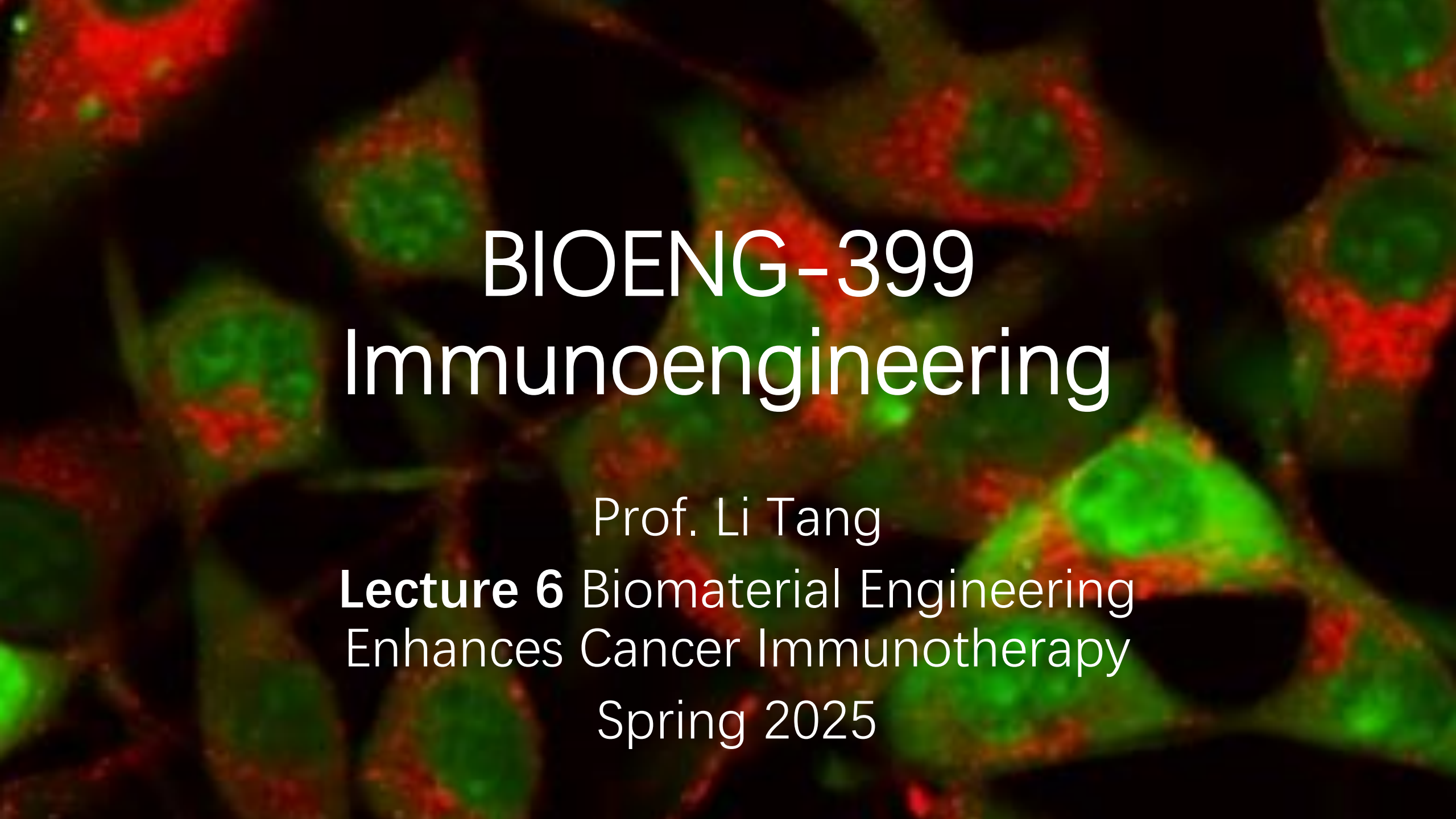


BIOENG-399 Immunoengineering

Prof. Li Tang

Lecture 6 Biomaterial Engineering Enhances Cancer Immunotherapy

Spring 2025

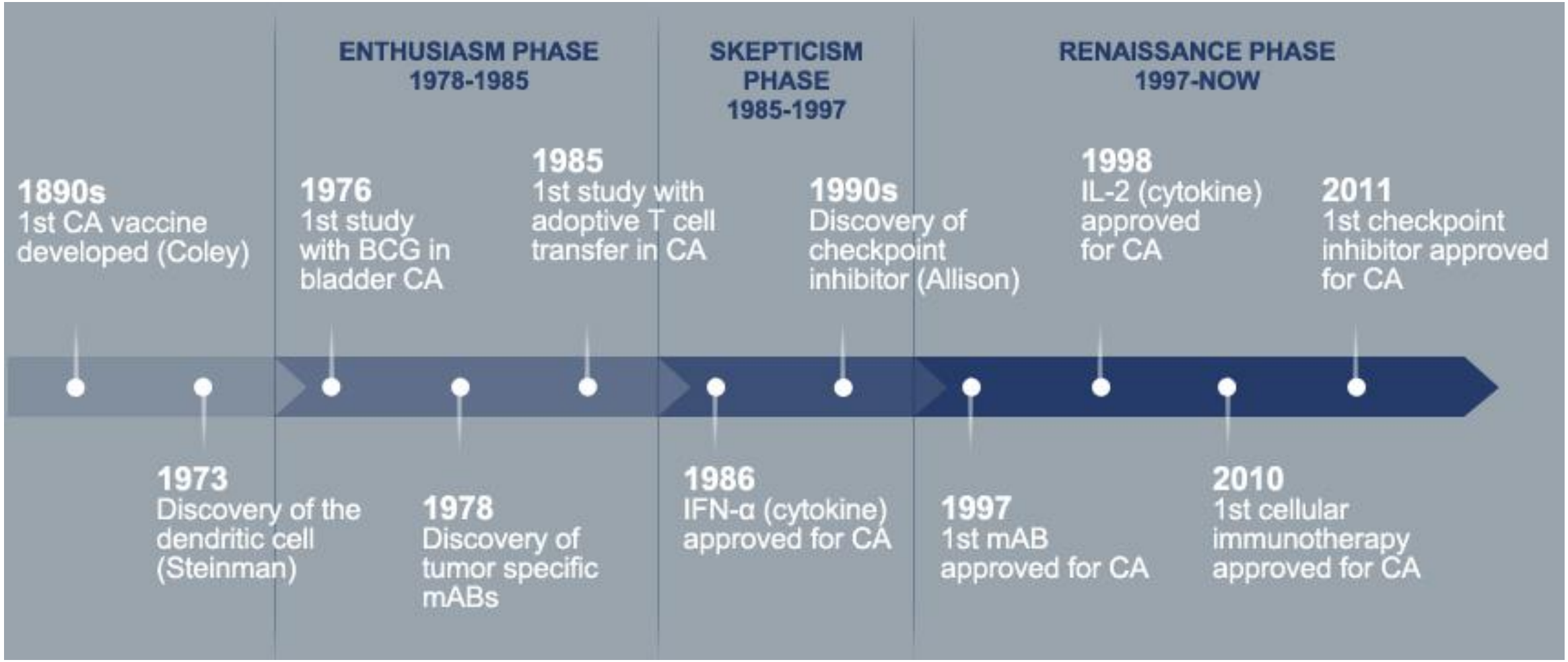
Can biomaterial engineering improve immunotherapies?

The key is drug delivery!

To deliver drug
INTACT
In the
RIGHT AMOUNT
At the
RIGHT PLACE
And at the
RIGHT TIME

https://www.aiche.org/chenected/2014/02/evolution-drug-delivery

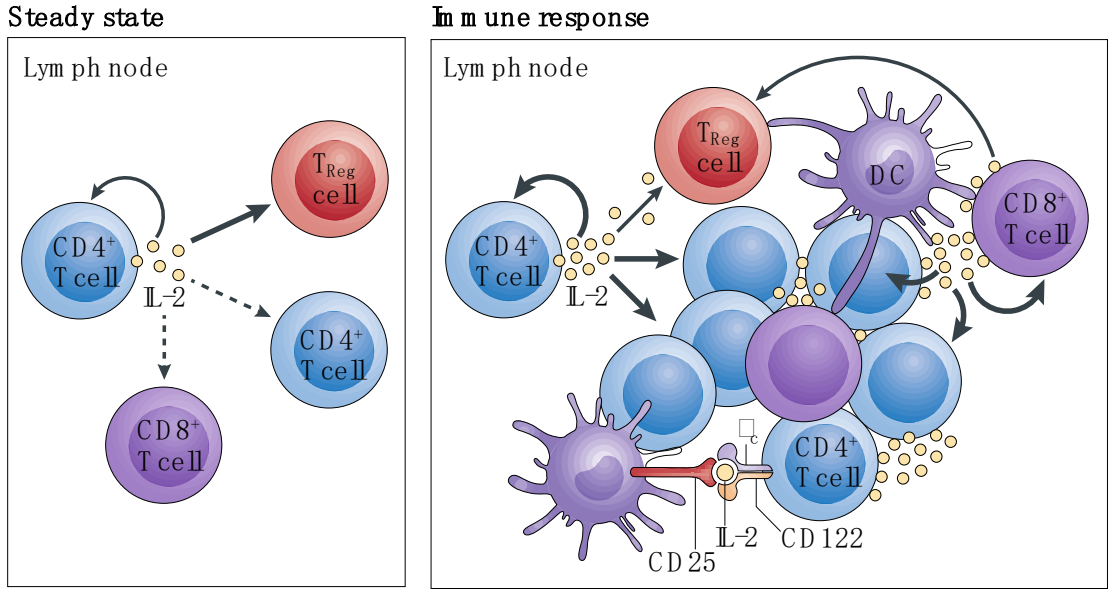
Early attempts: Using immune regulatory signals as immunotherapies



http://www.fightcancerwithimmunotherapy.com/ImmunotherapyAndCancer/StateOfCancerImmunotherapy

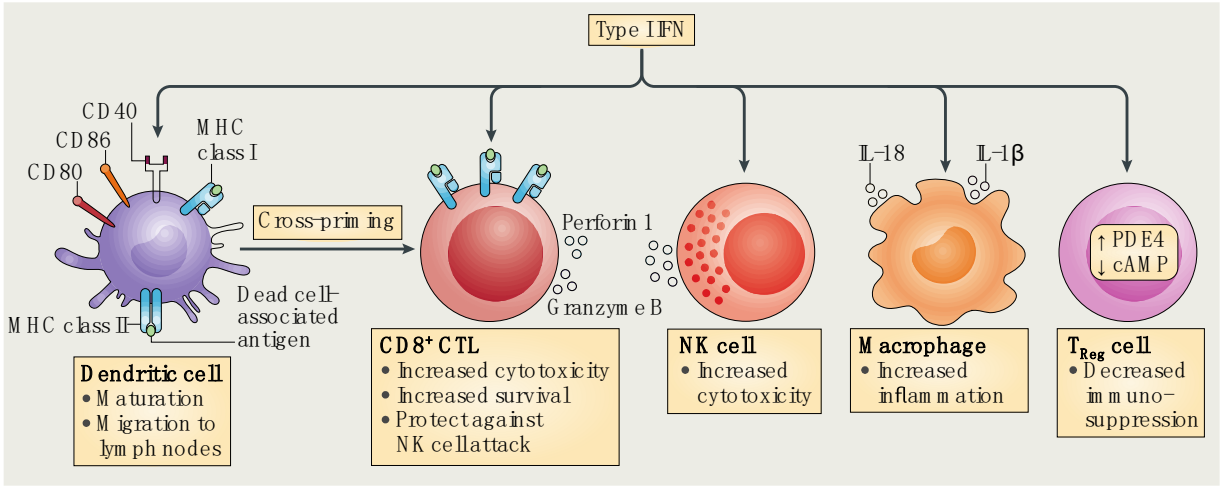
IL-2 and interferon-α: two critical signals for anti-tumor immunity; the first cytokine drugs

