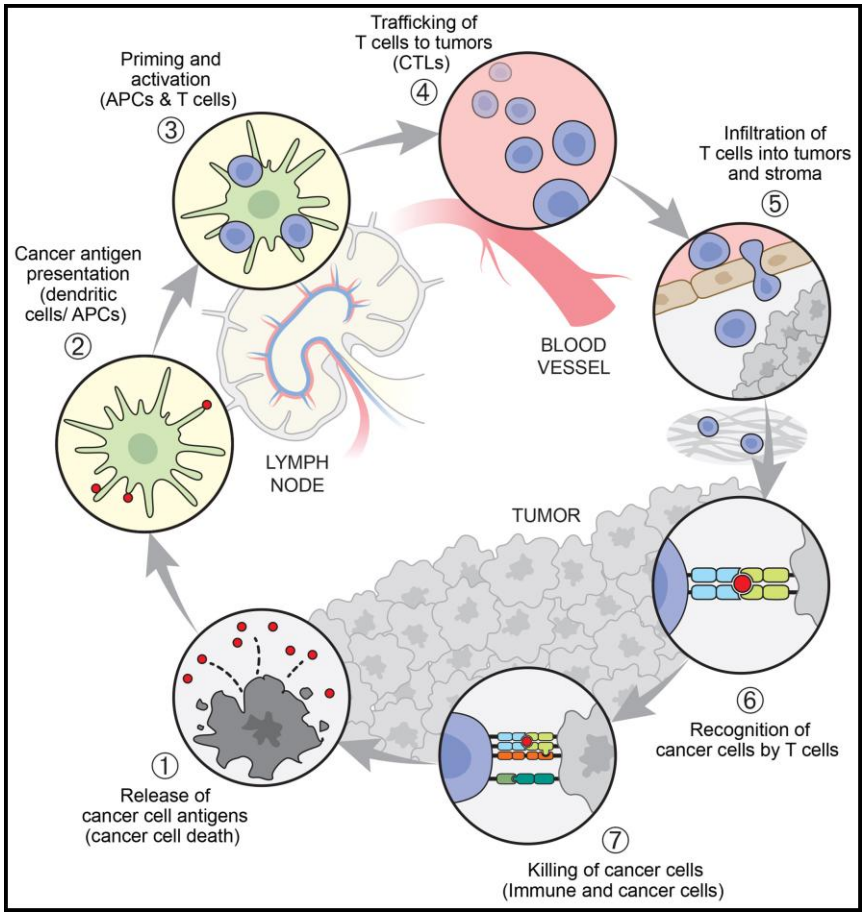
mRNA therapeutics for in situ cancer immunotherapy

Ji-Ho Park, PhD
Department of Bio and Brain Engineering

In situ cancer immunotherapy

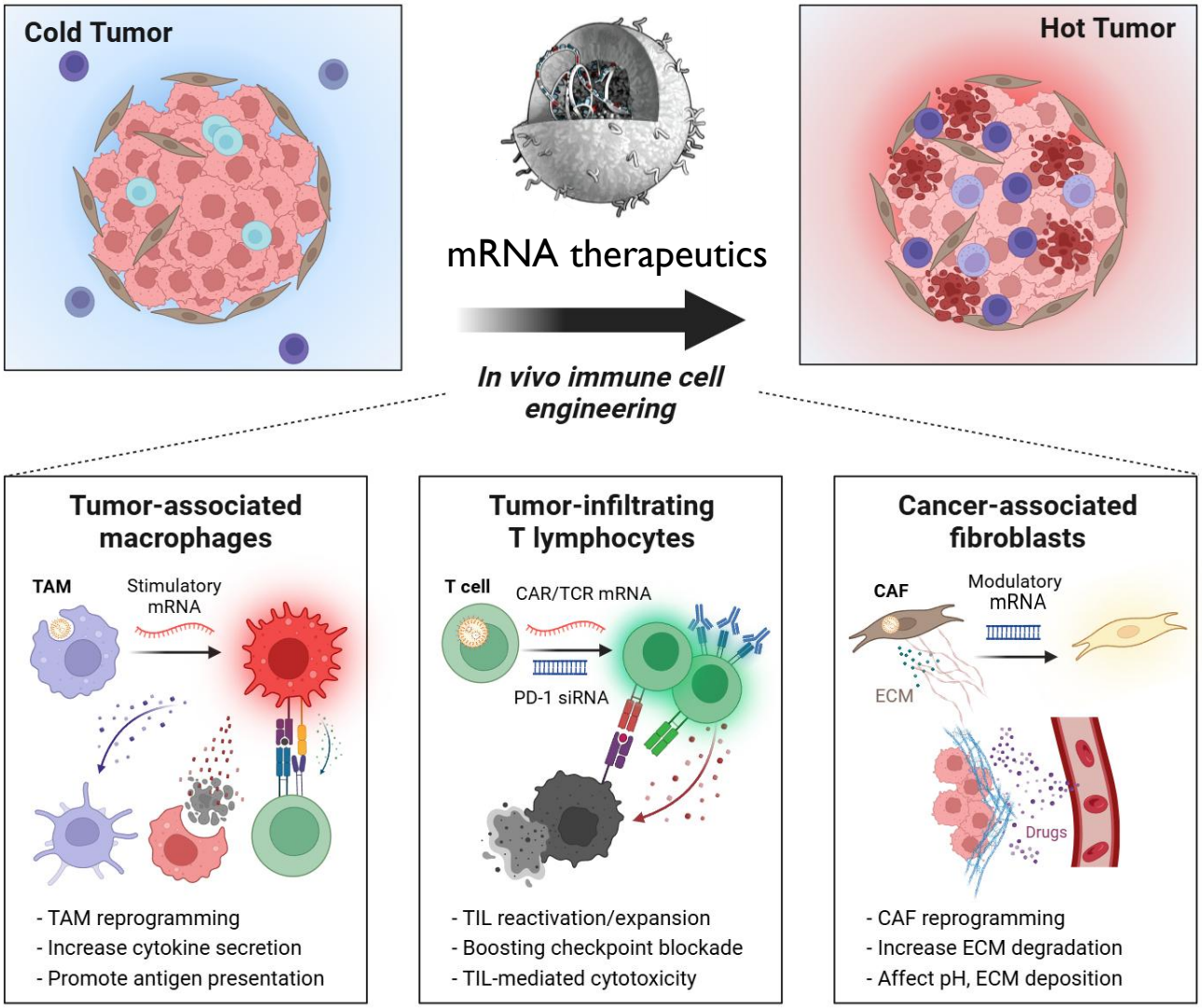
Cancer-Immunity Cycle



Mellman et al. *Immunity* (2023) 2188

Local immunotherapeutic strategy that **engineers immune cells directly within the tumor microenvironment (TME)** to amplify the Cancer-Immunity Cycle and thereby enhance antitumor immune responses.

Engineering immune cells in situ



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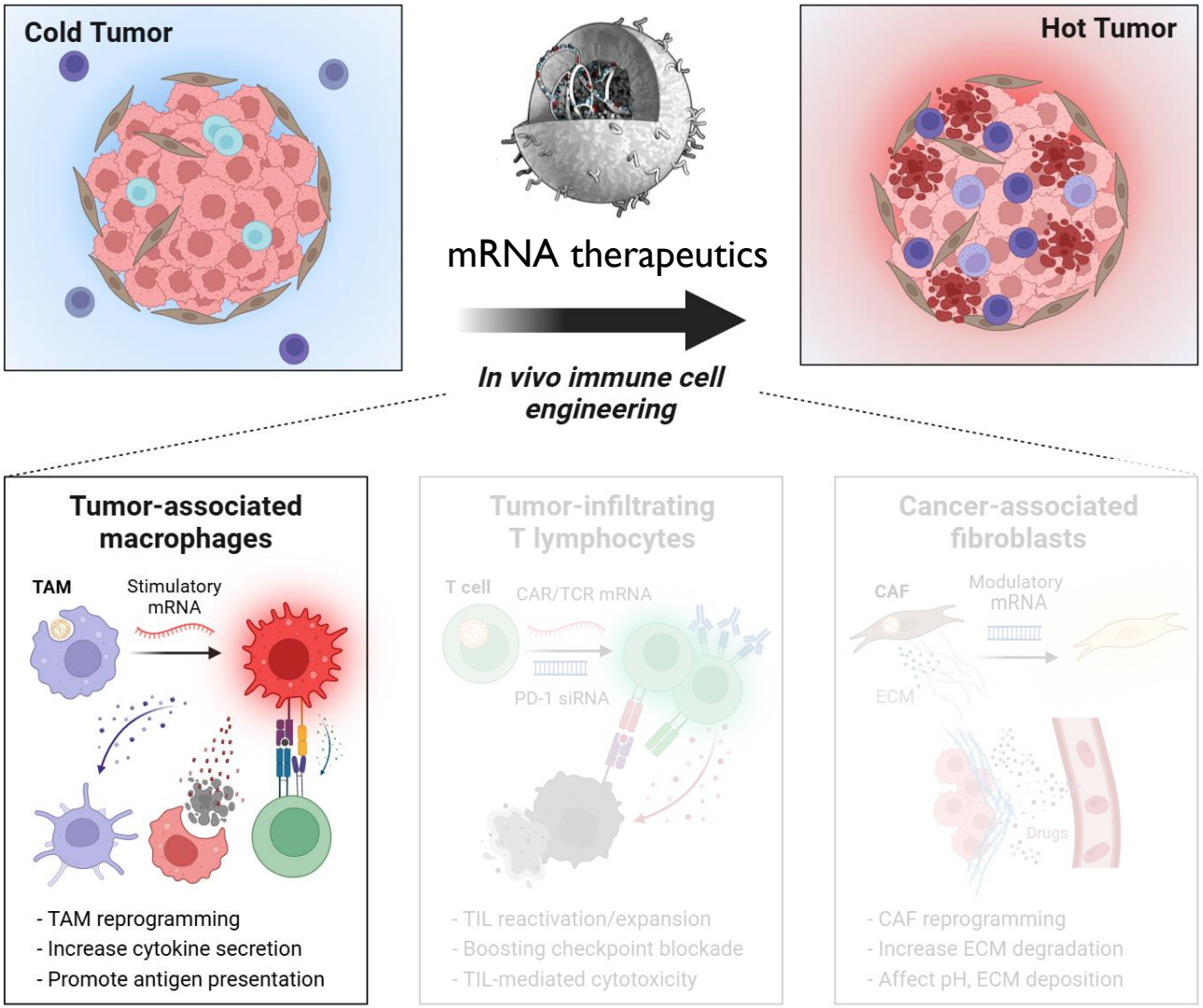
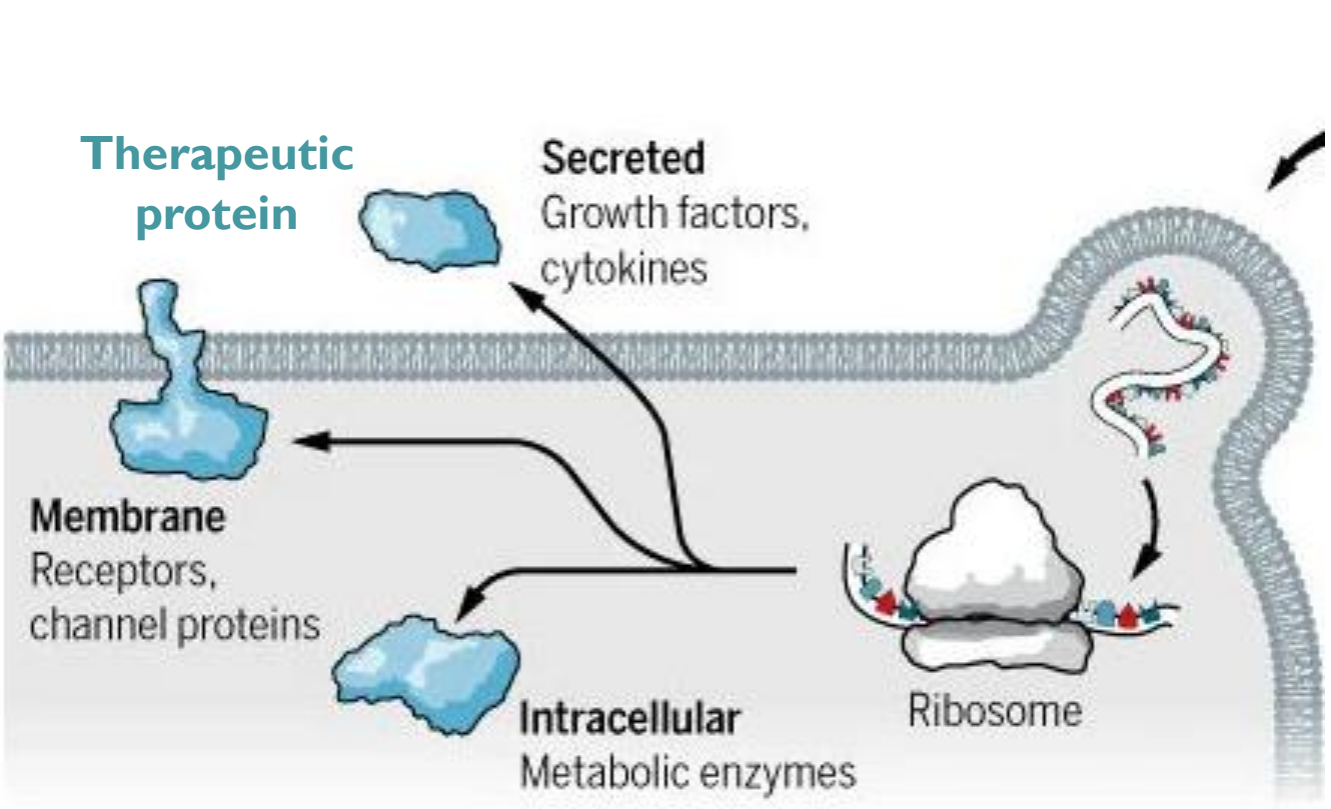
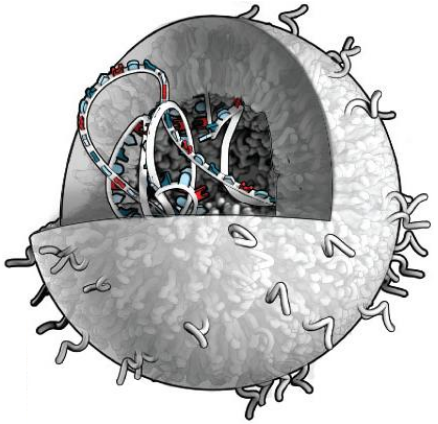


mRNA therapeutics

a class of medicines that deliver mRNA into cells, enabling the body's own cells to produce therapeutic proteins





Lipid nanoparticle (LNP)

Science 270 (2020) 1022

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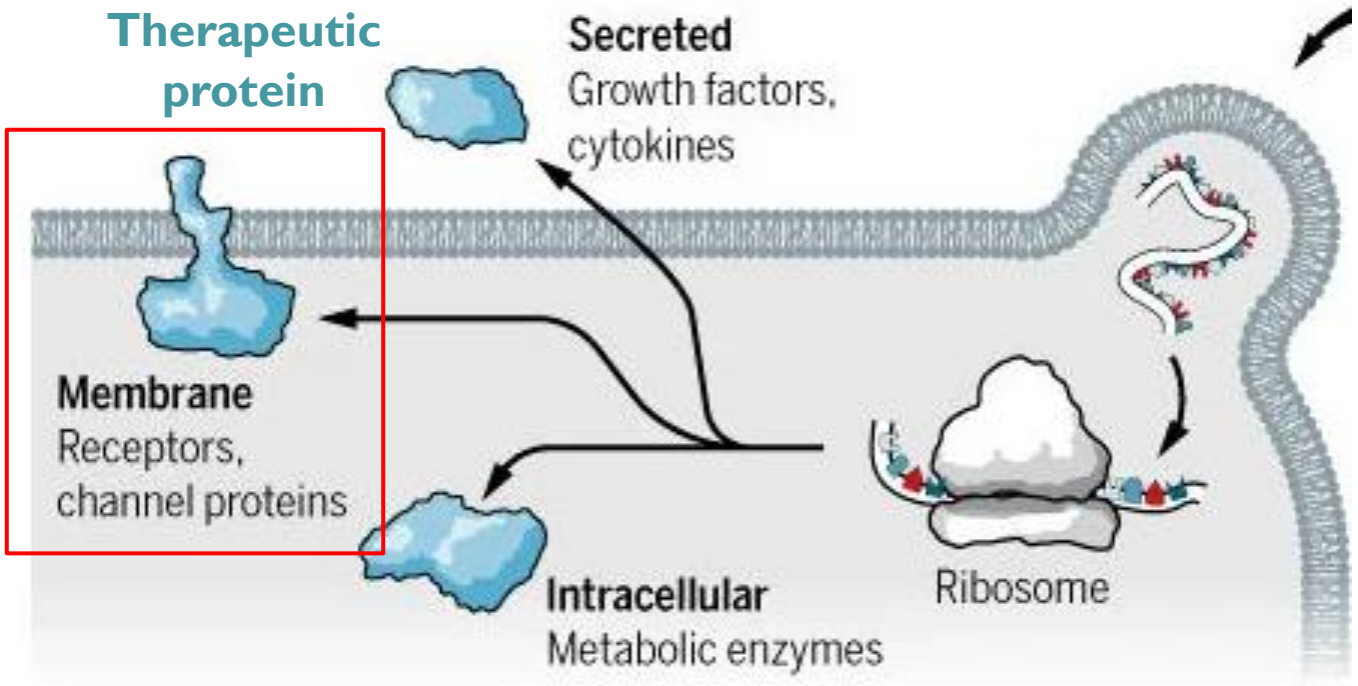

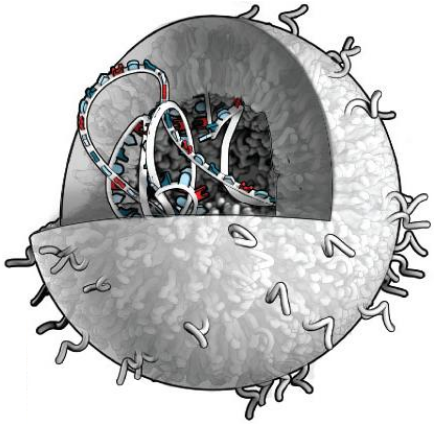

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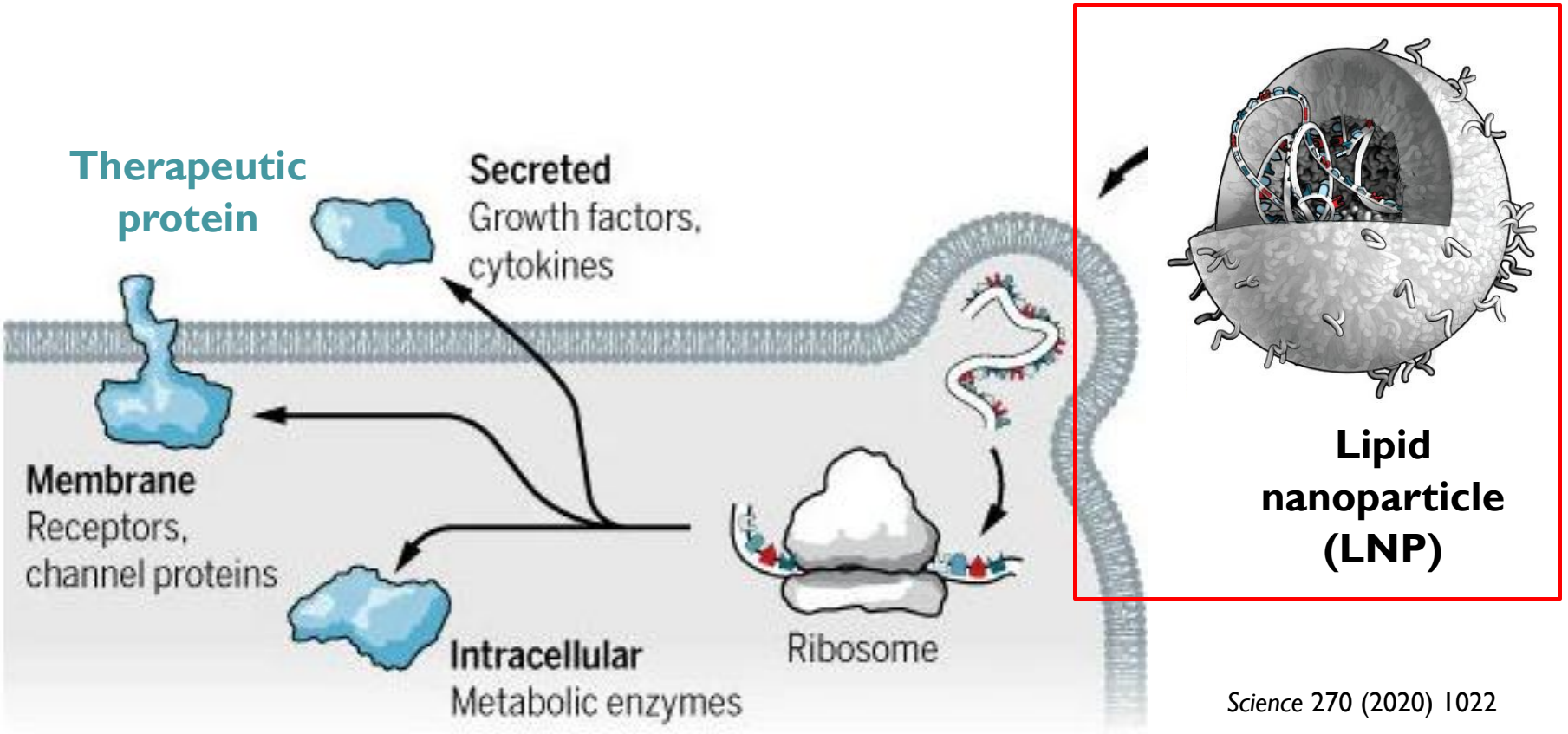

