Local Delivery of Immunoactive Complex for Systemic Therapy of Solid Tumors

Yoon Yeo

Industrial and Physical Pharmacy
& Biomedical Engineering

PURDUE UNIVERSITY

CRS 2022 Annual Meeting & Expo

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

Advanced Delivery Science

Yeo Lab

- **Intracellular delivery**
  - Gene therapeutics
  - Antimicrobial agents against intracellular pathogens
- **New methods of delivering anticancer drugs**
  - Nanocrystals; nanoparticles; nanocapsules
- **Immunomodulatory formulations**
  - Anti-inflammatory applications
  - Immunostimulating nanoparticles
- **Long-acting local drug delivery systems**
  - Anti-inflammatory implants
  - Ocular drug delivery systems

Yeo lab in lab shirts, May 2022

Current; *Past*

Grad students

- Soonbum Kwon
- Dhawal Chobisa
- Jianping Wang
- Hytham Gadalla
- Yanying He
- Yongzhe Li
- *Simseok Yuk*
- *Maie Taha*
- *Joonyoung Park*
- *Jun Xu*
- *Yihua Pei*
- *Hyesun Hyun*
- *Bo Sun*
- *Sara Abouelmagd*
- *Karen Liu*
- *Hillary Holback*
- *Basma Ibrahim*
- *Emily Gullotti*
- *Zohreh Amoozgar*

Post-docs & Visiting scholars

- Fanfei Meng
- Yun-Chu Chen
- Sheryhan Ahmed
- Woojun Kim
- *Hyungjun Kim*
- *Nisar Khaliq*
- *Simseok Yuk*
- *Kunyu Jiang*
- *Yihua Pei*
- *Jinho Park*
- *Kyung-Oh Doh*
- *Eun Jung Cho*
- *Gaurav Bajaj*
- *Peisheng Xu*
- *Yuanfen Liu*
- *Ning Han*
- *Bieong-kil Kim**
- *Ruoci Hu*
- *Hyesun Hyun*
- *Jianping Wang*
- *Liang Pang*
- *Marwa Elnaggar*
- *Hassan Tamam*

Collaborators

- Tim Ratliff (Purdue)
- Debbie Knapp (Purdue)
- Wooin Lee (SNU)
- Steve Kron (U Chicago)
- Chang H. Kim (Michigan)
- Mohamed Seleem (Virginia Tech)
- Tiffany Lyle (Purdue)
- Ick Chan Kwon (KIST)
- Pilhan Kim (KAIST)
- Michael D. Tsifansky (Children's National Medical Center)

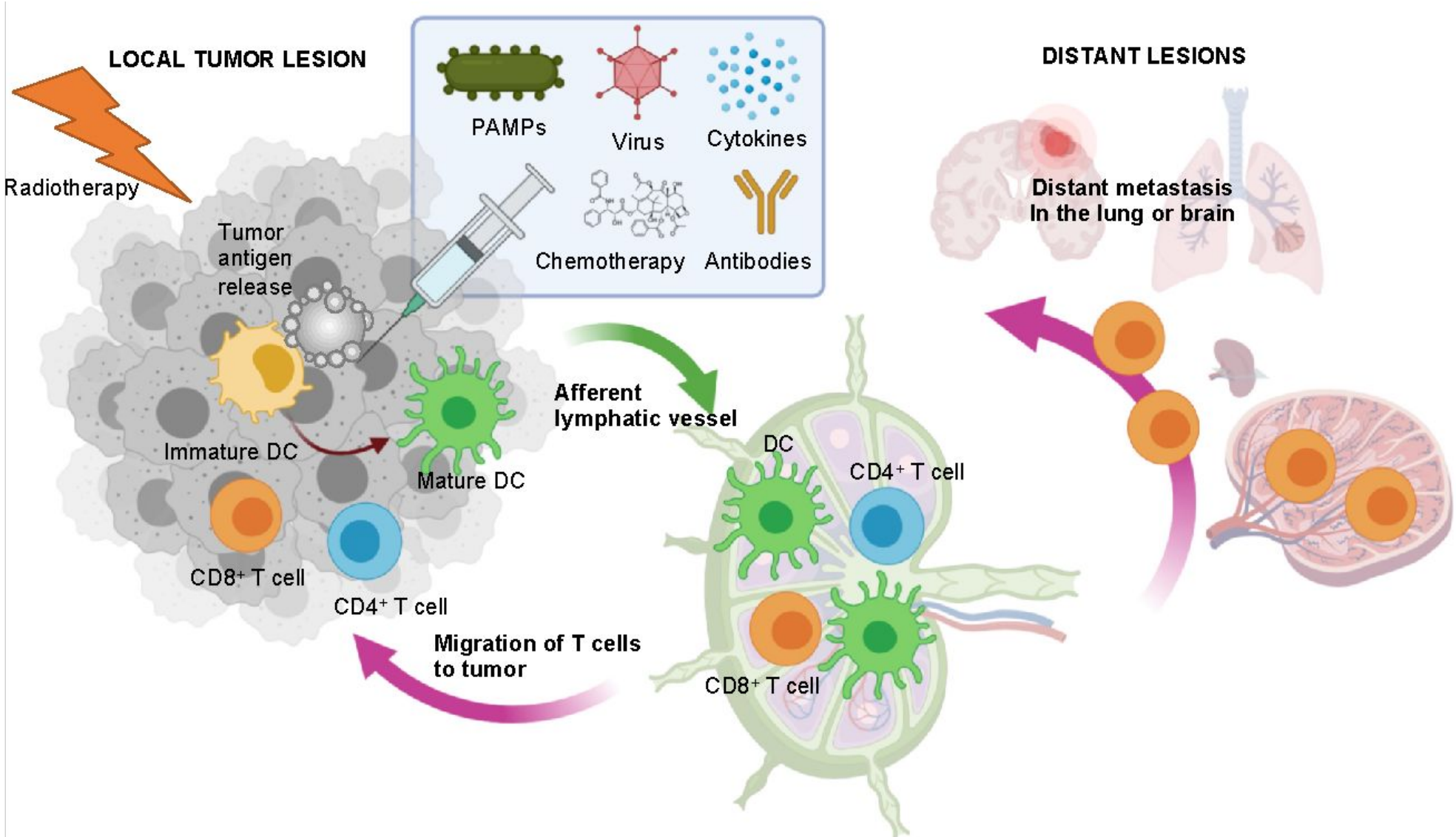
Yeo Lab @ 2020 Summer commencement ceremony

Funding

- NIH/NCI (R01 CA232419)
- NIH/NCI (R01CA258737)
- NIH/NCI (R01CA199663)
- *NIH/NIBIB (R01EB017791)*
- Purdue Institute for Drug Discovery
- Eli Lilly
- *NIH/NIAID (R21AI119479)*
- *NSF-DMR*
- *NSF/CAREER*
- *LIFA fellowship*
- *Showalter Trust*
- *Purdue Research Foundation*
- *Purdue Center for Cancer Research*

Intratumoral Delivery of Immunotherapy

"Act Locally, Think Globally" – <u>Act locally, function systemically</u>



Intratumoral Delivery of Immunotherapy

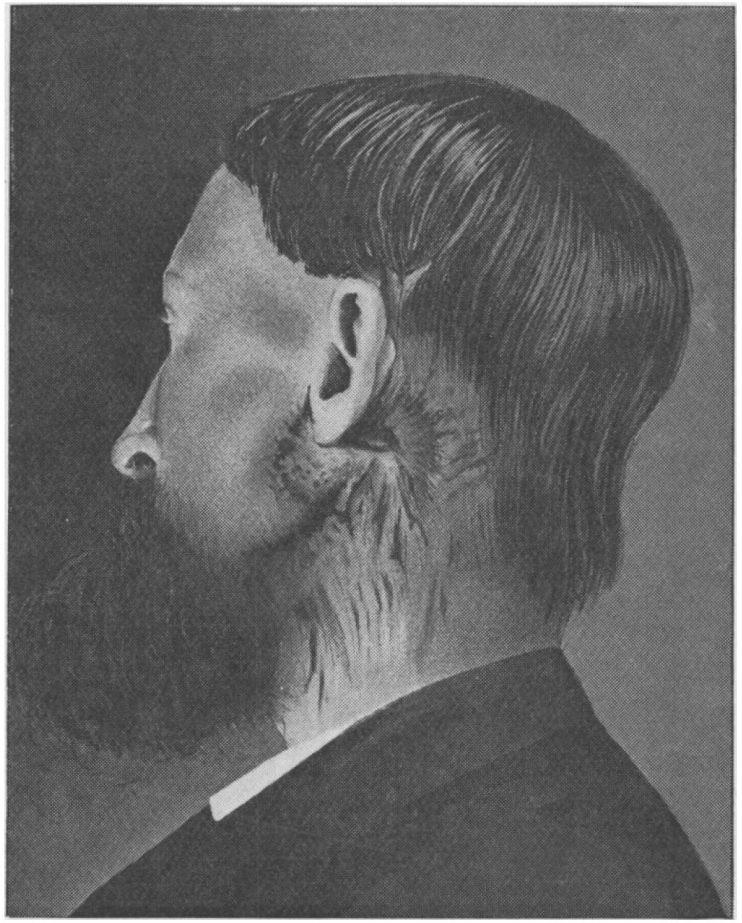


FIG. 1.

Recurrent round-celled sarcoma. Spontaneous recovery following accidental erysipelas. Photograph taken seven years after the cure.

Coley, W. B., The Treatment of Inoperable Sarcoma by Bacterial Toxins (the Mixed Toxins of the Streptococcus Erysipelas and the Bacillus Prodigiosus). *Proceedings of the Royal Society of Medicine* **1910,** *3* (Surg Sect), 1-48.

BIOTECHNOLOGY

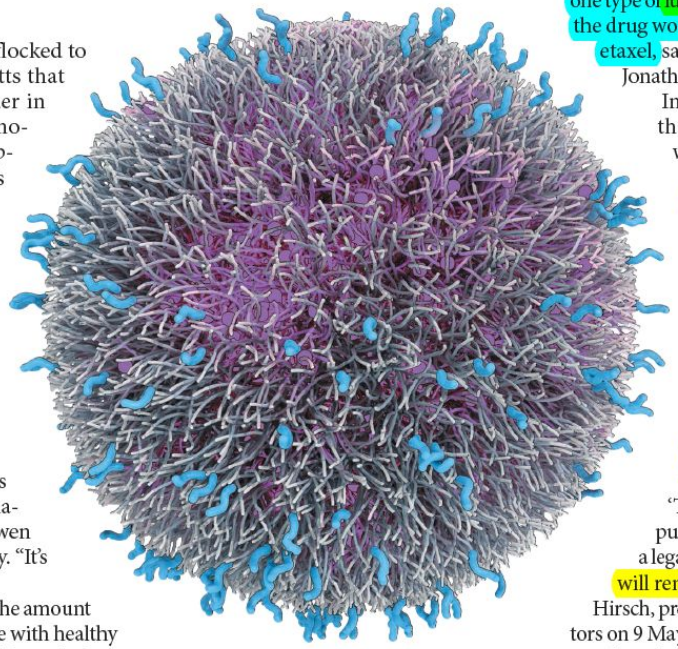
Bankruptcy of nanomedicine firm worries drug developers

Financial troubles of leading biotech firm highlight challenges of making innovative drugs.

BY HEIDI LEDFORD

Not long ago, investors flocked to a firm in Massachusetts that was hailed as the leader in a wave of next-generation nanotechnology companies developing ways to ferry cancer drugs to tumours. But on 2 May, the company — BIND Therapeutics — declared bankruptcy.