IL-2



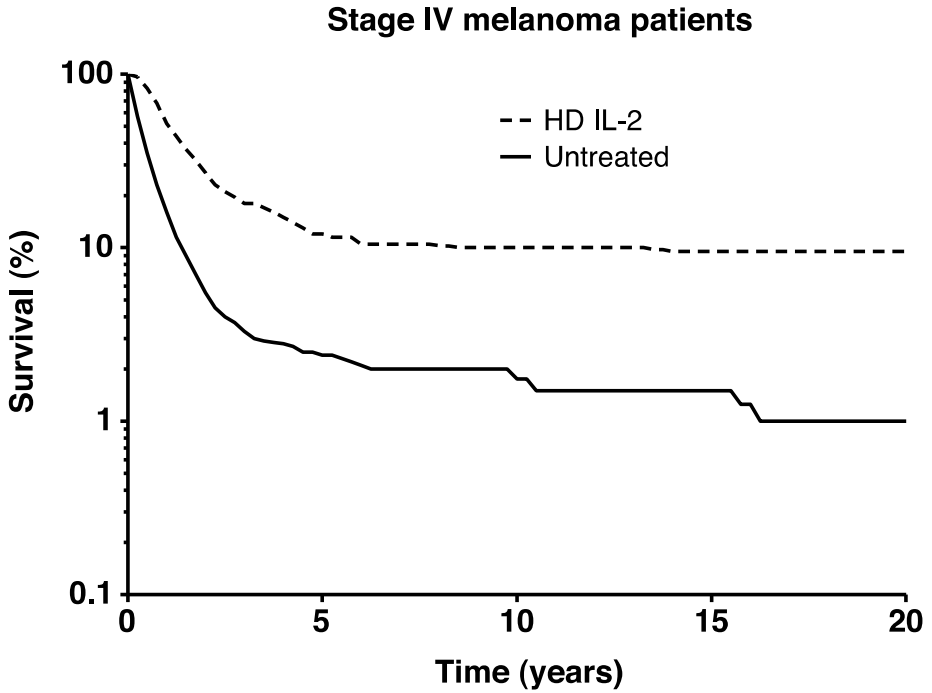
Boyman, O., & Sprent, J. (2012) Nature Reviews Immunology, 12(3), 180–190. http://doi.org/10.1038/nri3156

IFN-α



Zitvogel, L., Galluzzi, L., Kepp, O., Smyth, M. J., & Kroemer, G. (2015) Nature Reviews Immunology, 15(7), 405–414.

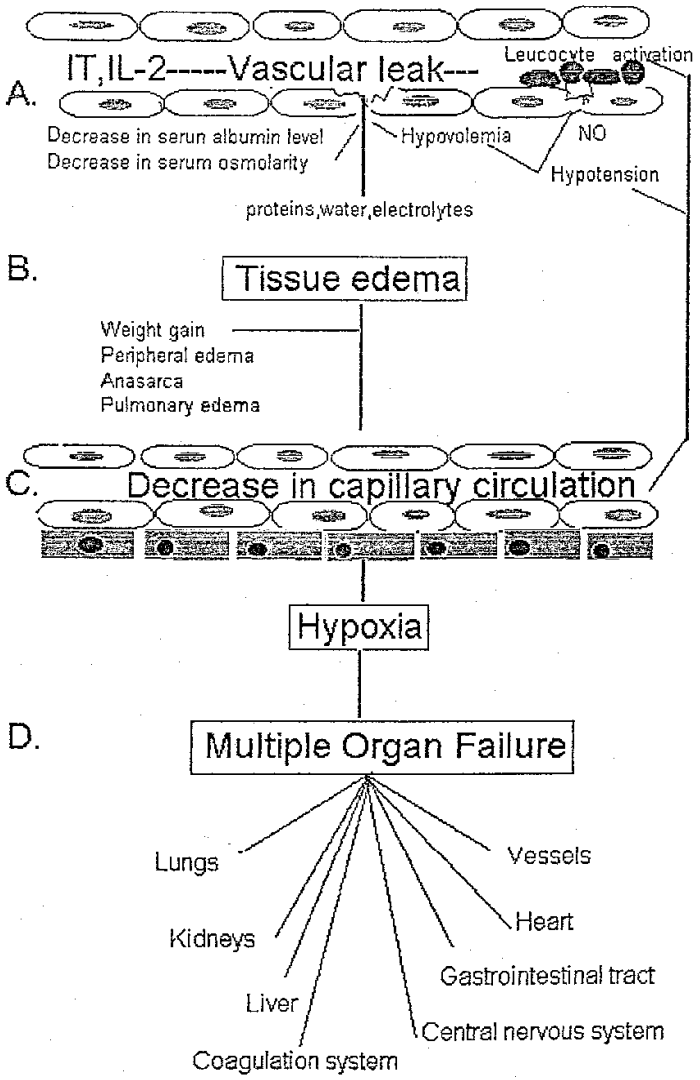
The good and bad of systemic cytokine administration



- "high dose" IL-2 promotes long term complete responses in 5-10% of melanoma and renal cell carcinoma patients
- Accompanied by serious toxicities
- Known to expand regulatory T-cells

http://doi.org/10.1016/j.cbpa.2014.09.006

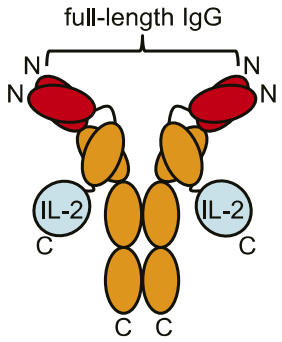
vascular leak syndrome (VLS)



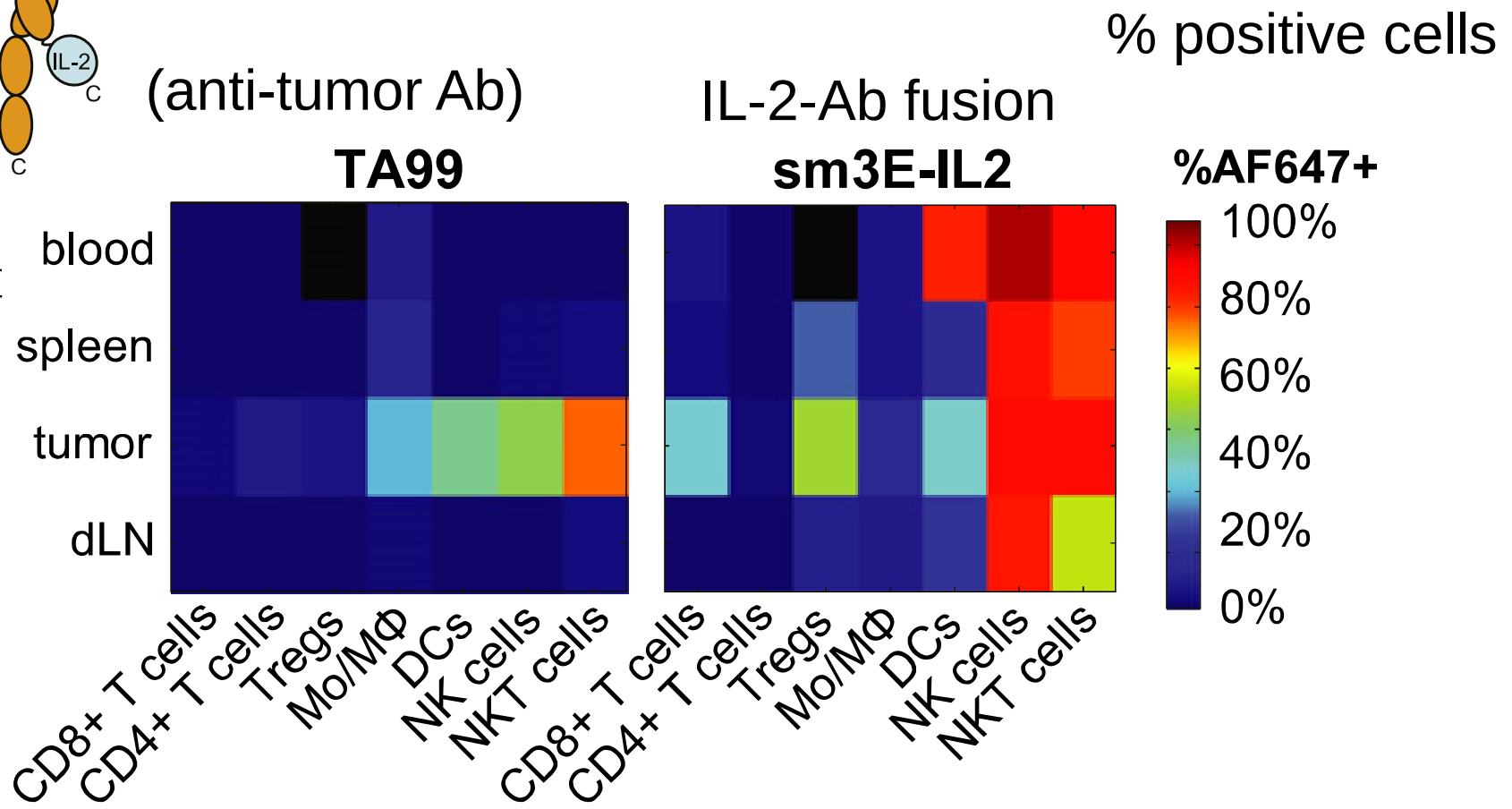
Tissue edema caused by vascular leak results in weight gain, peripheral edema, anasarca or pulmonary edema

Baluna, R., & Vitetta, E. S. (1997). Immunopharmacology, 37(2-3), 117–132.

How about using tumor-targeted antibody?



- Anti-TA99 Ab targets tumor well as expected
- However, IL-2-Ab fusion protein behaved very different from the Ab alone
- Likely, the fusion IL-2 still target IL-2 receptors (dominates!)
- Systemic cytokines find systemic leukocytes



Tzeng, A., Kwan, B. H., Opel, C. F., Navaratna, T., & Wittrup, K. D. (2015). Proceedings of the National Academy of Sciences, 112(11), 3320–3325.

