Tumor-targeted Nanocytokine Boosts Anticancer Immunity through pH-Triggered Inflammation

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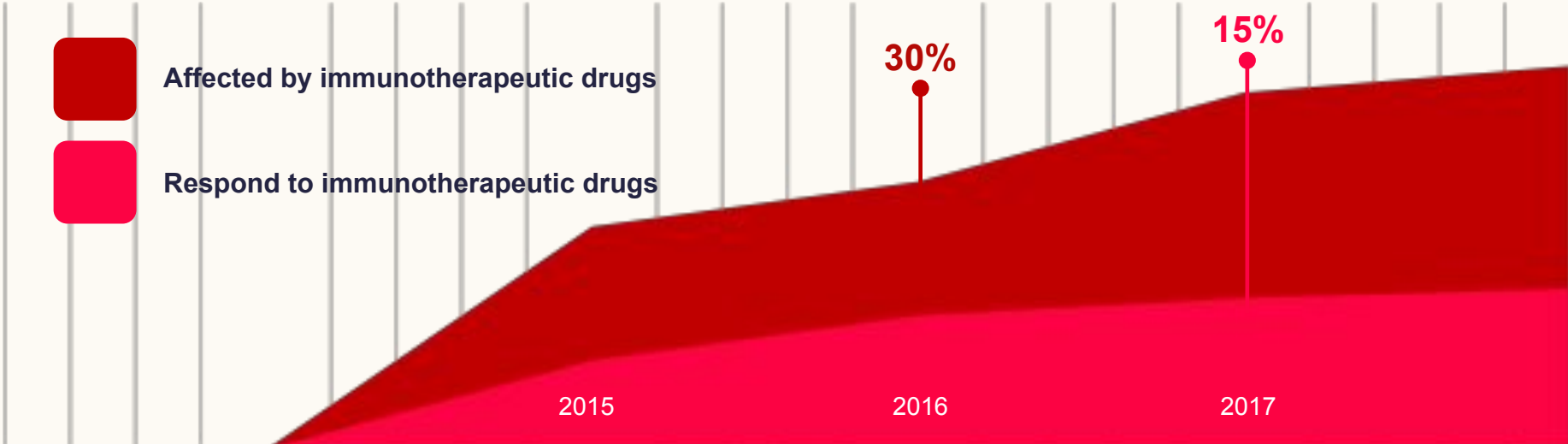
Immuno-Oncology

Less than 20% of cancer patients respond to traditional immunotherapy.

JAMA Netw Open. **2** (2019) e192535.



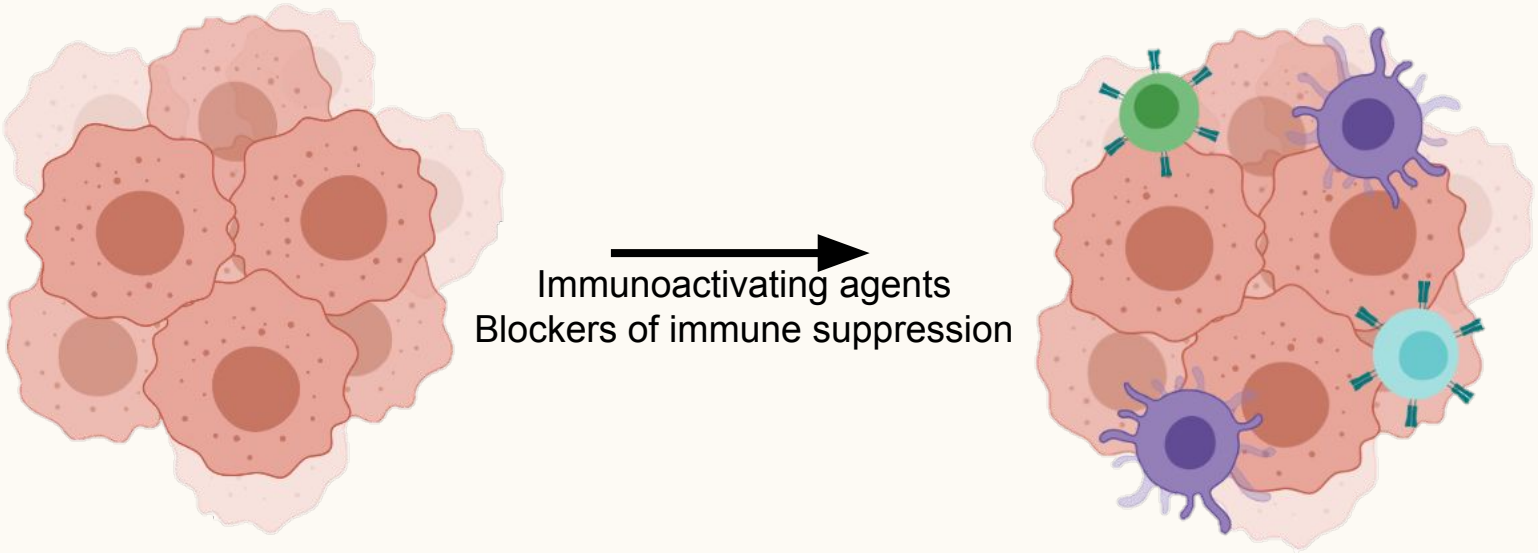
Converting cold tumors into hot tumors

Cold tumor

- Exclusion of CD8+ T cells and NK cells
- Immunosuppressive cells
- Poor response to immunotherapy

Hot tumor

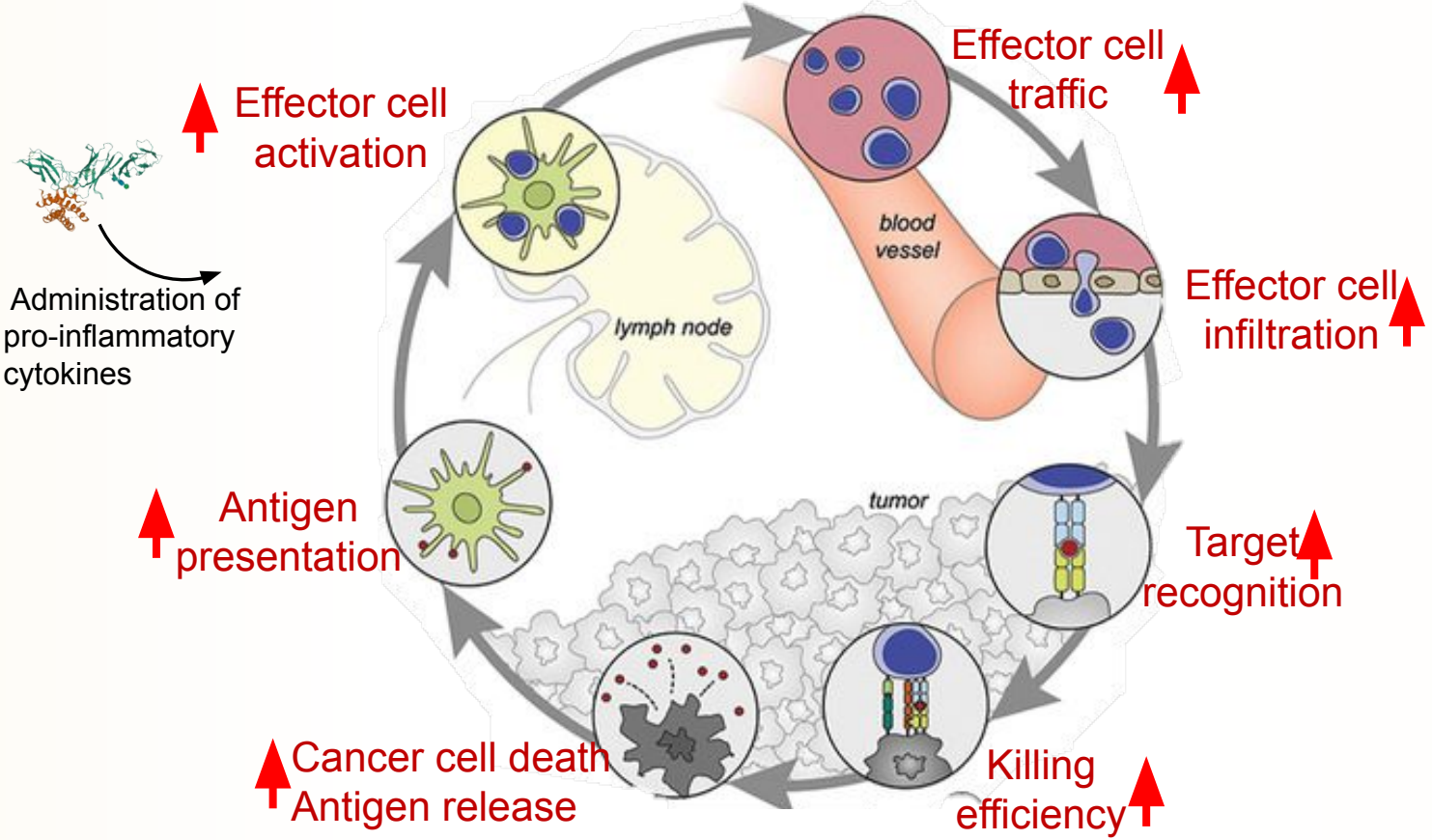
- CD8+ T cells and NK cells inside the tumor
- Elimination of immunosuppressive cells
- High antitumor immune response