Researchers in the field of nanomedicine are waiting anxiously to see whether the Cambridge-based firm will pull through its financial crisis — and whether its troubles will taint the swiftly evolving field of nanoparticle drug delivery. "It's been a rapid rise and fall," says Eric Schmidt, a biotechnology analyst at the investment bank Cowen and Company in New York City. "It's all unravelled pretty quickly."



COURTESY BIND THERAPEUTICS

BIND Therapeutics' nanoparticle is coated in molecules that target it to tumours.

Because nanoparticles lessen the amount of contact that cancer drugs have with healthy tissue, they offer a chance to deliver higher doses with fewer side effects. In 1995, the US Food and Drug Administration approved the first such treatment, Doxil, which packages a chemotherapy drug called doxorubicin in a lipid nanoparticle. The particles are too large to escape from normal blood vessels — and so are less toxic to the heart than naked doxorubicin — but they can seep out of the leaky blood vessels often found in tumours.

BIND's nanoparticles were designed to target tumours more precisely than liposome particles can. The company's lead product, BIND-014, involves a polymer particle coated with a molecule that steers the particle to a protein found in many tumours. The particle releases the chemotherapy drug it carries, called docetaxel, inside the tumour.

Early tests in animals and small clinical trials showed that the approach was safer than docetaxel alone — and fuelled BIND's US$70.5-million initial public offering in 2013. But later clinical trials disappointed. BIND-014 failed against cervical and head-and-neck cancers. Although it was somewhat effective against one type of lung cancer, it was not clear whether the drug worked any better than regular docetaxel, says BIND's chief scientific officer Jonathan Yingling.

In April, the company announced that it would cut back on its work with BIND-014, and Yingling says that the firm will now explore new targets. It cut the number of employees by 38% and aims to trim its expenses to $6 million per quarter — a dramatic decrease for a company that spent $11 million on research and development alone in the first quarter of 2016.

After one of its creditors demanded that BIND repay a loan ahead of schedule, the company filed for bankruptcy (see 'Troubled times'). It plans to dispute the need for early repayment at a legal hearing on 18 May. "BIND is and will remain open for business," Andrew Hirsch, president of the company, told investors on 9 May.

Schmidt says that BIND remains at the technological forefront of nanoparticle drug delivery, but waited too long to move away from BIND-014. By then, the investor enthusiasm for biotechnology that had driven BIND's initial public offering had cooled. "People are not interested in funding technology right now," Schmidt says. "They're interested in funding later-stage projects. And the one at this company didn't have what it takes."

In the time since BIND-014 was developed, researchers have also realized that differences between tumours — such as size, density and leakiness of the blood vessels that lace through

what's new

Results of Phase III study of NK105, a novel macromolecular micelle encapsulating an anticancer drug

2016/07/05

Tokyo, Japan, July 05, 2016 – Nippon Kayaku Co., Ltd. (Head Office: Tokyo; President: Masanobu S hereinafter referred to as "Nippon Kayaku") announced that in a phase III clinical study of its in-ho developed polymeric micelle anti-cancer drug NK105 in patients with metastatic or recurrent brea cancer, the primary endpoint of the study, progression free survival (PFS), did not meet the prespe statistical criteria. The study is a randomized, multinational study comparing weekly administratio NK105 versus Paclitaxel in terms of efficacy and safety in patients with metastatic or recurrent bre cancer. The primary endpoint of the study is statistical non-inferiority of PFS. Detailed efficacy anc analyses from this study are expected to be presented at an upcoming scientific congress. Future the development of NK105 will be further examined.

About NK105
NK105 is a novel DDS (Drug Delivery System) formulation encapsulating active ingredient paclitaxe macromolecular micelles.

Active-NanoFect — Co-Research with Chugai Pharmaceutical and In-house research

NK105 Paclitaxel Micelle
- Breast Cancer (Japan・Asia)※
- Stomach Cancer (Japan) — Out-licensed to Nippon Kayaku

Carrier consideration

- Retain immunotherapeutic agents locally to maximize their pharmacological effects in tumors and prevent systemic side effects
- Co-deliver multiple drugs, which share little physicochemical features and would otherwise not colocalize
- Stimulate antitumor immunity to leverage immunostimulatory effects of therapeutic agents

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Nucleic acid and oligonucleotide delivery for activating innate immunity in cancer immunotherapy

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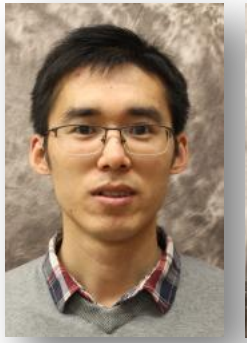
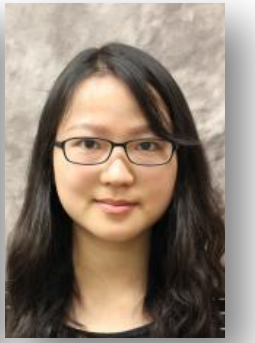
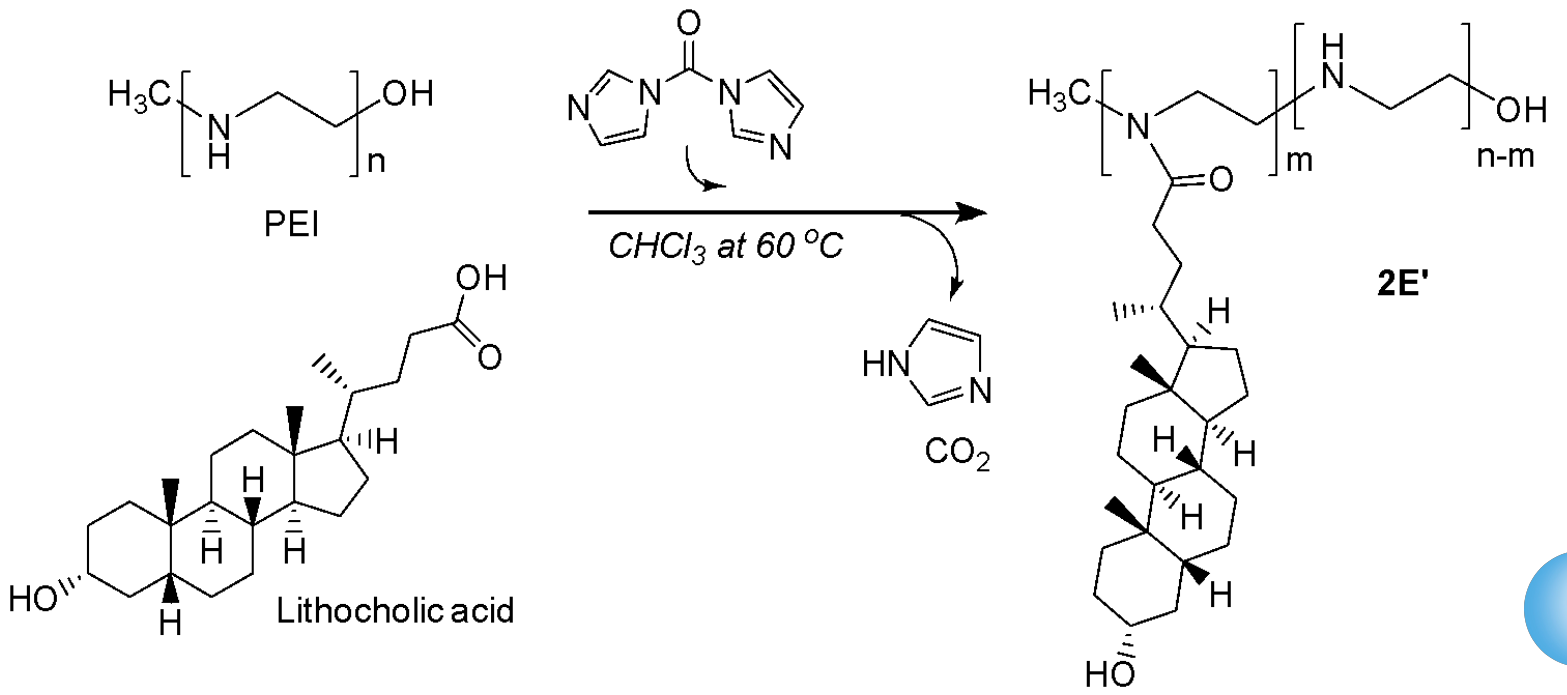
ABSTRACT

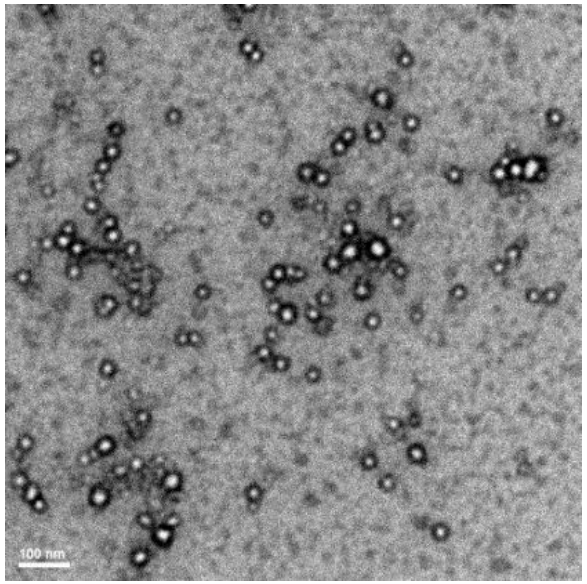
A group of nucleic acids and oligonucleotides play various roles in the innate immune system. They can stimulate pattern recognition receptors to activate innate immune cells, encode immunostimulatory proteins or peptides, or silence specific genes to block negative regulators of immune cells. Given the limitations of current cancer immunotherapy, there has been increasing interest in harnessing innate immune responses by nucleic acids and oligonucleotides. The poor biopharmaceutical properties of nucleic acids and oligonucleotides make it critical to use carriers that can protect them in circulation, retain them in the tumor microenvironment, and bring them to intracellular targets. Therefore, various gene carriers have been repurposed to deliver nucleic acids and oligonucleotides for cancer immunotherapy and improve their safety and activity. Here, we review recent studies that employed carriers to enhance the functions of nucleic acids and oligonucleotides and overall immune responses to cancer, and discuss remaining challenges and future opportunities in the development of nucleic acid-based immunotherapeutics.