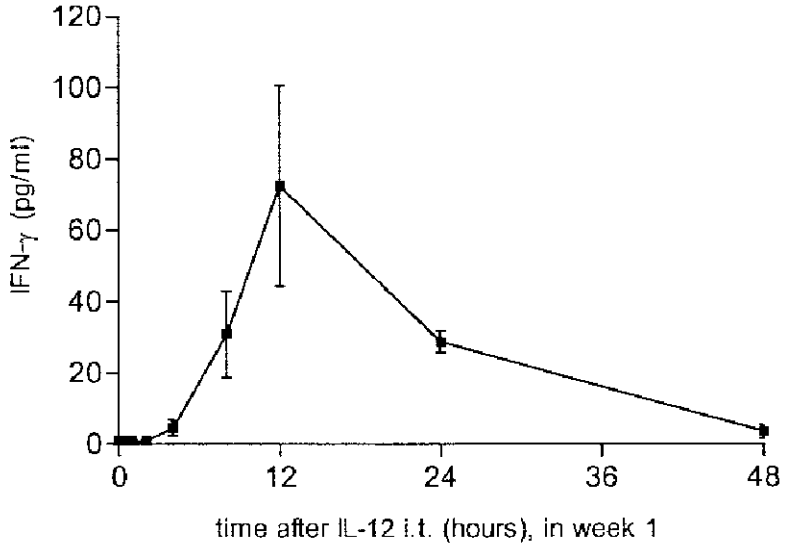
Does intra-tumoral injection help?

local injections ≠ local retention

https://theodora.com/rodent_laboratory/injections.html

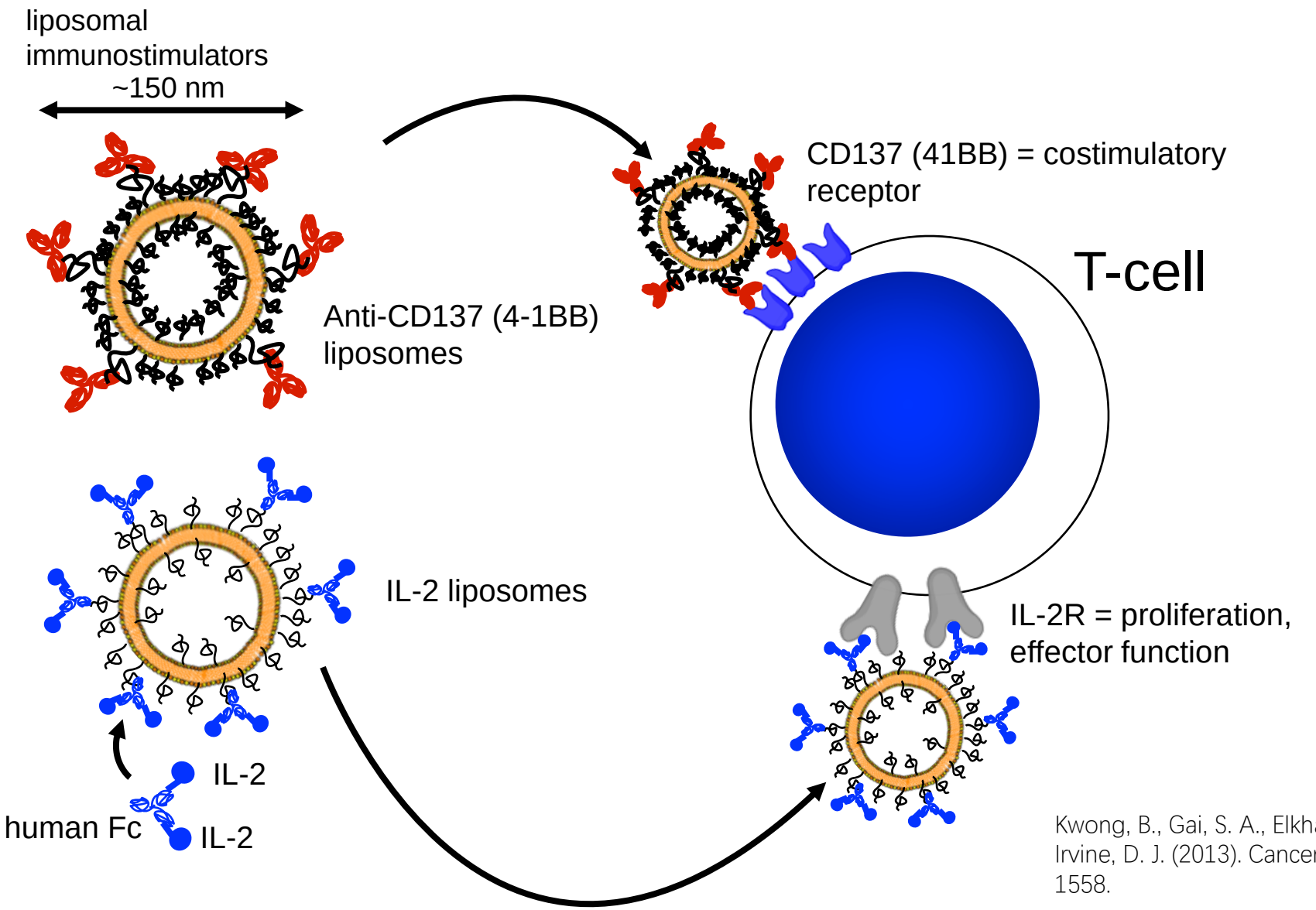
In humans with head and neck cancer:
PK parameters for intratumoral IL-12:

	100 ng/kg	
	Week 1 (n = 3)	Week 6 (n = 2)
C_{max} (pg/ml)	362 (74.7)	763 (62.2)
T_{max} (h)	3 (1.4)	0.5 (0)
$t_{1/2}$ (h)	6.8 (0.1)	5.1 (0.4)
AUC (pg × h/ml)	4361 (1199)	6045 (2068)



van Herpen, Huijbens, R., Looman, M., & de Vries, J. (2003). Clin. Cancer Res.

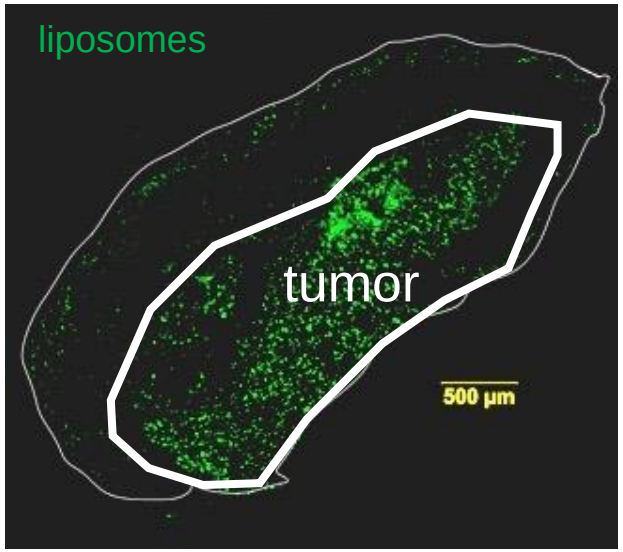
A possible solution: Nanoparticle (NP) immune agonists



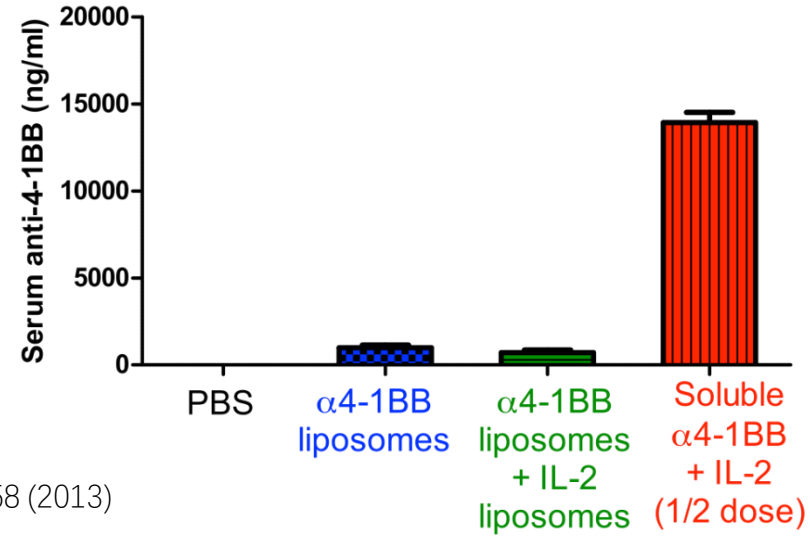
Kwong, B., Gai, S. A., Elkhader, J., Wittrup, K. D., & Irvine, D. J. (2013). Cancer Research, 73(5), 1547–1558.

Intratumoral NP injection confines immunostimulators to the tumor and draining lymph nodes

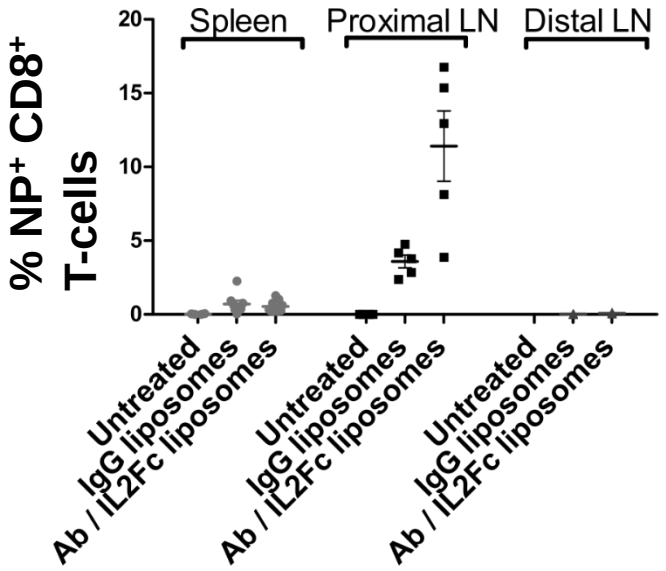
Anti-CD137 and IL-2Fc immunoliposomes are locally confined following intratumoral administration, minimizing systemic exposure.



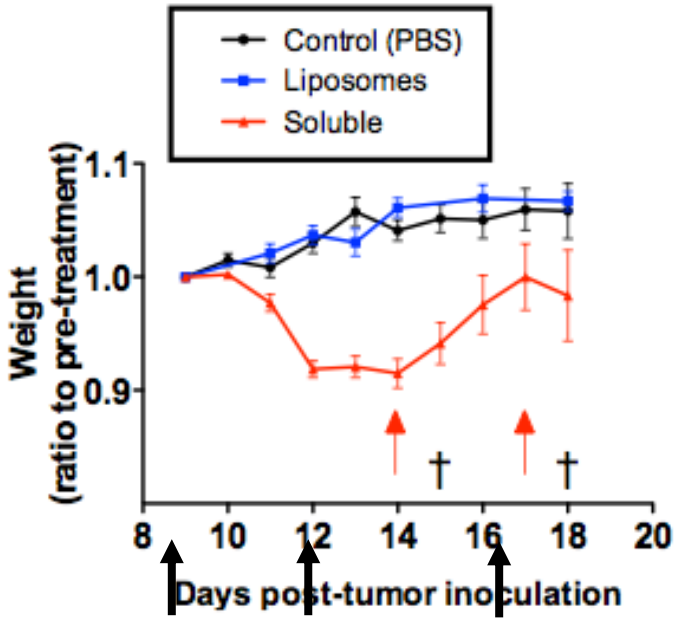
Anti-4-1BB serum level at 24h p.i.