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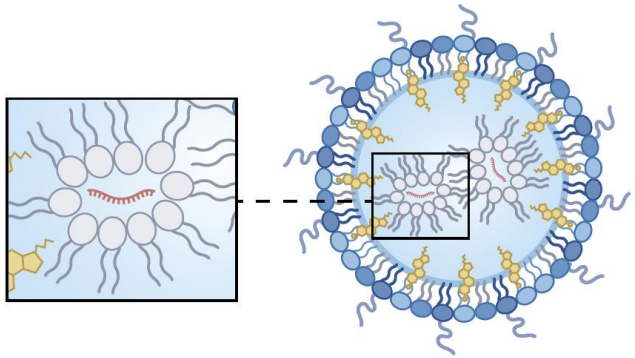
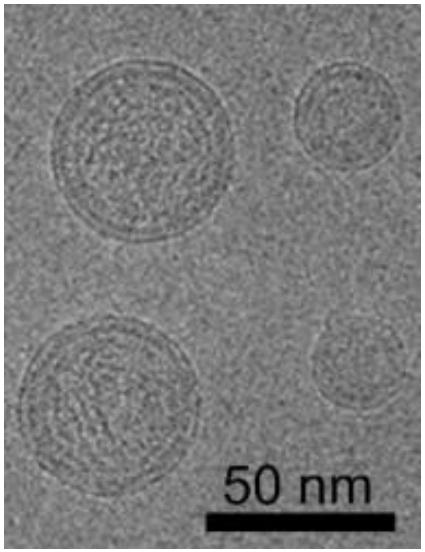
Lipid nanoparticles for mRNA delivery



Ionizable lipid
PEG-lipid
Cholestrol
Helper lipid (DOPE or DSPC)

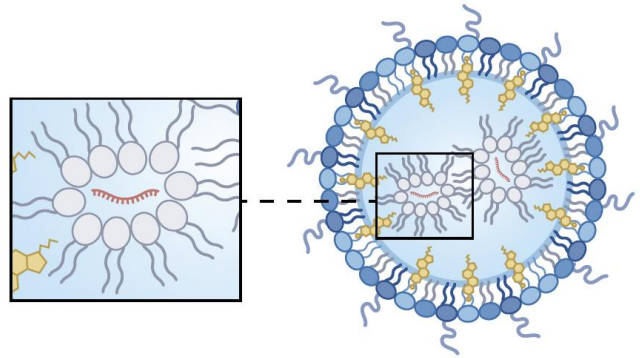
LNP



Onpattro (*Alnylam*)

- siRNA therapeutics for hereditary TTR amyloidosis
- Approved in 2018
- Intravenous injection of LNP
- Hepatocyte targeting

Lipid nanoparticles for mRNA delivery



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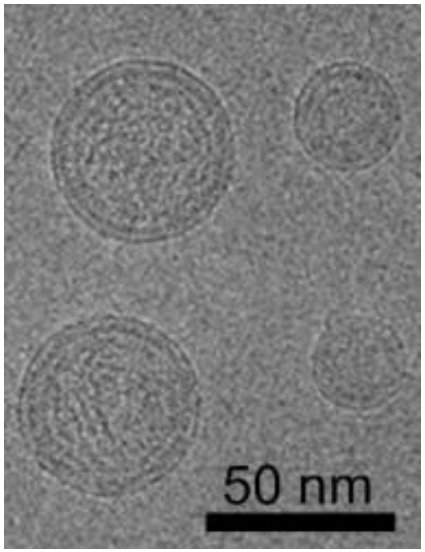

mRNA vaccine

- COVID-19
- Approved in 2020
- Immune cell targeting

Onpattro (*Alnylam*)

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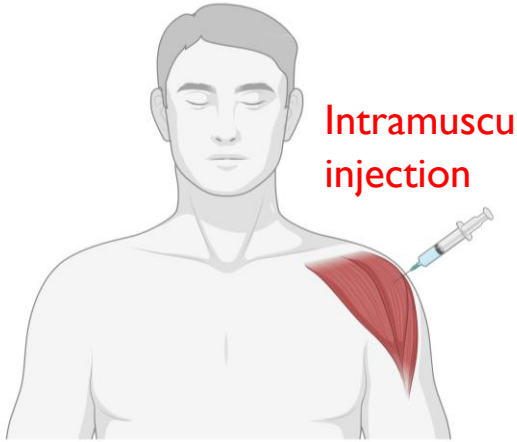
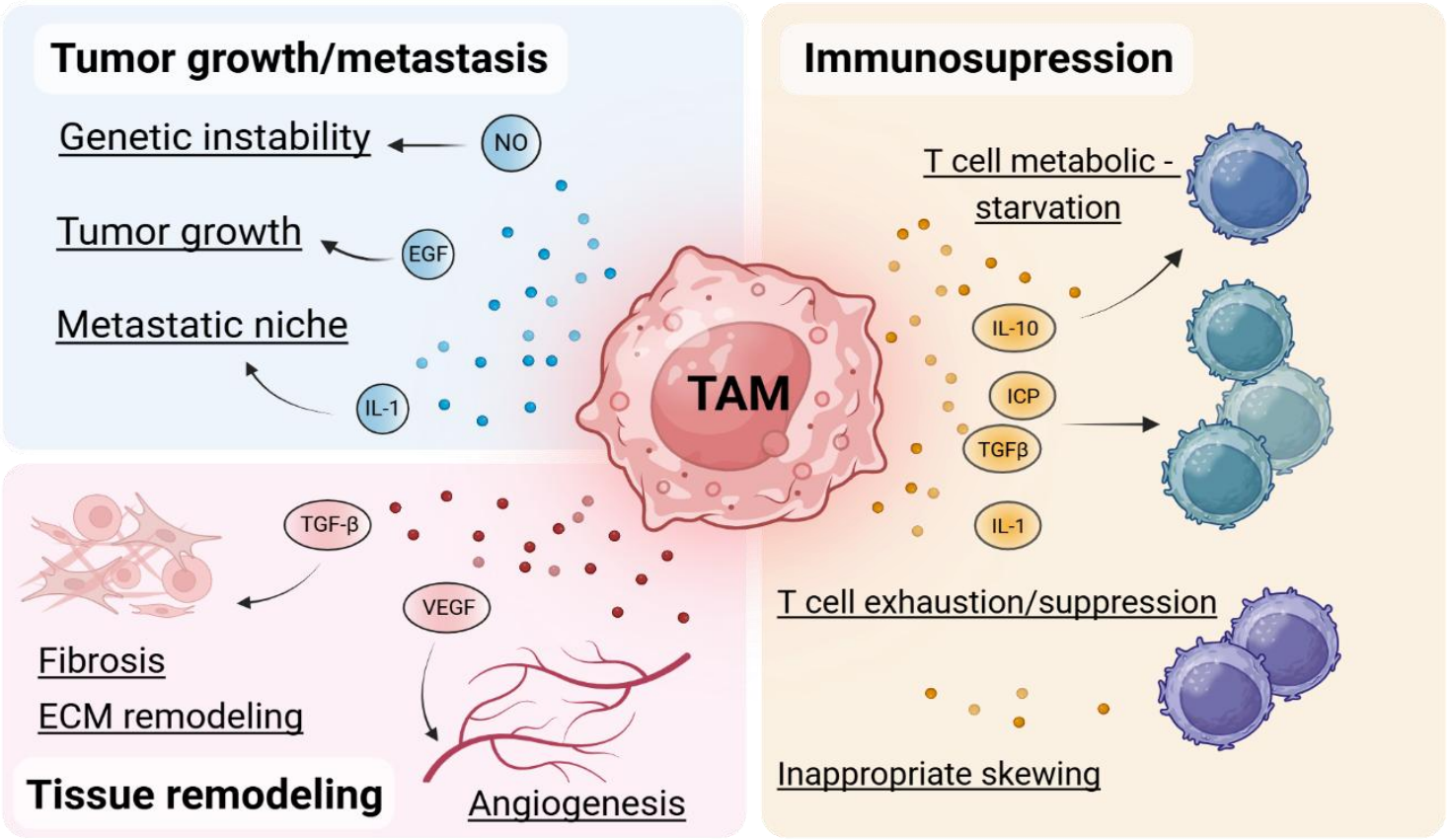


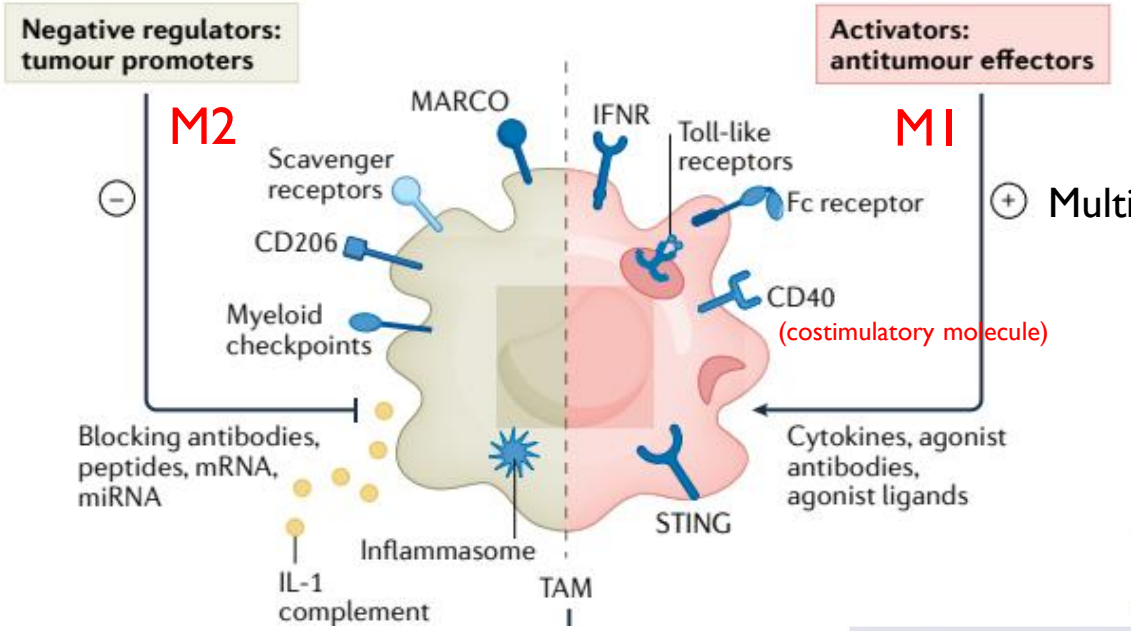
Intramuscular injection

Tumor-associated macrophages (TAM)



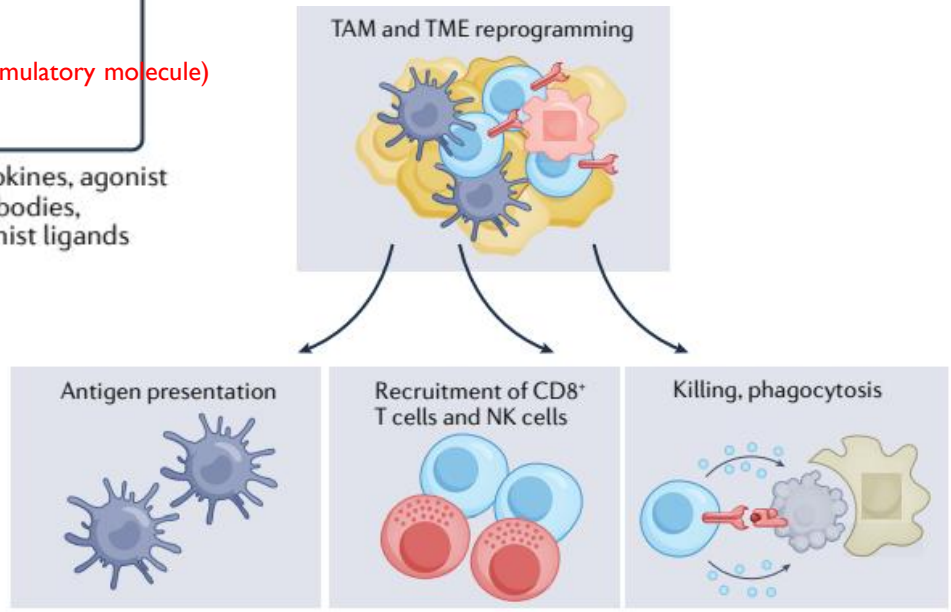
The most abundant immune cell populations in the tumor microenvironment (TME) and generally promote **tumor progression and immunosupression.**

Engineering TAMs for cancer immunotherapy



M2 → M1

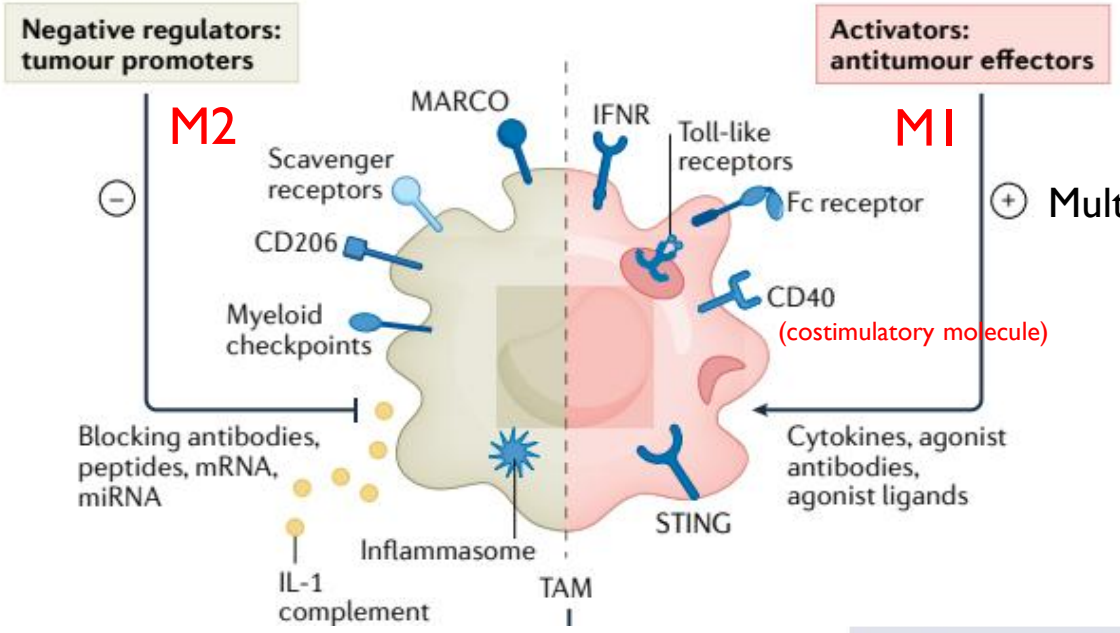
Multipronged anti-tumor effects



Nature Reviews Drug Discovery 21(2022) 799–820

Highly plastic and reprogrammable: Tumor-promoting M2-like macrophages can be converted into M1-like macrophages with a multipronged antitumor effects such as antigen presentation, recruitment of $CD8^+$ T cells, and killing tumor cells through phagocytosis.

Engineering TAMs for cancer immunotherapy



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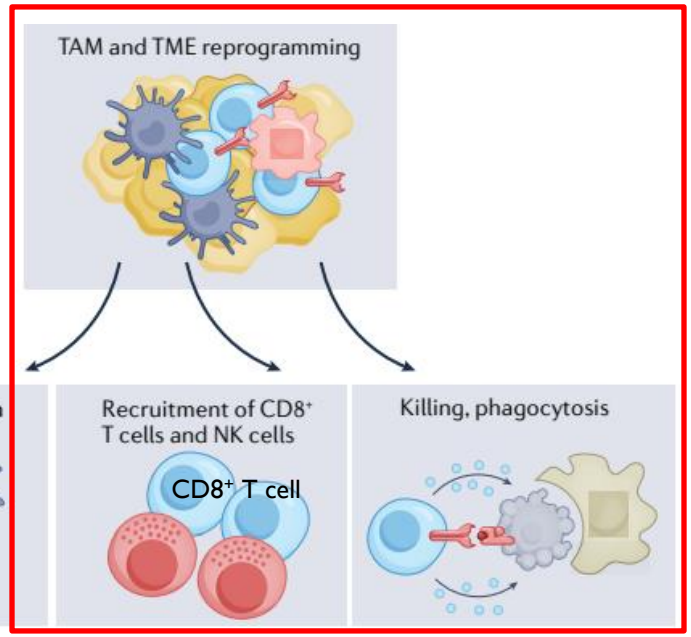

M2 → M1

M1 + CD8⁺T cell

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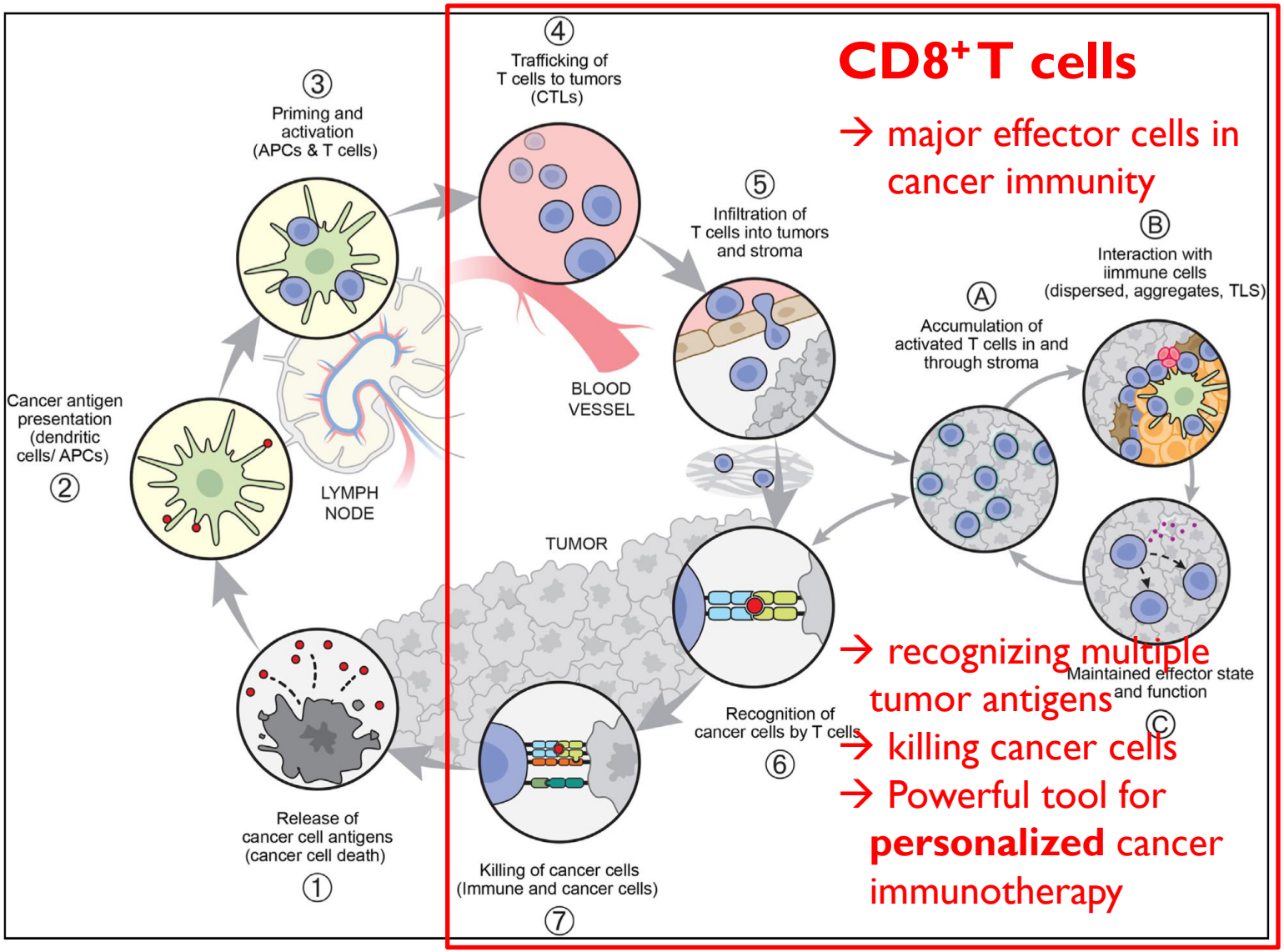
Tumor-infiltrating lymphocyte (TIL)