Immunoactive complexes


Fanfei Meng

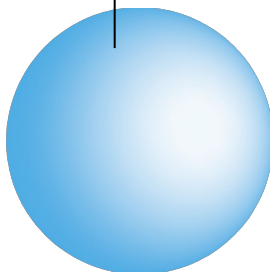

Jianping Wang



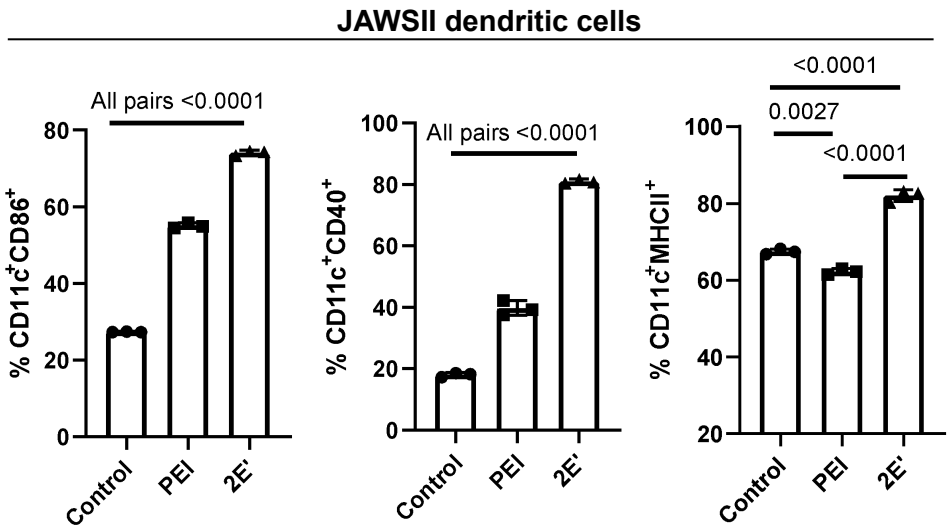


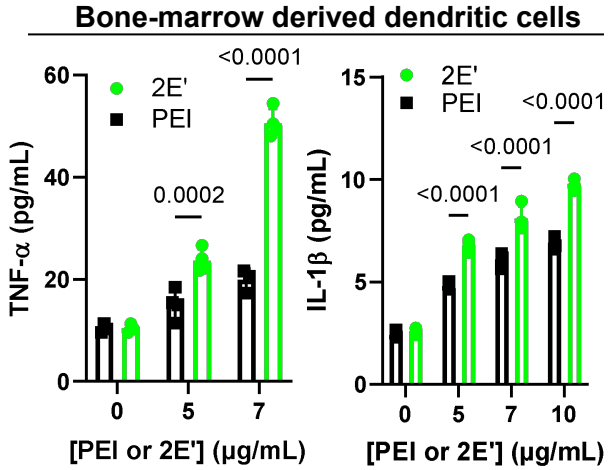
Fanfei Meng, Jianping Wang, Yanying He, Gregory M. Cresswell, Nadia A. Lanman, L. Tiffany Lyle, Timothy L. Ratliff, Yoon Yeo. *PNAS.* 2022 (119) 22: e2122595119

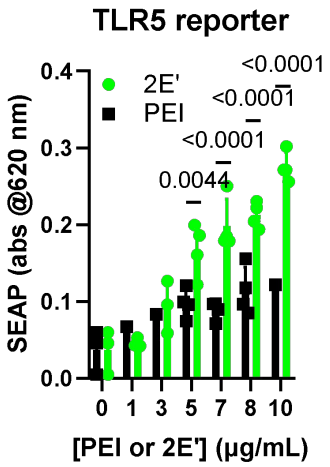
Polyethyleneimine derivative (2E')

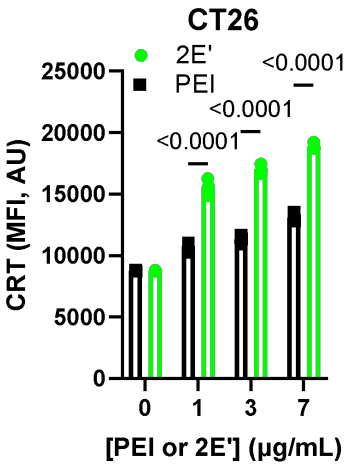


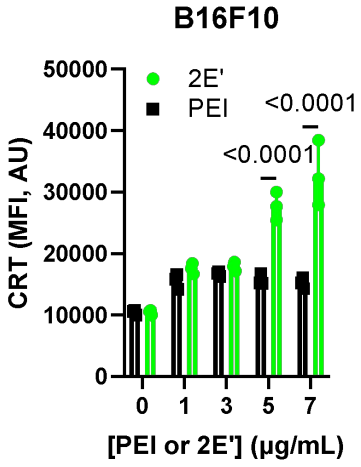
2E' serves as an immunoadjuvant and enhances cancer cell uptake by antigen presenting cells (APCs)

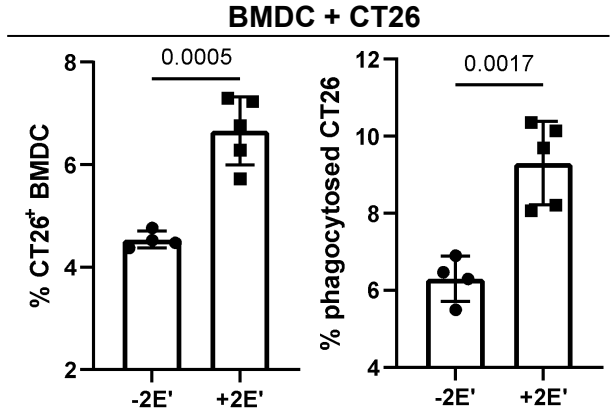


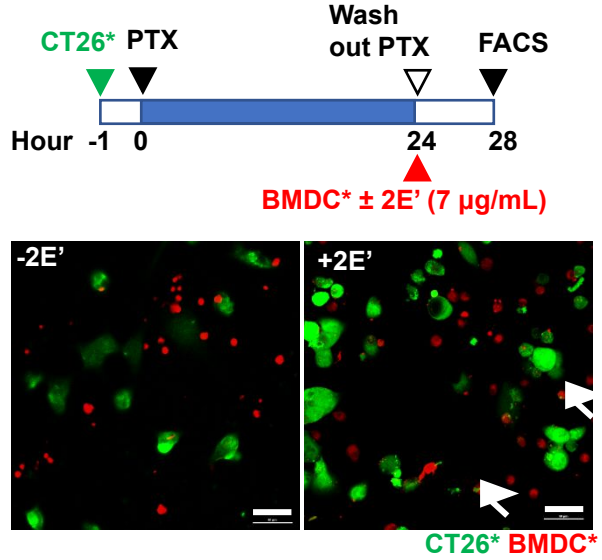




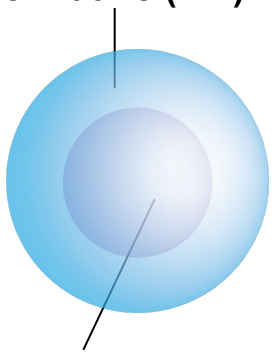




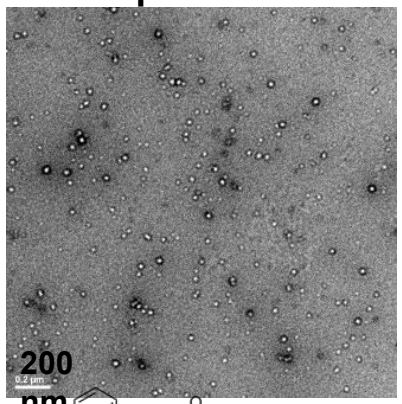




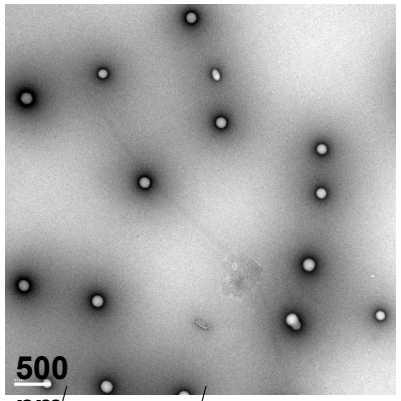
2E’ forms nanoparticulate self-assemblies with hydrophobic drugs