Cancer Res. 73, 1547-1558 (2013)

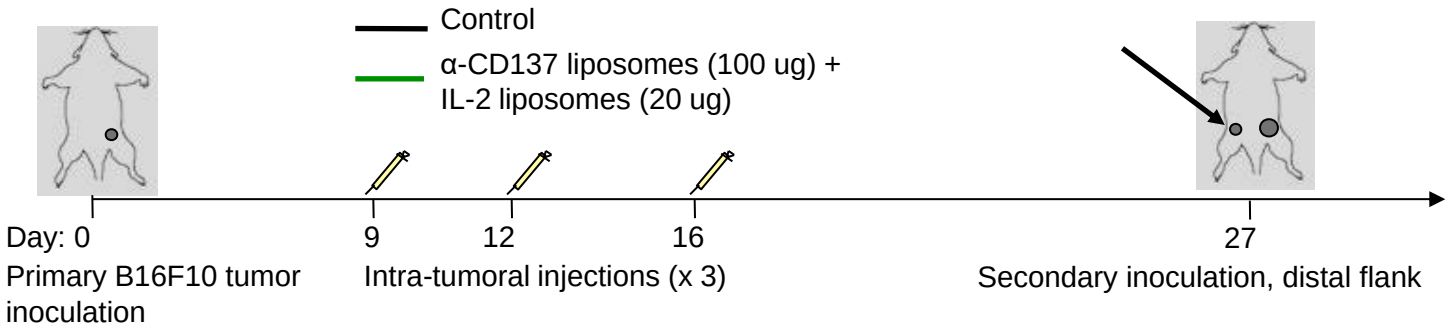


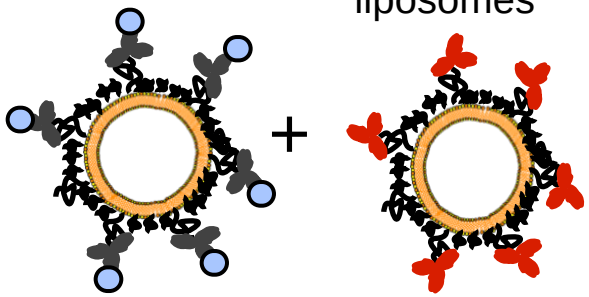
Immunoliposomes also bound to 5% to 15% of CD8 T cells in the proximal tumor draining LNs but were not detectable in spleens or distal lymph nodes following intratumoral injections, **confirming that lymphatic drainage of liposomes was confined to the treatment-proximal LN.**

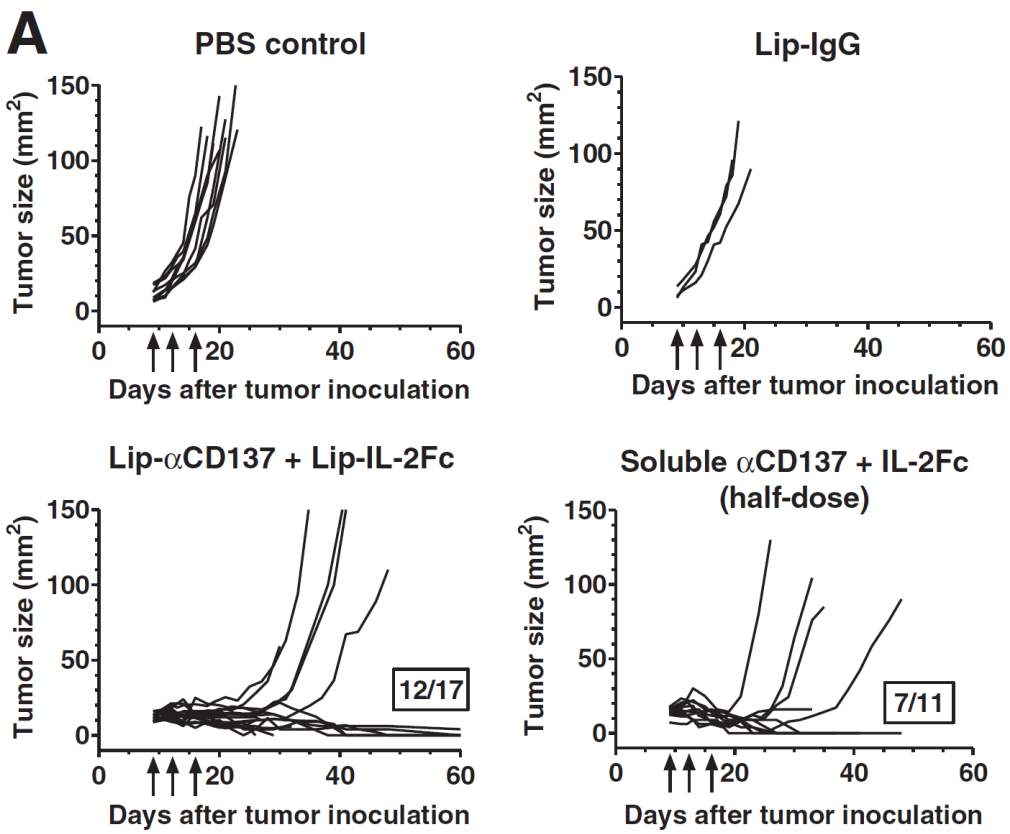


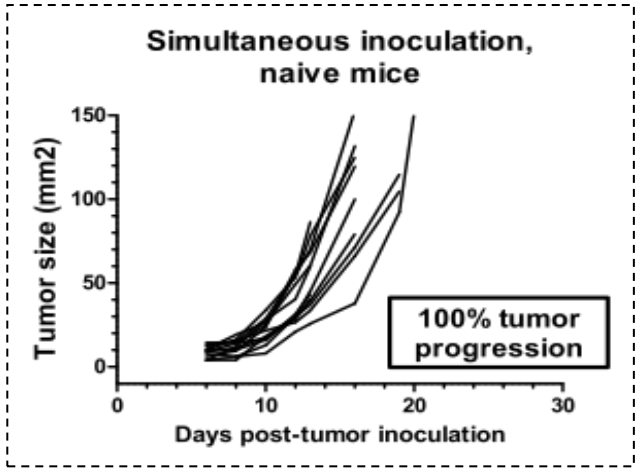
25% of animals treated with soluble IL-2-Fc + anti-CD137 died of treatment toxicity

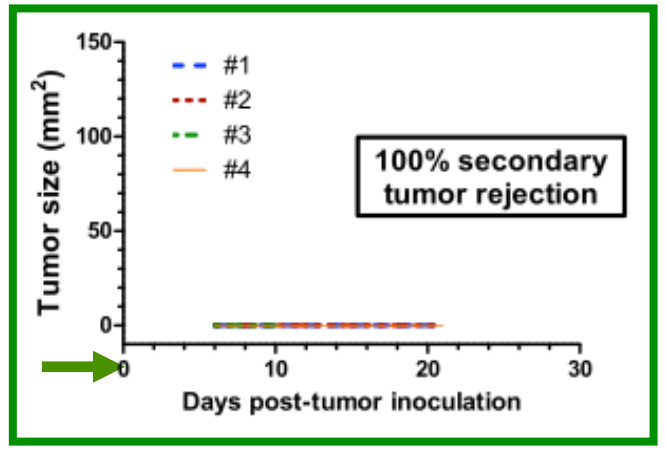
Intratumoral NP injection showed enhanced efficacy against cancer











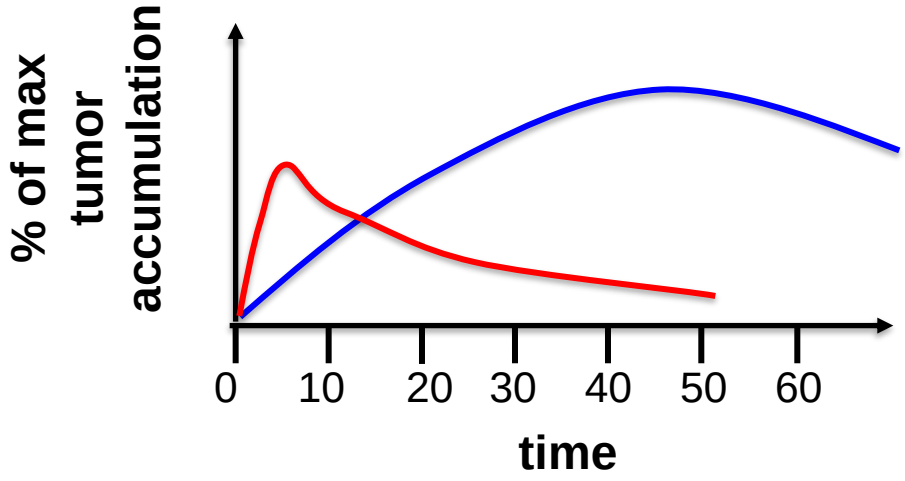
systemic memory established in protected animals

Cancer Res. 73, 1547-1558 (2013)

Tumor-localized immune agonists can be effective and safe. How do we then target immune agonists to metastatic tumors?

- Passive targeting
  - ➢ Enhanced permeation and retention (EPR) effect in tumors
- Active targeting
  - ➢ Antibody-based targeting

EPR effect is driven by size and circulation time



antibody

nanoparticles

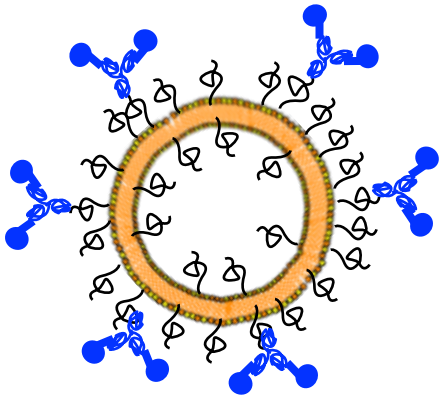
IL-2

Rapid clearance from blood, poor tumor accumulation

IL-2-Fc fusion

or

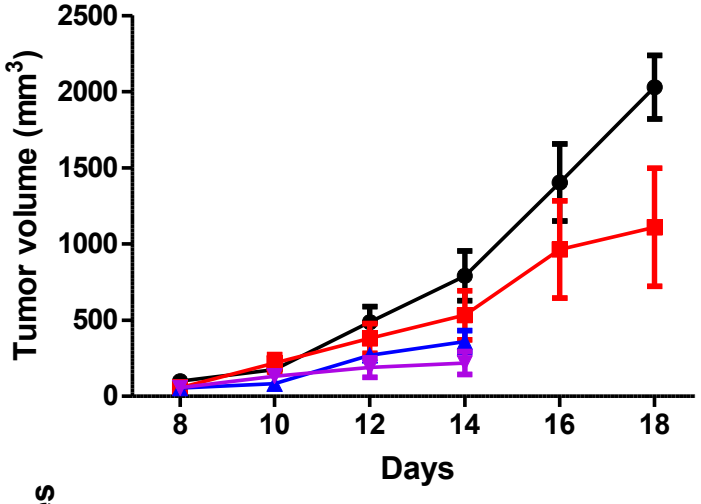
IL-2-Fc liposomes

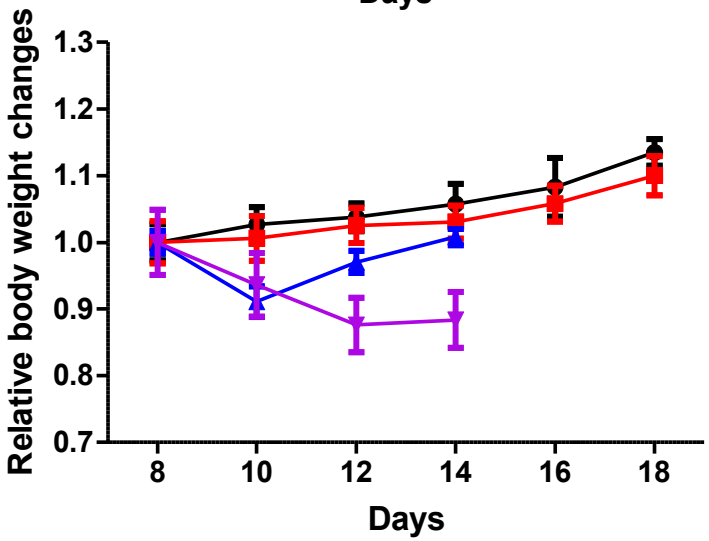


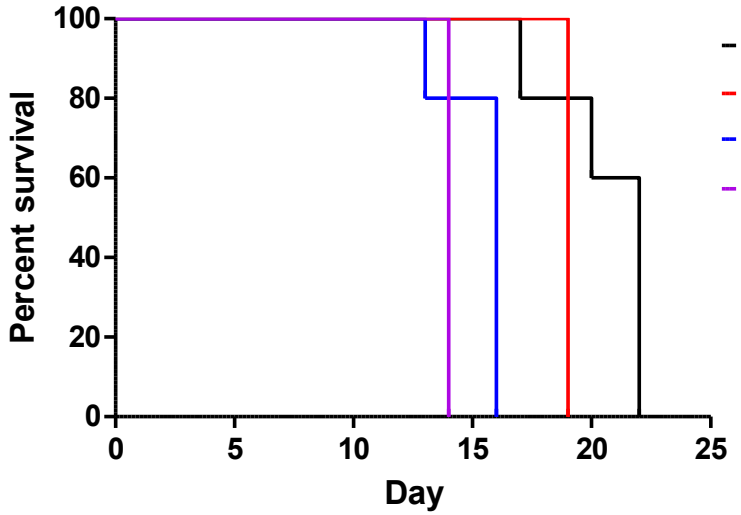
Strategy 1: long half-life antibody-sized agonists for EPR-based accumulation

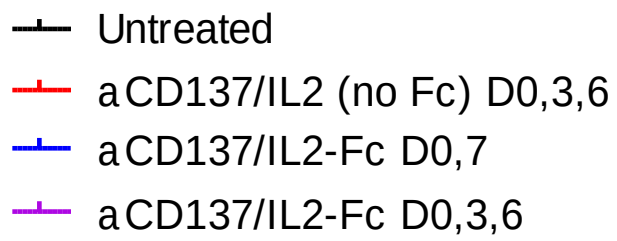
IL-2-Fc fusion

Anti-CD137 and IL-2-Fc combination immunotherapy effectively arrests tumor growth, but elicits lethal in vivo toxicity.