CD8⁺ T cells

→ major effector cells in cancer immunity

→ recognizing multiple tumor antigens

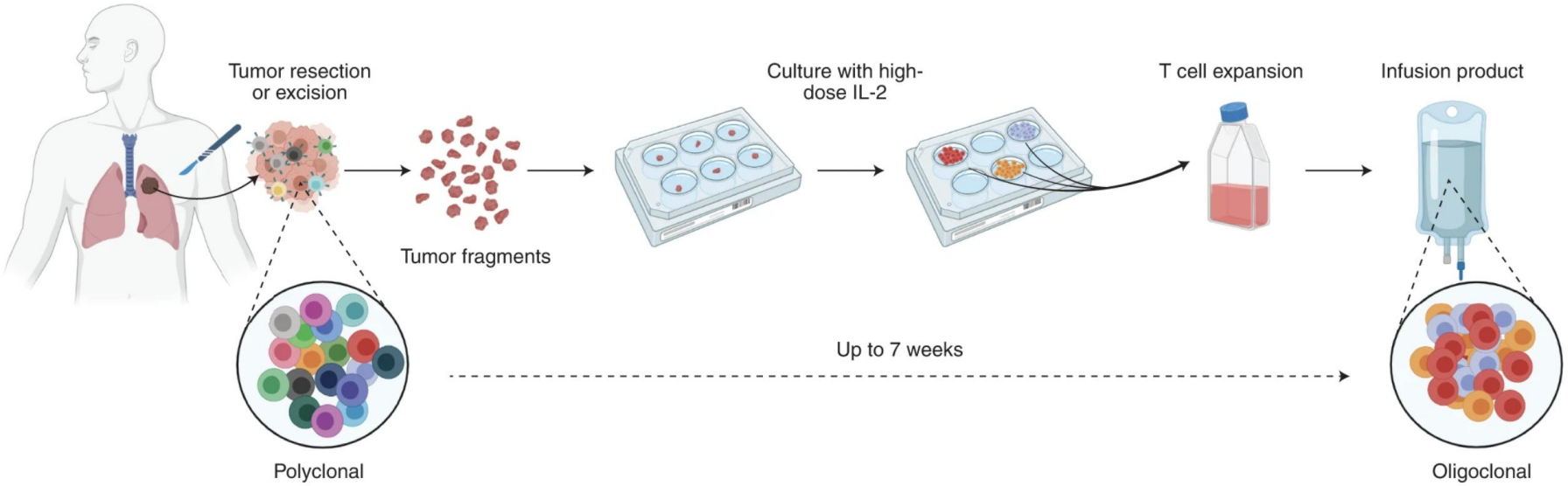
→ killing cancer cells

→ Powerful tool for **personalized** cancer immunotherapy

Mellman et al. *Immunity* (2023) 2188

TIL therapy for solid tumors

: a type of immunotherapy that uses a patient's own T cells to treat cancer



AMTAGVI™ (lifileucel) Suspension for IV infusion

: a prescription treatment for adults with **advanced melanoma** that has spread or cannot be removed by surgery (**2024 Feb**)

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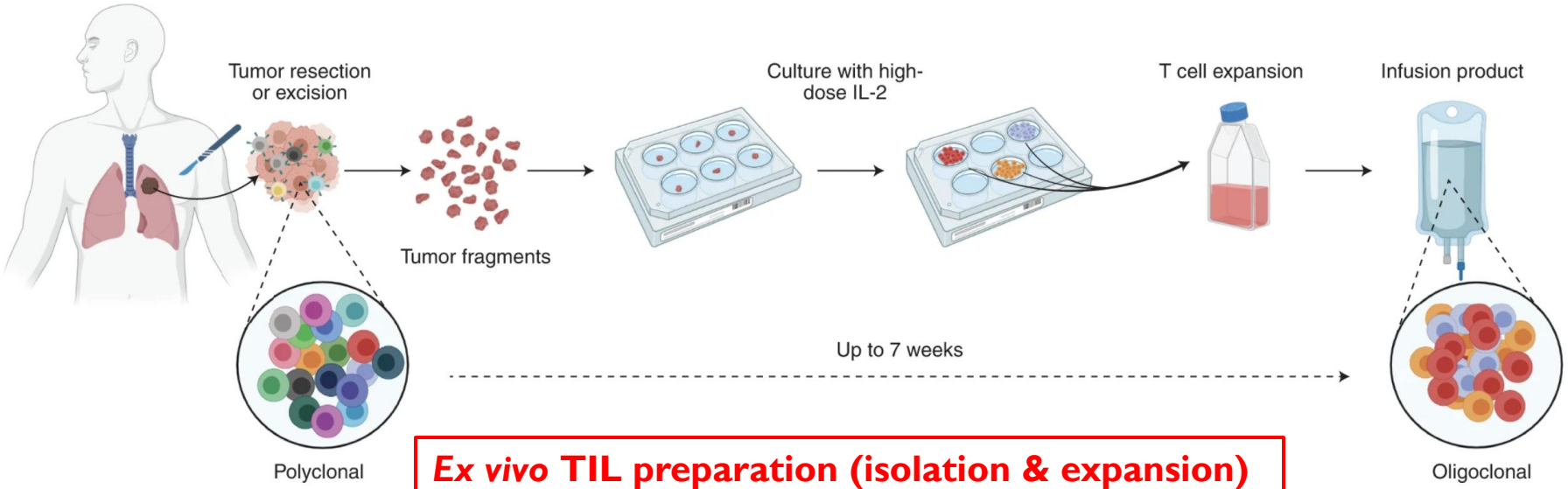

***Ex vivo* TIL preparation (isolation & expansion)**

1) Long cell manipulation process (1~2 months)
2) Hard to grow TIL *ex vivo* from tumor digests

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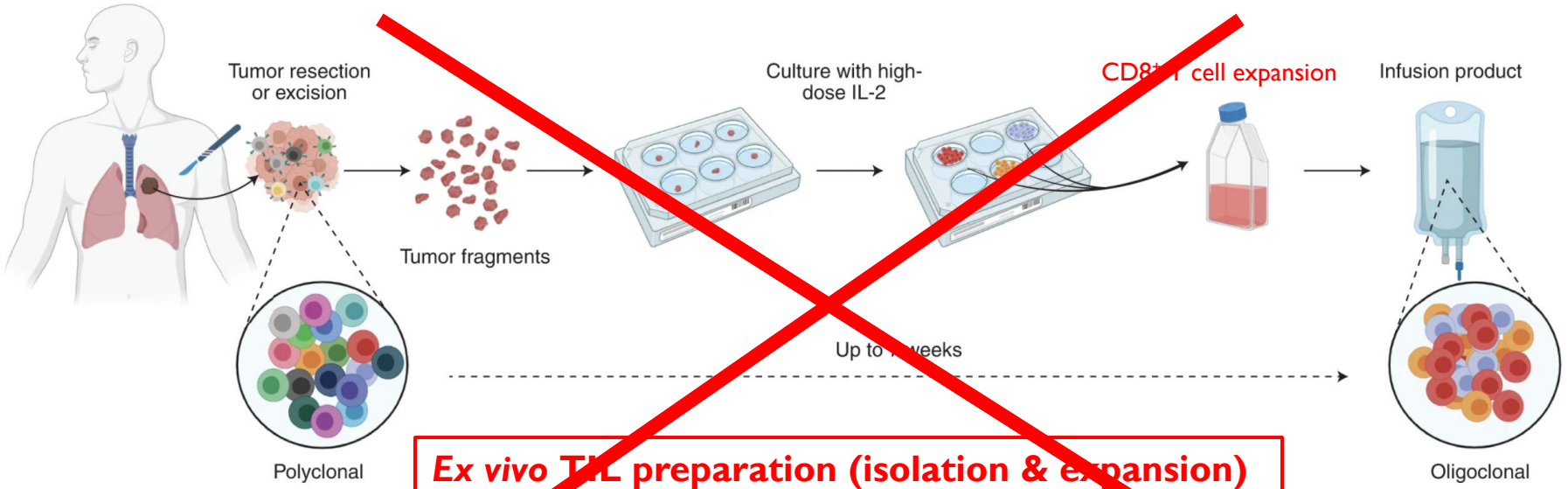

***Ex vivo* TIL preparation (isolation & expansion)**

1) Long cell manipulation process (1~2 months)
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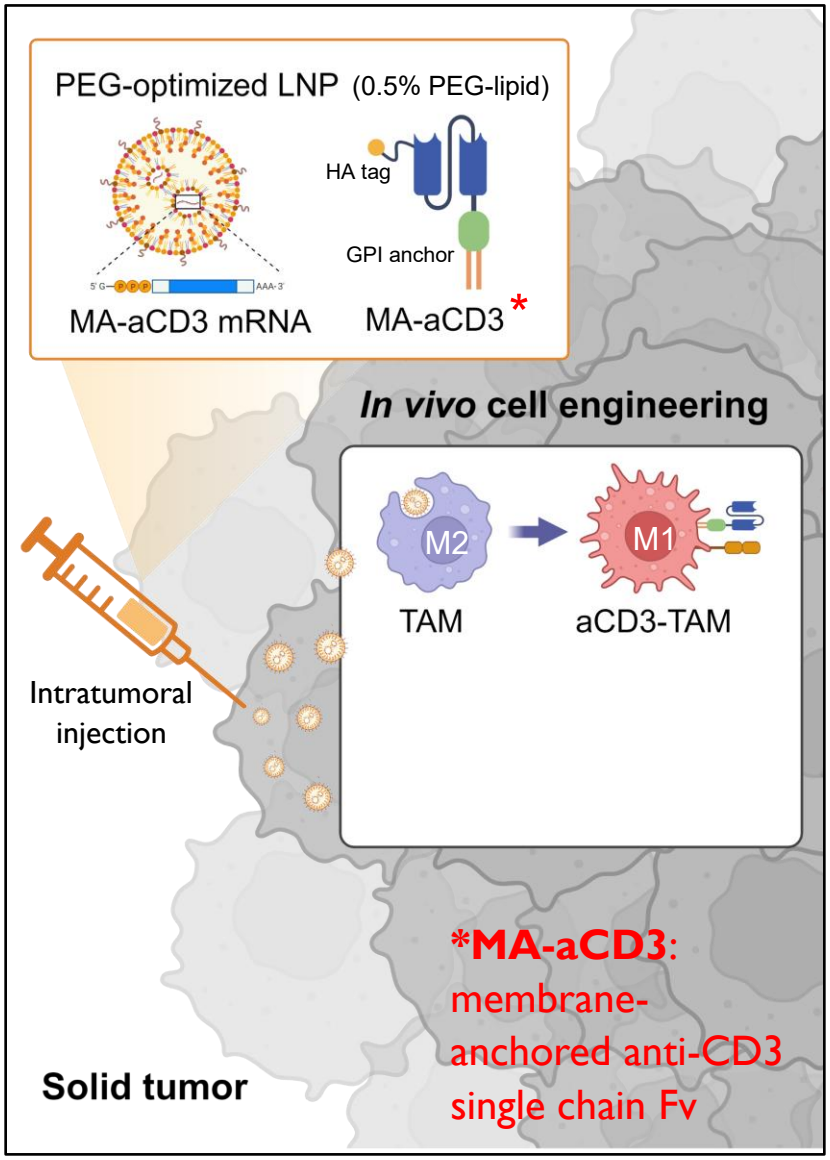
Can we achieve the benefits of TIL therapy without the need for *ex vivo* TIL isolation and expansion?

In situ TIL therapy via mRNA delivery



Yoon et al. *ACS Nano* 18 (2024) 32401-32420

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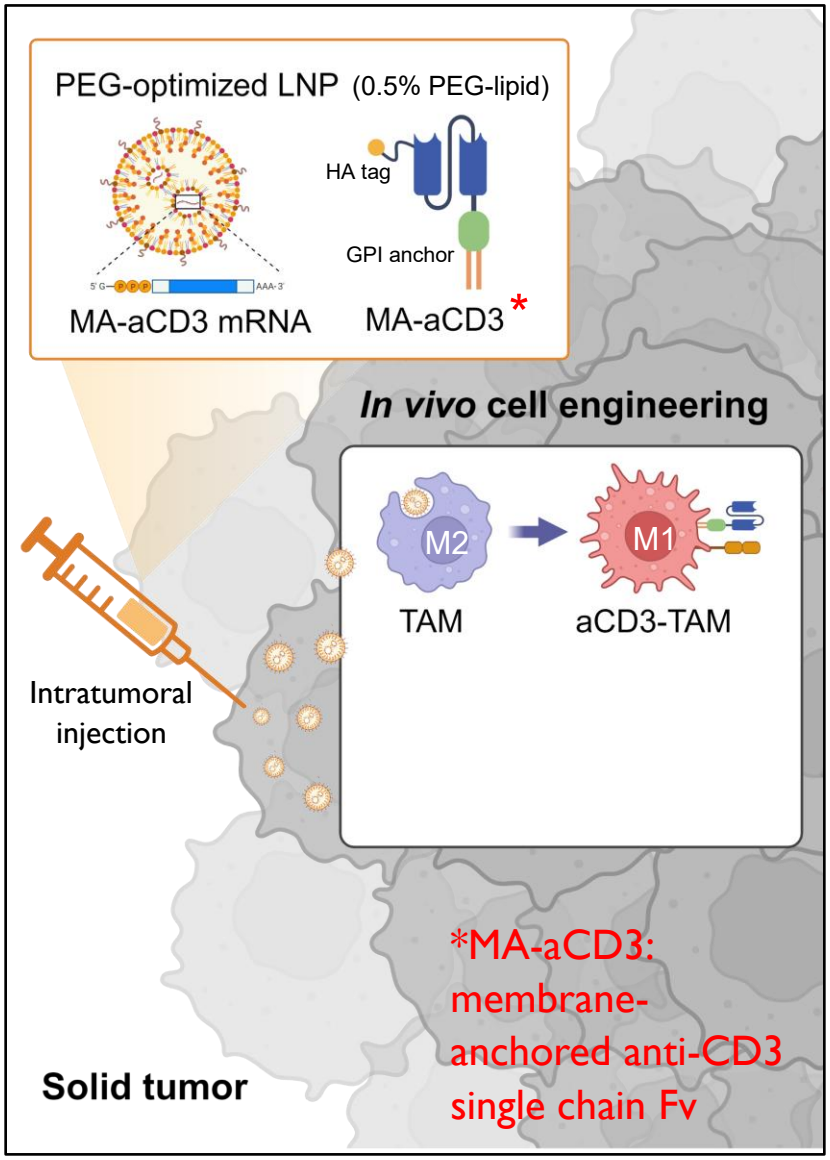
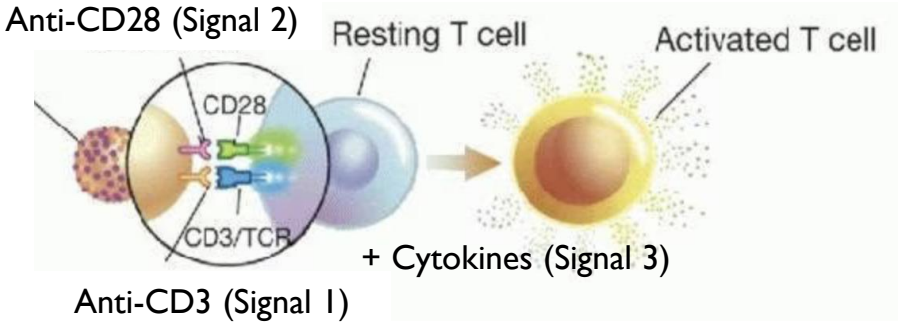


Dynabeads®



Signal 1: TCR signaling

Signal 2: Co-stimulatory interaction

Signal 3: Cytokine signaling

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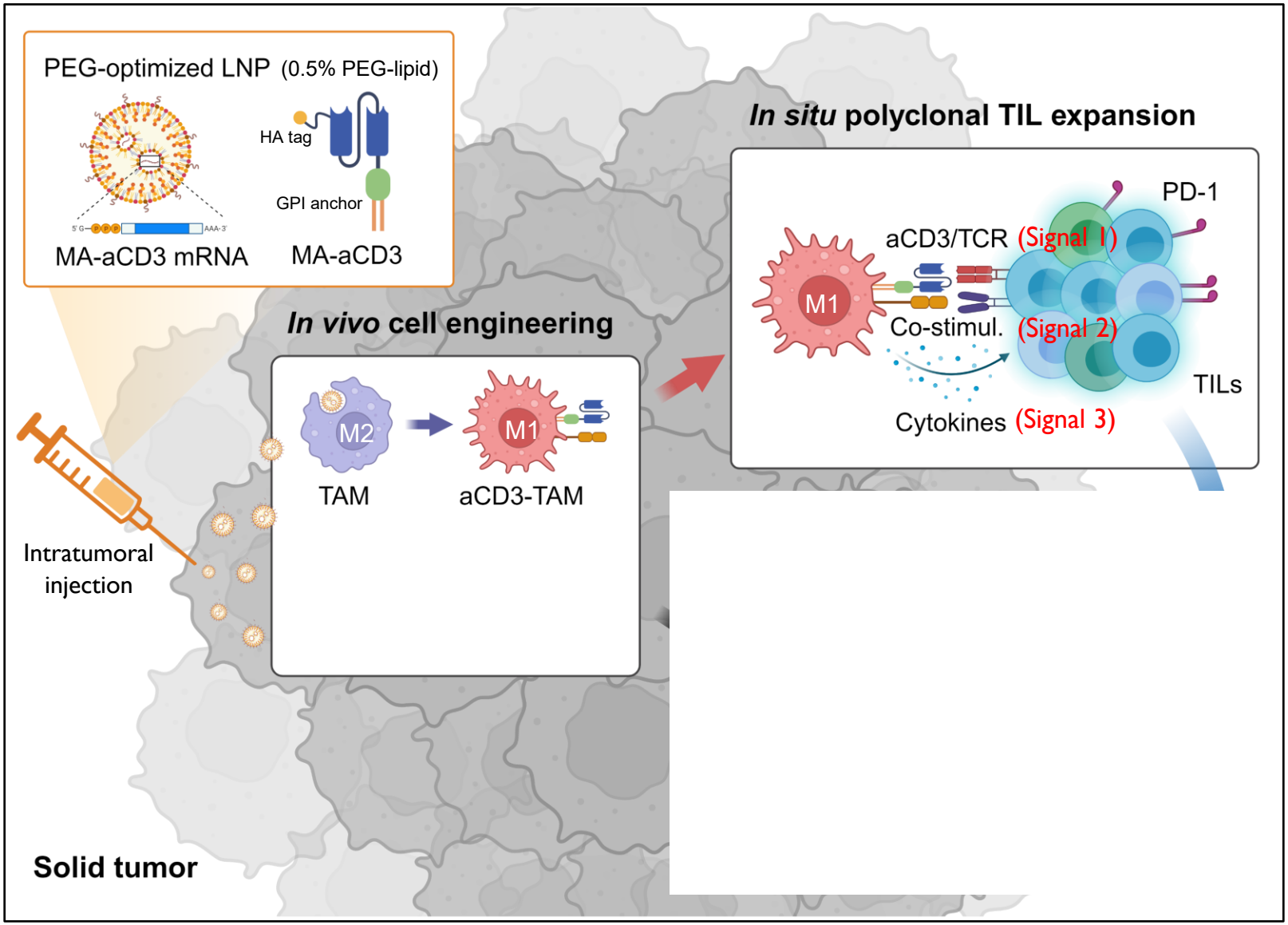

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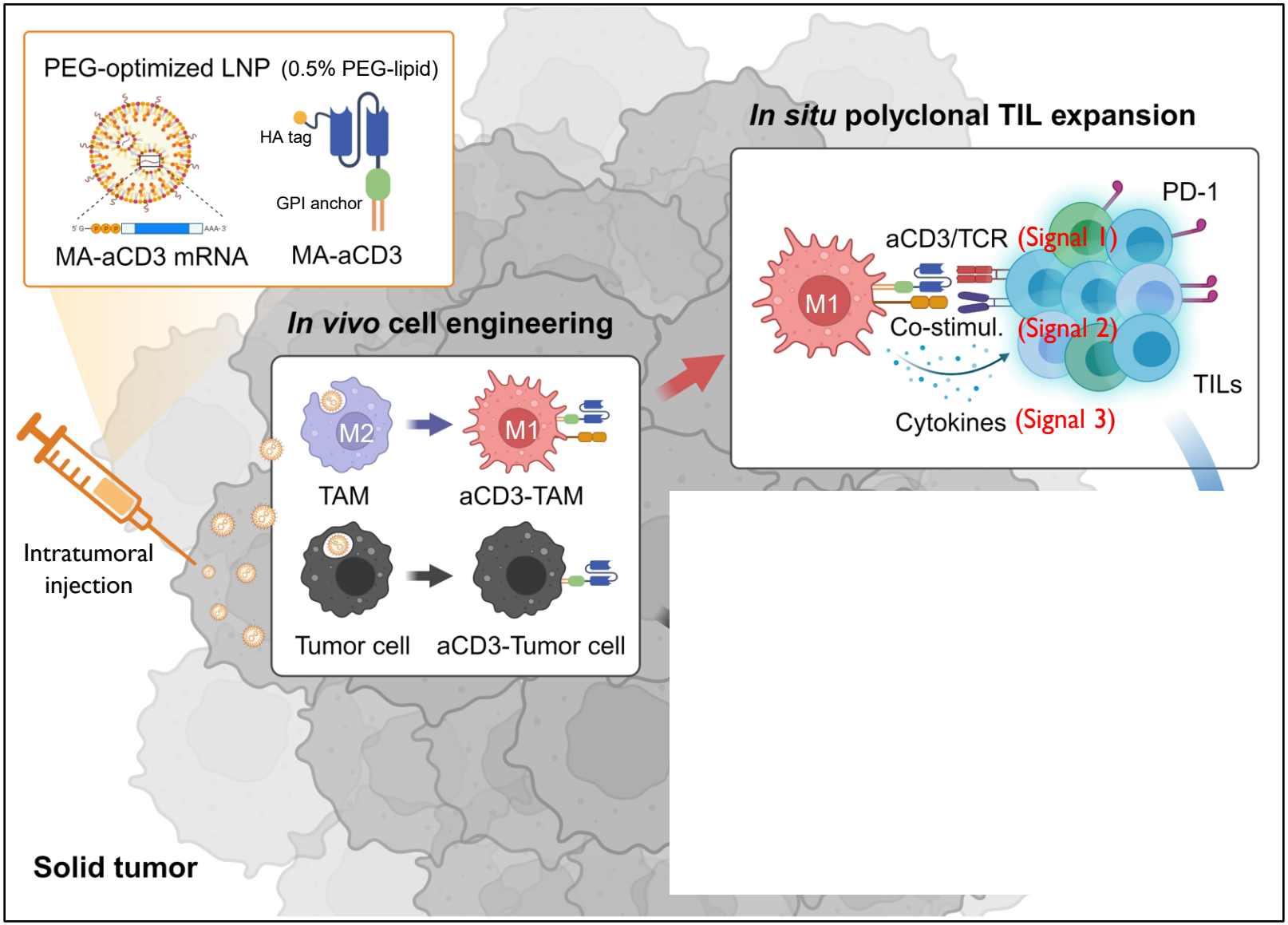