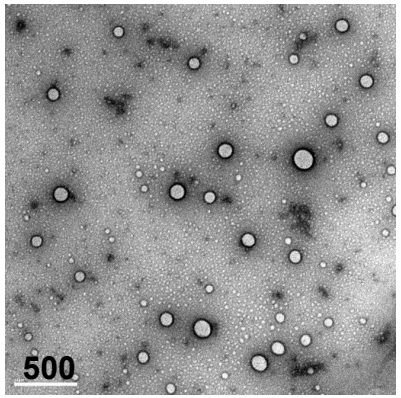
2E’/paclitaxel



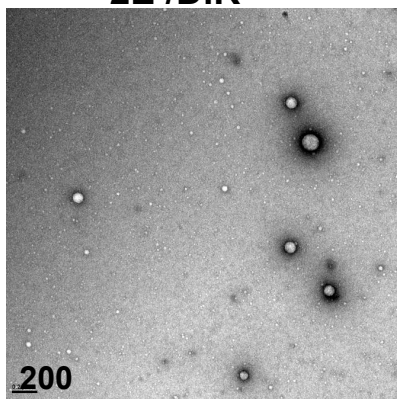
2E’/carfilzomib



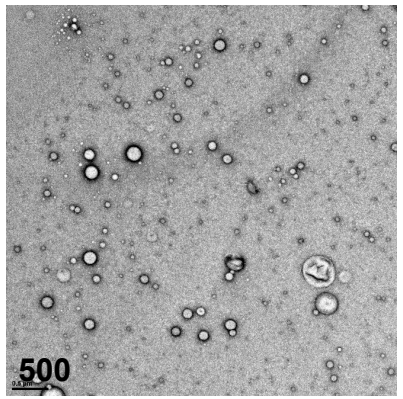
2E’/camptothecin



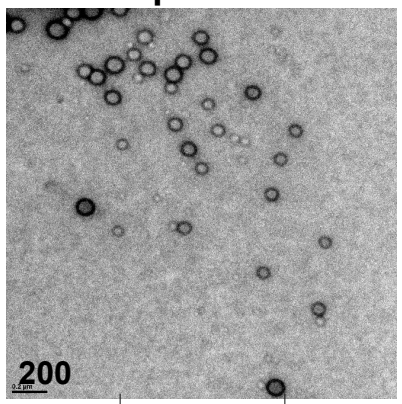
2E’/DiR



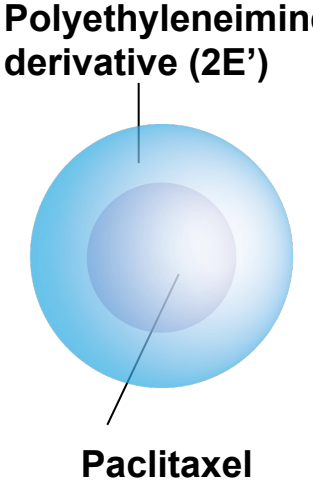
2E’/niflumic acid

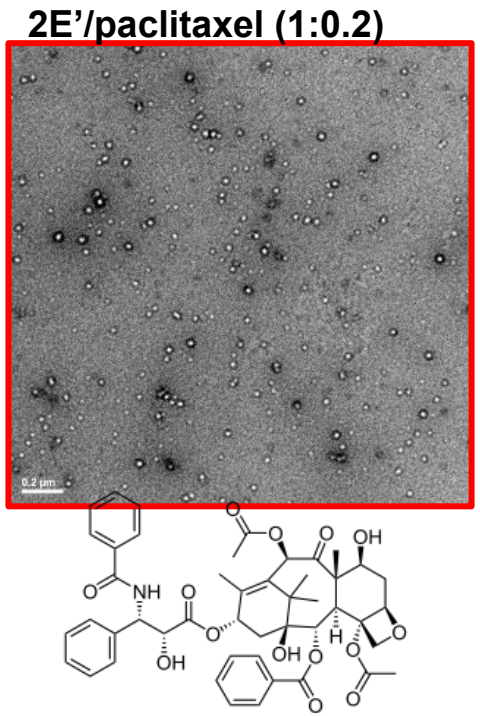


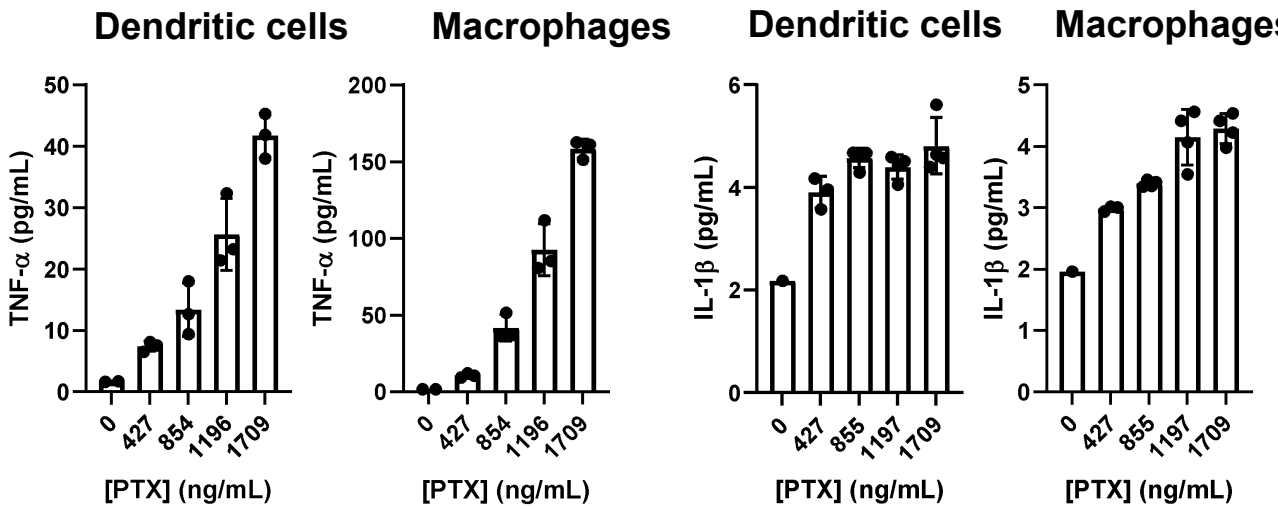
2E’/probucol

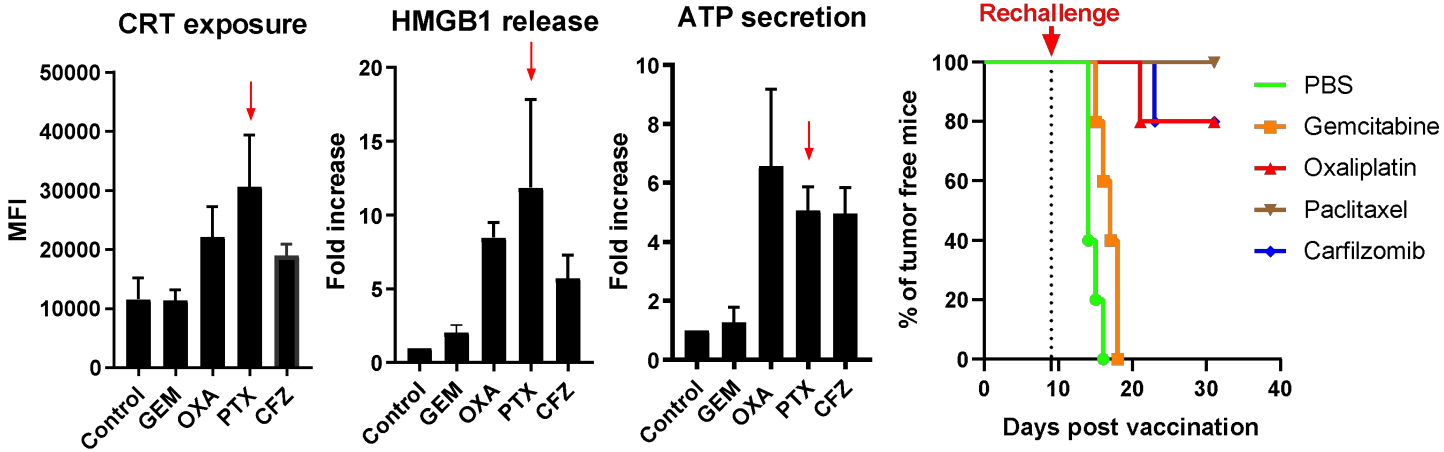


Paclitaxel (PTX) stimulates antigen-presenting cells and induces immunogenic death of tumor cells



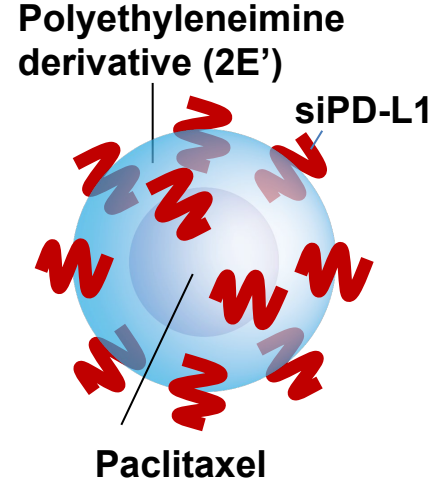


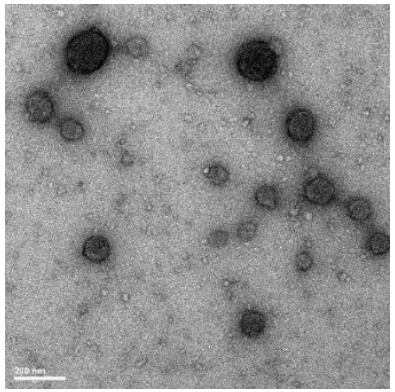




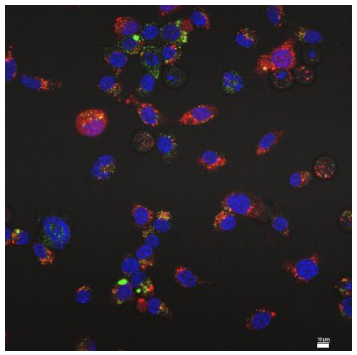
Soonbum Kwon

2E’ carries paclitaxel and siPD-L1, retaining its immunostimulatory effects.