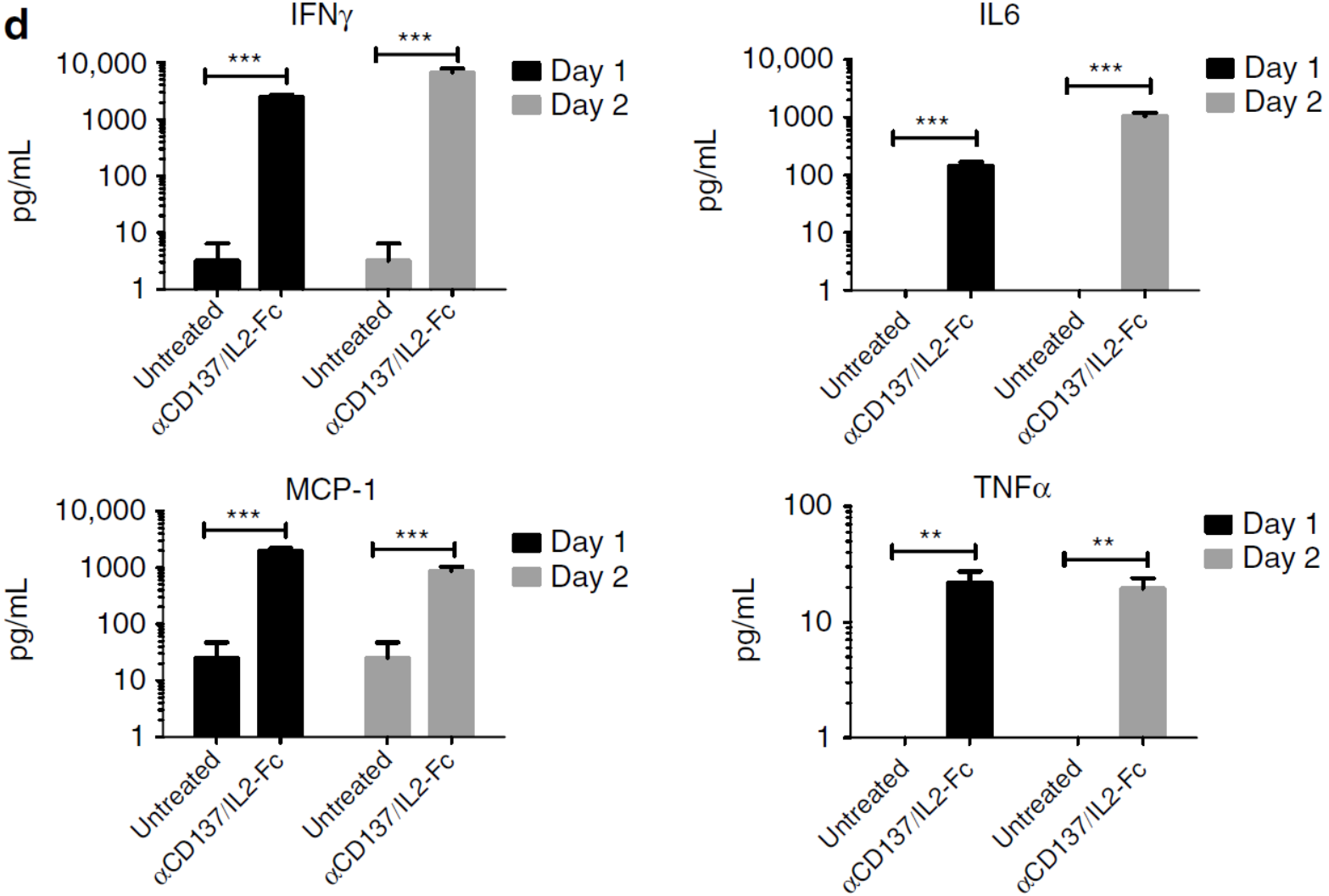




Nature Communications, volume 9, Article number: 6(2018)
doi:10.1038/s41467-017-02251-3

Hypothesis: toxicity of cytokines is caused by stimulation of circulating immune cells (and possibly endothelial cells)
How to test this hypothesis?

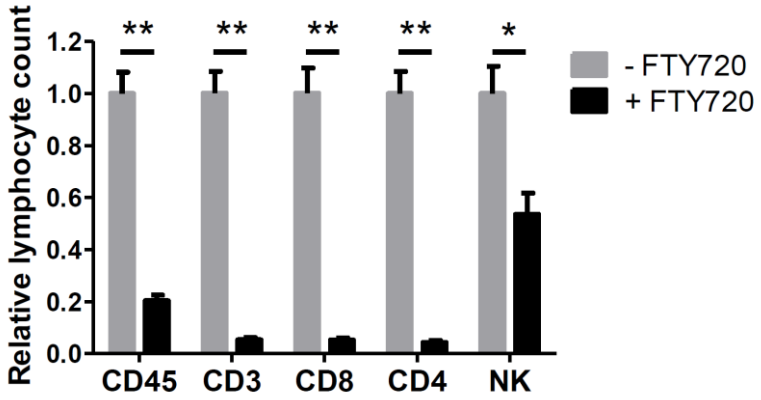
B16F10 tumor-bearing mice received i.v. injections of αCD137 + IL-2-Fc. One and 2 days later, sera from peripheral blood were collected, and **inflammatory cytokine and chemokine levels in sera were measured** by luminex cytokine assays. $^{**}p < 0.005$, $^{***}p < 0.0005$.



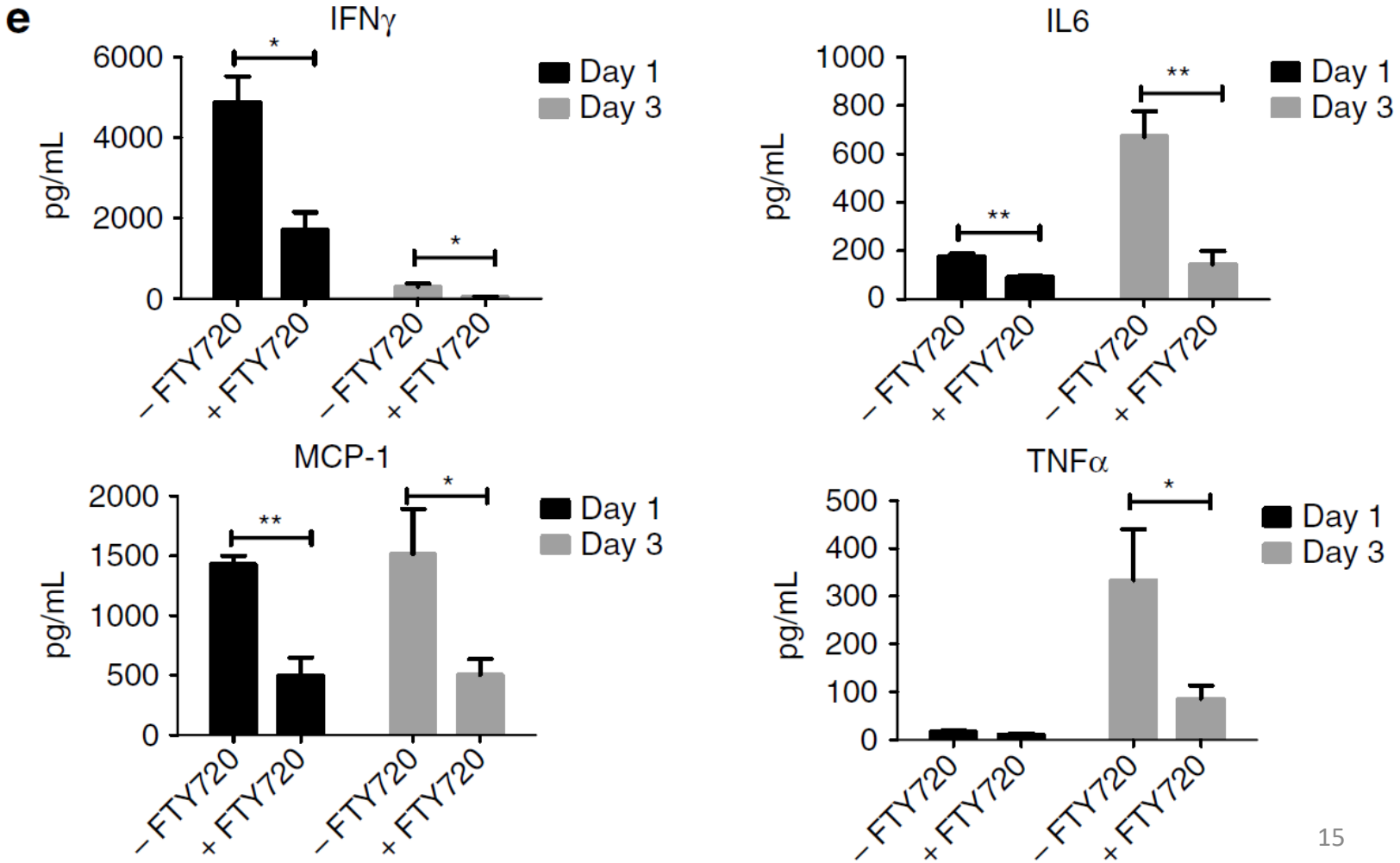
Hypothesis: toxicity of cytokines is caused by stimulation of circulating immune cells (and possibly endothelial cells)
How to test this hypothesis?

This part is not required.