Immunity **52** (2020) 17-35

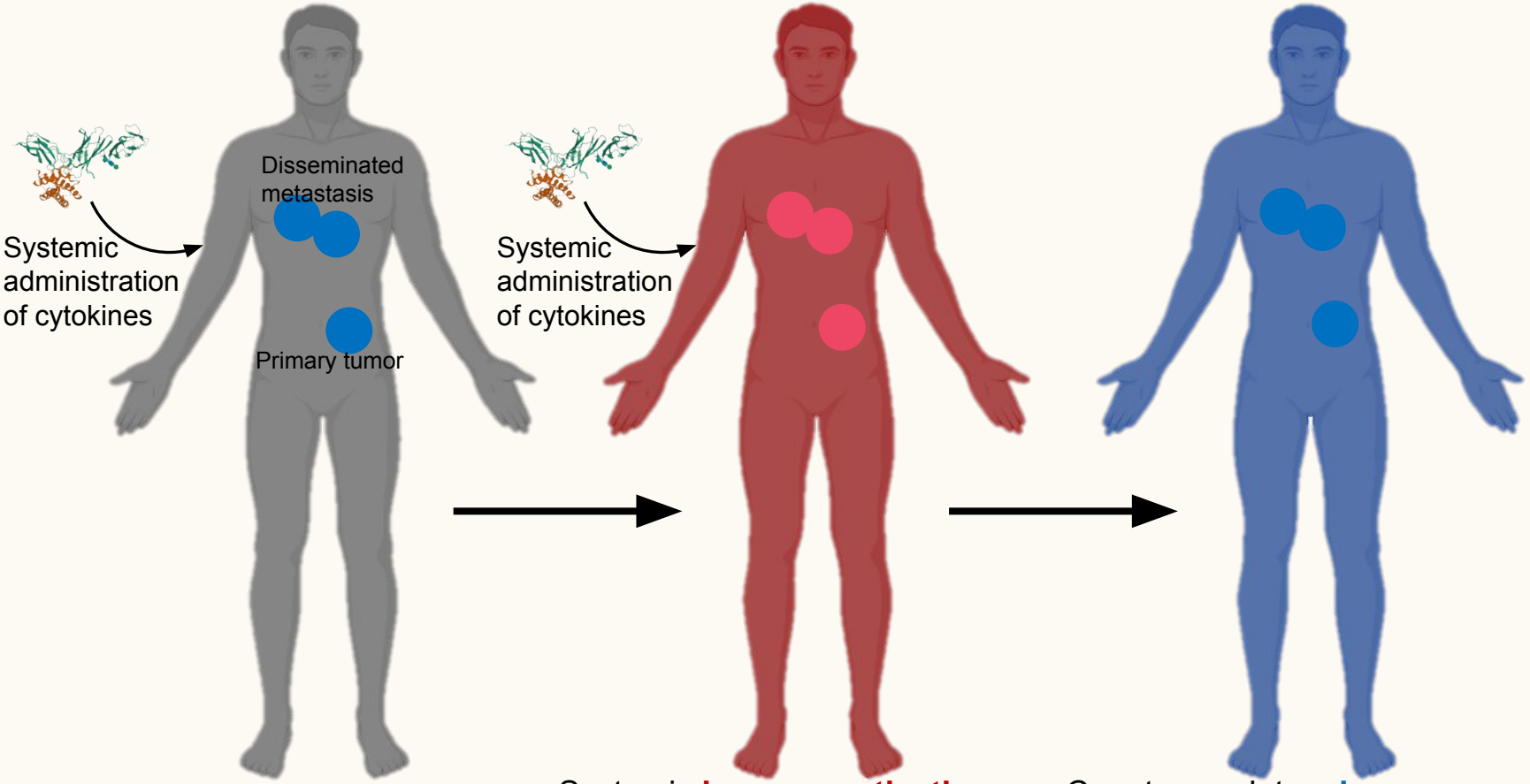
Enhancing inflammatory response and immune cells in tumors is key

Pro-Inflammatory cytokines synergize the cancer immunity cycle



Immunity **39** (2013) 1-10

Systemic cytokine treatment leads to severe side effects



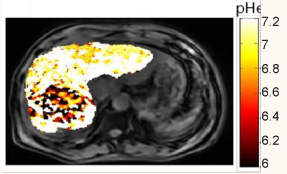
- Systemic **immune activation**
- Organ damage

- Counterregulatory **immunosuppression**
- Anaphylaxis

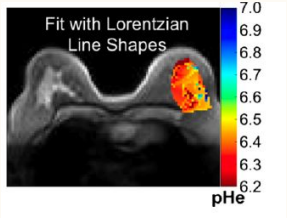
Our technology: Tumor-activatable nanocytokines

Tumor acidity is a hallmark trigger

pH mapping of human liver cancer

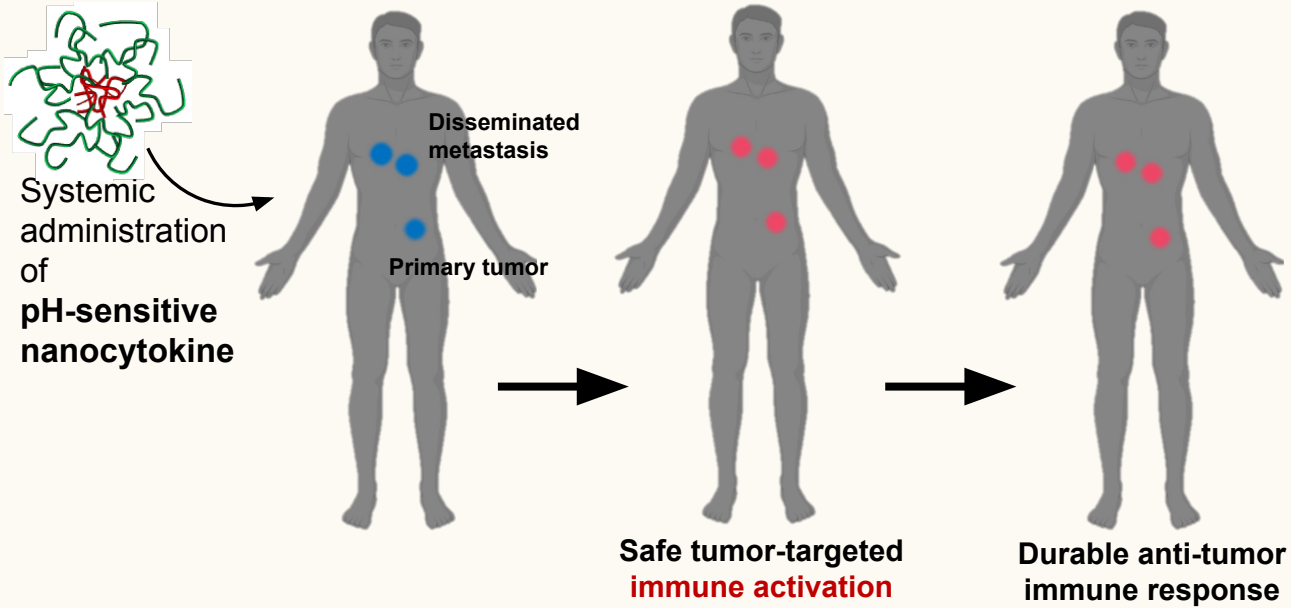


pH mapping of human breast cancer



Front. Oncol. 578985 (2020)
Pharmaceuticals **14**, 11 (2021)

