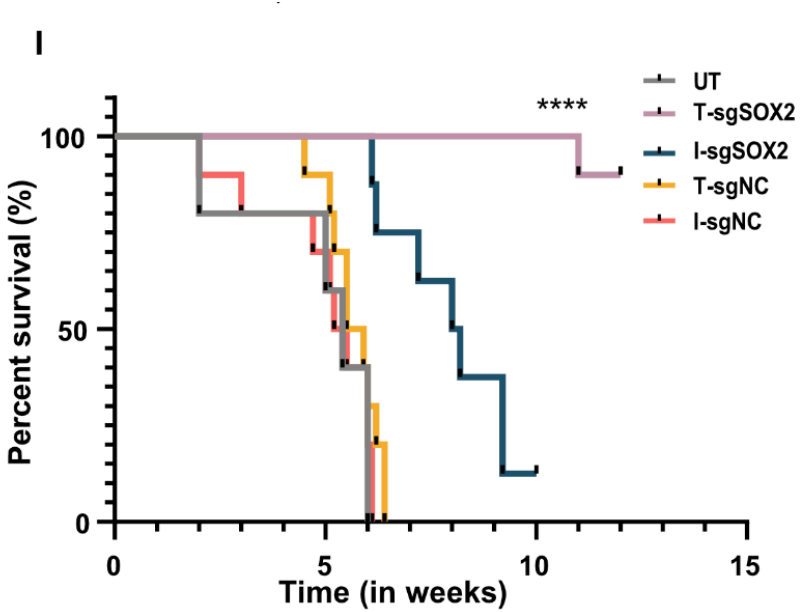
EGFR targeted sgSOX2-cLNPs Mediates Therapeutic Genome Editing in a Head and Neck Tumor post IT administration





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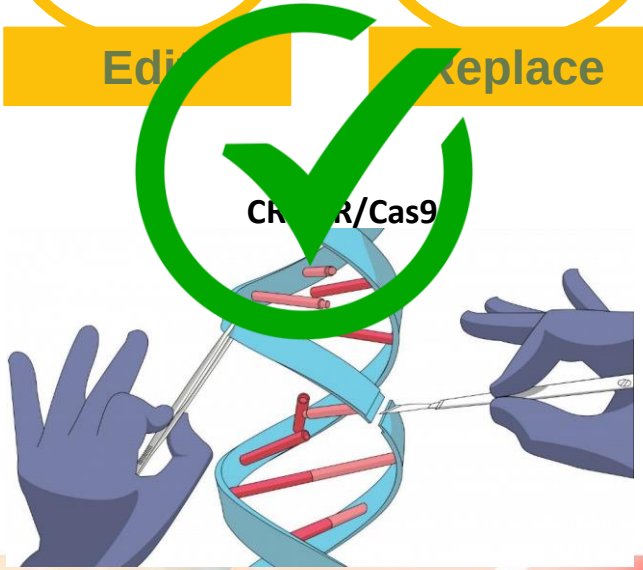
RNA based therapeutics