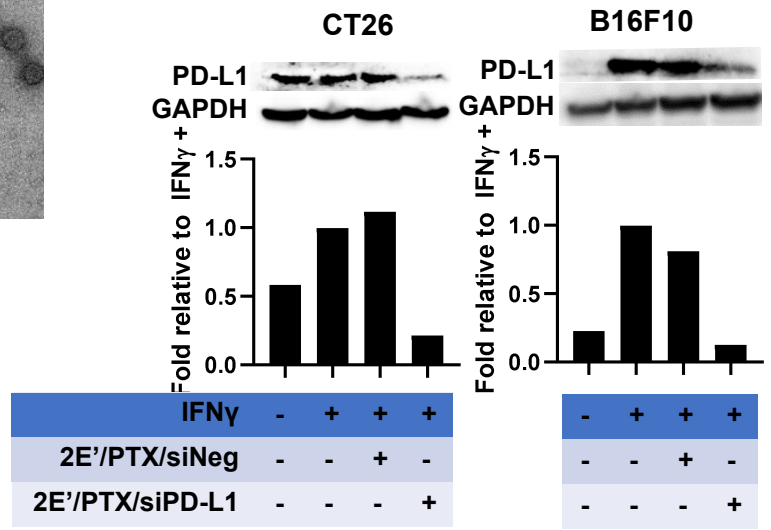


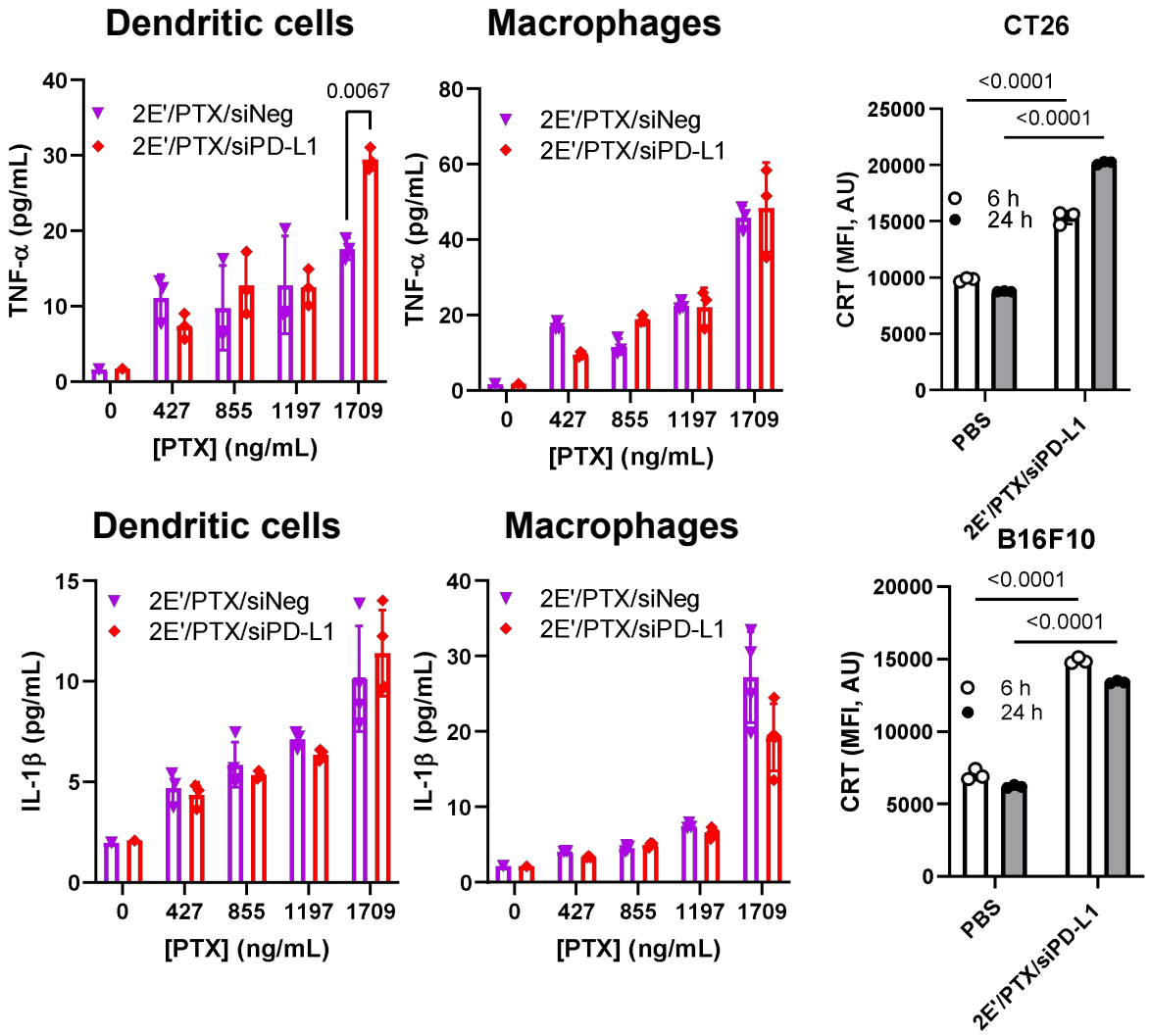


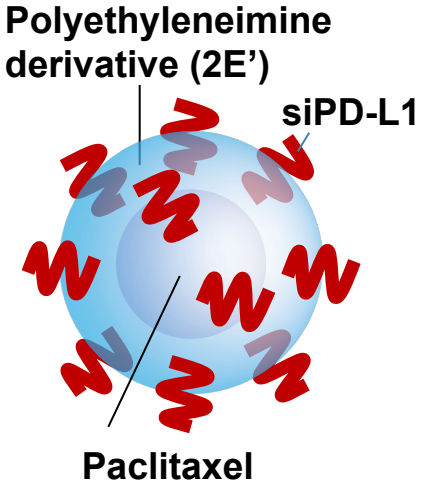
2E’/PTX/siPD-L1



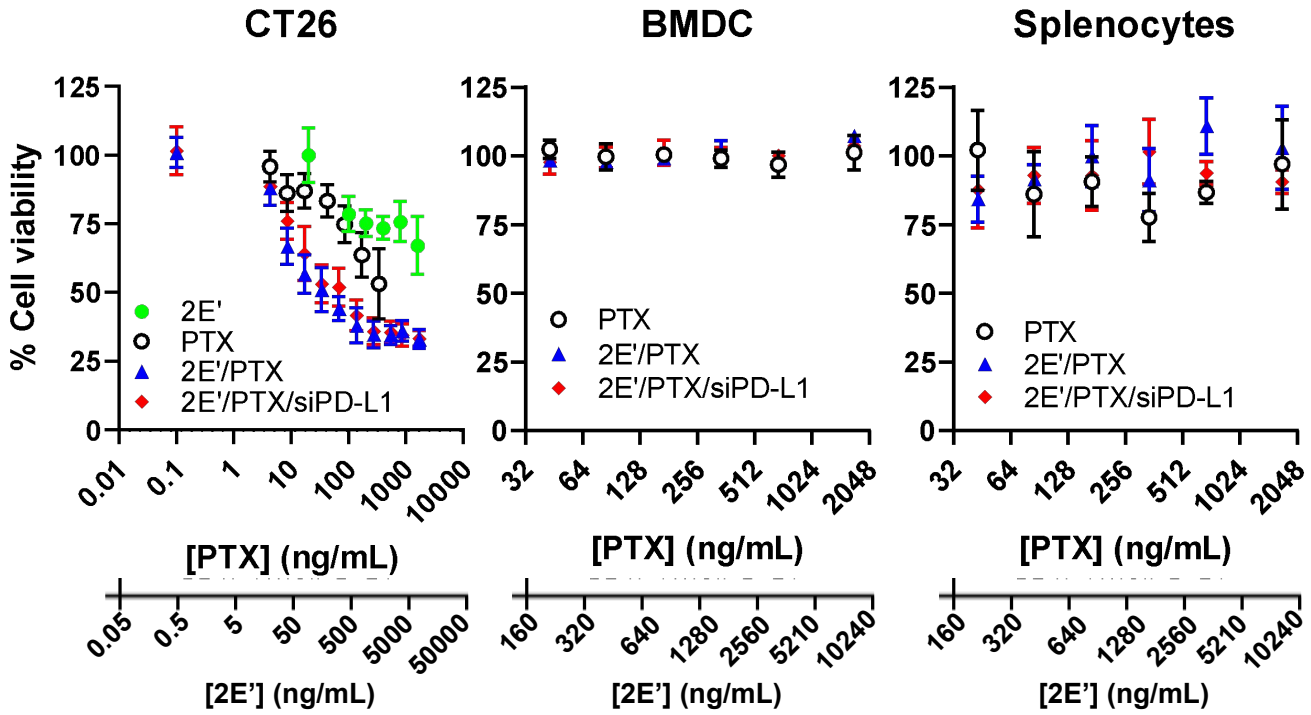
Nuclei Lysosome siPD-L1

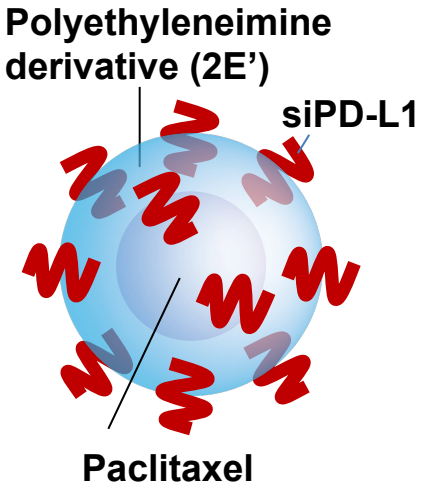




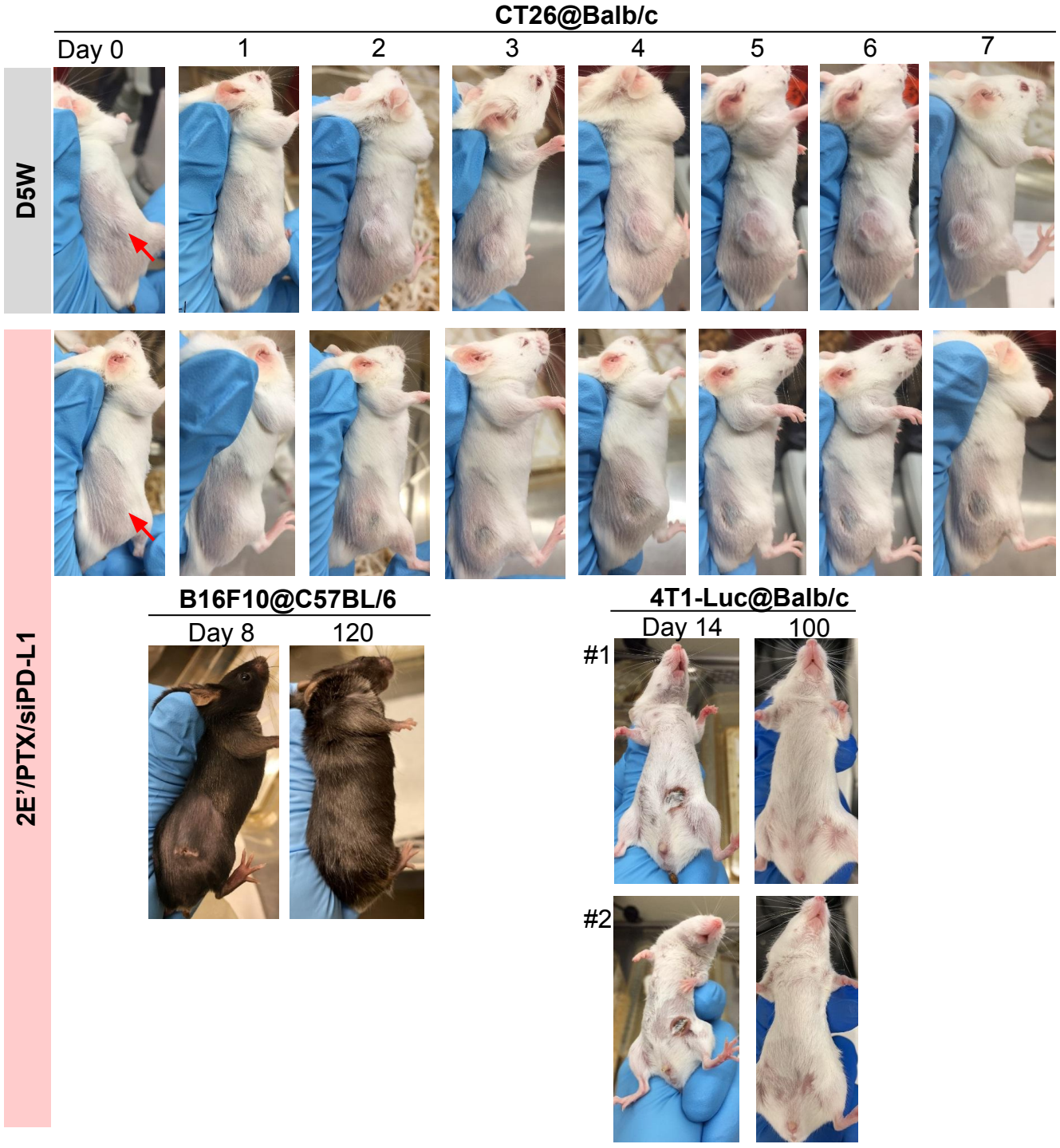


2E'/PTX shows selective toxicity to tumor cells

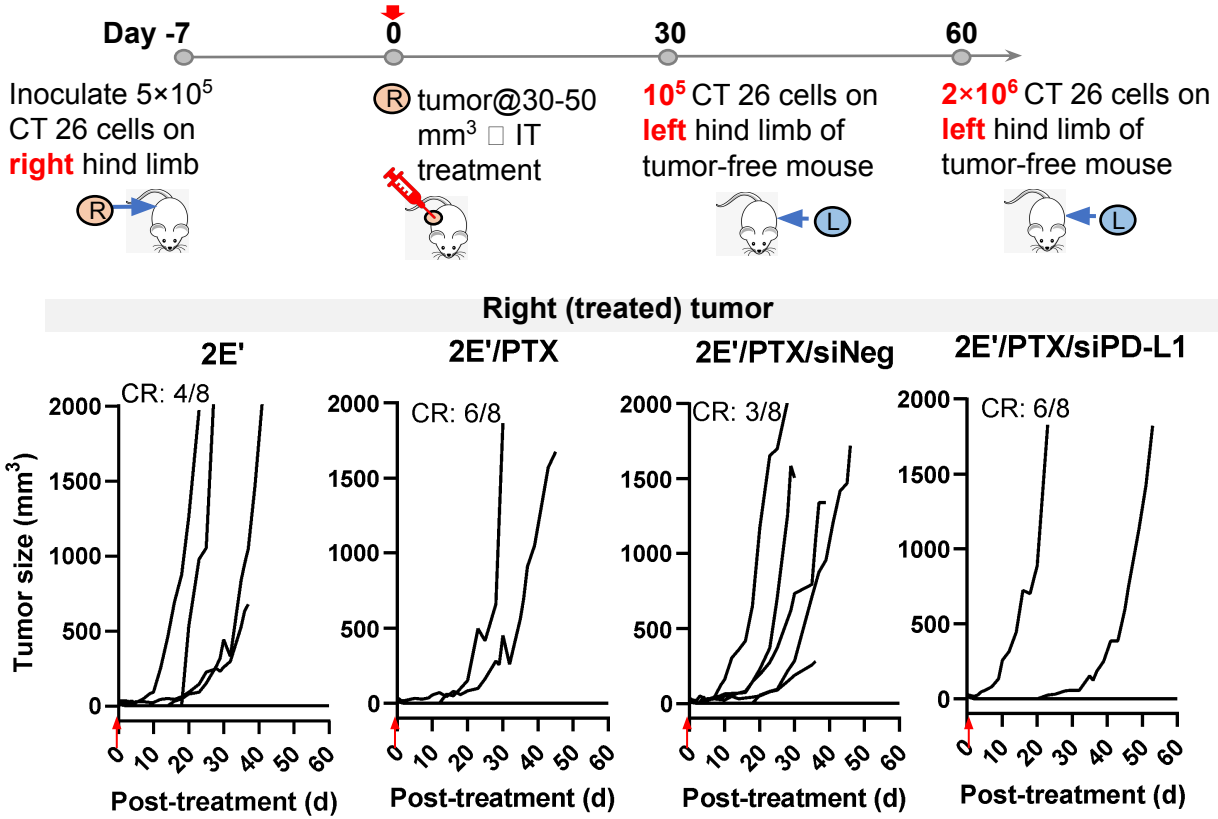


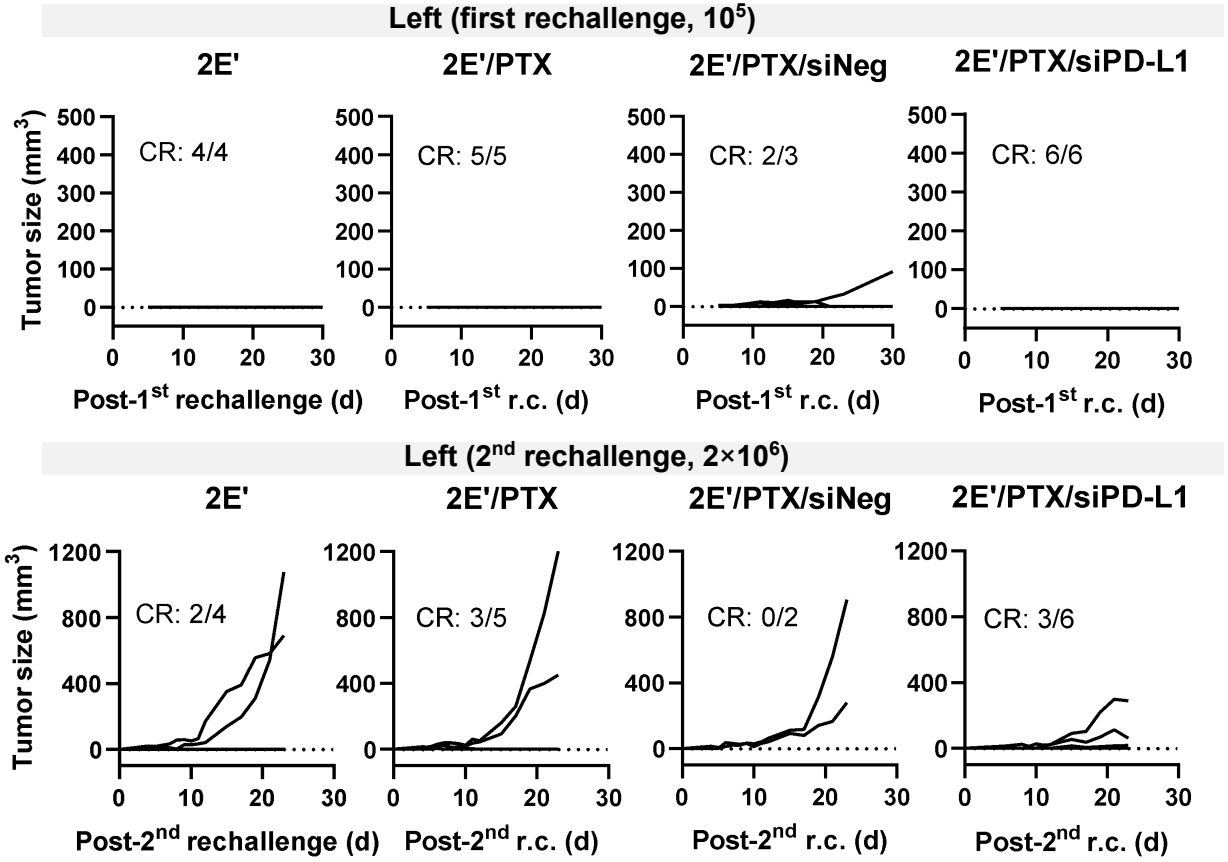