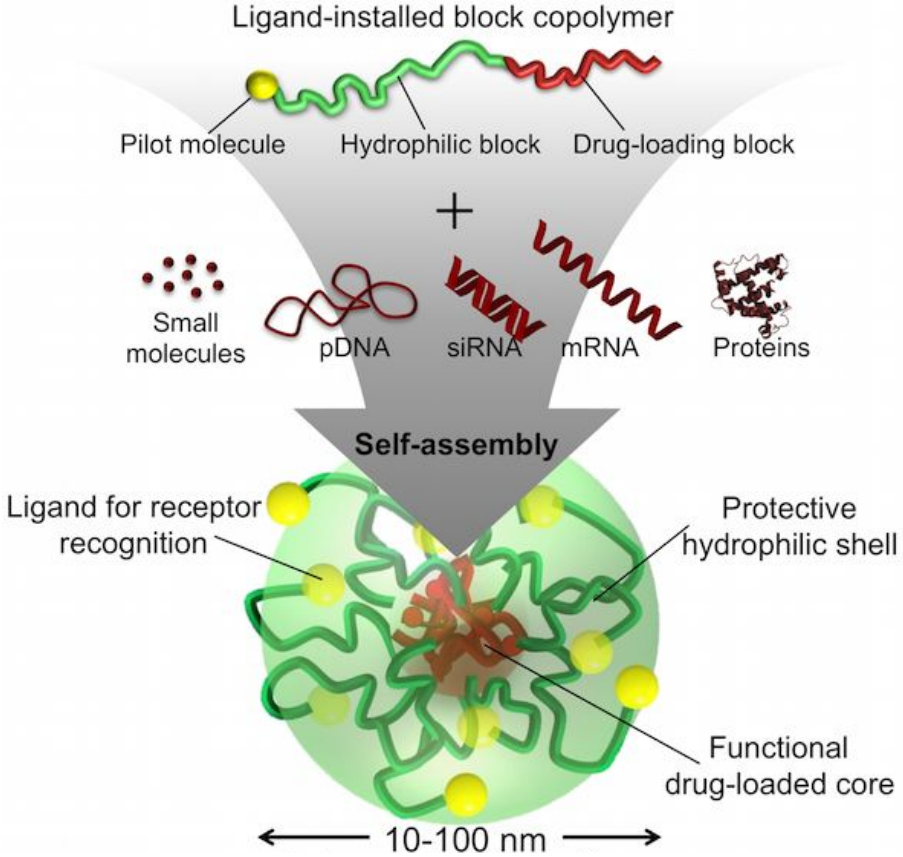
Tumor pH is below 7.0



Nanocytokine delivers a cytokine after sensing **acidic tumor microenvironment**

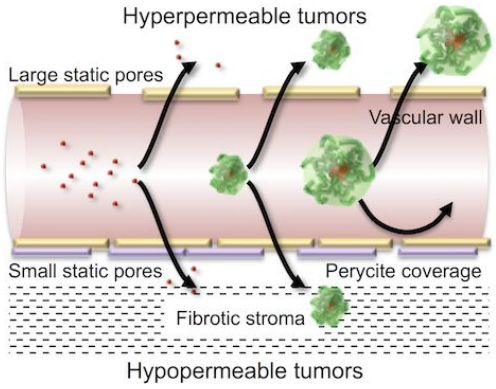
Our technology **improves PK** and **selectively delivers cytokine(s) to tumors**

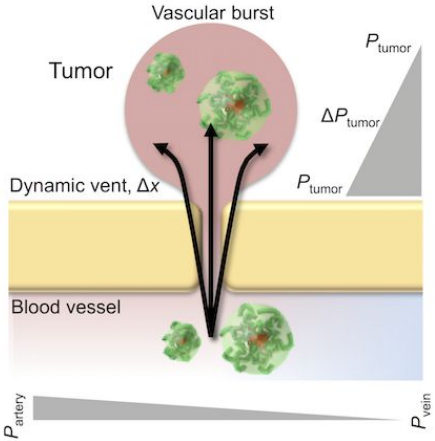
Polymeric self-assemblies as nanocarriers

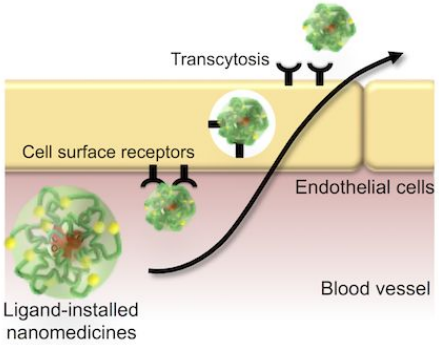


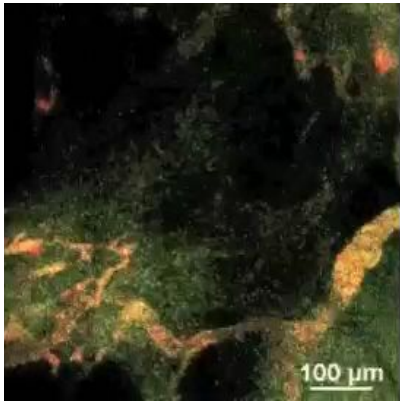
Polymeric Nanocarriers *H. Cabral, et al. Chem. Rev. 118 (2018) 6844*

Enhanced access into solid tumors



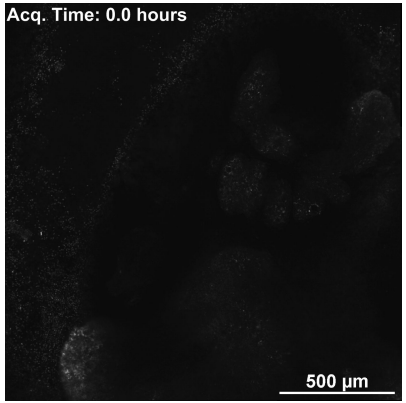






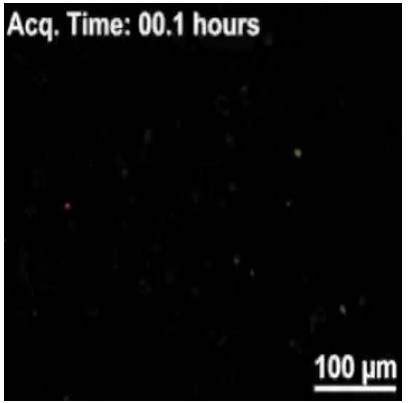
30-nm micelles/70-nm micelles/Colocalization