FTY720 to deplete circulating lymphocytes

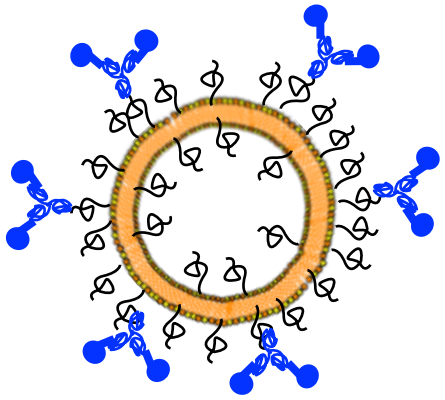


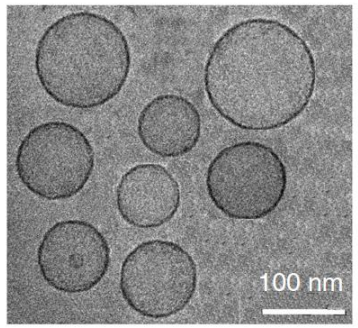
FTY720, a sphingosine-1-phosphate analog that depletes both T-cells and NK cells from the peripheral blood by blocking their egress from lymph node



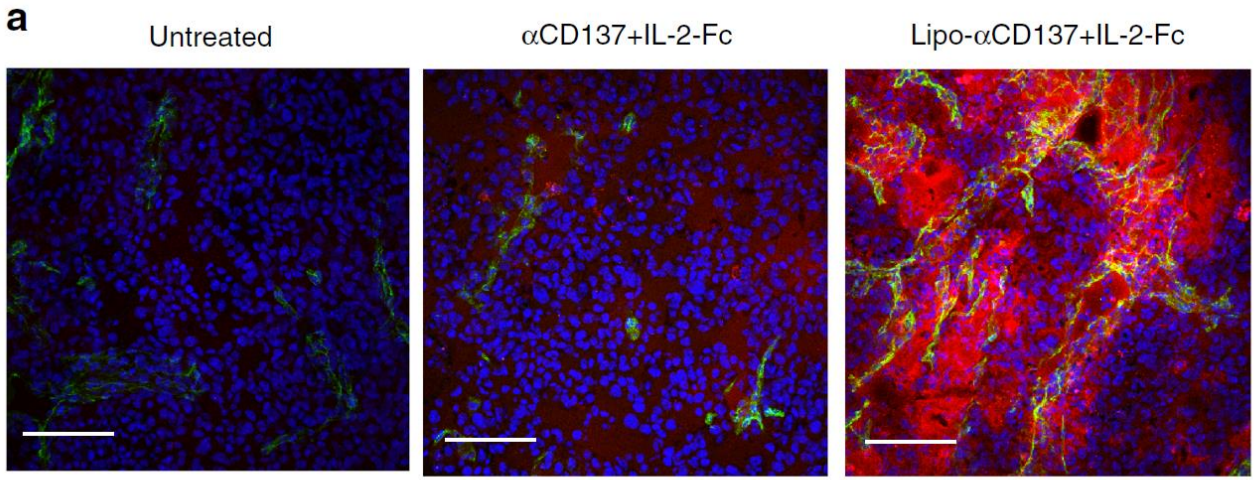
Strategy 2: nanoparticle-agonists for EPR-based accumulation (systemic delivery)

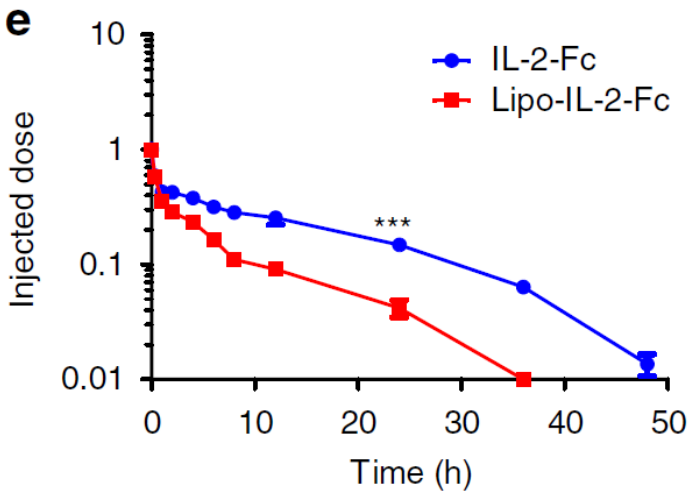
IL-2-Fc liposomes



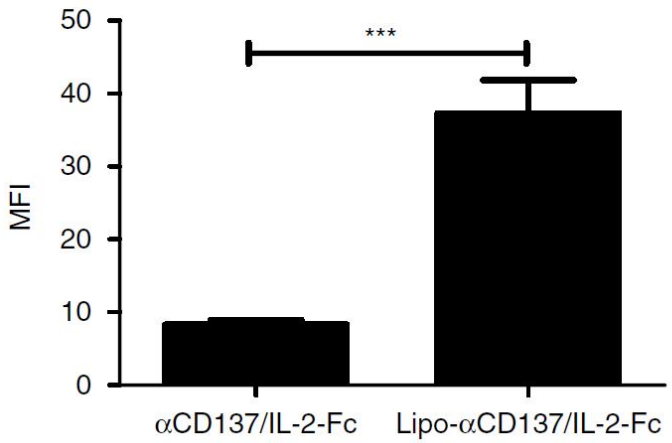


Tumor tissue



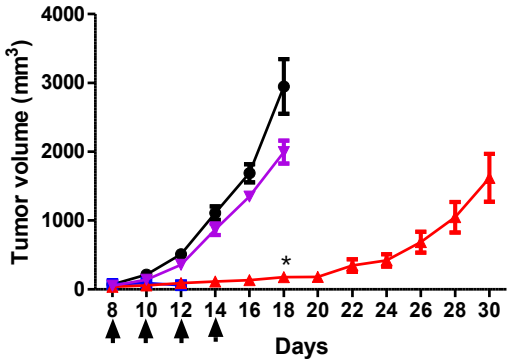


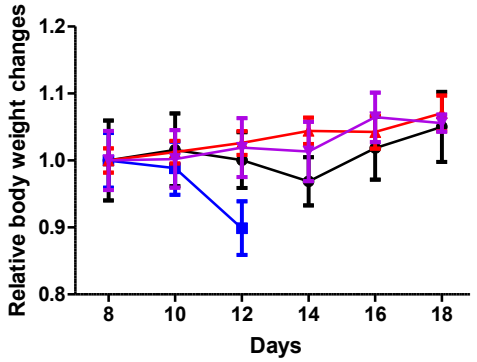
Pharmacokinetics of the labeled proteins were followed over time in the blood

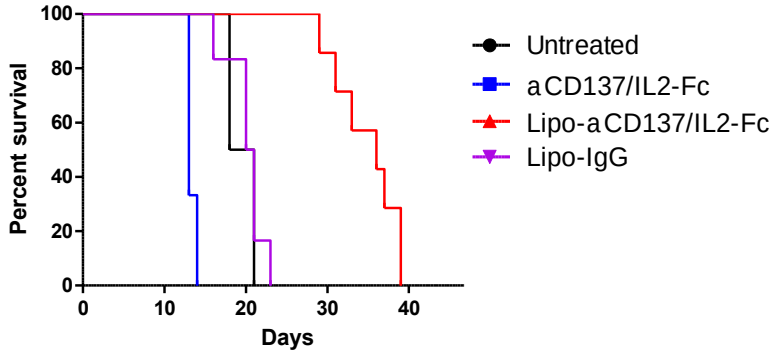


Liposome-anchored anti-CD137 and IL-2-Fc rapidly accumulate in tumors. a, **b** Groups of C57Bl/6 mice (n = 3/group) were inoculated with 5×10^5 B16F10 tumor cells on day 0, and received i.v. injections of **Alexa-568-labeled αCD137 and IL-2-Fc**, Lipo-αCD137 + Lipo-IL2-Fc, IL-2-Fc alone, or Lipo-IL-2-Fc alone on day 10. One hour later, tumors were collected, and frozen sections were stained for CD31 and DAPI and imaged by confocal microscopy.

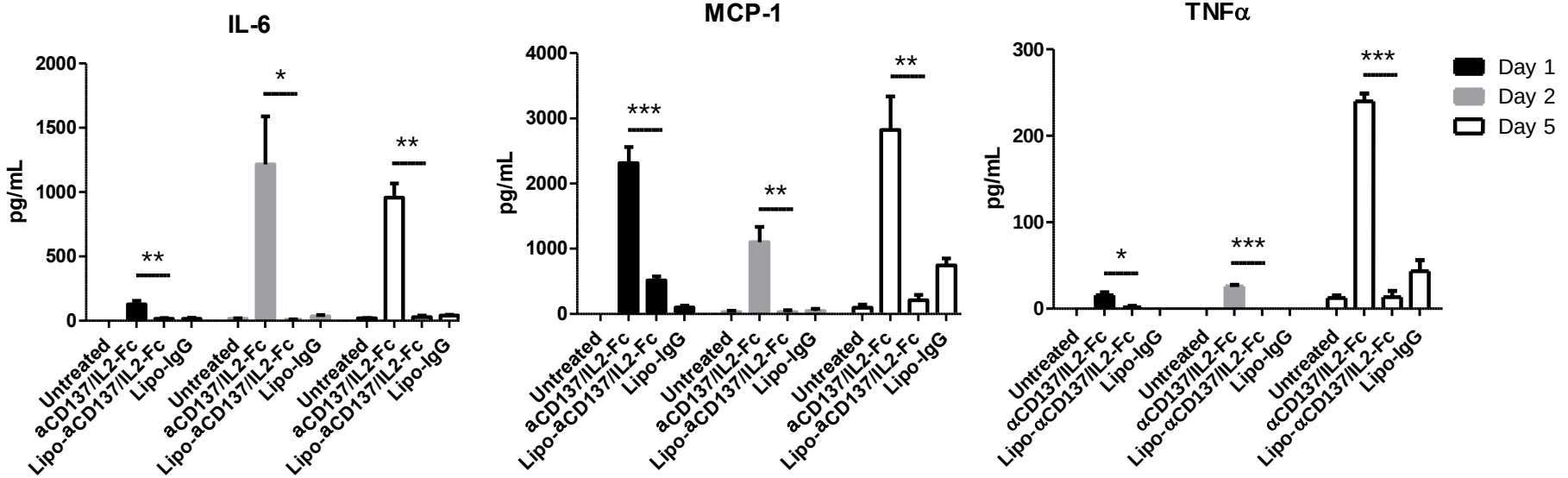
Strategy 2: nanoparticle-agonists for EPR-based accumulation (systemic delivery)







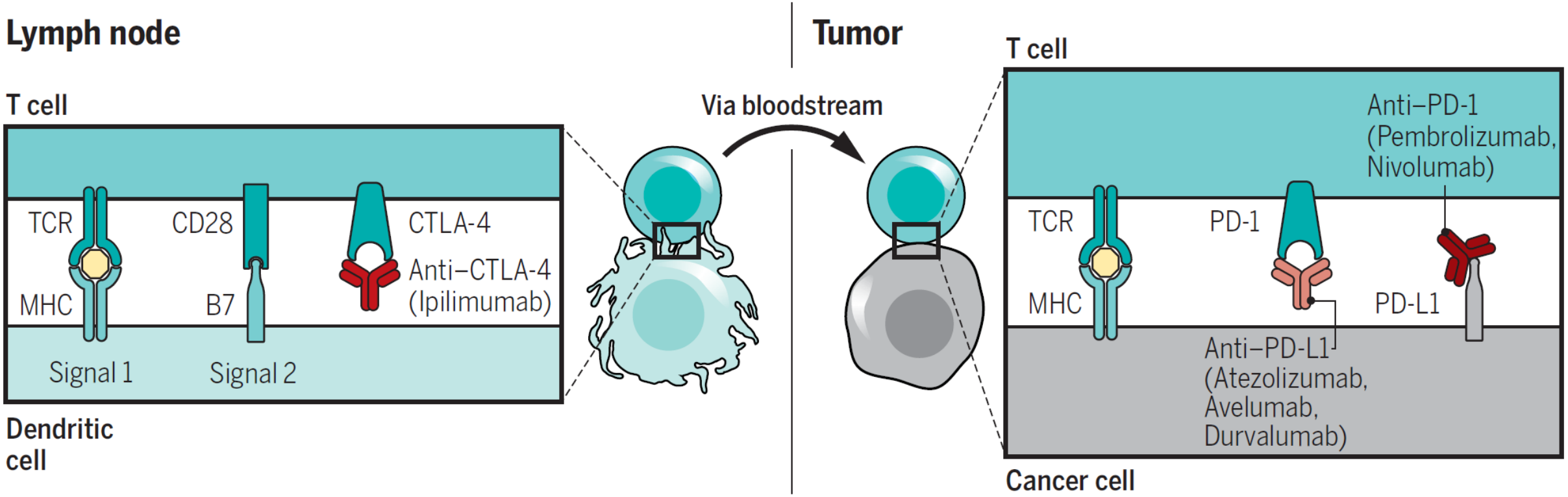
Immunoliposome IL-2-Fc/αCD137 therapy inhibits melanoma tumor growth and prolongs survival without toxicity.