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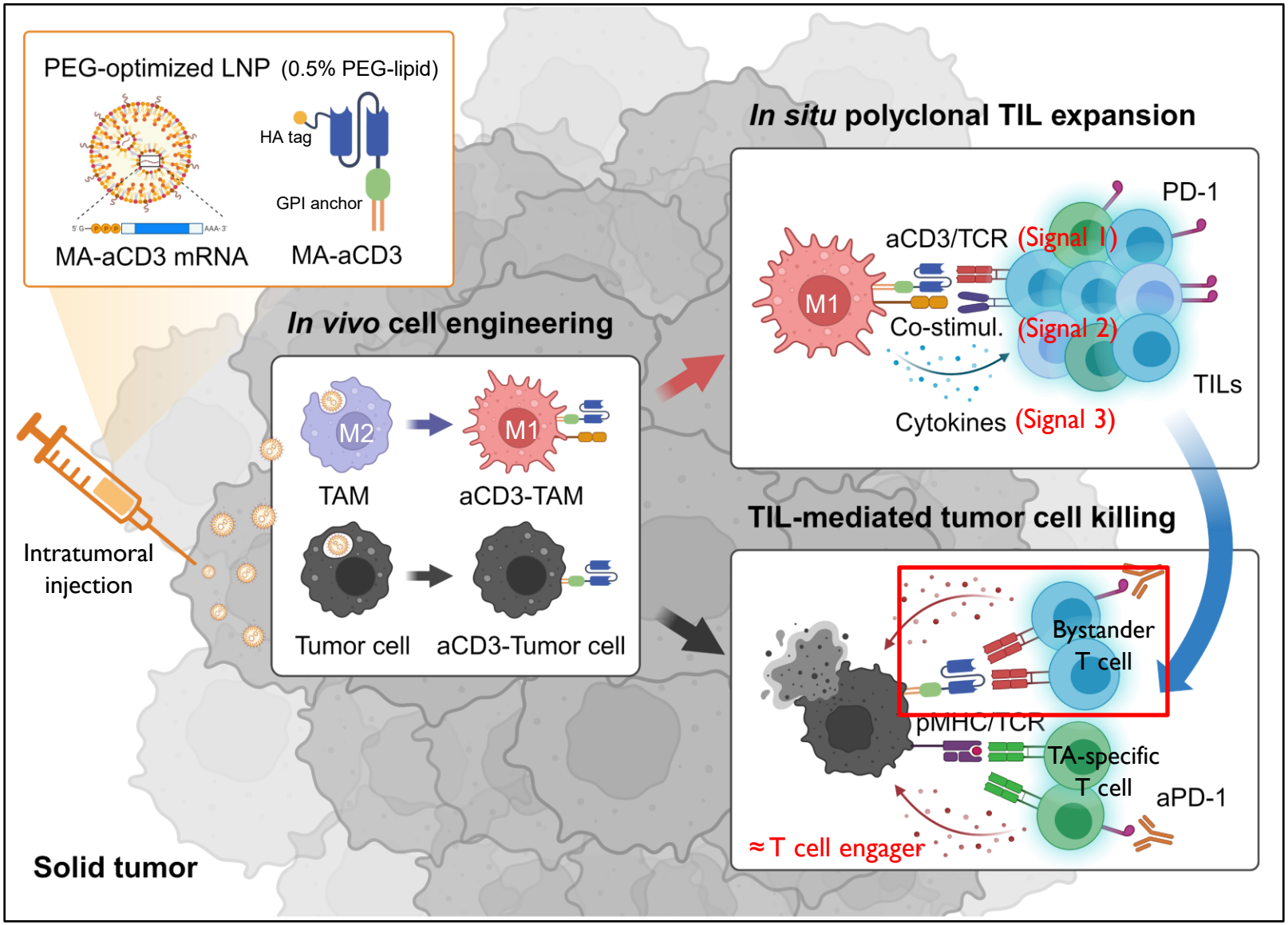

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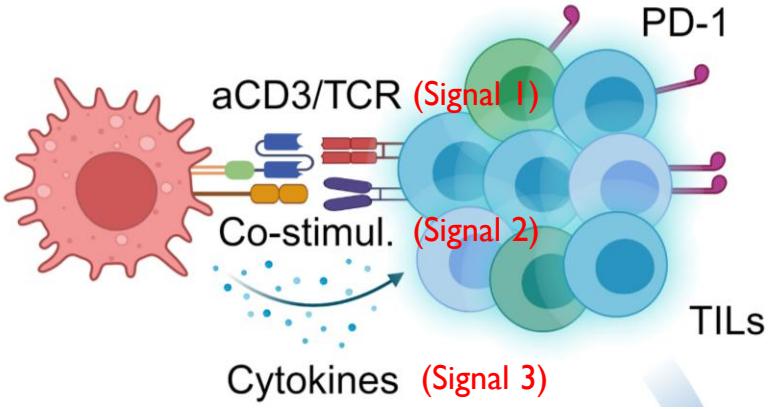
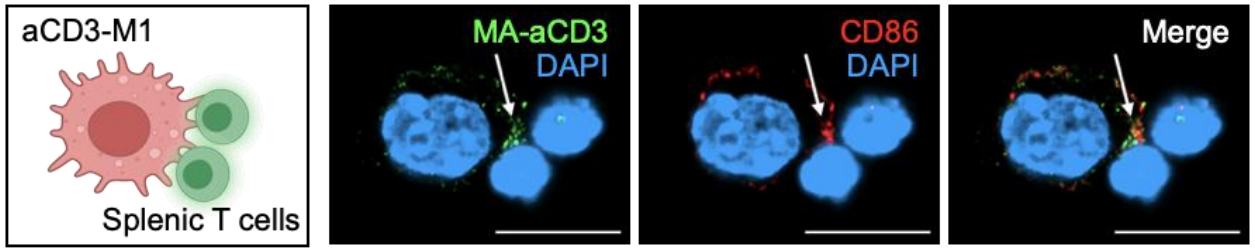
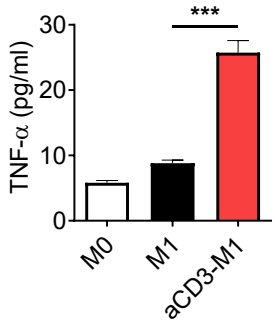
Three signals from engineered macrophages







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Yoon et al. *ACS Nano* 18 (2024) 32401-32420

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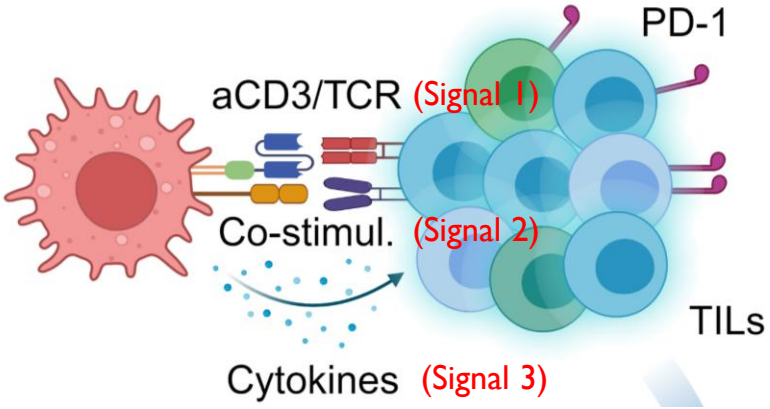

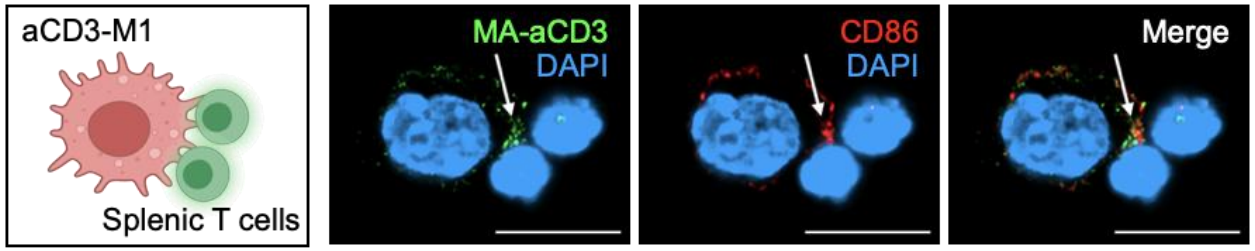

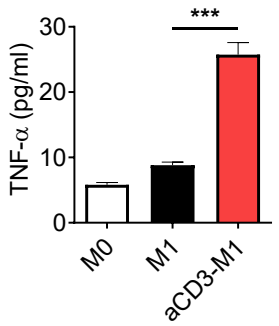

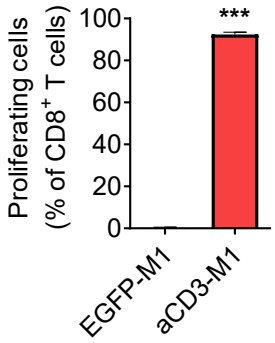

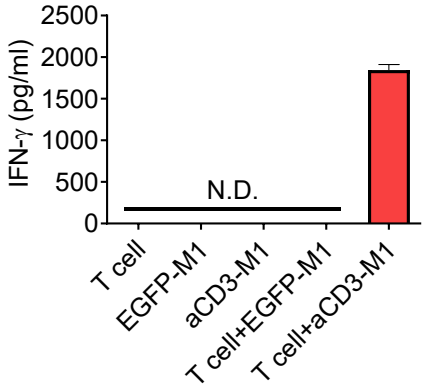

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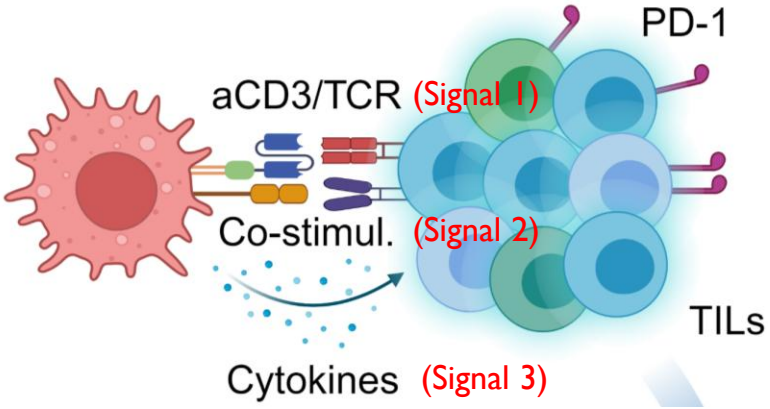

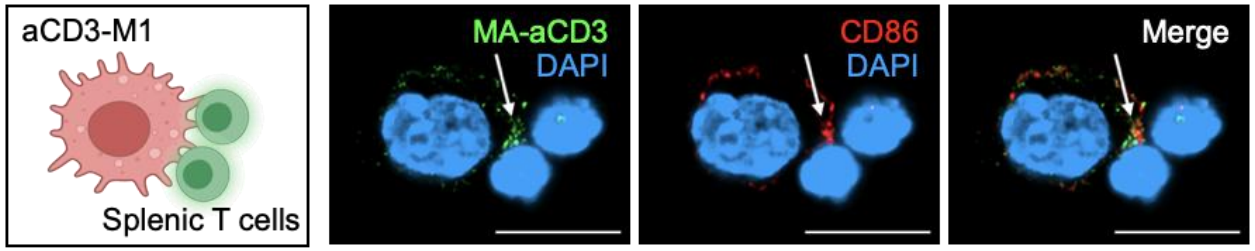

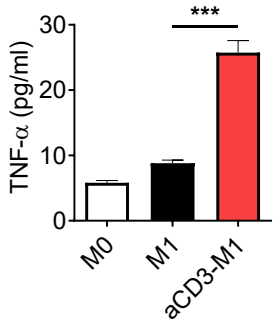

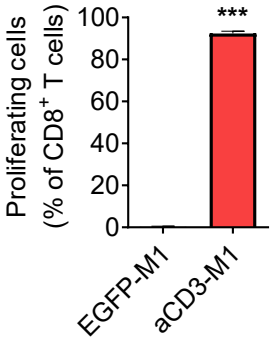

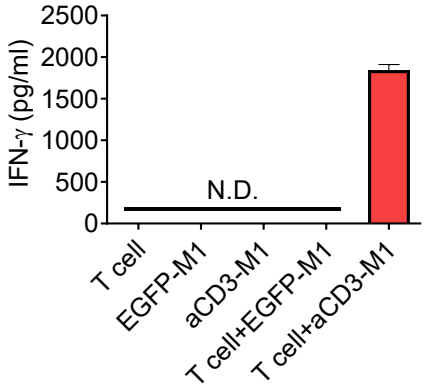

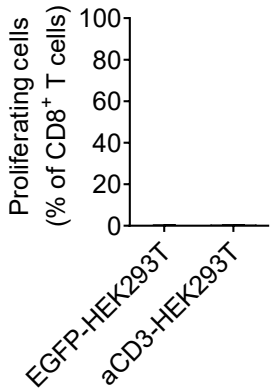

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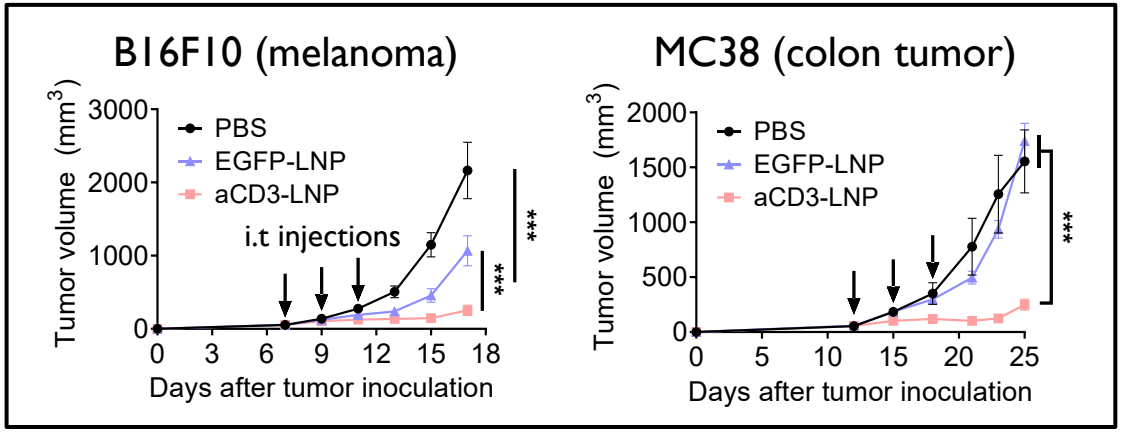
Anti-tumor effects of in situ TIL therapy

Tumor growth suppression



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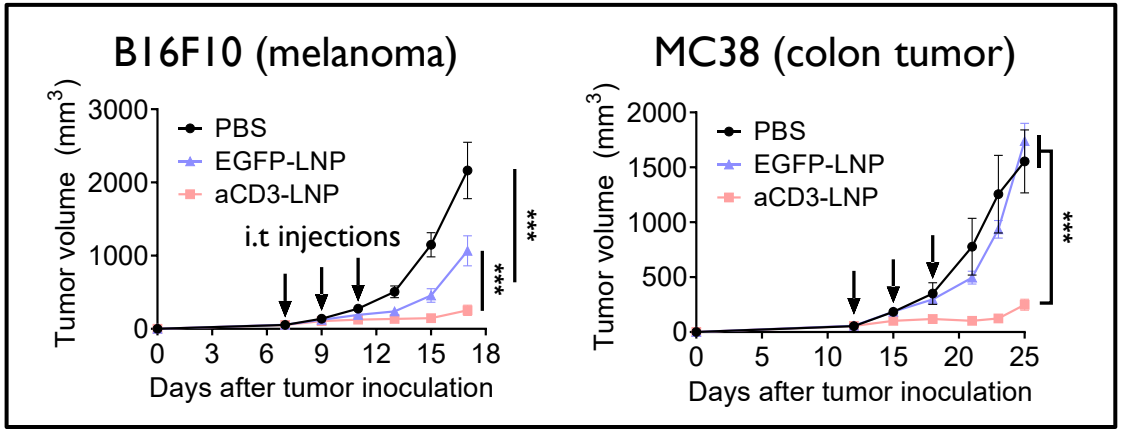

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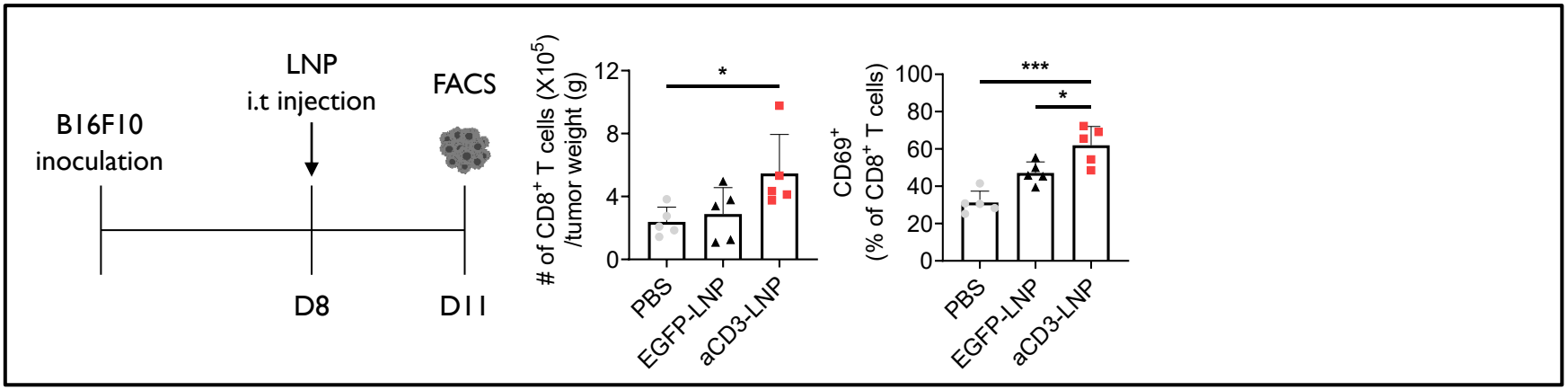
In situ polyclonal CD8+ TIL expansion



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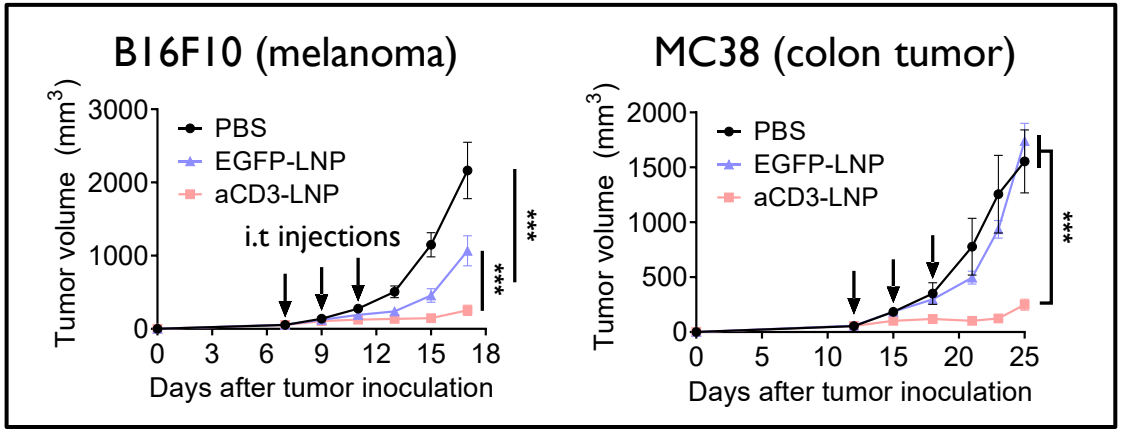