H. Cabral, et al. Nat Nanotechnol (2011)



70-nm micelles
Vascular Burst

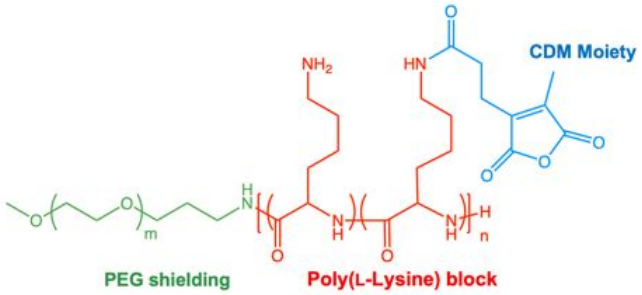
Y. Matsumoto, et al. Nat Nanotechnol (2016)



cRGD micelles/cRAD micelles/Colocalization

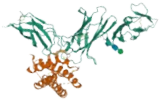
Y. Miura, et al. ACS Nano (2013)

Nanocytokines with rapid intratumoral pH response



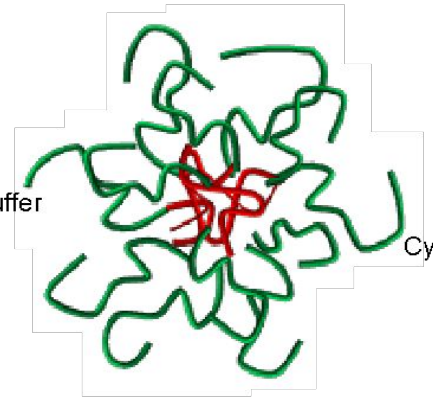
CDM-modified PEG-poly(L-Lysine(CDM) [PEG-PLL(CDM)]

+



Cytokine

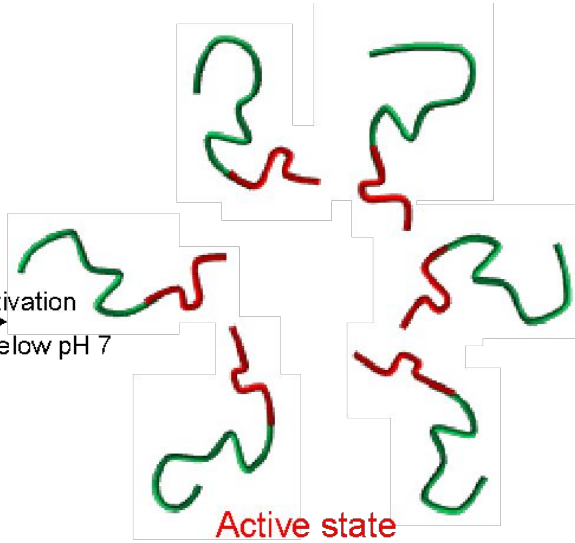
Association in buffer
pH 7.4



Silent state

Nanoparticle-based cytokine (Nanocytokine)

Intratumoral activation
Cytokine release below pH 7

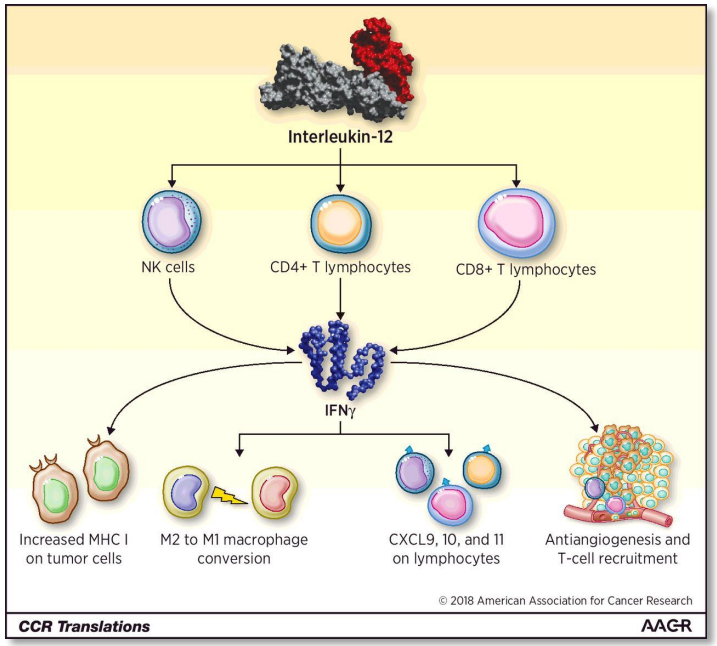


Active state

Polymer detachment and cytokine release

Polymer associate with protein just by mixing in buffer by ion complexation and pH-sensitive conjugation of the surface amines in the protein

Interleukin-12 delivery



Strong antitumor immunity

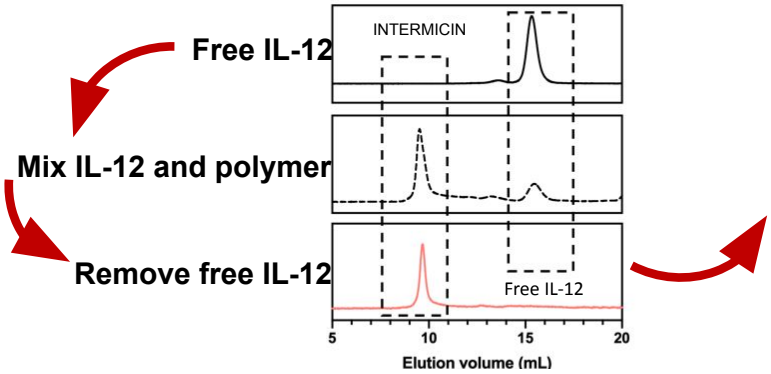
1. Enhances MHC I **antigen presentation** in tumor cells
2. **Attracts NK, Th1, and CD8$^+$ T cells**
3. Reprogram **antitumor M1 macrophages**
4. **Antiangiogenesis**
5. Increase **homing receptors for T-cell recruitment**

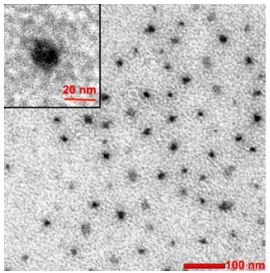
Clin Cancer Res **24** (2018) 2716–8.

IL12 has poor blood circulation and a narrow therapeutic window that limits its application to local delivery

Preparation of IL-12-based nanocytokines (Nano-IL-12)

- **IL-12-based Nanocytokines (Nano-IL-12) are made just by mixing the protein with polymers**



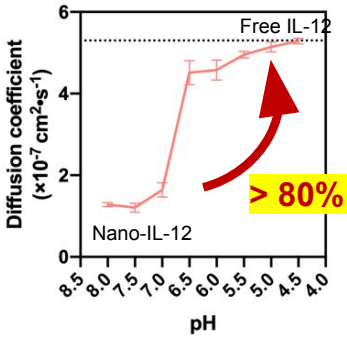


Nano-IL-12

Size: 40 nm (core: 20 nm)
Loading: 70% of feeding
Capacity: 1 cytokine per Nano-IL-12