Single local administration of 2E'/PTX/siPD-L1 induces immediate regression of large established tumors

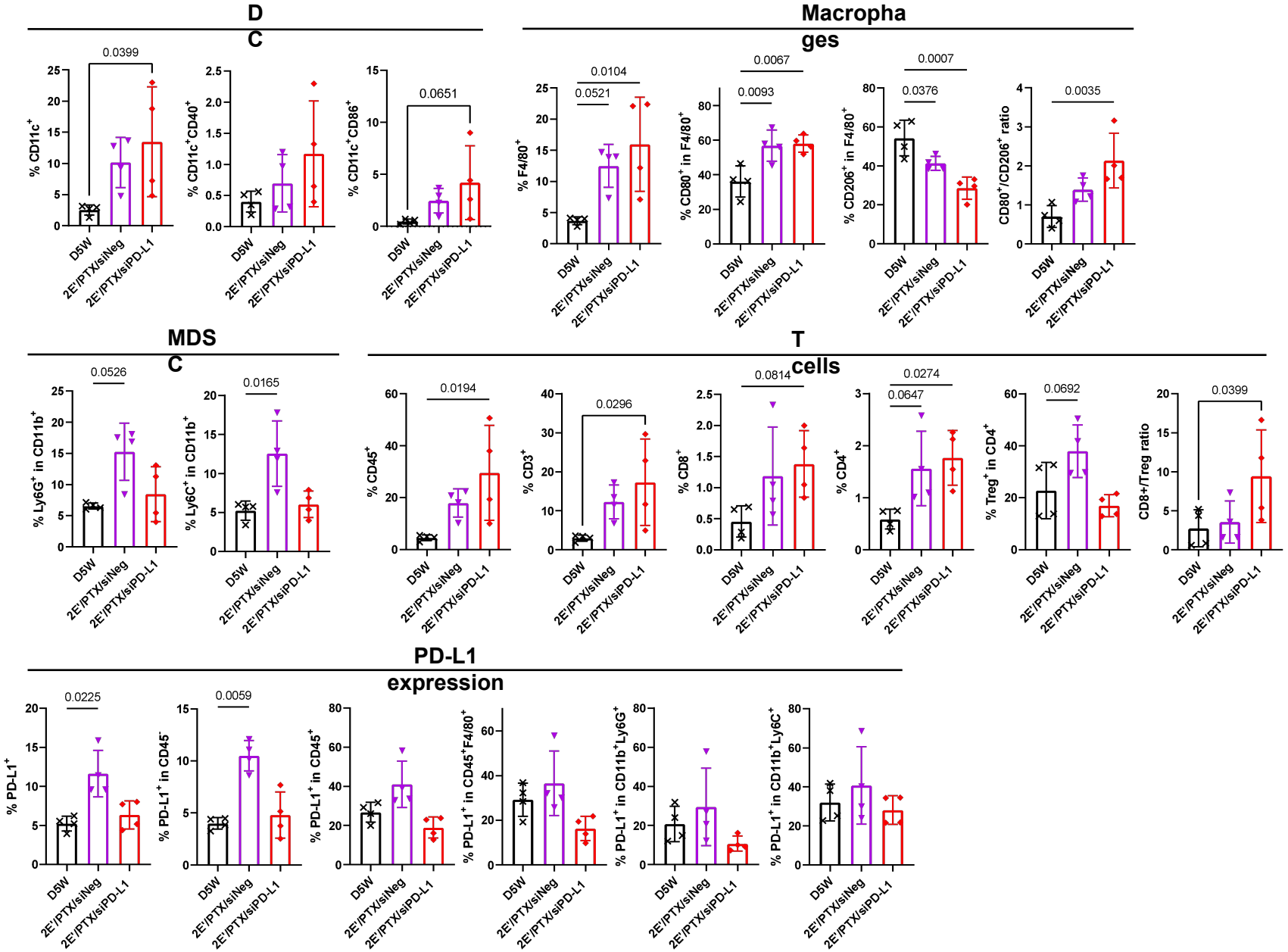


Single intratumoral injection of 2E'/PTX/siPD-L1 induces tumor regression and protects animals from repeated tumor challenge in CT26@Balb/c model.

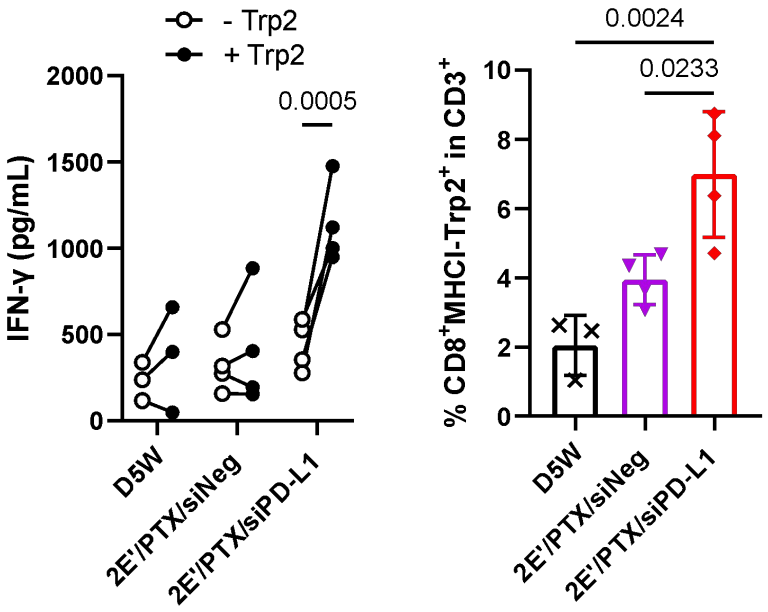


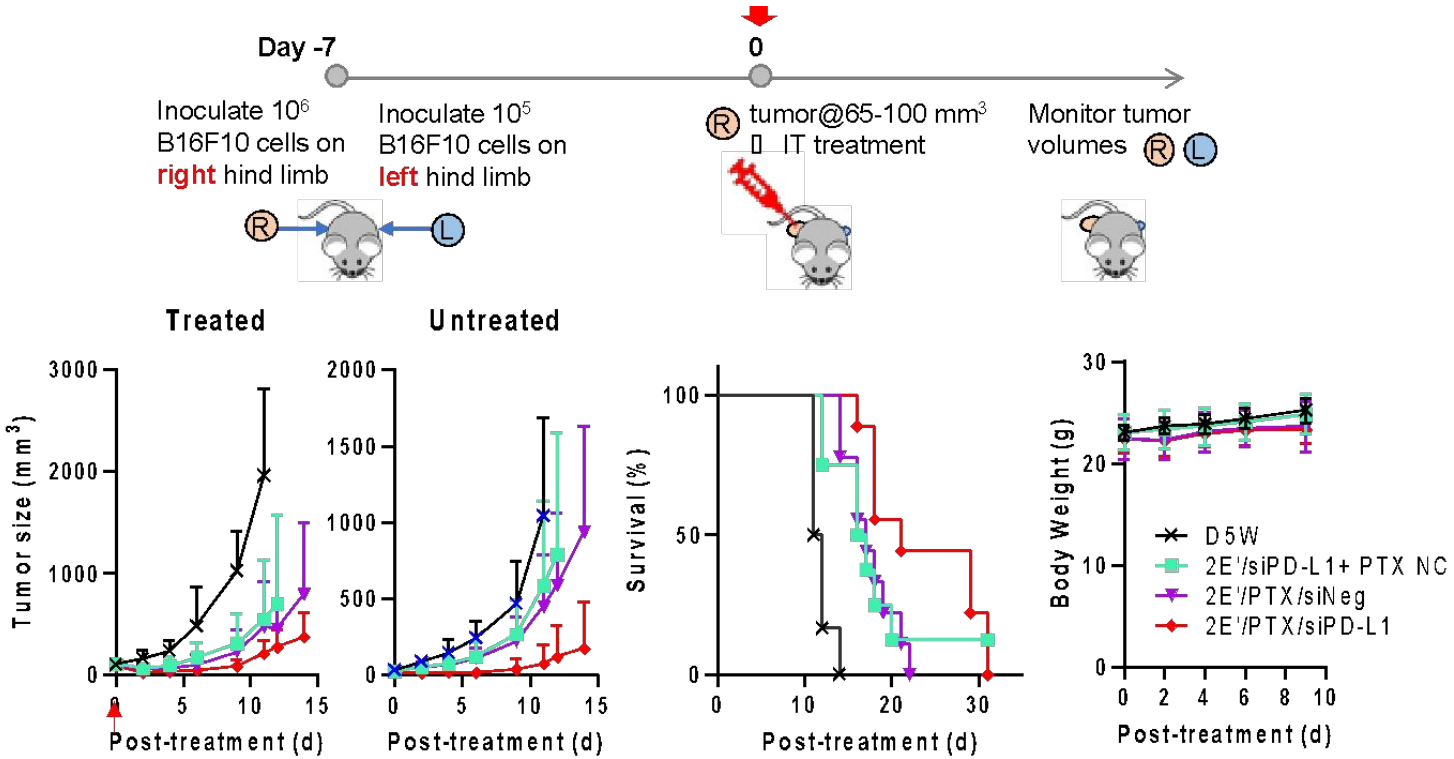


Single intratumoral injection of 2E'/PTX/siPD-L1 induces and maintains immunoactive phenotype in poorly immunogenic B16F10 tumors.