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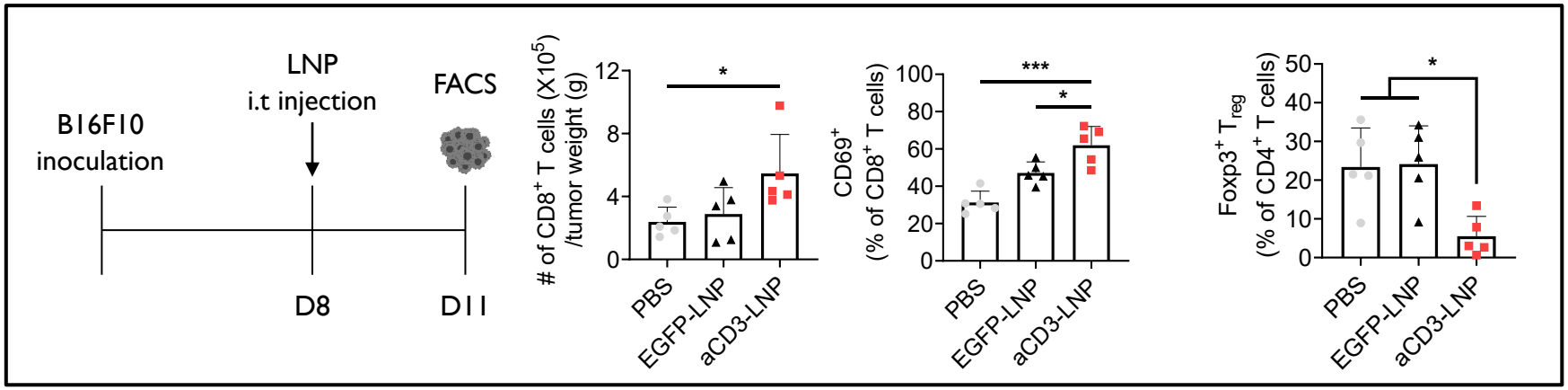
In situ polyclonal CD8+ TIL expansion (& Foxp3$^+$ T_{reg} reduction)



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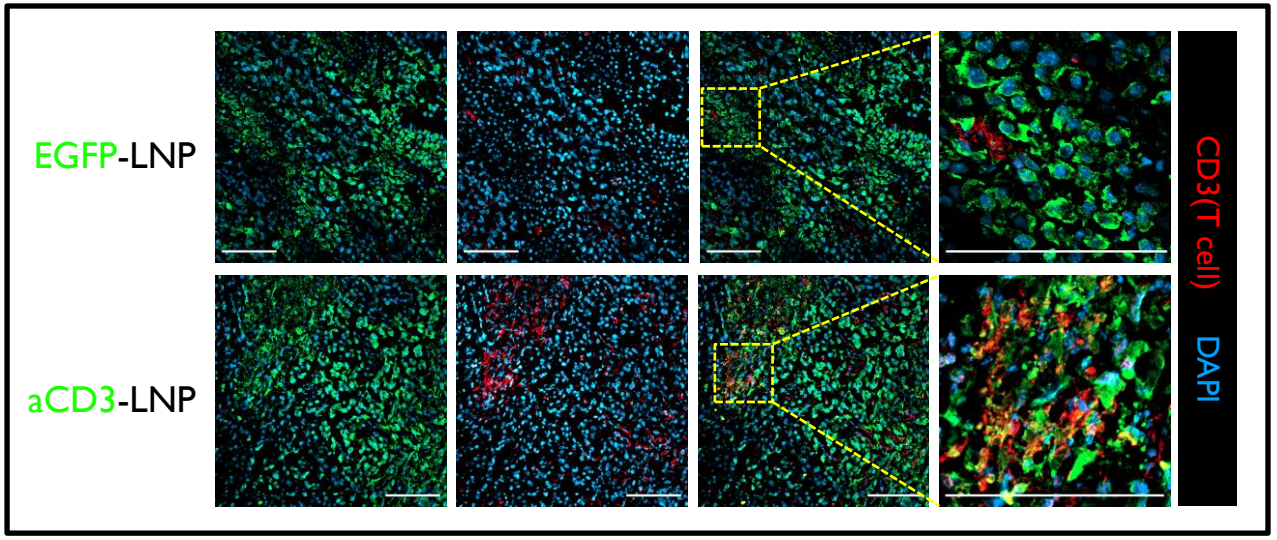
Anti-tumor effects of in situ TIL therapy

Co-localization of *in situ* engineered cells with T cells in tumor tissues



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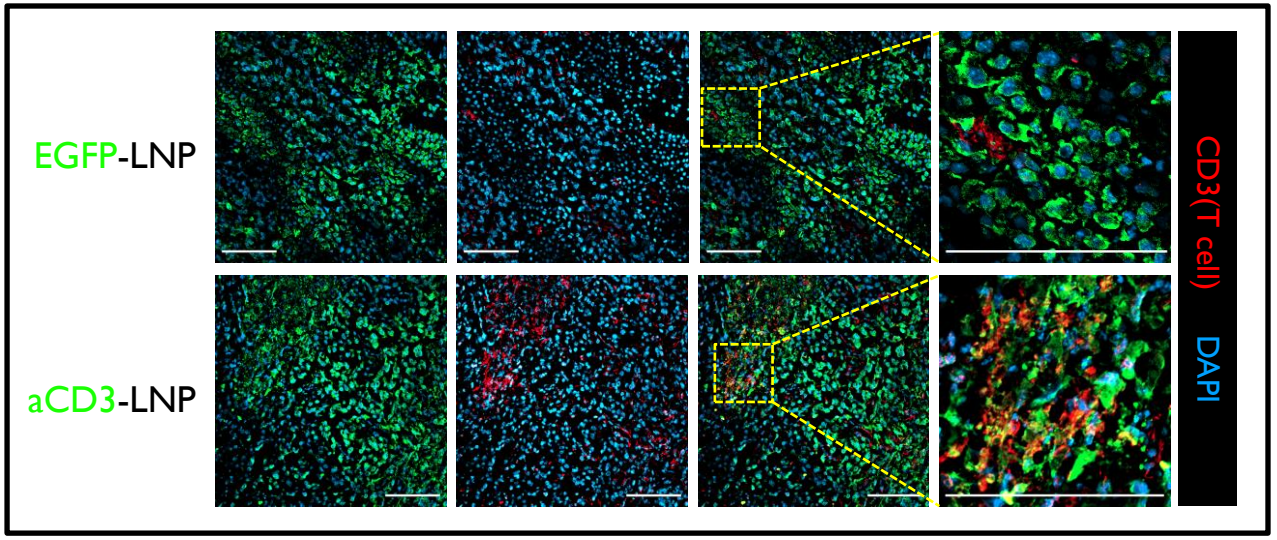
Anti-tumor effects of in situ TIL therapy

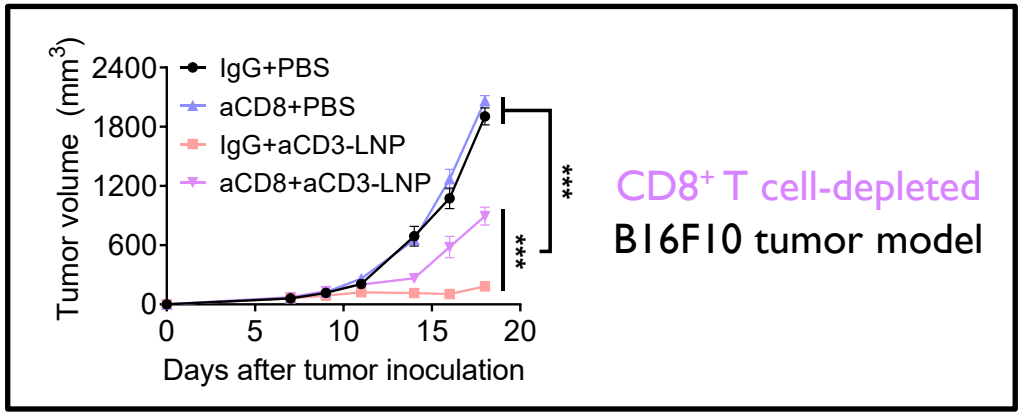
Co-localization of *in situ* engineered cells with T cells in tumor tissues



EGFP-LNP: LNP loaded with MA-EGFP mRNA

aCD3-LNP: LNP loaded with MA-aCD3 mRNA

CD8$^+$ T cell-mediated anti-tumor effects

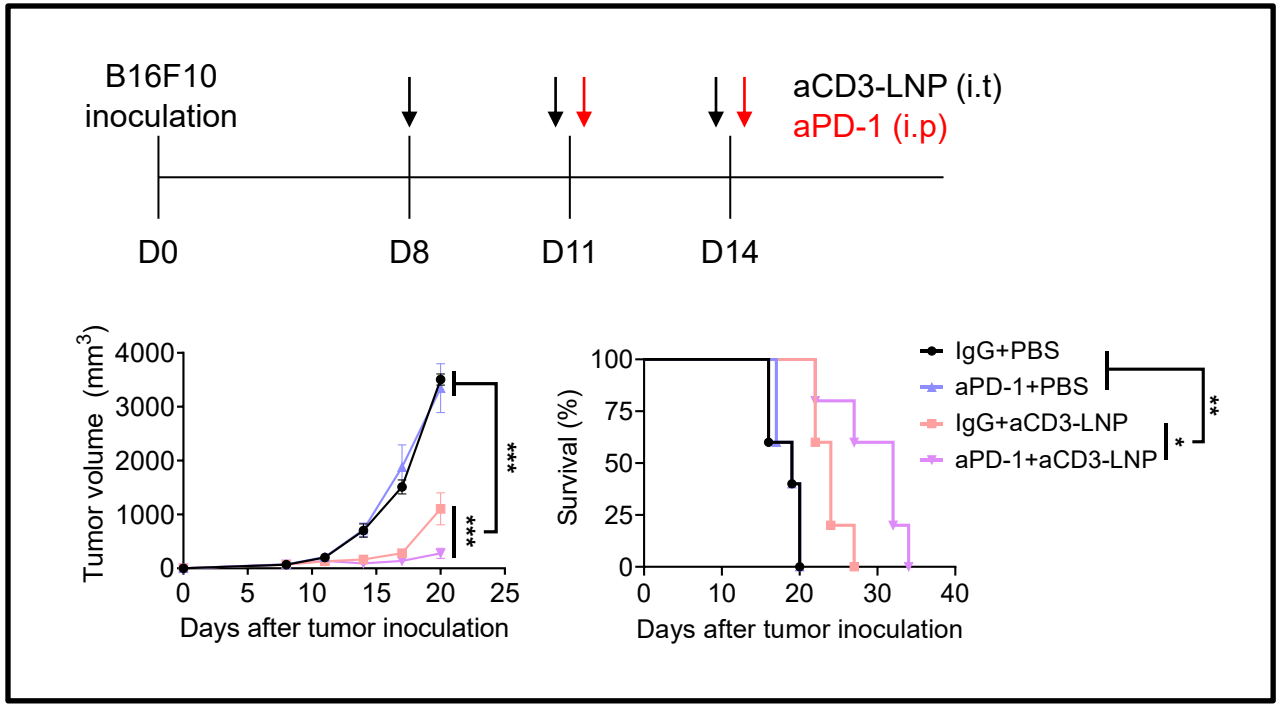


CD8$^+$ T cell-depleted B16F10 tumor model

→ aCD3-mRNA treatment induces antitumor immunity through the expansion of polyclonal CD8$^+$ T cells and their subsequent engagement with tumor cells.

Yoon et al. *ACS Nano* 18 (2024) 32401-32420

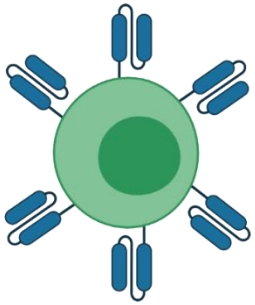
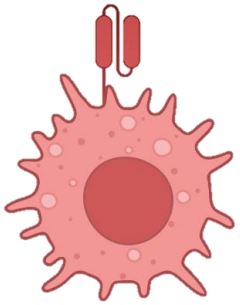
Combination therapy with anti-PD-1



→ Combinatorial treatment of aCD3 mRNA and anti-PD-1 resulted in synergistic anti-tumor effects in anti-PD-1-refractory B16F10 tumors.

Yoon et al. *ACS Nano* 18 (2024) 32401-32420

CAR-T cell vs **CAR-Macrophage**

	CAR-T cell	**CAR-Macrophage (CAR-M)**
Cell type		
Primary Function	• Direct CAR-specific tumor cell killing	• Phagocytosis of tumor cells • TME modulation • Antigen cross-presentation
Challenges	• Poor activation of adaptive immunity • Exhaustion in TME*	• Prone to repolarization in the TME (M1 → M2)*

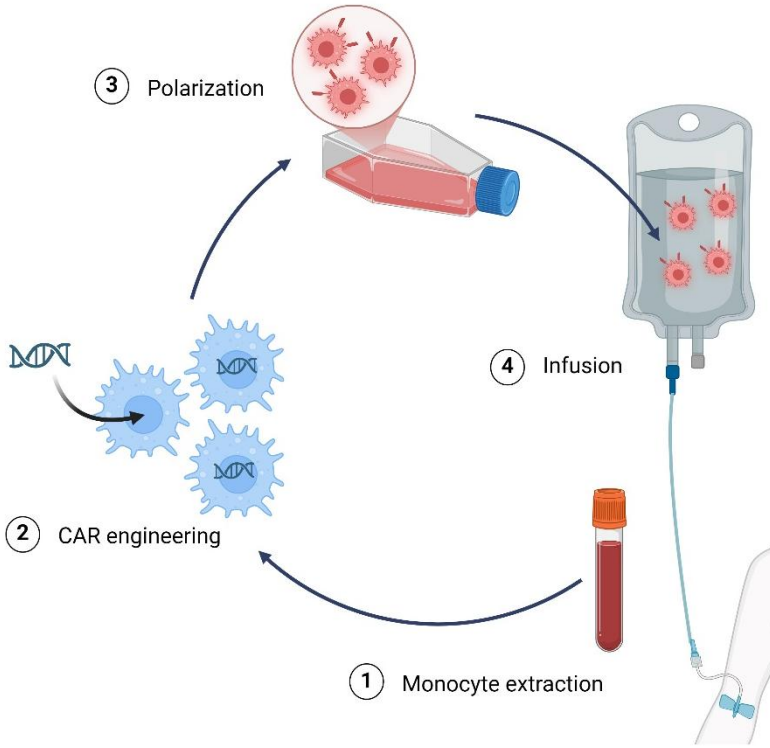
*__TME__, tumor microenvironment

*__M1, anti-tumor (pro-inflammatory) phenotype__; M2, pro-tumor (anti-inflammatory) phenotype

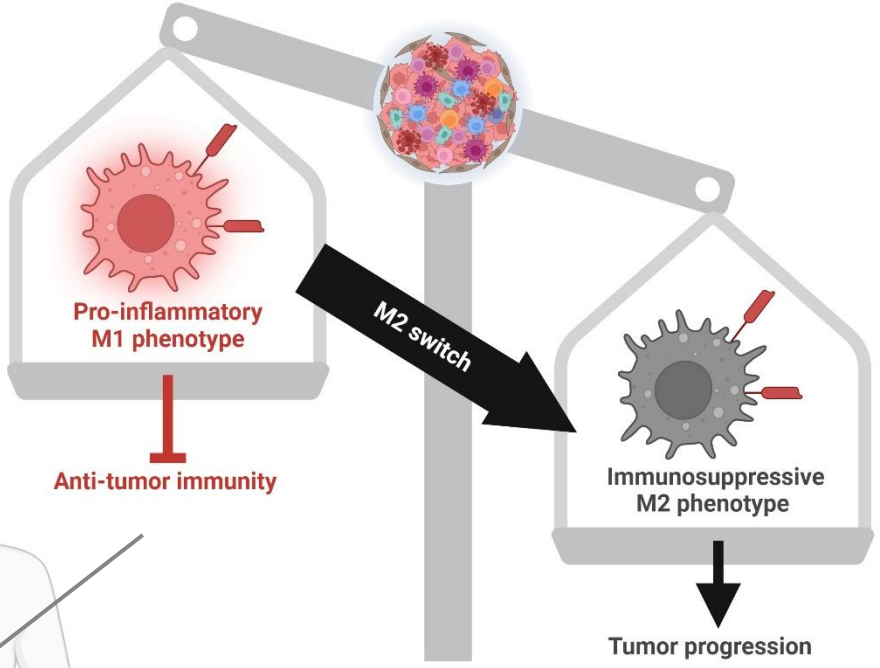
→ CAR-M therapy addresses key challenges faced by CAR-T cell therapy by engaging both the innate and adaptive immune systems thereby launching a multipronged attack against tumors.

CAR-macrophage (CAR-M) therapy

Difficulties in ex vivo bioengineering



Prone to repolarization in TME

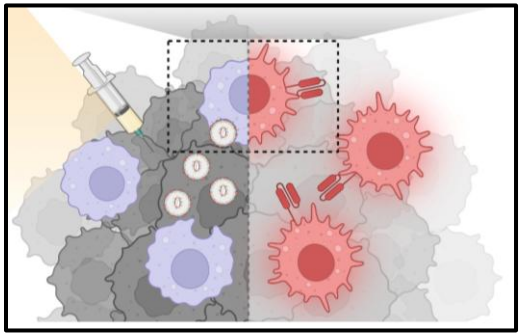


Current CAR-M therapy

- ✓ Ex vivo bioengineering
- ✓ Repolarization in TME

Reduced therapeutic efficacy

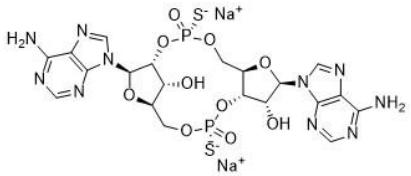
In situ CAR-M therapy for melanoma

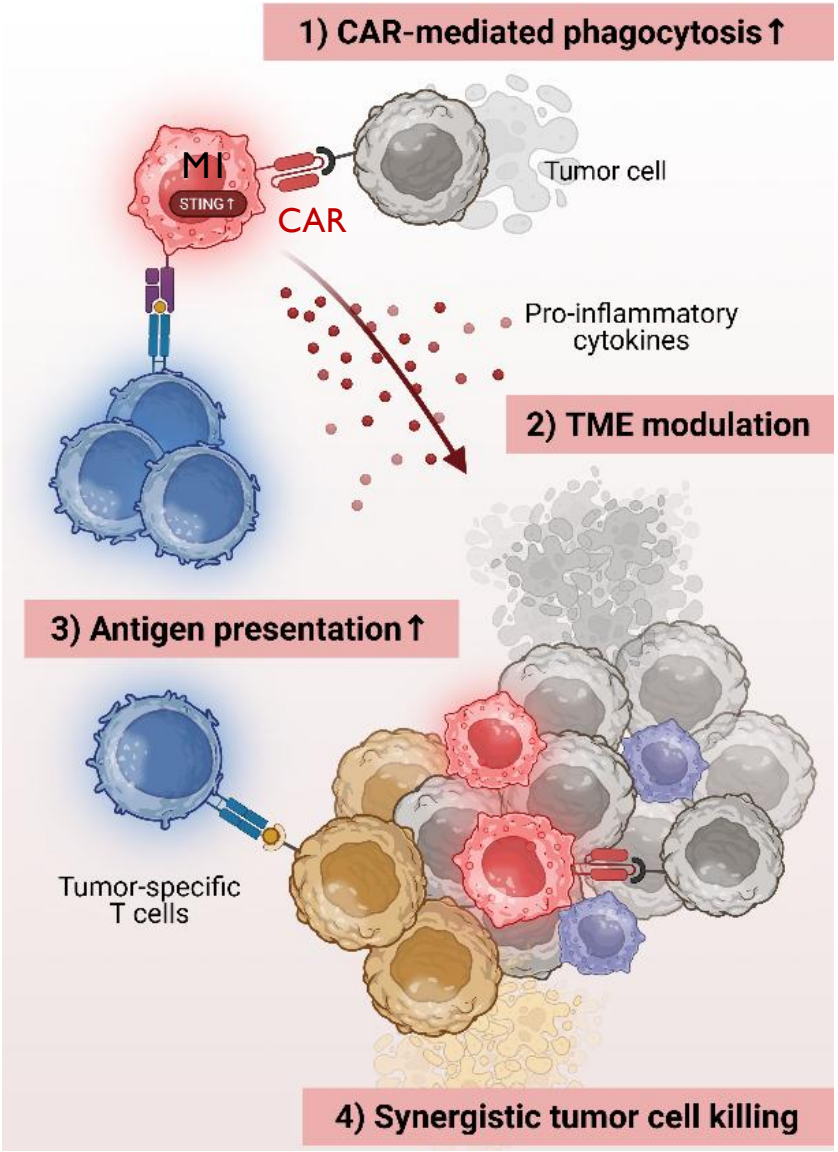


Intratumoral administration of **CAR mRNA & STING agonist** using macrophage-targeted LNPs

CAR target: mouse tumor-associated antigen glycoprotein 75 (GP75) abundant in melanoma

STING agonist: ADU-S100 (ST-RR in this study)