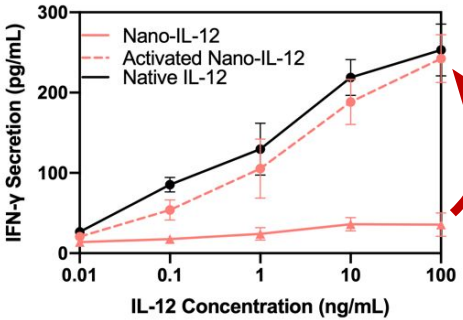
- **The IL-12 activity is muted at physiological pH, but fully recovered at intratumoral pH**

pH activation profile



> 80% release at pH < 7

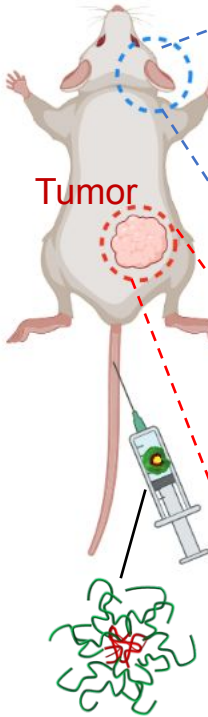
IFN-g induction



Similar to native IL-12

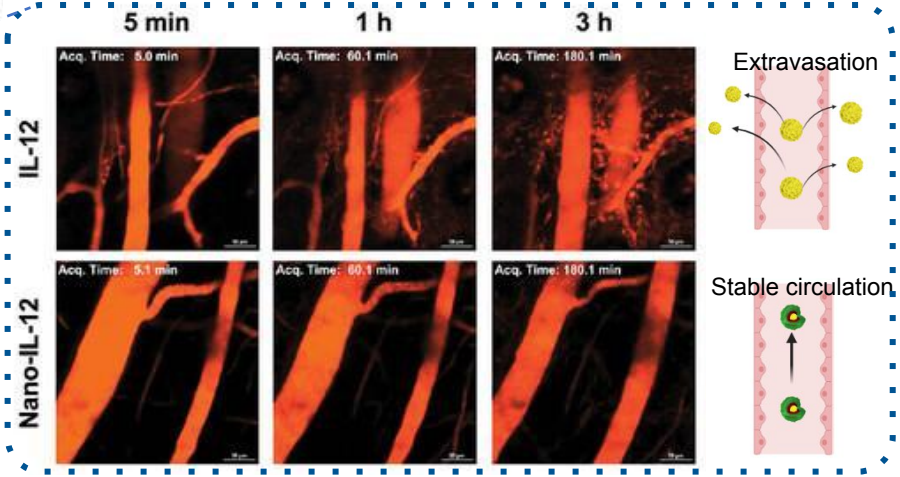
Nano-IL-12 are stable at pH 7.4, but release active cytokines at intratumoral pH

In vivo blood circulation and tumor accumulation

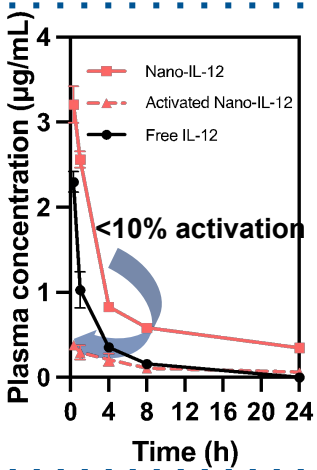


Nano-IL-12 made with
PEG_{12k}-$pLL_{40}(CDM)_{18}$
10μg e.q.; i.v. injection

In-vivo confocal observation of skin vessels

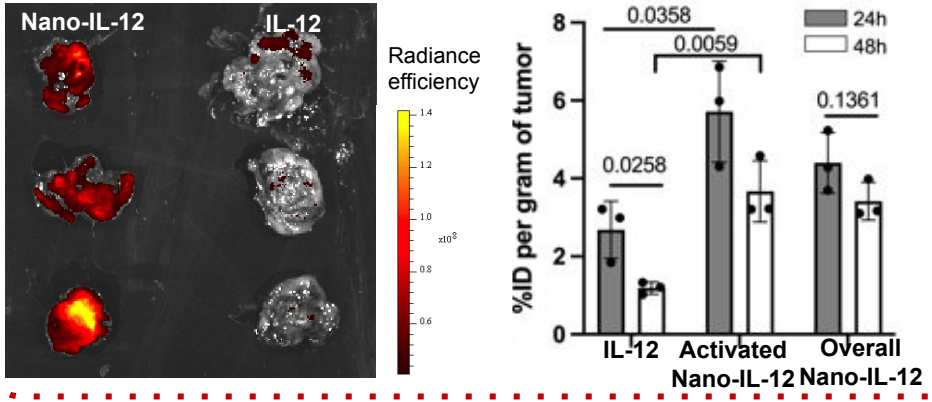


Circulation profile (by ELISA)



Adv. Sci. **10** (2023) 2205139

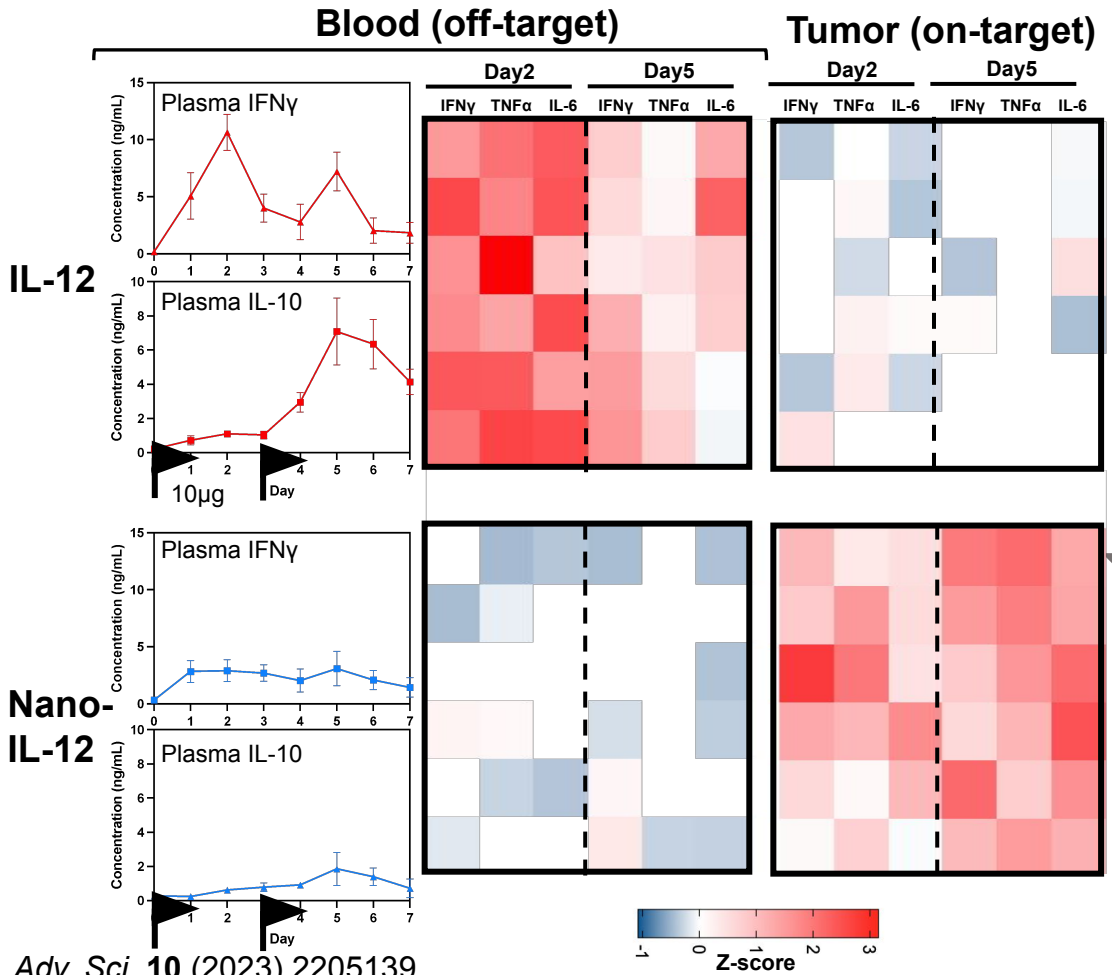
Intratumoral accumulation and activation



Nano-IL-12 showed:

- **Long circulation and low activation in blood**
- **High accumulation in tumor**
- **High activation in tumor**

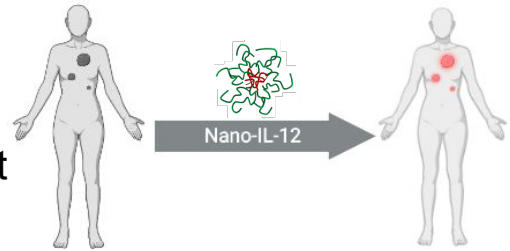
Inflammation markers in blood and tumors