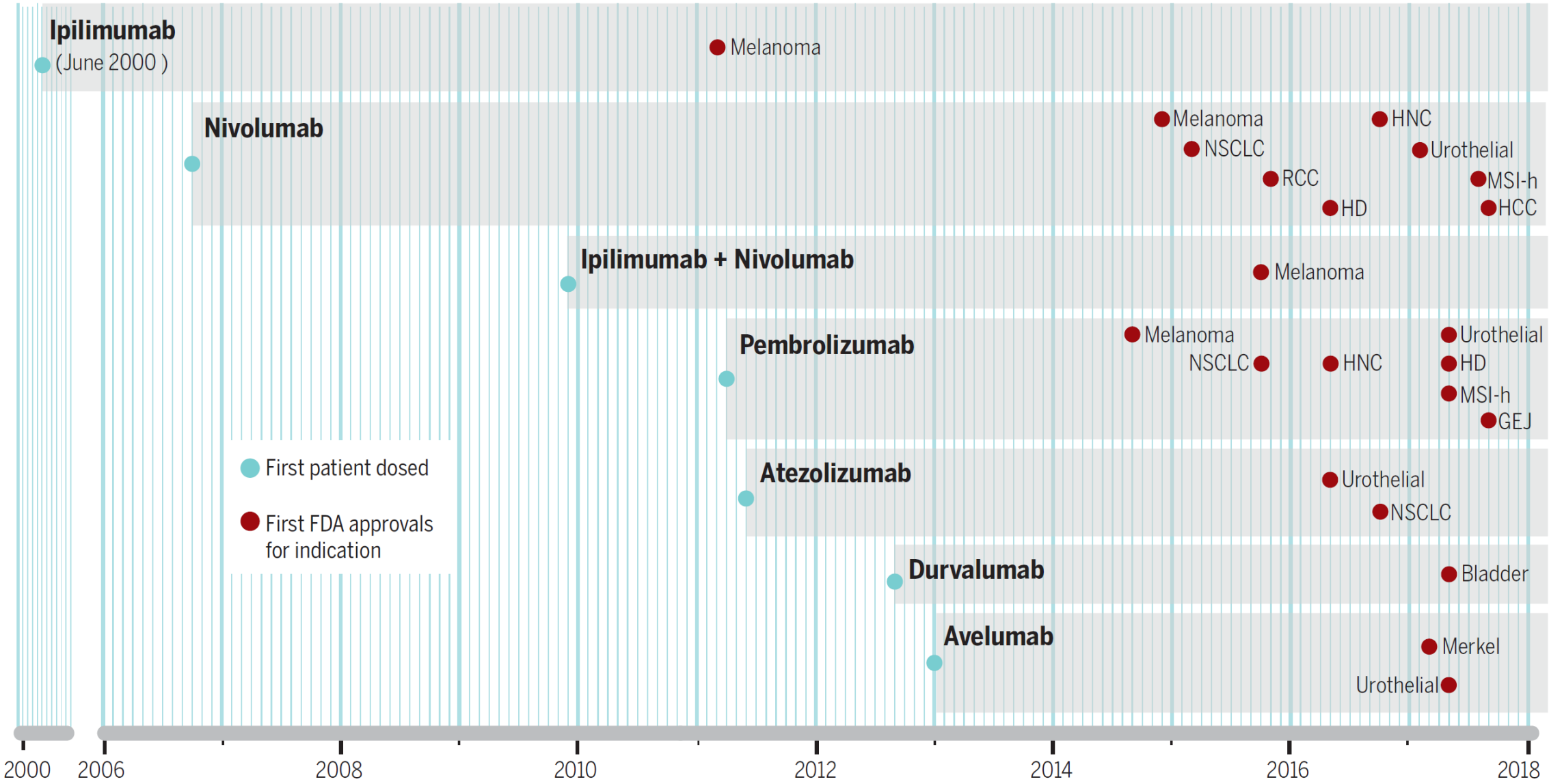
On days 9, 10, and 13, sera were collected from peripheral blood and analyzed for levels of cytokines and chemokines by luminex assays.

Nature Communicationsvolume 9, Article number: 6(2018)
doi:10.1038/s41467-017-02251-3

Checkpoint Inhibitors

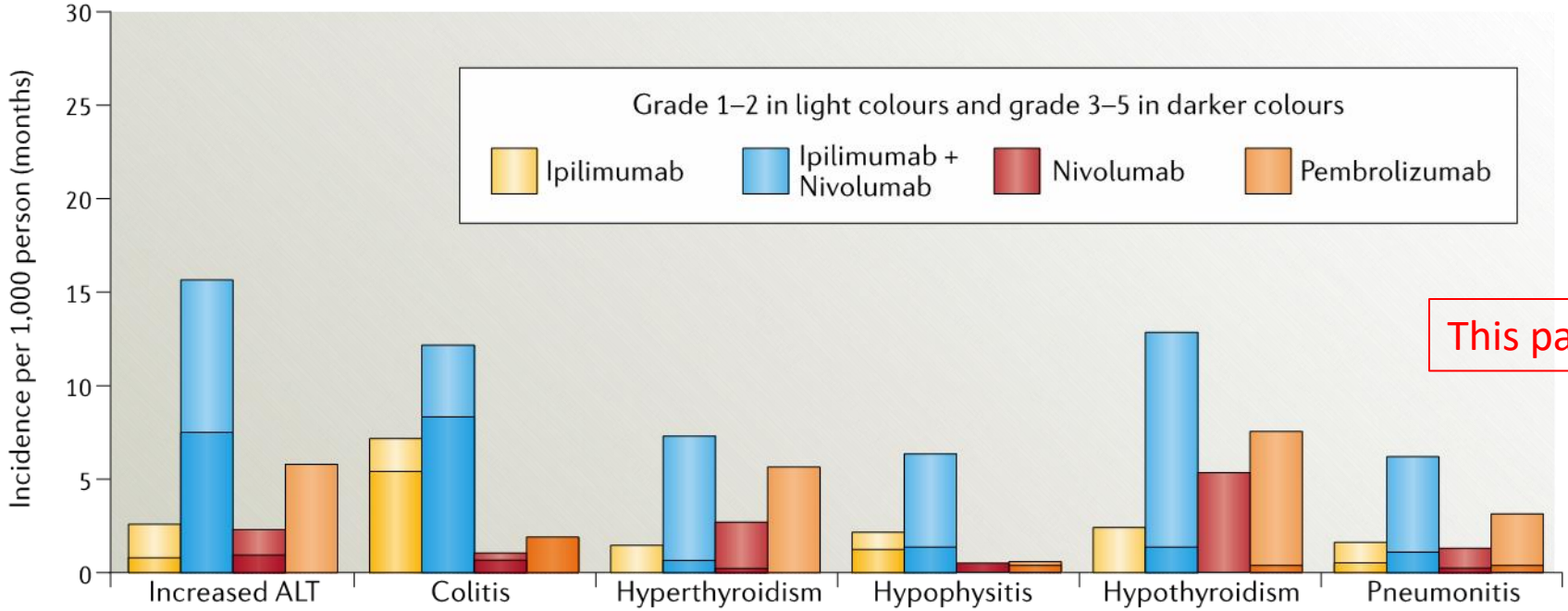


Science 23 Mar 2018:
Vol. 359, Issue 6382, pp. 1350-1355
DOI: 10.1126/science.aar4060



Science 23 Mar 2018:
Vol. 359, Issue 6382, pp. 1350-1355
DOI: 10.1126/science.aar4060

Not without toxicities…

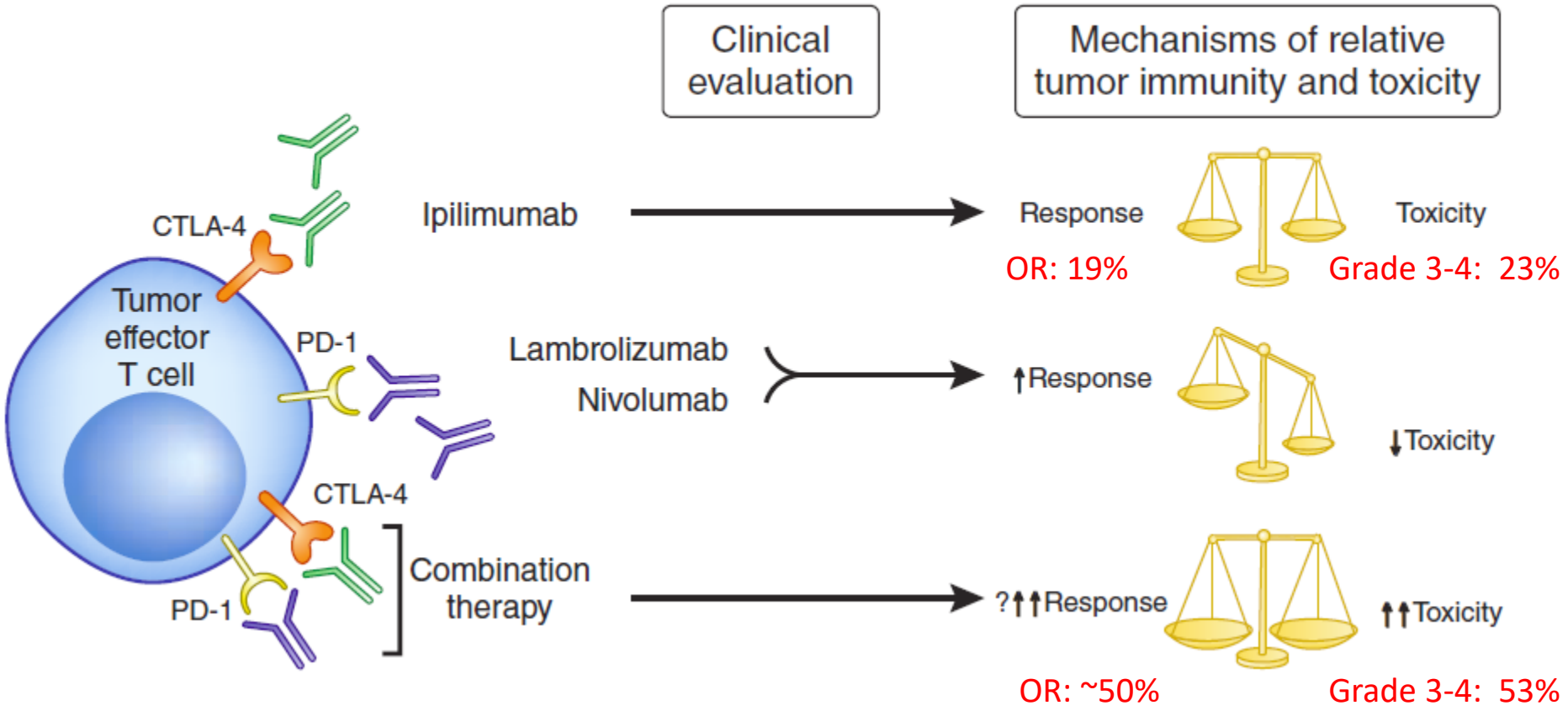


This part is not required.