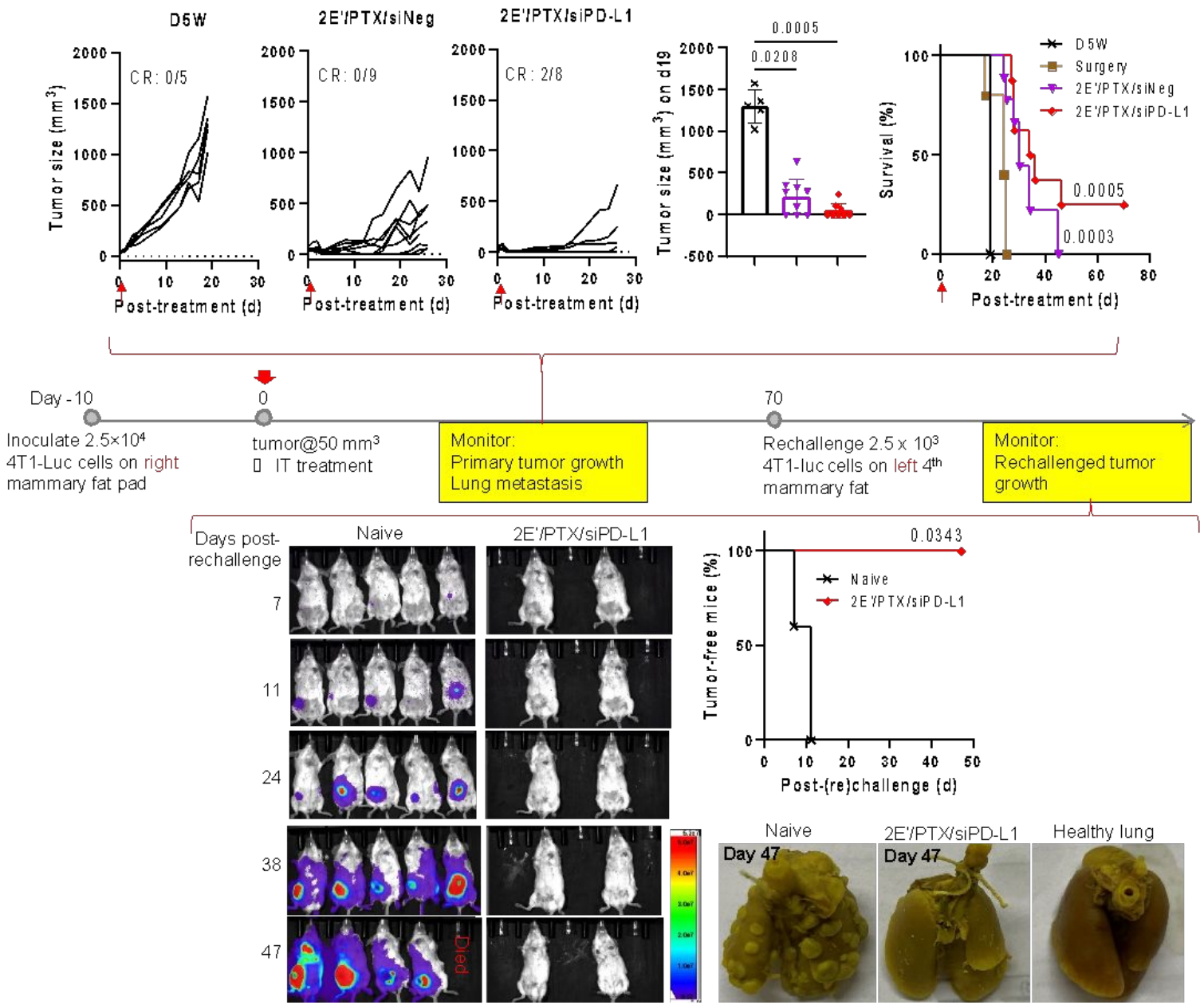


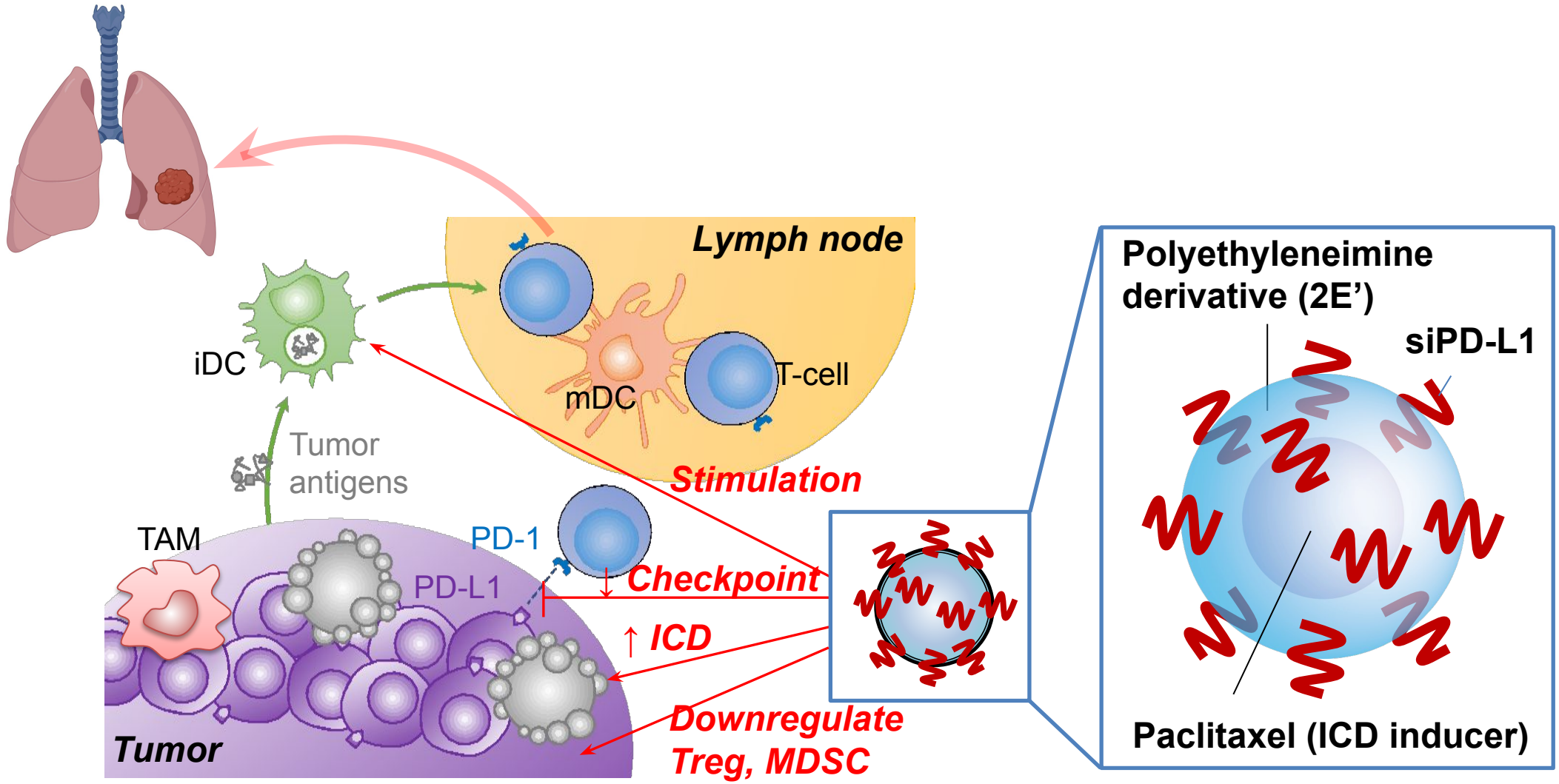
Single intratumoral injection of 2E'/PTX/siPD-L1 primes systemic antitumor immunity in B16F10 tumors.



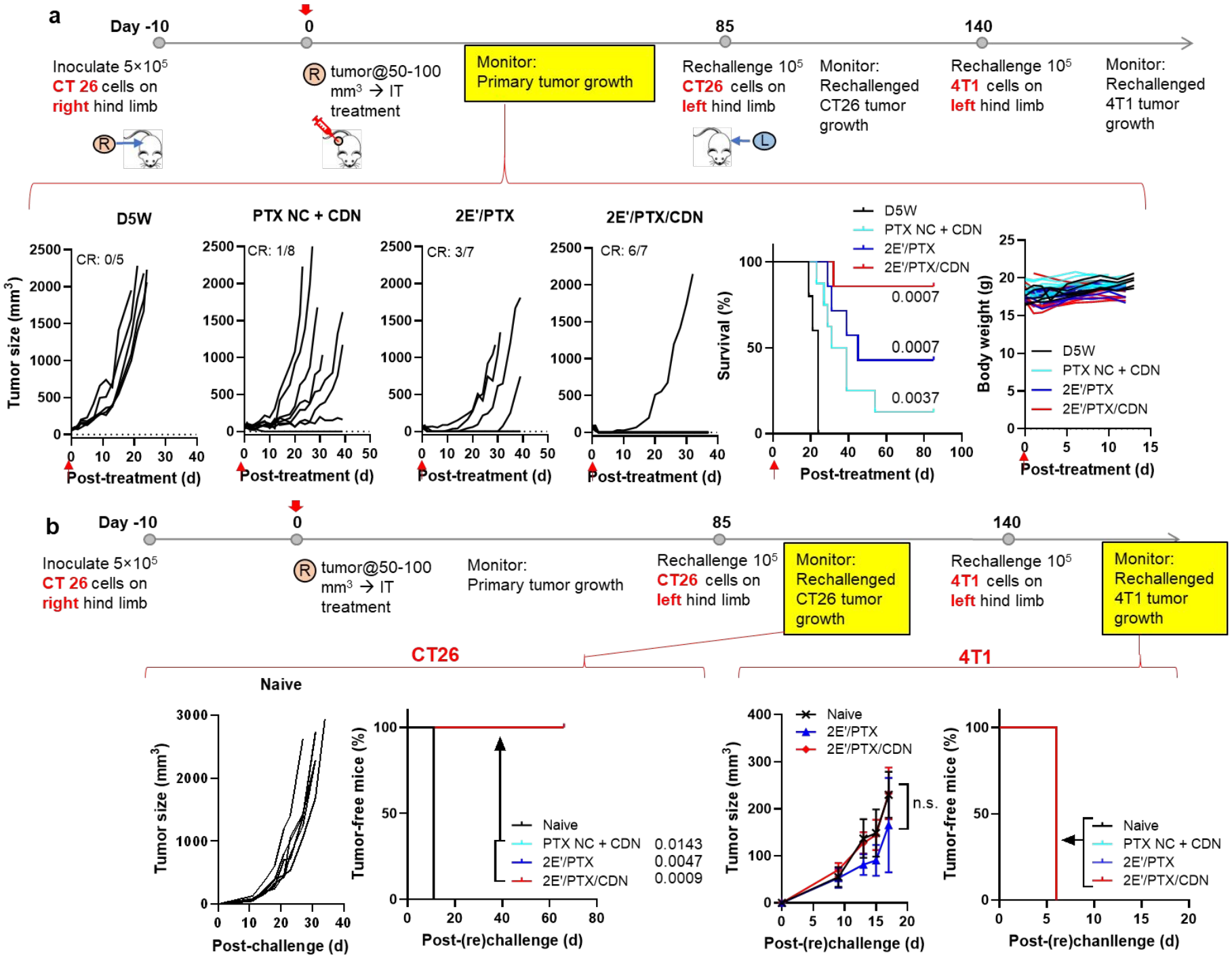


Intratumoral injection of 2E'/PTX/siPD-L1 inhibits growth and metastasis of 4T1 tumors