- High off-target
- Low on-target

Polarizing the immune activation

- Low off-target
- High on-target



Nano-IL-12 avoided systemic response and increased intratumoral inflammation

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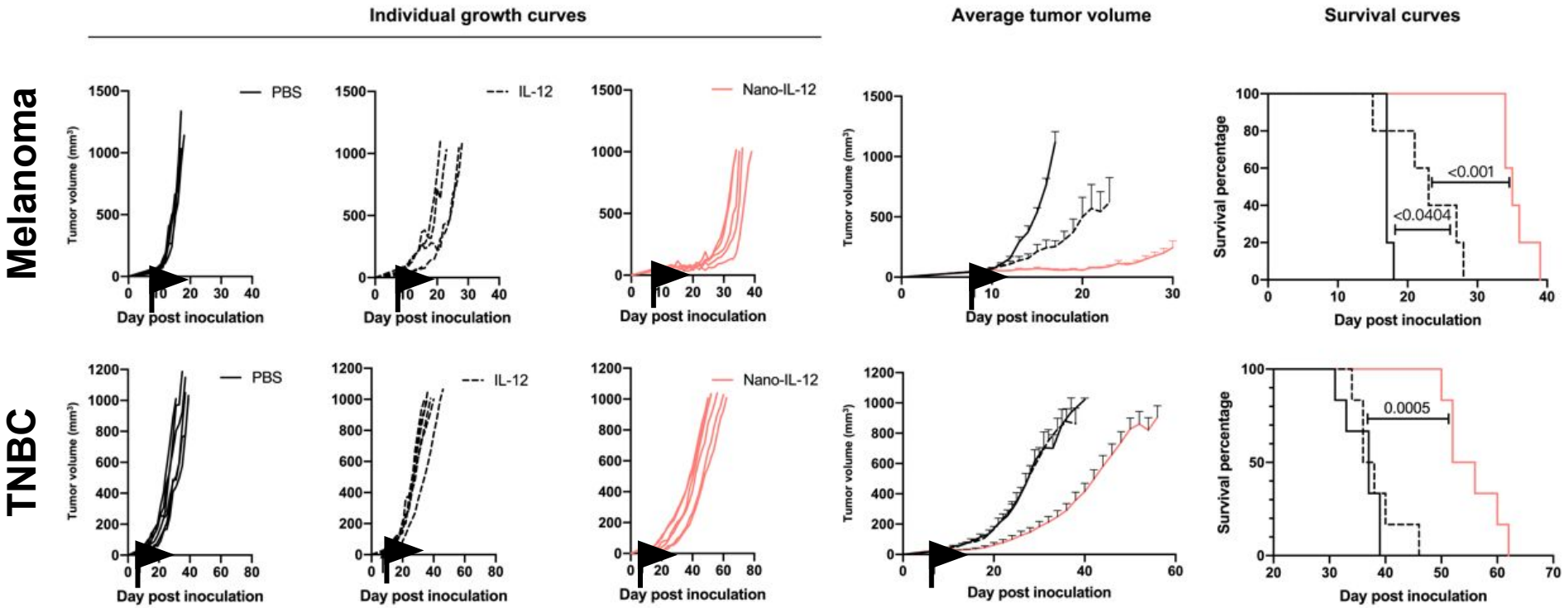
Efficacy of Nano-IL-12 as single agent



Nano-IL-12 increase the efficacy of IL-12 against cold tumor models

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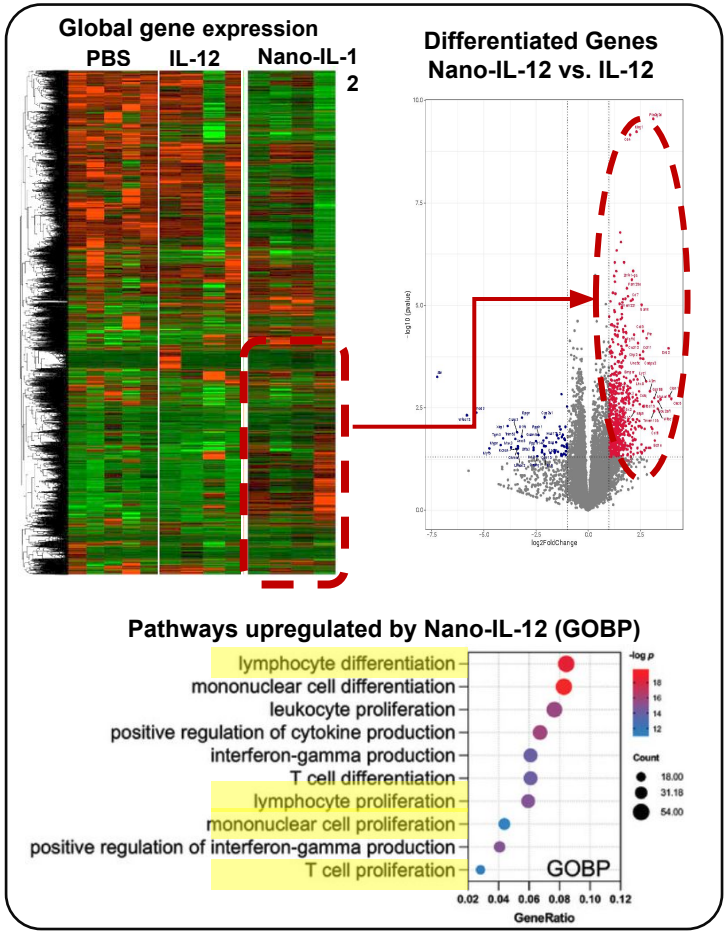
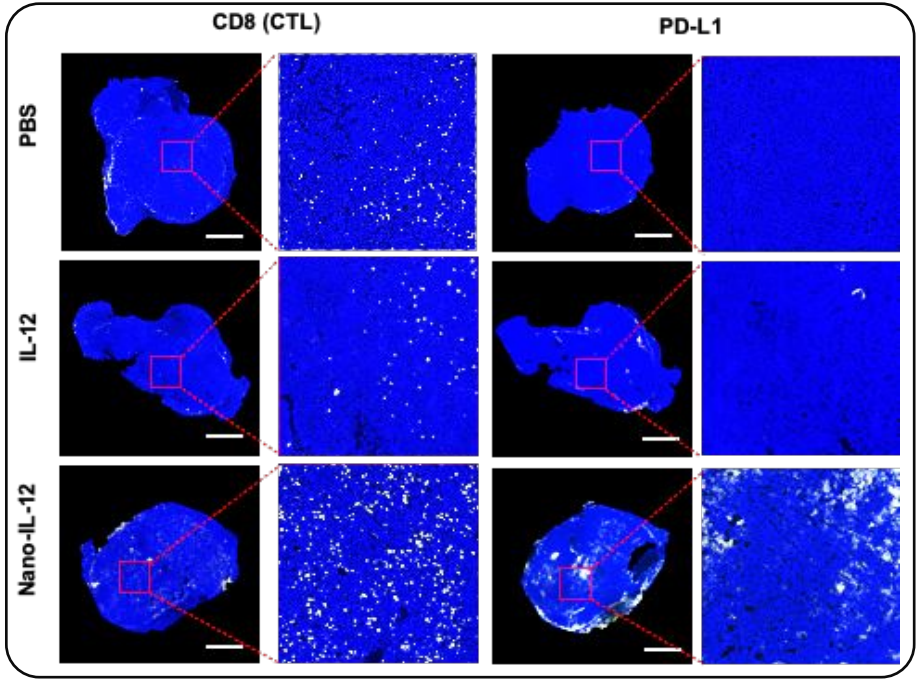
Potentiation of intratumoral immunity