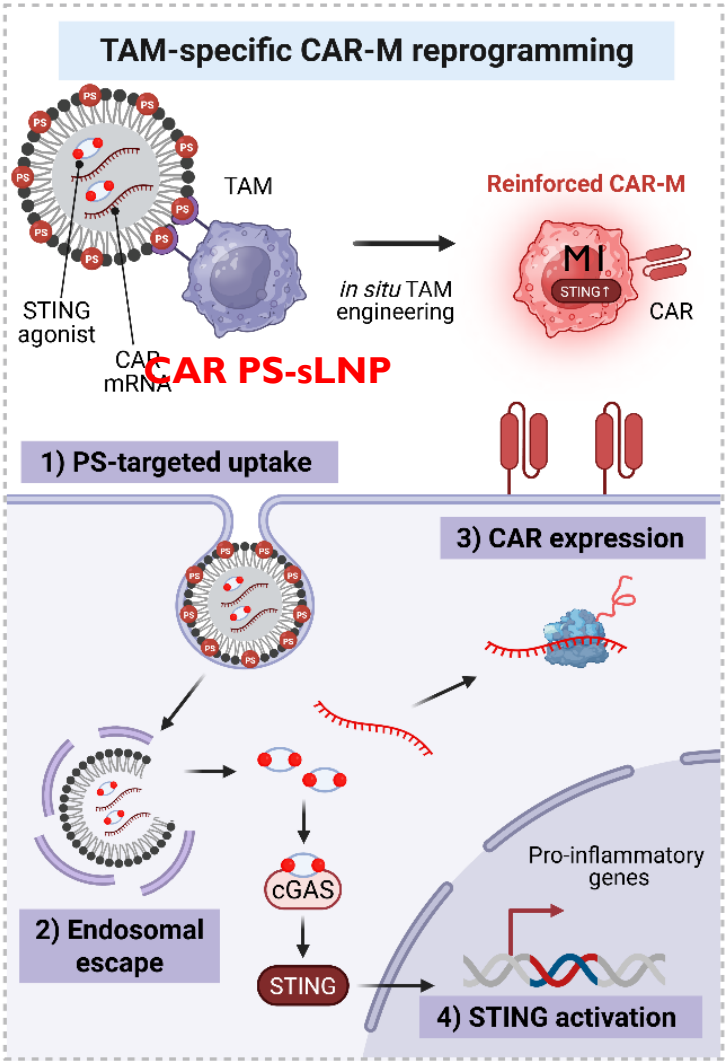


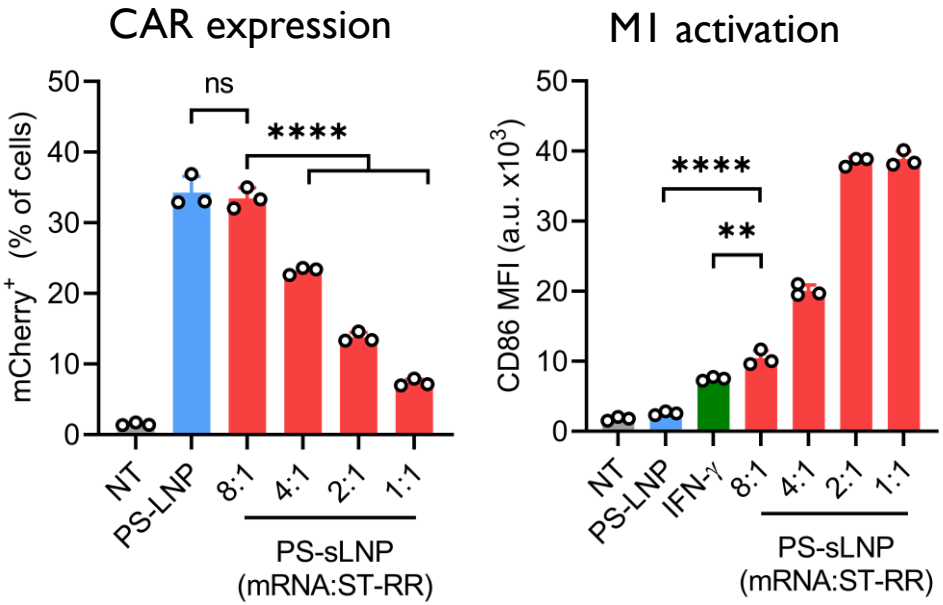


Han et al. *ACS Nano* 19 (2025) 40798-40816

TAM-specific delivery of CAR-mRNA & STING agonist

*PS: phosphatidylserine

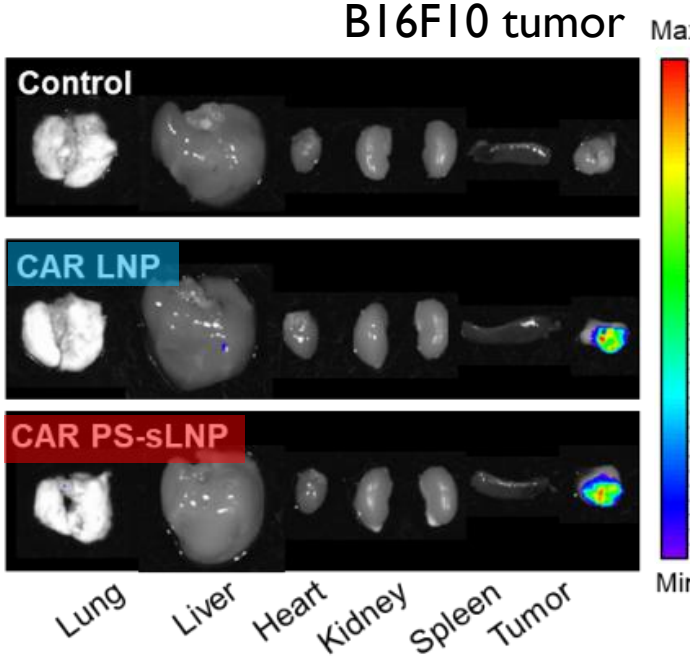




→ LNPs were optimized for selective co-delivery of CAR mRNA and a STING agonist to TAMs, with the goal of achieving **both efficient CAR expression and robust M1 polarization**.

Han et al. *ACS Nano* 19 (2025) 40798-40816

Targeted macrophage engineering *in vivo*



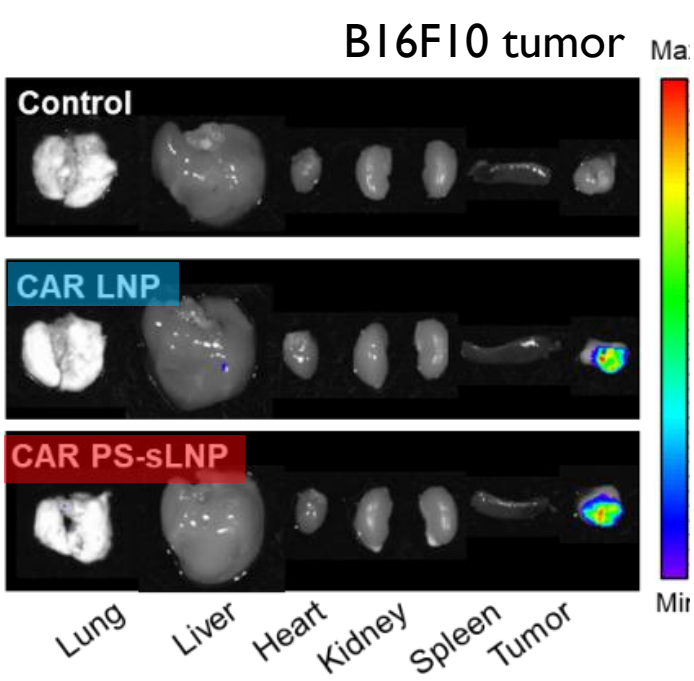
*intratumoral injection

CAR LNP: CAR-mRNA-loaded LNP

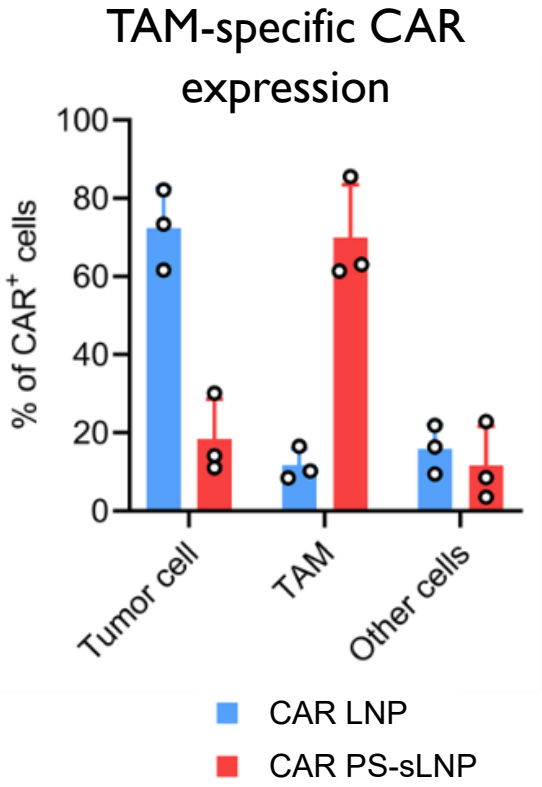
CAR PS-sLNP: CAR-mRNA & STING agonist-loaded PS-incorporated LNP

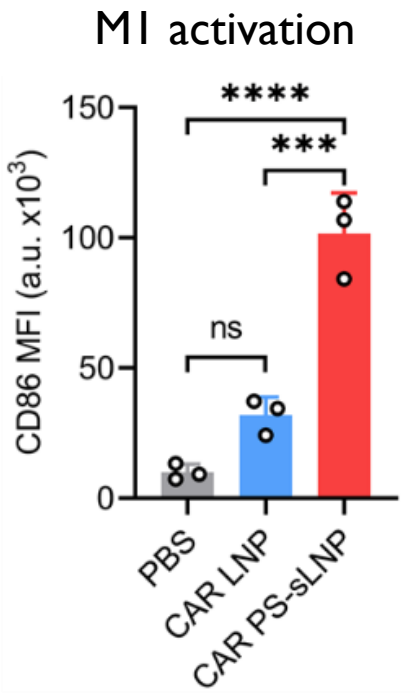
Han et al. *ACS Nano* 19 (2025) 40798-40816

Targeted macrophage engineering *in vivo*



*intratumoral injection





CAR LNP: CAR-mRNA-loaded LNP

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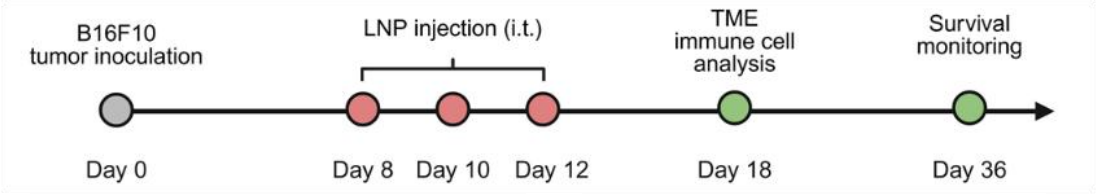
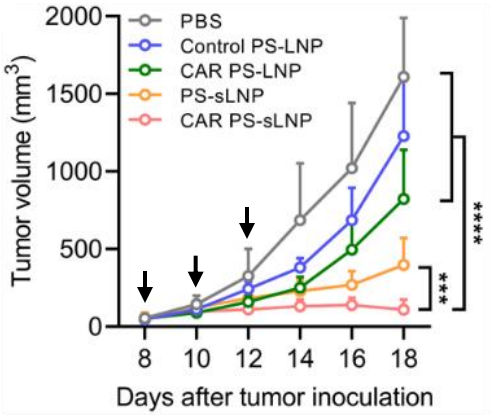
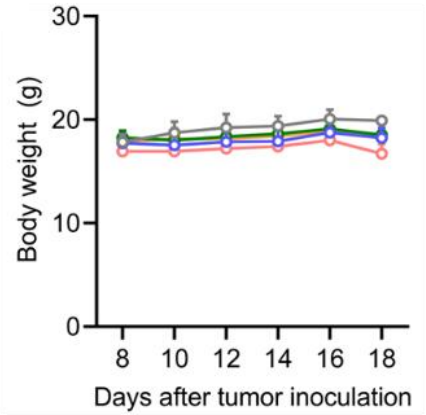
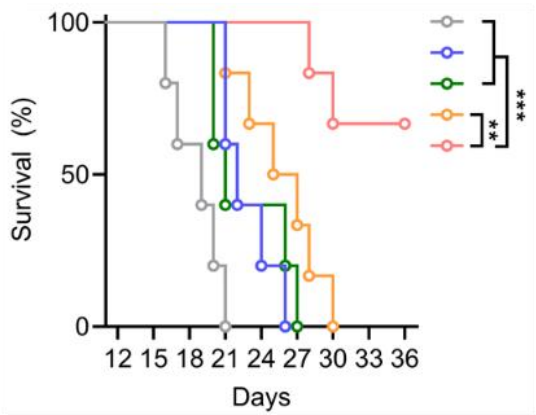
Han et al. *ACS Nano* 19 (2025) 40798-40816

Antitumor effects of in situ CAR-M therapy









Control PS-LNP: EGFR mRNA-loaded PS-LNP
CAR PS-LNP: CAR mRNA-loaded PS-LNP
PS-sLNP: STING agonist-loaded PS-LNP
CAR PS-sLNP: CAR mRNA & STING agonist-loaded PS-LNP

Han et al. *ACS Nano* 19 (2025) 40798-40816

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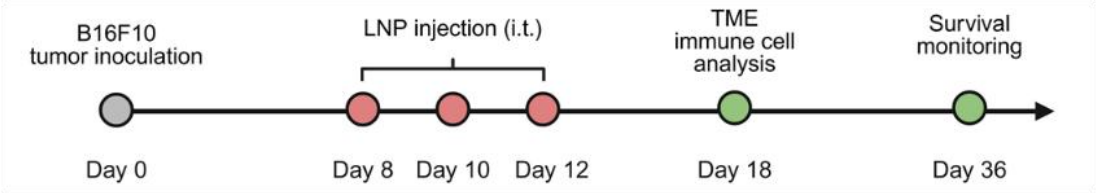
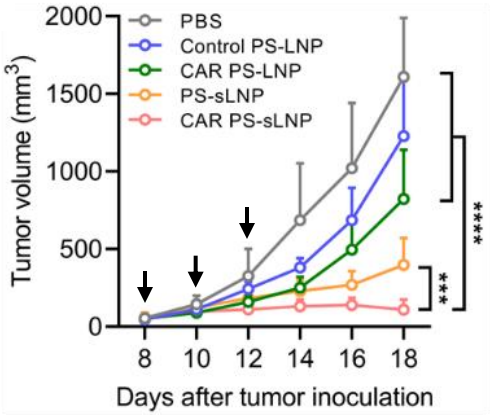
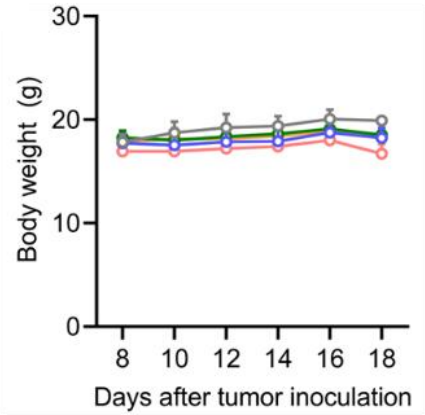
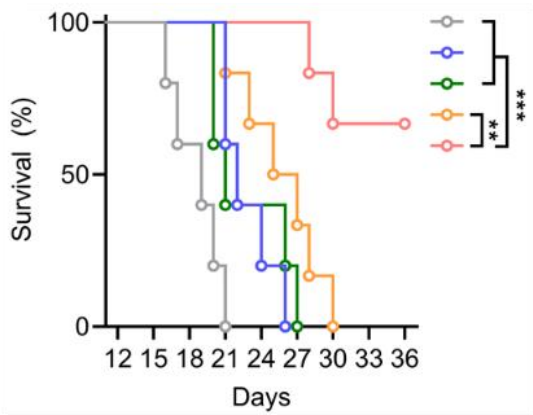
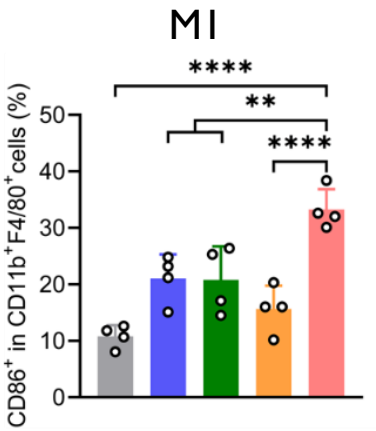
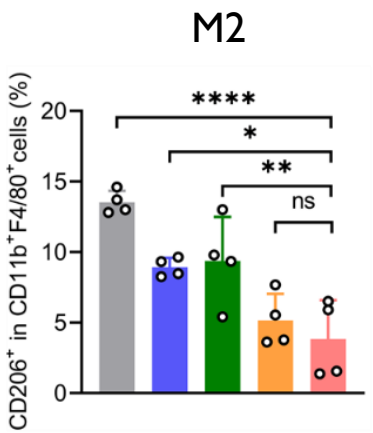


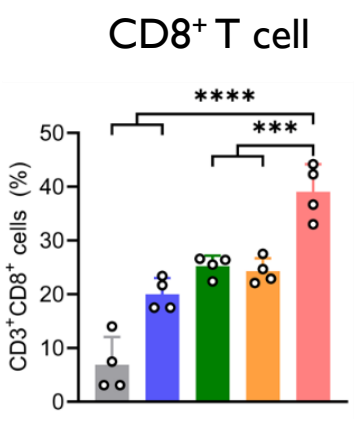


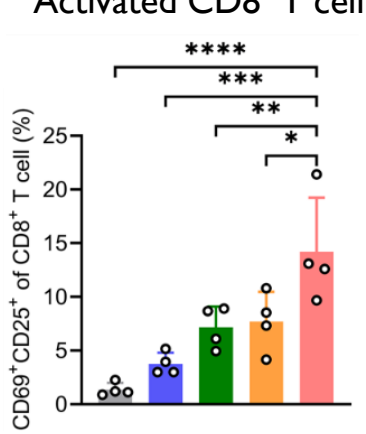






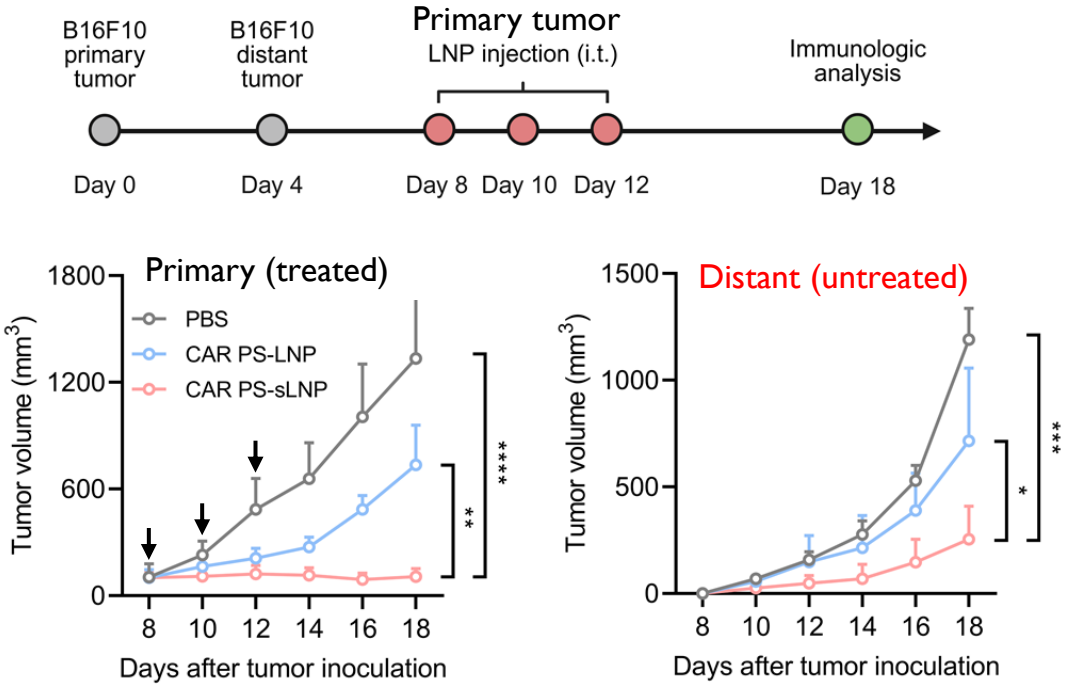






Han et al. *ACS Nano* 19 (2025) 40798-40816

Systemic immunity of in situ CAR-M therapy

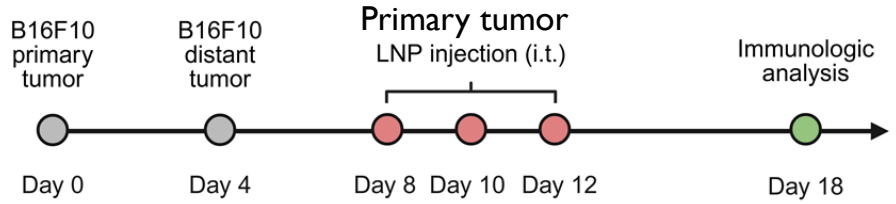


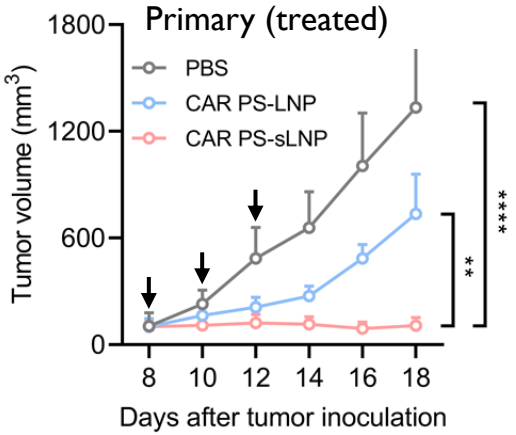
CAR PS-LNP: CAR mRNA-loaded PS-LNP

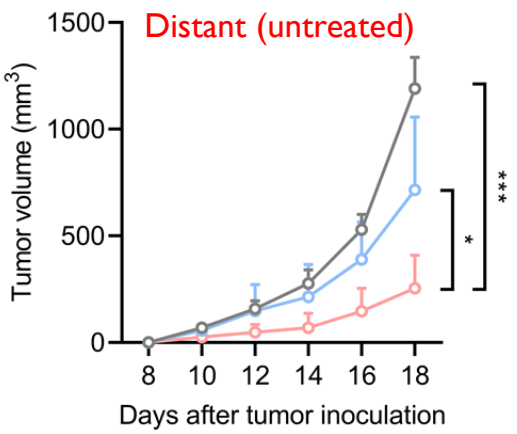
CAR PS-sLNP: CAR mRNA & STING agonist-loaded PS-LNP