ICD: Immunogenic cell death


a
Day -10
0
85
140
Inoculate 5×10^5 CT 26 cells on right hind limb
R tumor@50-100 mm^3 → IT treatment
Monitor: Primary tumor growth
Rechallenge 10^5 CT26 cells on left hind limb
Monitor: Rechallenged CT26 tumor growth
Rechallenge 10^5 4T1 cells on left hind limb
Monitor: Rechallenged 4T1 tumor growth
D5W
PTX NC + CDN
2E'/PTX
2E'/PTX/CDN
CR: 0/5
CR: 1/8
CR: 3/7
CR: 6/7
Tumor size (mm^3)
Post-treatment (d)
Survival (%)
0.0007
0.0007
0.0037
Body weight (g)
b
Day -10
0
85
140
Inoculate 5×10^5 CT 26 cells on right hind limb
R tumor@50-100 mm^3 → IT treatment
Monitor: Primary tumor growth
Rechallenge 10^5 CT26 cells on left hind limb
Monitor: Rechallenged CT26 tumor growth
Rechallenge 10^5 4T1 cells on left hind limb
Monitor: Rechallenged 4T1 tumor growth
CT26
4T1
Naive
Tumor size (mm^3)
Post-challenge (d)
Tumor-free mice (%)
Post-(re)challenge (d)
0.0143
0.0047
0.0009
n.s.
Post-(re)chanllenge (d)

Summary

- An immunoactive nanoparticle, 2E’ carrying PTX and siPD-L1, induced immediate regression of established tumors upon a single local administration and enhanced the activation of antitumor immunity, leading to systemic, long-term protection of surviving animals.
- Each component played distinct roles to activate antitumor immunity, with **2E’** activating innate immunity against tumor due to the immunoadjuvant effect and **PTX** inducing immunogenic cell death, which then activates T-cell immunity against tumors. **siPD-L1** contributed to the later step by preventing tumor expression of PD-L1 that would otherwise engage in immune checkpoint interaction and MDSC and Treg recruitment.
- 2E’ provides a simple and versatile platform for local immunotherapy by accommodating combinations of chemotherapeutic drugs and nucleic acids that address multiple events involved in the antitumor immunity.

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Development of immunofunctional nanocarrier for cancer immunotherapy

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Abstract

…cer immunotherapy aims to selectively activate host's immune response against tumors. Immunogenic cell …h (ICD)-inducing chemotherapy can contribute to cancer immunotherapy by increasing the antigenicity of … tumor cells and facilitating the immune recognition of tumor antigens. However, poor drug retention and …cient recruitment of antigen presenting cells (APCs) to the tumor limit the efficacy of ICD inducers. …tic-co-glycolic acid) (PLGA) nanoparticles (NP) were developed to enhance the retention and availability of … at the tumor. Furthermore, PLGA NPs were surface-modified with adenosine triphosphate (ATP), a chemotactic signal to APCs. The ATP-modified PLGA NPs enhanced the recruitment of APCs to the tumors by improving the stability and local availability of ATP. The ATP-modified PLGA NPs, loaded with paclitaxel (ICD inducing chemotherapy), provided the chemoattractant activity and enhanced the antitumor activity of the drug, leading to a significant delay in tumor growth. When combined with immune checkpoint blockade therapy, the paclitaxel-loaded, ATP-modified NPs induced complete tumor regression in 75% of the CT26 tumor-bearing Balb/c mice.

Objective

To develop a carrier of an ICD inducer that enhances the retention and stability of a drug and an APC attractant to activate antitumor immune response

Immunogenic Cell Death

Immunogenic apoptosis of cancer cells can activate dendritic cells (DCs) and tumor-specific T cells to induce effective anti-tumor immune responses.
Endoplasmic reticulum stress and reactive oxygen species production are key attributes of ICD induction.
ICD is mediated by damage-associated molecular patterns (DAMPs)

- Calreticulin (CRT) exposure
- High Motility Group Box 1 (HMGB1) release
- Adenosine triphosphate (ATP) release

Paclitaxel and Carfilzomib

Paclitaxel (PTX)

- Microtubule inhibitor
- Low water solubility (8.5 – 17 μg/mL)
- Cell cycle arrest in G2/M phase by interference with spindle formation
- Indicated for breast, ovarian, pancreatic, and lung cancer as Taxol® or Abraxane®
- In clinical trials as a combination with anti-PD-L1 antibodies

Carfilzomib (CFZ)

- 2nd generation of irreversible proteasome inhibitor
- Low water solubility (0.7-3.6 μg/mL)
- Antiproliferative and proapoptotic activities in tumor cells.
- Indicated for multiple myeloma as Kyprolis®

Validation of ICD inducers

PTX and CFZ induce ICD

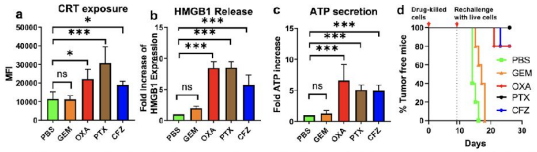
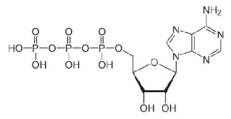


Fig. 1. Release of damage-associated molecular patterns from CT26 cells treated with cytotoxic agents at IC50. Gemcitabine (GEM): non-ICD inducer, negative control; oxaliplatin (OXA): known ICD inducer, positive control. (a) CRT exposure on the cell surface, (b) HMGB1 in the medium, (c) ATP in the medium, (d) percentage of tumor-free mice after the vaccination study.

Immunofunctional Nanoparticle Design

ATP

- Adenine based nucleotide
- MW: 507 g/mol
- Solubility: 50 mg/mL
- Binds to P2Y2 or P2X7 receptors of dendritic cells
- Act as a "find me" signal
- Promote phagocytic clearance of dying cells



ATP is loaded to the surface of polydopamine layer-coated PLGA NP (NP-pD-ATP) to recruit dendritic cells

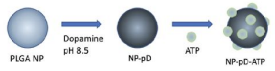


Fig. 2. Schematic illustration of ATP conjugation to PLGA NPs. PLGA-NPs were coated with polydopamine (pD) layer in pH 8.5 to produce PLGA-pD. The amine group of ATP was conjugated to the hydroxyl group of pD.

Immunofunctional Activity of Nanoparticle

NP-pD-ATP recruits dendritic cells. NP conjugation increases the stability of ATP.

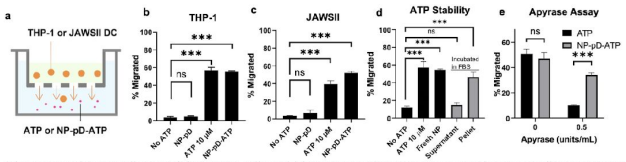


Fig. 3. (a) Diagram of Transwell setup; (b) % THP-1 cells and (c) % JAWSII cells migrating across the Transwell in response to treatments. (d) % JAWSII cells migrating across the Transwell in response to NPs incubated in FBS or the supernatant. ATP, NP-pD-ATP: 10 μM ATP equivalent. Free ATP and fresh NP are tested as references. (e) % JAWSII cells migrating across the Transwell in response to ATP or NP-pD-ATP receiving apyrase challenge.

In vivo evaluation of PTX loaded NP-pD-ATP (PTX@NP-pD-ATP)

PTX@NP-pD-ATP shows greater antitumor effect than a mixture of ATP and PTX@NP-pD in CT26 tumors and B16F10 melanoma in the immunocompetent syngeneic hosts, while the difference is not observed in immunodeficient nude mice

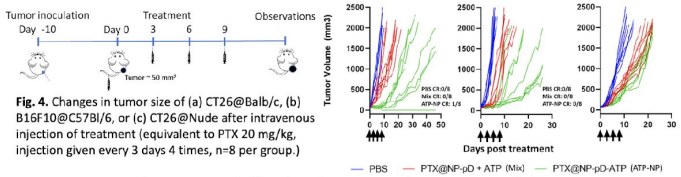


Fig. 4. Changes in tumor size of (a) CT26@Balb/c, (b) B16F10@C57Bl/6, or (c) CT26@Nude after intravenous injection of treatment (equivalent to PTX 20 mg/kg, injection given every 3 days 4 times, n=8 per group.)

PTX@NP-pD-ATP enhances tumor infiltration of DCs, macrophages, CD8⁺ T cells better than the mixture of ATP and PTX@NP-pD, but also increases Treg populations

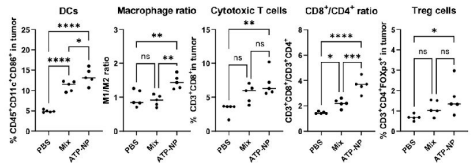


Fig. 5. % Immune cells and the ratio of cells in tumor at 7 days after single intratumoral injection of treatment (equivalent to PTX 5 mg/kg. Mix: a mixture of PTX@NP-pD and ATP; ATP-NP: PTX@NP-pD-ATP)

Combination therapy of PTX@NP-pD-ATP with anti-PD-1 antibodies

PTX@NP-pD-ATP combined with anti-PD-1 antibodies results in complete regression of CT26 tumor in 75% of the treated mice

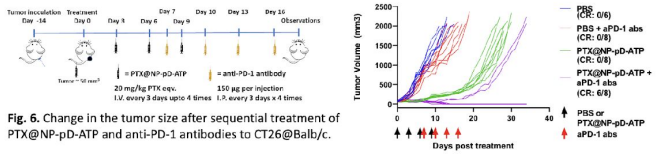


Fig. 6. Change in the tumor size after sequential treatment of PTX@NP-pD-ATP and anti-PD-1 antibodies to CT26@Balb/c.

Summary

- In vitro DAMP screening and in vivo vaccination study support that PTX and CFZ are ICD inducers.
- NP-conjugated ATP retained chemoattractant activity. The conjugated ATP remained conjugated in serum and showed greater stability than free ATP in apyrase.
- PTX@NP-pD-ATP showed greater antitumor effect than a mixture of ATP and PTX@NP-pD, in a manner dependent on an intact immune system.
- Immunophenotyping of CT26 tumors treated with PTX@NP-pD-ATP suggests that the enhanced antitumor activities have been mediated by the increased infiltration of dendritic cells, macrophages, and CD8+ T cells in response to PTX-generated tumor antigens and ATP presented by the NPs.
- Treg population also increased in the PTX@NP-pD-ATP-treated CT26 tumors, which may explain the tumor growth in the later phase.
- PTX@NP-pD-ATP combined with anti-PD-1 antibodies led to complete regression of CT26 tumors by inhibiting Treg interaction with CD8+ T cells.

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Boyang Dong

At the Poster Pub:

- *Wednesday, July 13 @ 7-9 PM*
- *Thursday, July 14 @ 5-6 PM*