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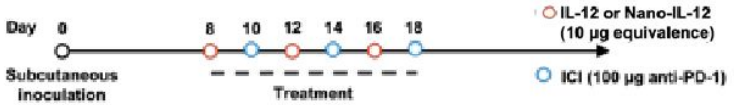
Nano-IL-12 can

- **Promote the intratumoral immunity gene expression**
- **Enhance effector cells infiltration into tumors**
- **Upregulate PD-L1 (synergy with PD-1/PD-L1 blockers)**

Nano-IL-12 plus immune checkpoint inhibitors (ICI) in melanoma



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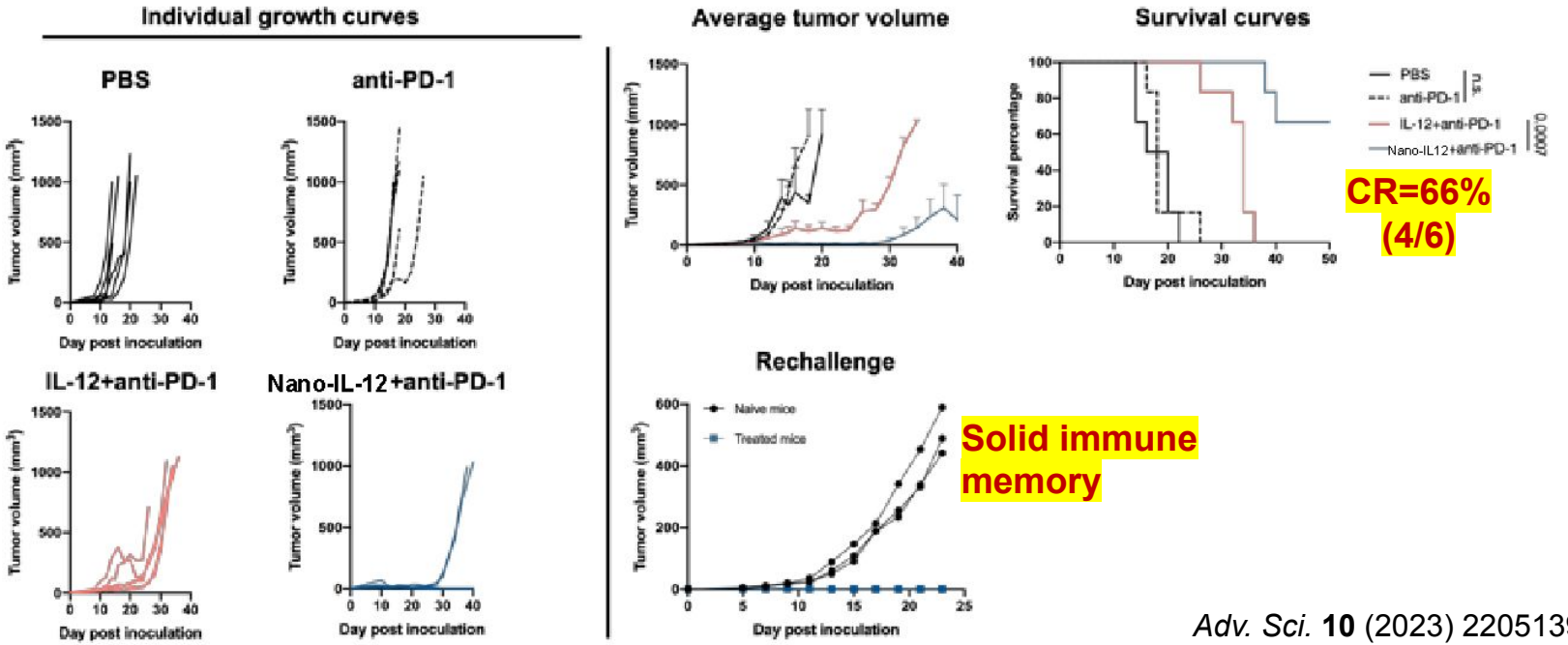

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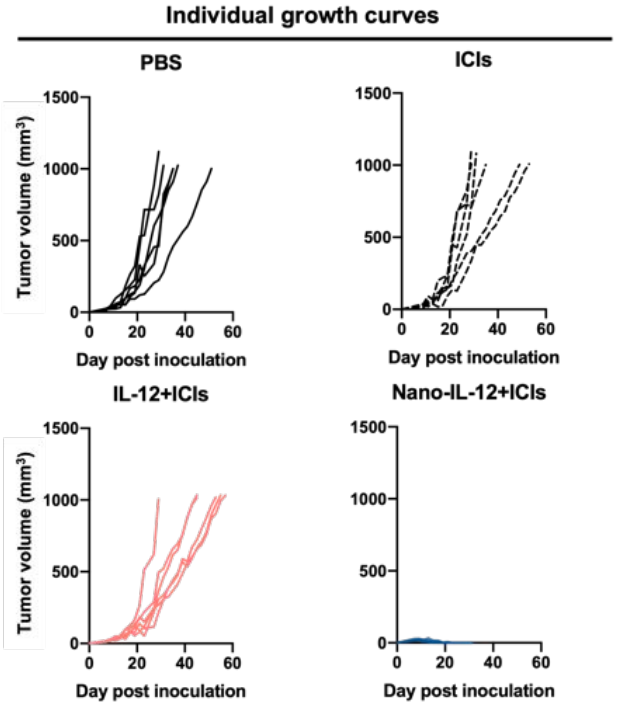
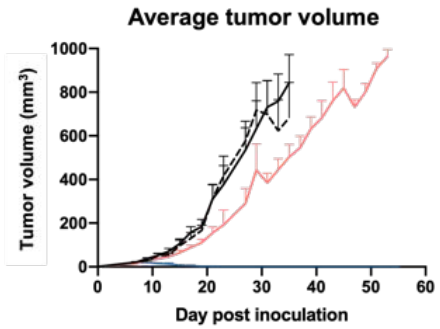
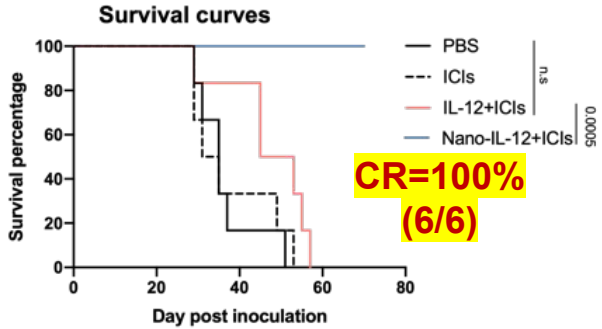
Nano-IL-12 plus ICIs eradicates melanoma

Nano-IL-12 plus ICI against primary TNBC

Primary Immunosuppresive TNBC (4T1)







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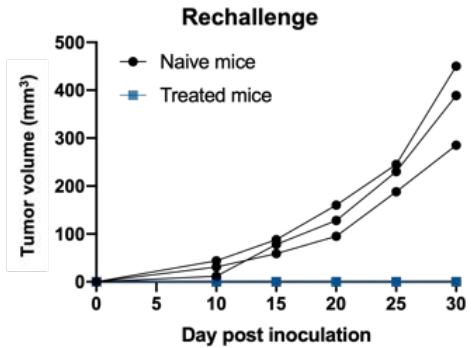