Silence

Activate

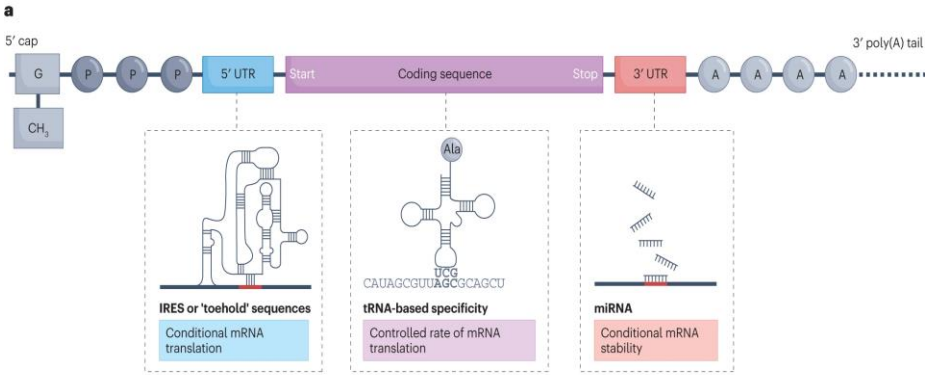
Edi

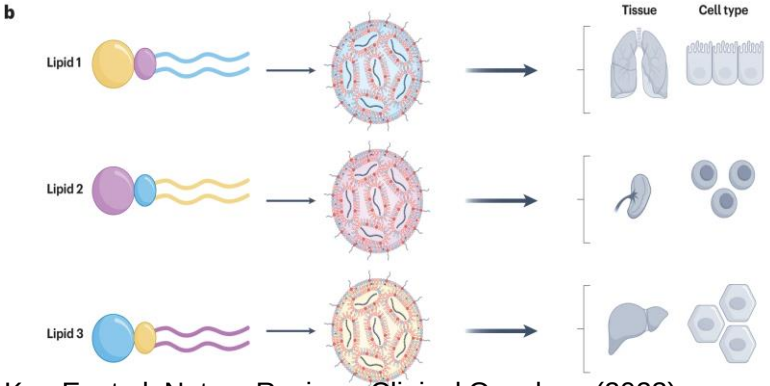
Replace



Vision

Tailoring the expression of specific genes in diseased cells





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

Dr. Edo Kon

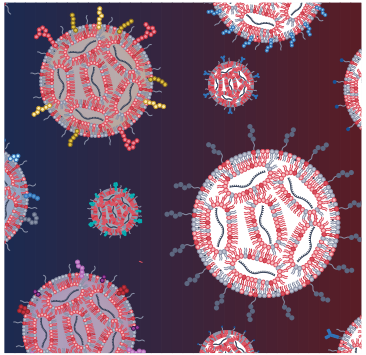
Dr. Inbal Hazan-Halevi

Lior Stostky-Oterin

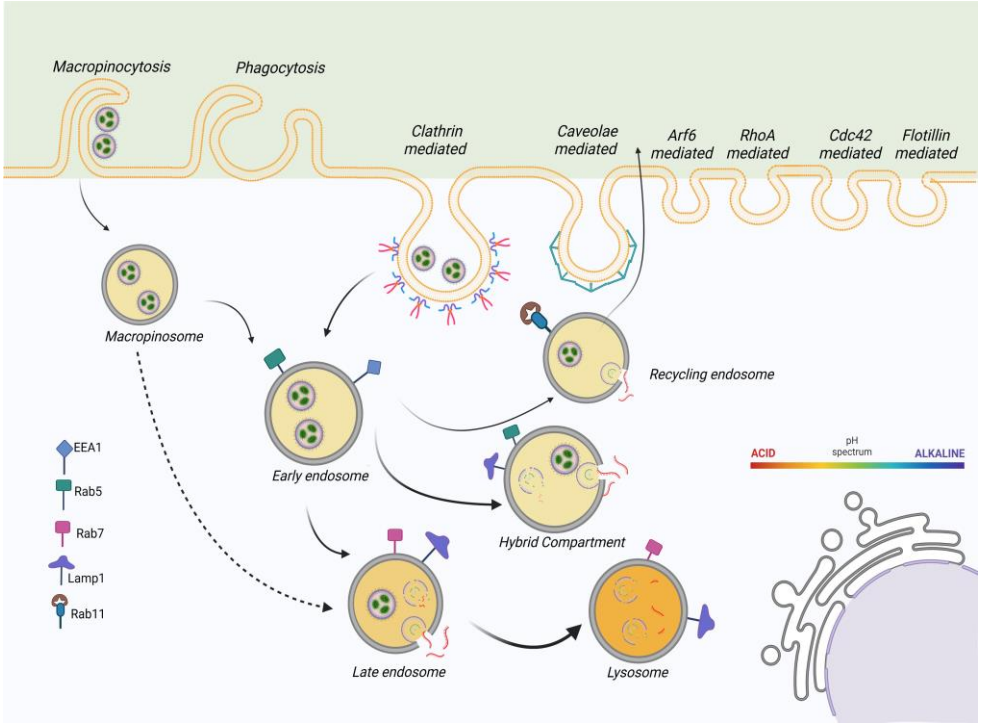
Nitay Ad-El

nature reviews
clinical oncology

November 2023

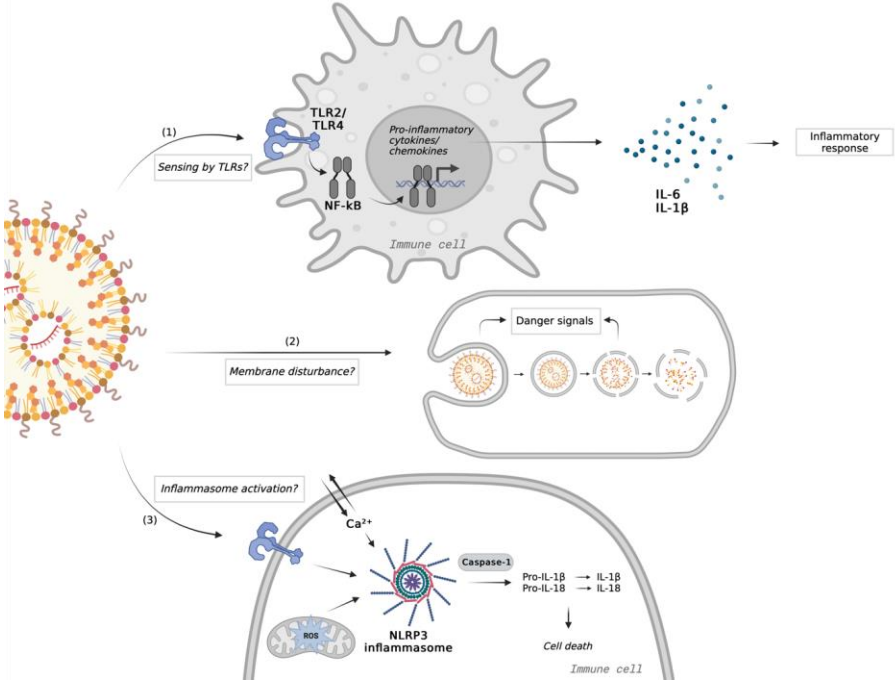


Endosomal Escape: A Bottleneck for LNP Mediated Therapeutics ?