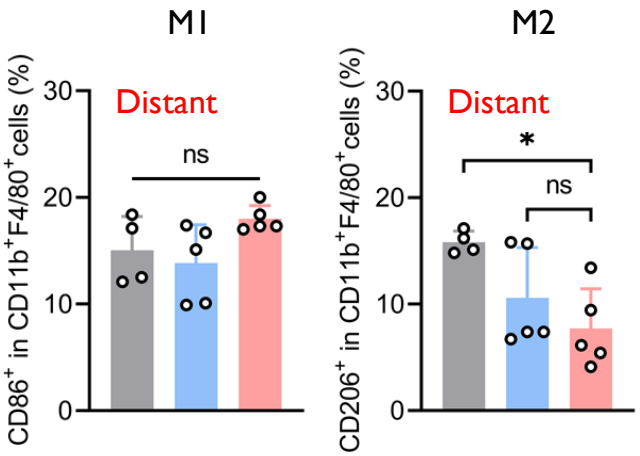
Han et al. *ACS Nano* 19 (2025) 40798-40816

Systemic immunity of in situ CAR-M therapy









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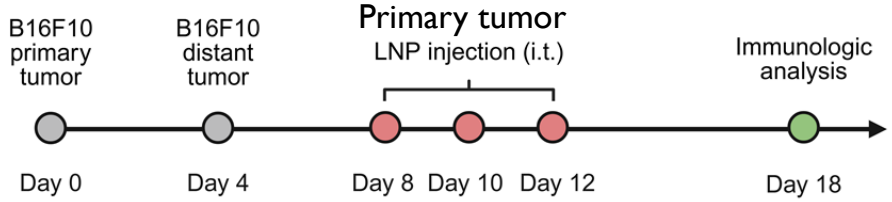
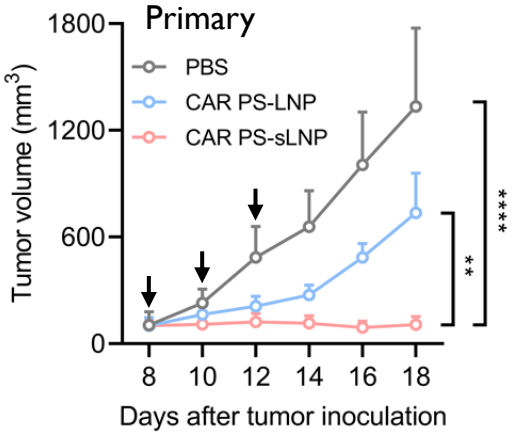
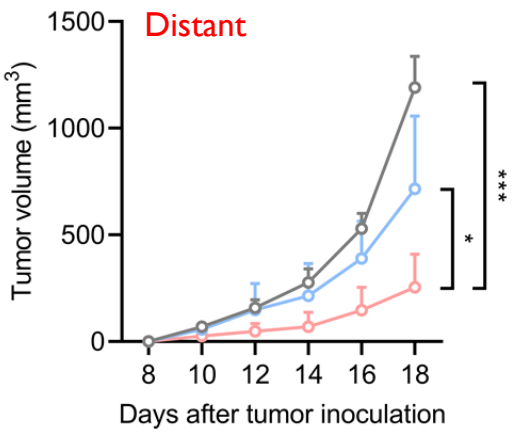
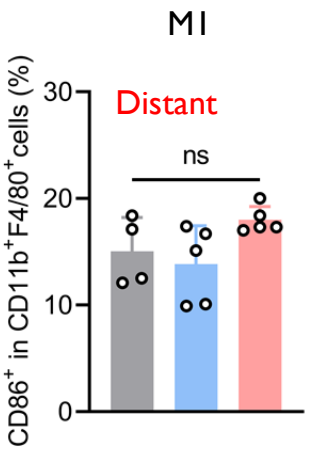
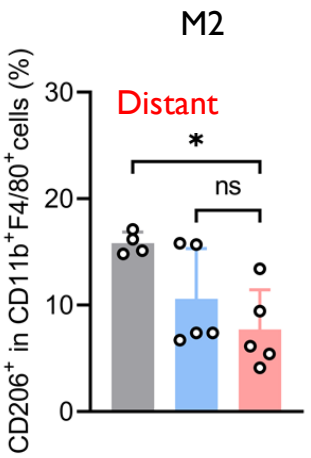
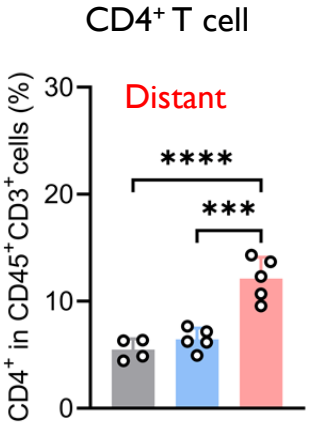


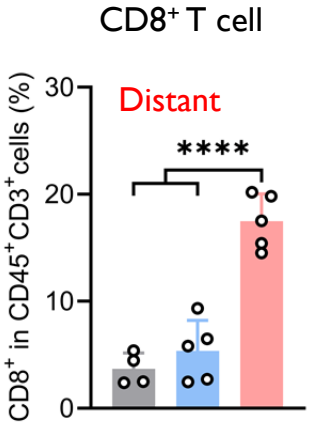






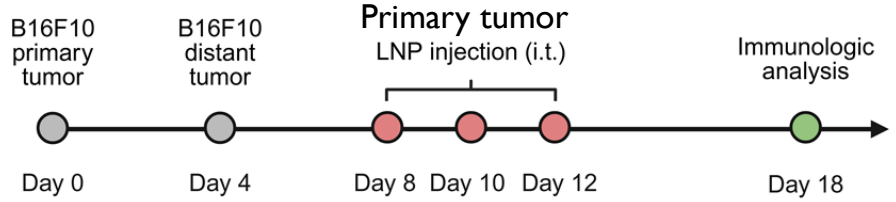


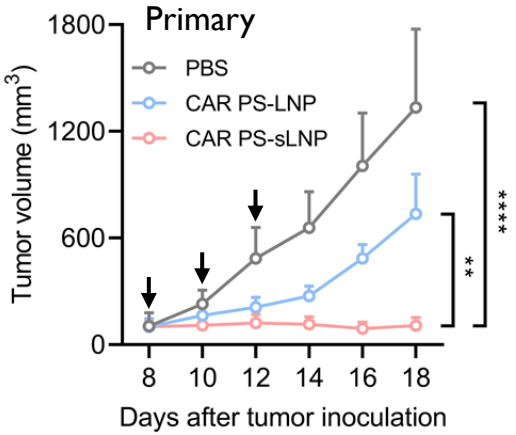


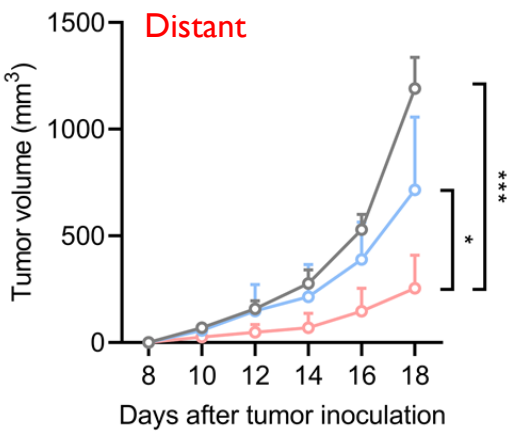


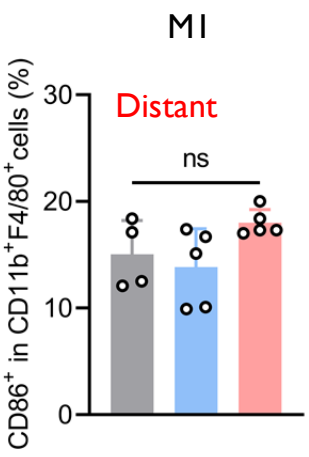
Han et al. *ACS Nano* 19 (2025) 40798-40816

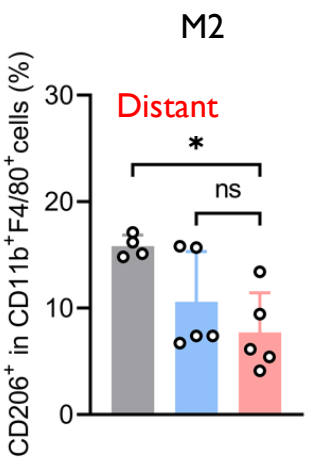
Systemic immunity of in situ CAR-M therapy

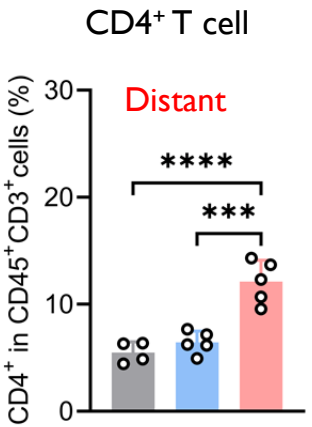


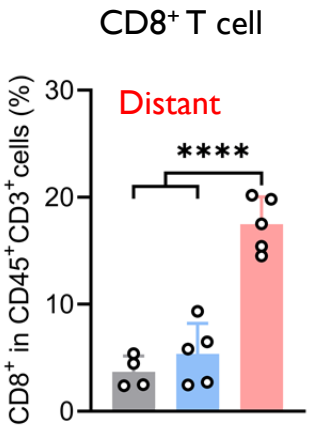


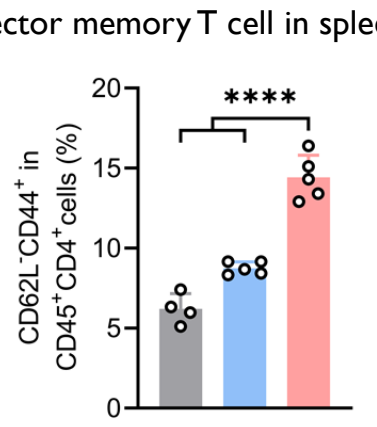








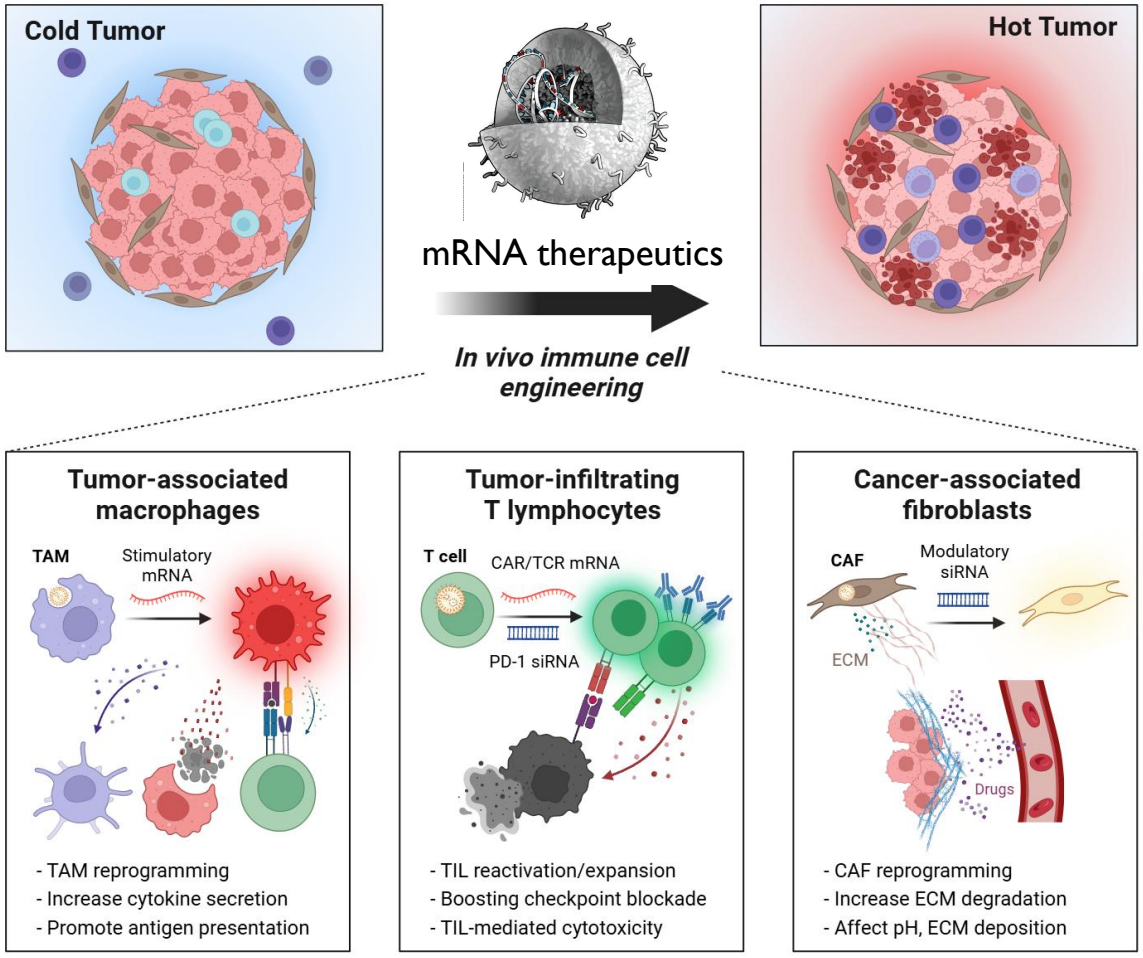




Han et al. *ACS Nano* 19 (2025) 40798-40816

Summary

mRNA therapeutics can be leveraged to **in situ engineer tumor-associated macrophages** for effective **cancer immunotherapy**



Thank you!

[Co-founder]

De novo Biotherapeutics

EXOPERT

Jun-Hee Han

Erinn Fagan

[Collaborators]

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Jieun Oh, MD/PhD

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Hyun Koo Kim, MD/PhD

SNU Hospital:
Hyun-Jik Kim, MD/PhD

Nagoya University (Japan):
Hiroshi Abe, PhD

JFCR (Japan):
Takatsugu Ishimoto, MD/PhD

Georgia Tech (USA):
Gabe Kwong, PhD

[Email] jihopark@kaist.ac.kr

[Funding]

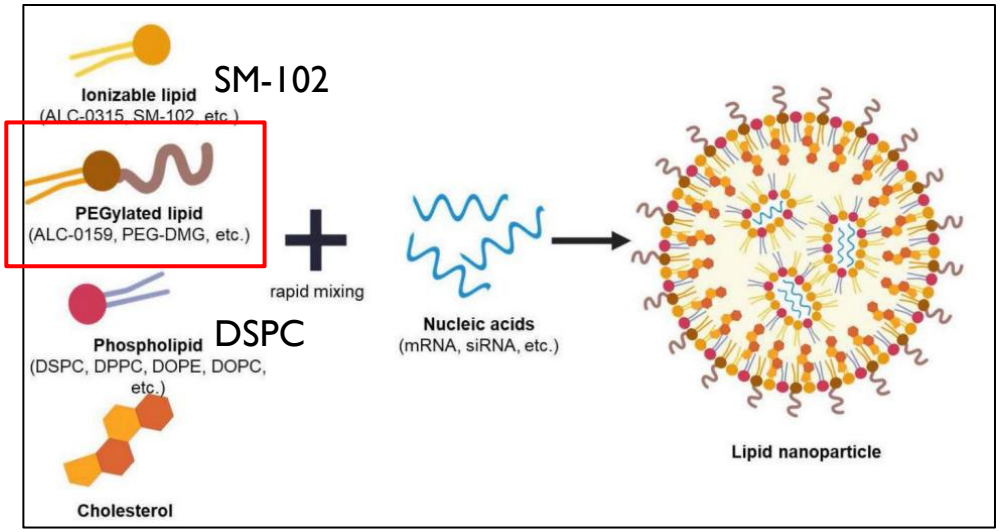
Ministry of Agriculture, Food and Rural Affairs

Ministry of Health and Welfare

NRF National Research Foundation of Korea

국방과학연구소 Agency for Defense Development

LNP for engineering TAM and tumor cells



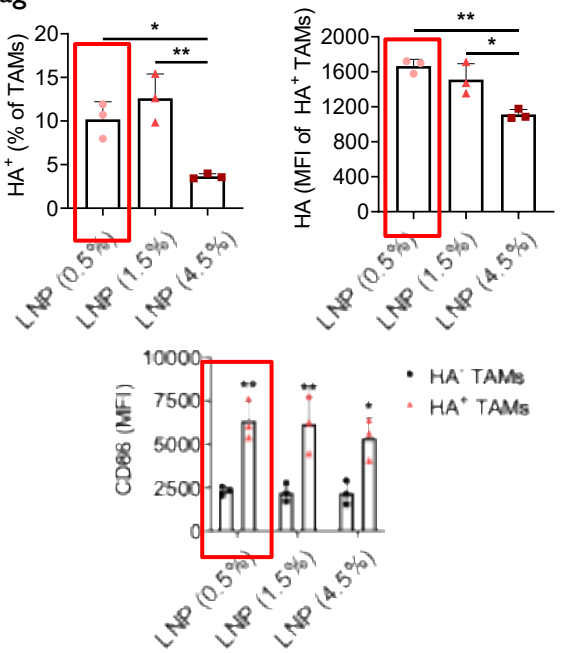
Theranostics (2022)

LNP	Size (d.nm)	PDI	Encapsulation efficiency (%)
LNP (PEG 0.5%)	189.57±2.45	0.09	96.10
LNP (PEG 1.5%)	117.92±1.41	0.14	92.38
LNP (PEG 4.5%)	105.88±1.41	0.20	94.75

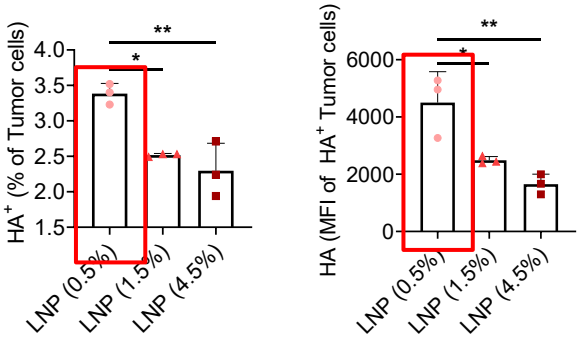
HA+: HA-tag

B16F10 tumor

TAM



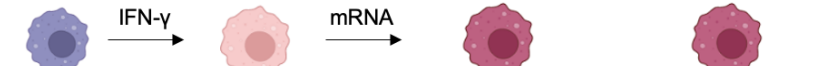
Tumor cell



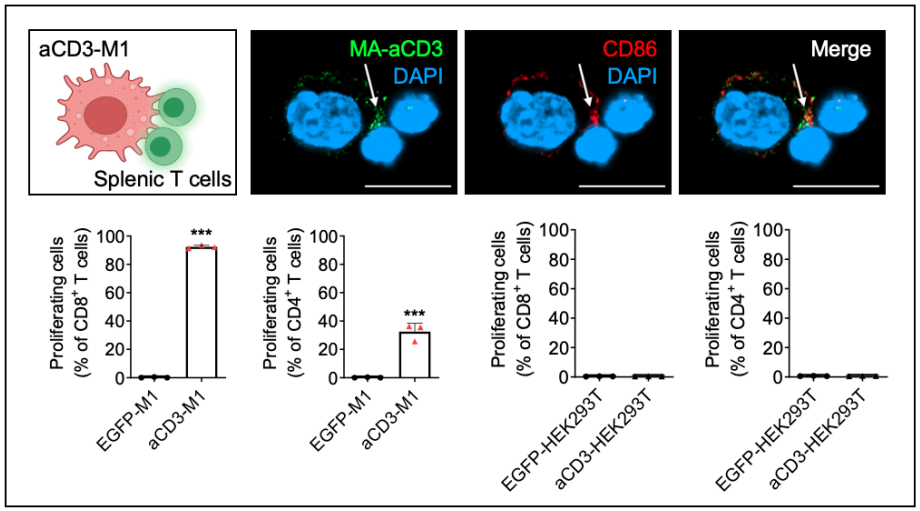
→ LNP with 0.5% PEG-lipids was selected for *in vivo* engineering of both TAM and tumor cells