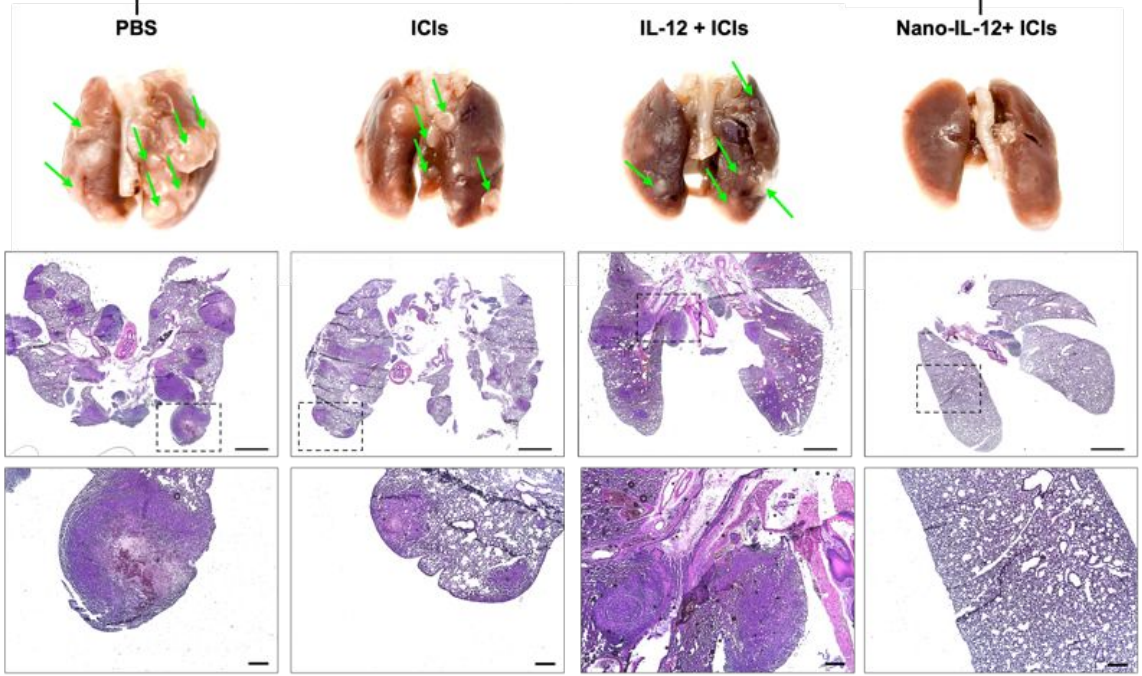
Solid immune memory

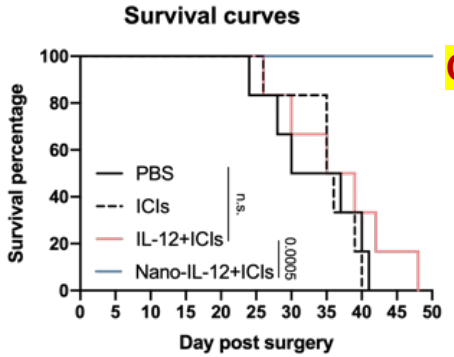
Adv. Sci. **10** (2023) 2205139

Nano-IL-12 plus ICIs eradicates TNBC

Nano-IL-12 plus ICI eradicates lung metastasis of TNBC

Metastatic immunosuppresive TNBC (4T1)





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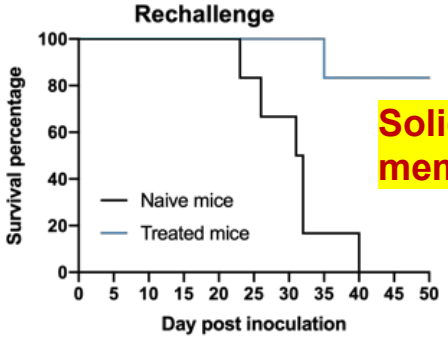

Solid immune memory

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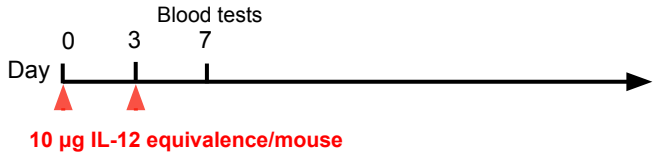
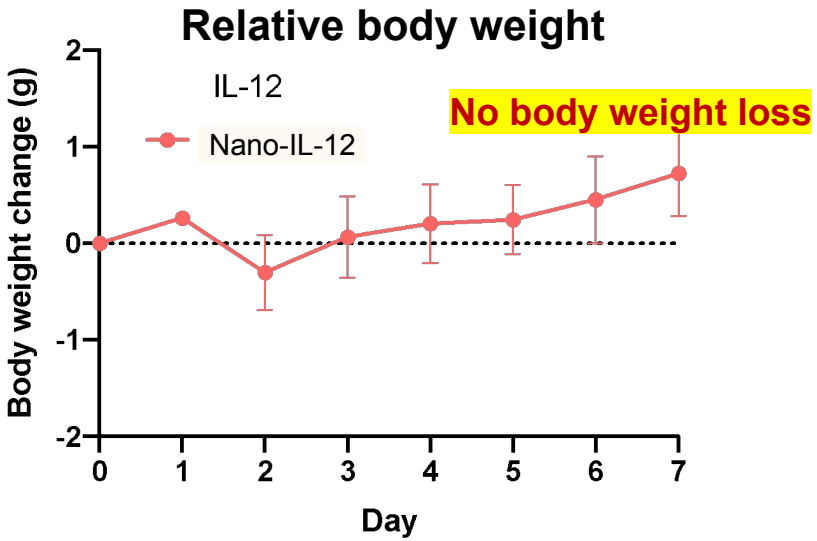
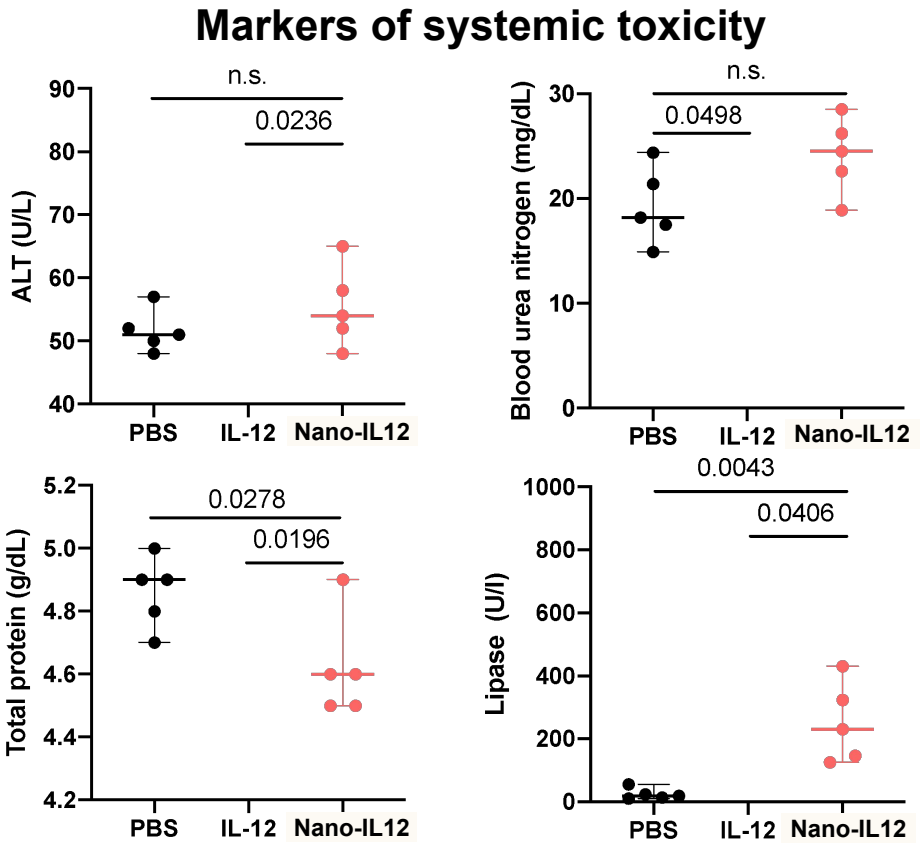
Nano-IL-12 plus ICIs eradicates TNBC lung metastasis

Toxicity evaluation







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Nano-IL-12 avoids the systemic toxicity of free IL-12

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