Chatterjee S. et al. PNAS (2024)

Understanding intracellular immune response of LNPs



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

The Peer Team

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על שם ורדה ובעז דותן

National Cancer Institute

National Institute of Allergy and Infectious Diseases

erc
European Research Council

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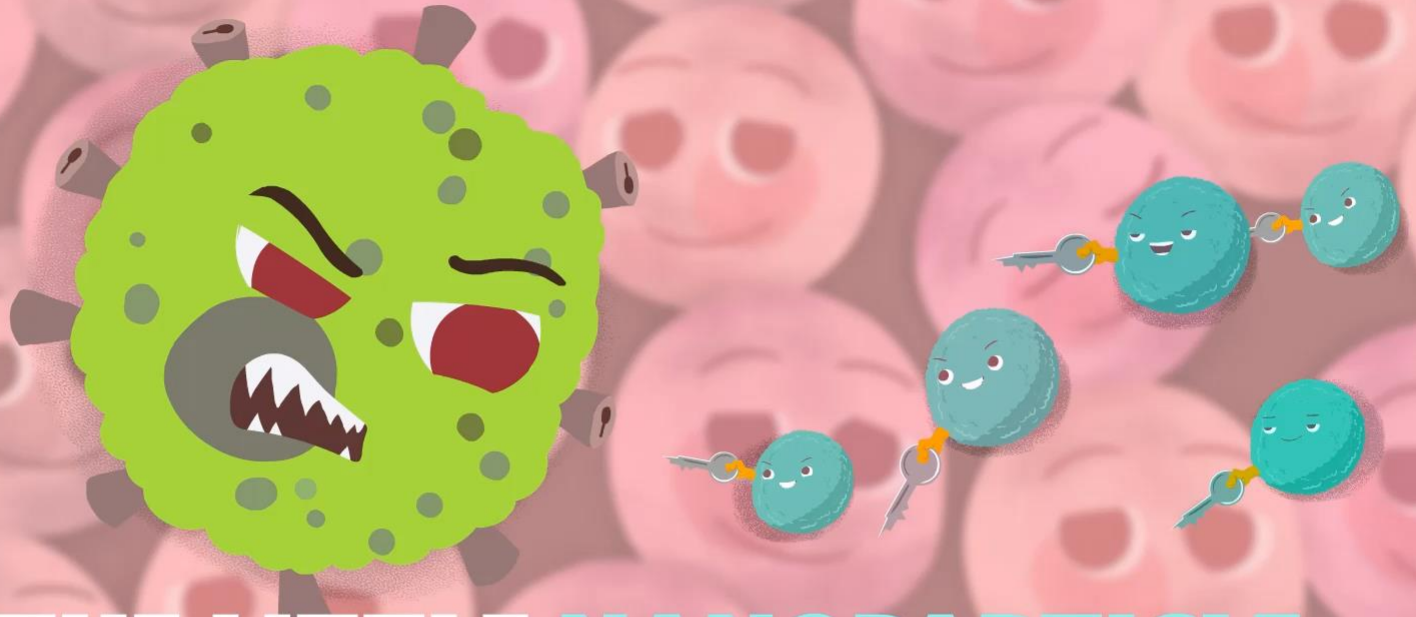
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS



THE LITTLE NANOPARTICLE THAT CAN DEFEAT CANCER FROM WITHIN

Peer Lab
Precision Nanomedicine