Yoon et al. *ACS Nano* 18 (2024) 32401-32420

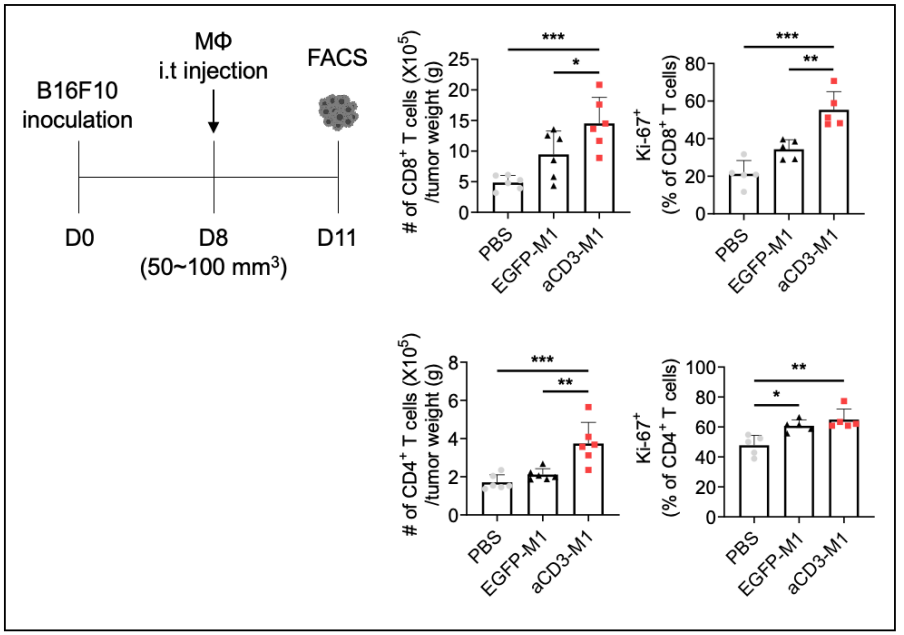
aCD3-TAM-mediated T cell expansion



In vitro polyclonal T cell expansion by aCD3-M1



In situ polyclonal TIL expansion by aCD3-M1



Yoon et al. *ACS Nano* 18 (2024) 32401-32420

Screening for TAM-targeting mRNA lipid nanoparticles

Screening workflow

1. Variables for LNP formulation

ionizable lipid (pKa, amine head)

Targeting lipid

lipid molar ratio

+ luciferase mRNA

2. Targeted LNP library

Physicochemcal properties

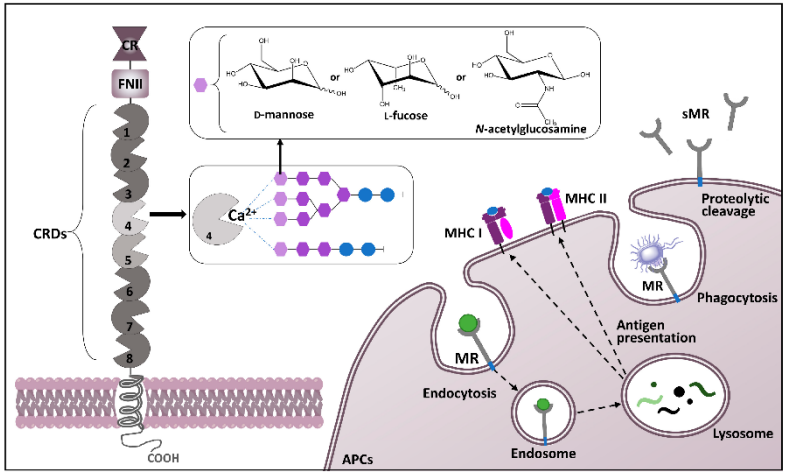
3. Transfection in M2 macrophages

Transfection efficiency

BMDM

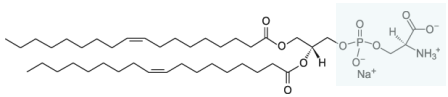
TAM-targeting moiety

Mannose

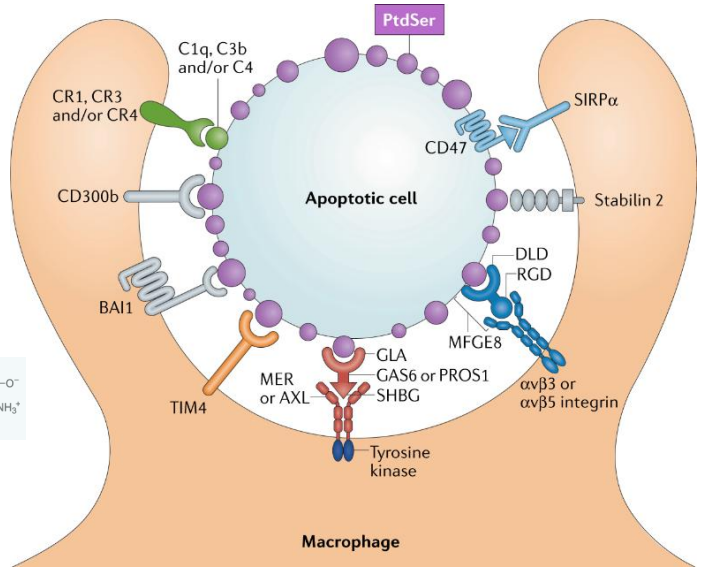


Int. J. Mol. Sci. (2024)

Phosphatidylserine (PS)



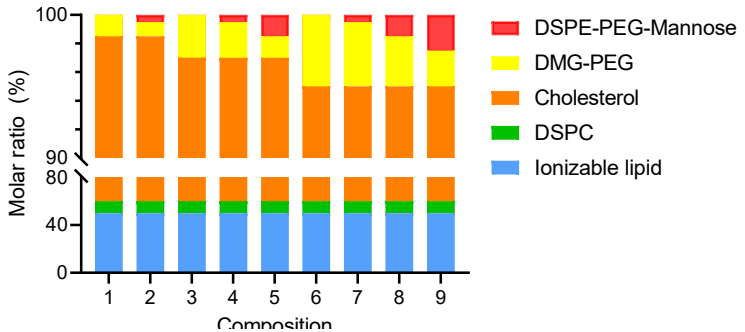
DOPS (18:1 PS)

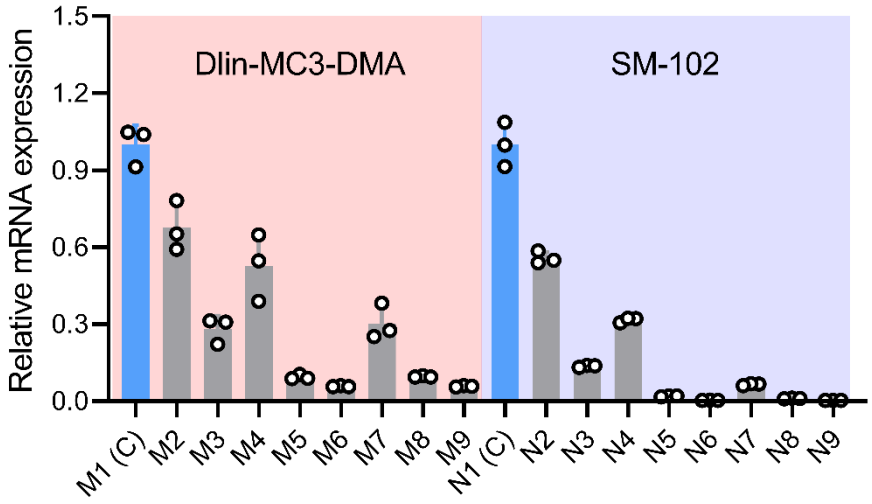


Nat Rev Immunol (2019)

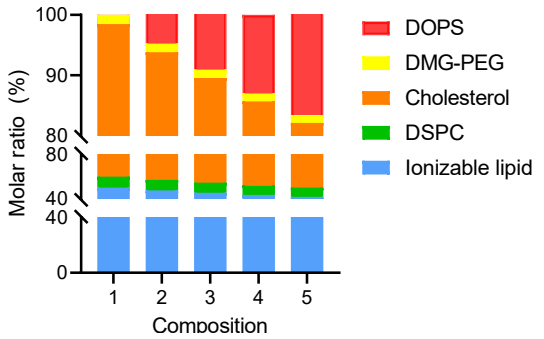
Screening for TAM-targeting mRNA lipid nanoparticles

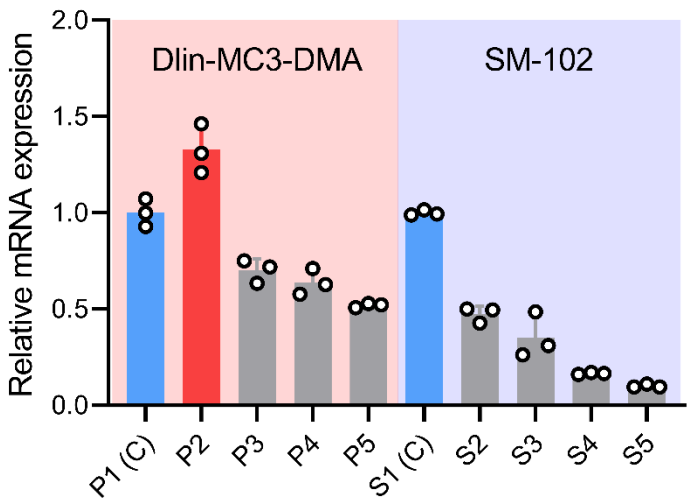
Mannose targeting moiety





PS targeting moiety

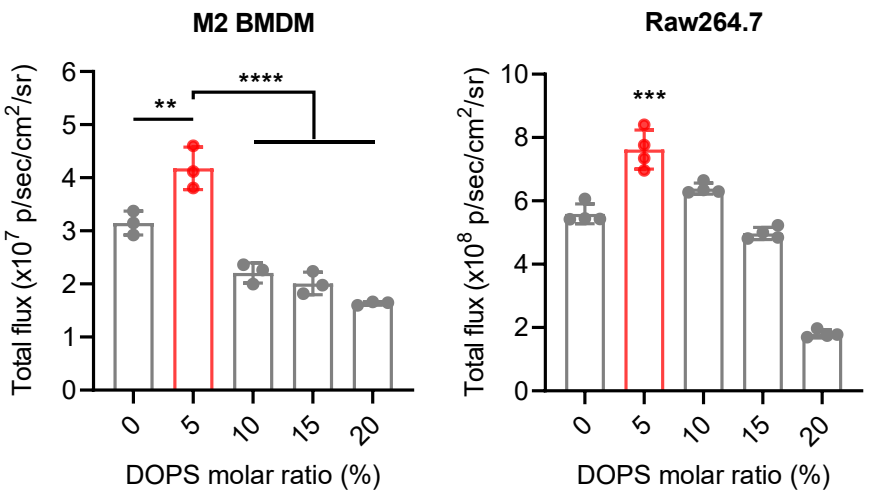




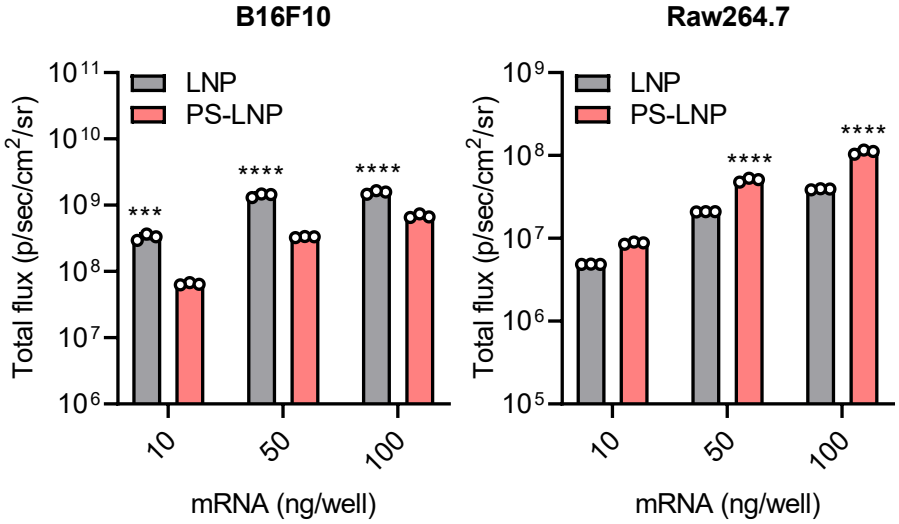
- Only PS targeting moiety with MC3 ionizable lipid combination outperforms conventional LNPs.

TAM-targeting mRNA lipid nanoparticles

Optimization of PS molar ratio in LNP



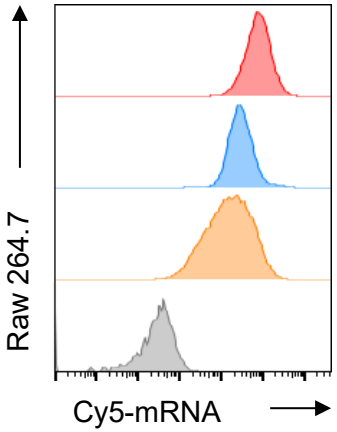
In vitro fLuc expression of PS-LNP

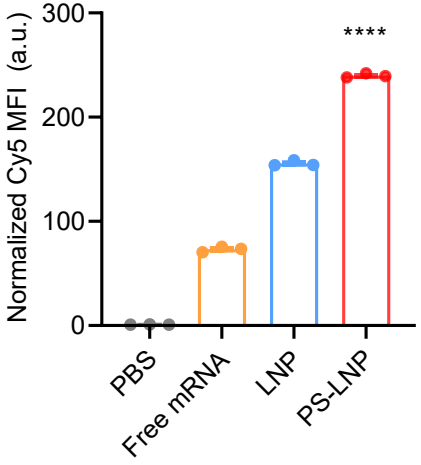


- PS-LNP showed a significant increase in mRNA expression in macrophages.
- PS-LNP outperformed in mRNA expression specifically in macrophages, but not in tumor cells.

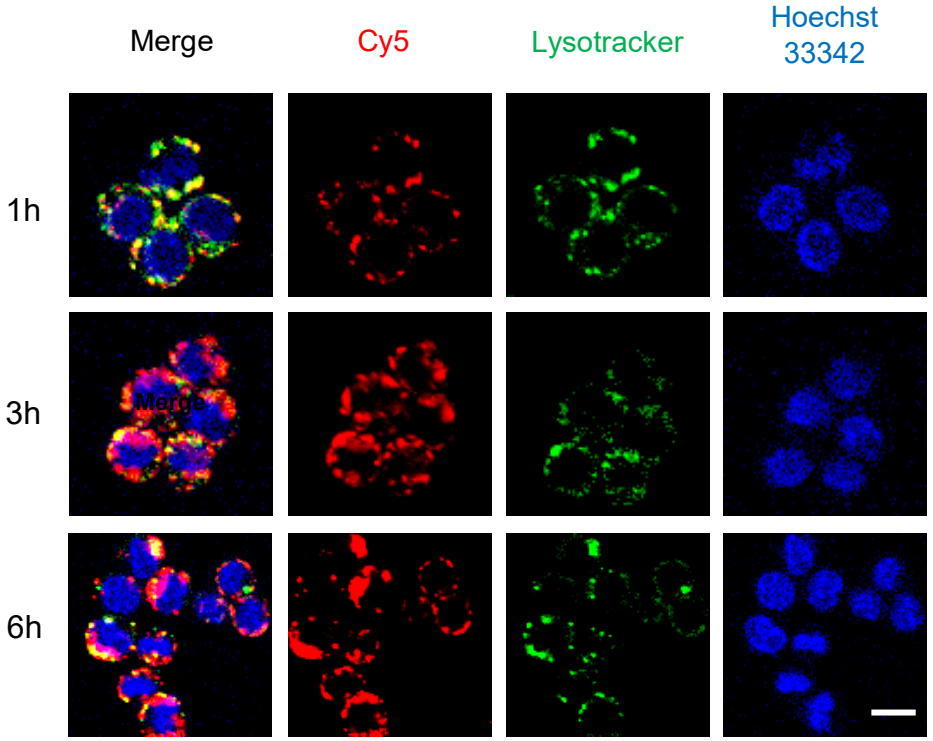
Cellular uptake and endosomal escape of PS-LNP

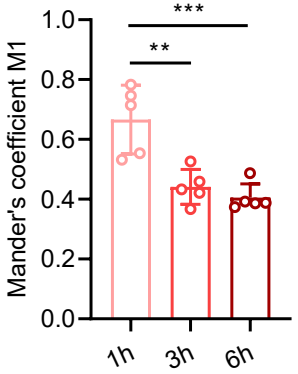
Cellular uptake

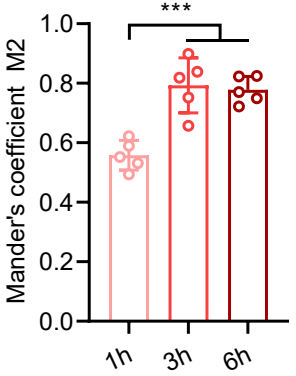




Endosomal escape



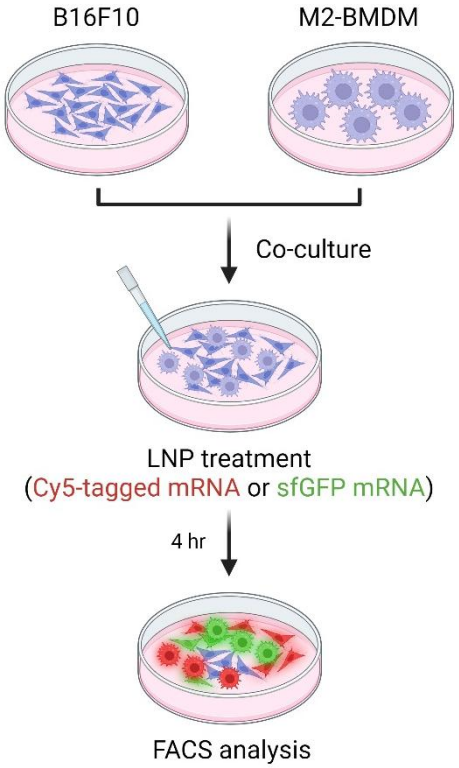




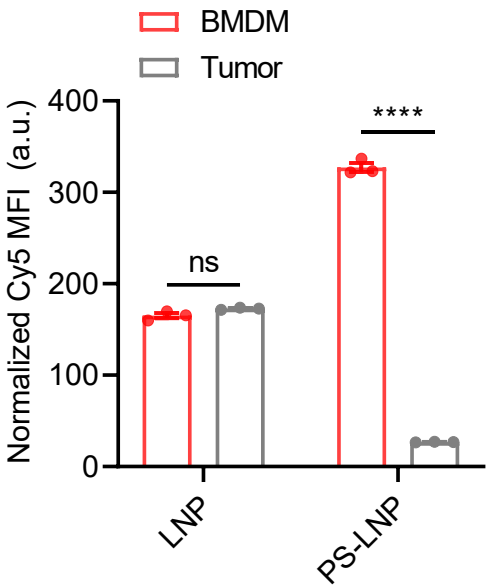
• PS-LNP has a potential for MΦ engineering related to intracellular delivery and cytosolic release of mRNAs.

Macrophage-targeted mRNA delivery and expression of PS-LNP

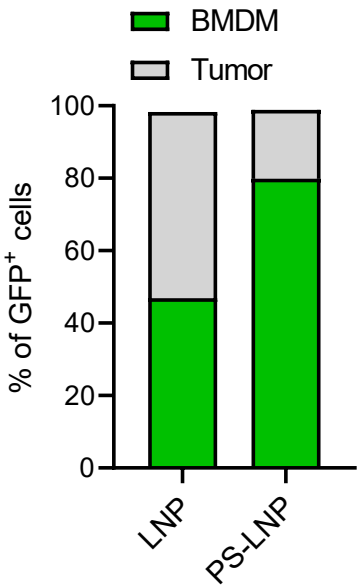
Co-culture assay



Cy5-mRNA LNP uptake



sfGFP mRNA expression



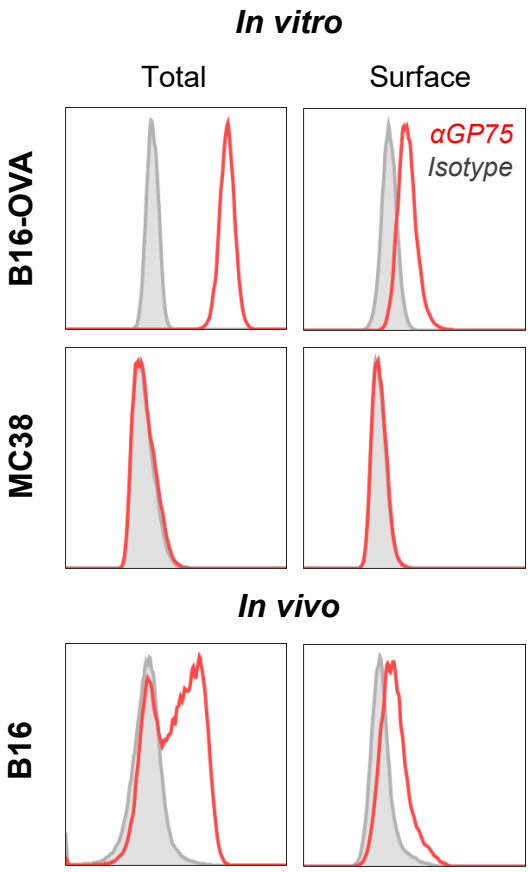
• PS-LNP is preferentially taken up by BMDMs and selectively induces mRNA expression in BMDMs.

Design of model CAR construct

Model target antigen: Mouse tumor-associated antigen glycoprotein 75 (GP75)

- Most abundant glycoprotein synthesized by pigmented melanocytes and melanomas
- GP75 is specific for melanocytes with both primary and metastatic melanomas makes it attractive therapeutic target

Target antigen expression



Anti-GP75 CAR design (2nd gen)