Figure 3 | **Adverse events of special interest noted with immune-checkpoint inhibitors.** These adverse events are a direct result of activation of the immune system, as reported in patients treated with ipilimumab, pembrolizumab, nivolumab or ipilimumab plus nivolumab. Incidence per 1,000 person-months; these incidences include data from the following studies: CA-184-002 (REF. 16), KEYNOTE-001 (REF. 30), KEYNOTE-001 (randomized cohorts[31]), KEYNOTE-002 (REF. 32), KEYNOTE-006 (REF. 33), CheckMate-037 (REF. 100), CheckMate-066 (REF. 29), CheckMate-067 (REF. 45), and CheckMate-069 (REF. 44).

Di Giacomo, A. M., Biagioli, M., & Maio, M. (2010).
Seminars in Oncology, 37(5), 499–507.
http://doi.org/10.1053/j.seminoncol.2010.09.007

A looming challenge in cancer immunotherapy: balancing immunity and toxicity



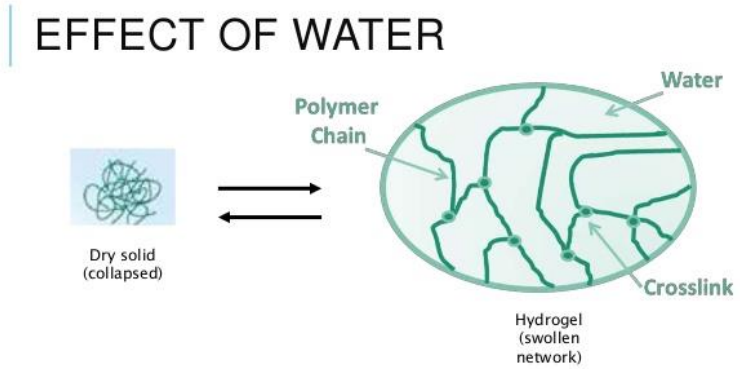
Nat. Med. 19, 1100-1101 (2013)

How to better deliver checkpoint blockade antibodies tumors?

- Local delivery
- Systemic delivery

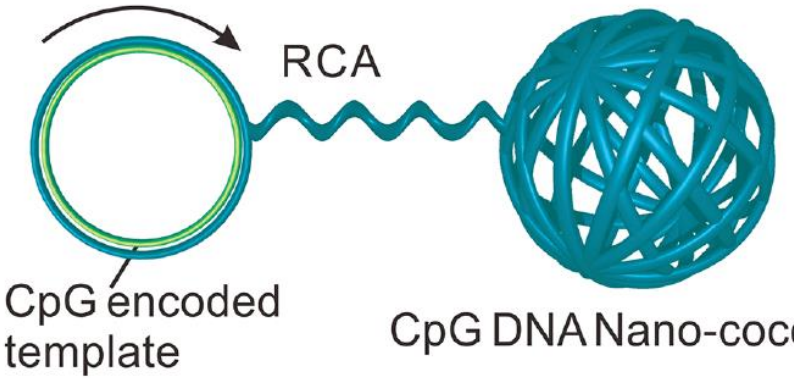
Hydrogel for deliverer of checkpoint blockade antibodies

A **hydrogel** is a network of polymer chains that are hydrophilic, sometimes found as a colloidal gel in which water is the dispersion medium. **Hydrogels** are highly absorbent (they can contain over 90% water) natural or synthetic polymer



ONCOIMMUNOLOGY
2016, VOL. 5, NO. 2, e1074374 (12 pages)
http://dx.doi.org/10.1080/2162402X.2015.1074374

How to better control the drug release?



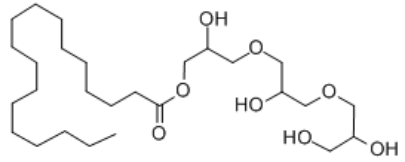
Loading

aPD1

Cargo: anti-PD1 antibody (aPD1)
Carrier: DNC

An enzyme that can degrade the carrier (DNC) to release aPD1

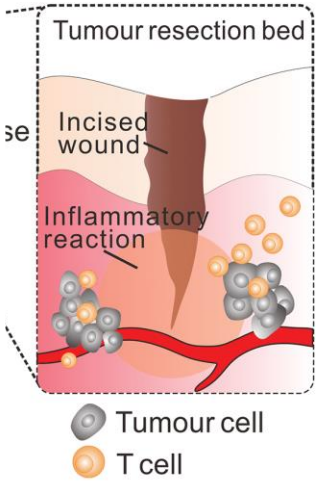
Restriction enzyme

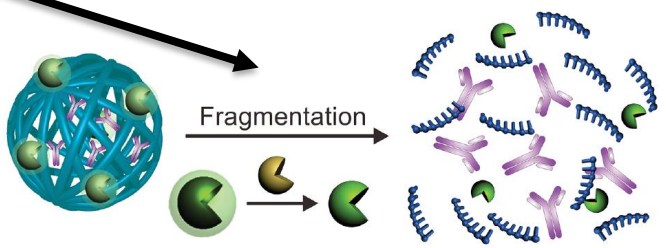


Caged enzyme by TGMS

- Triglycerol monostearate (**TGMS**) is an amphiphile.
- TGMS can be degraded by esterases and matrix metalloproteinases (MMPs) that are highly expressed at the wound sites for developmental tissue remodeling.

esterases and matrix metalloproteinases (MMPs)

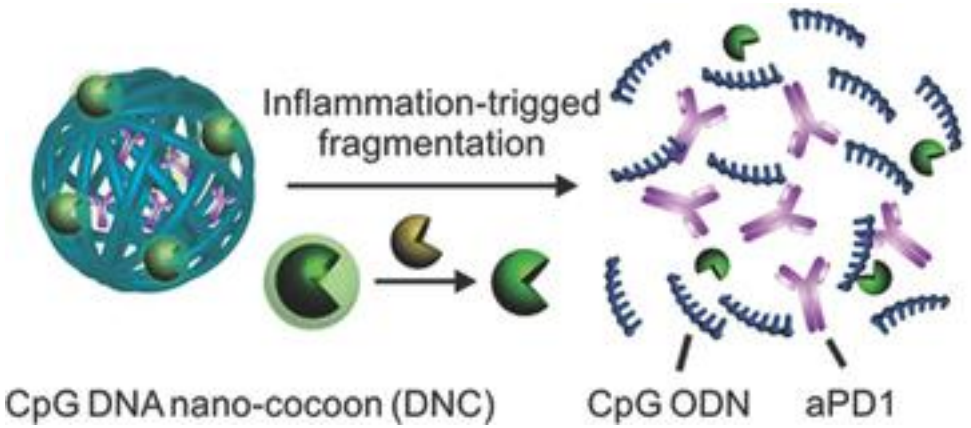


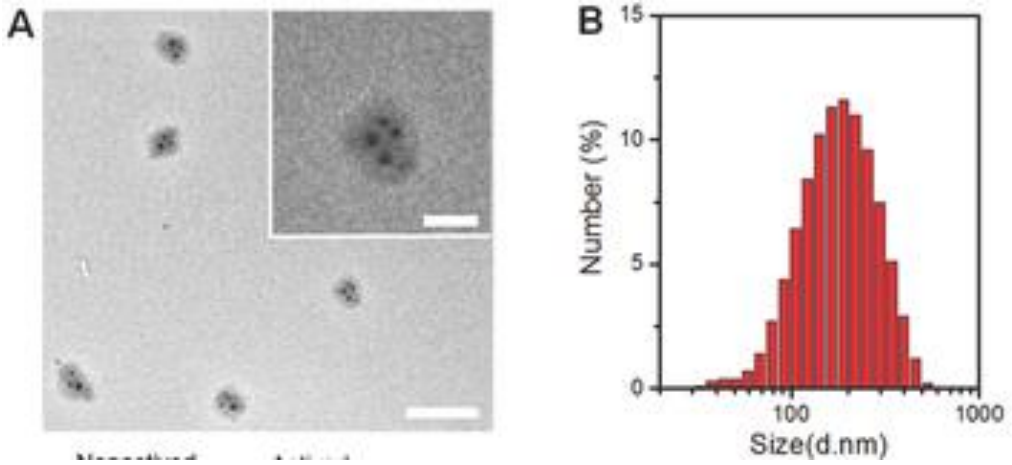


Degradation of the carrier (DNC) and release of aPD1

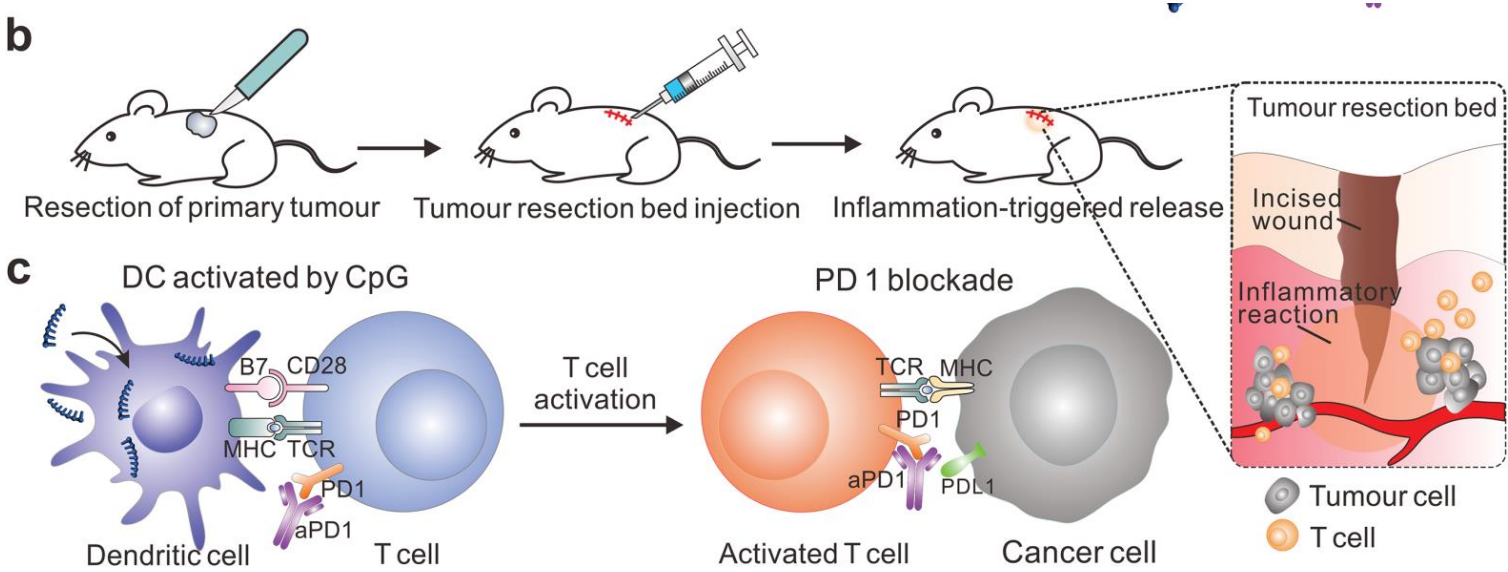
Adv Mater. 2016 Oct; 28(40): 8912–8920.

Drug release in response to inflammation conditions





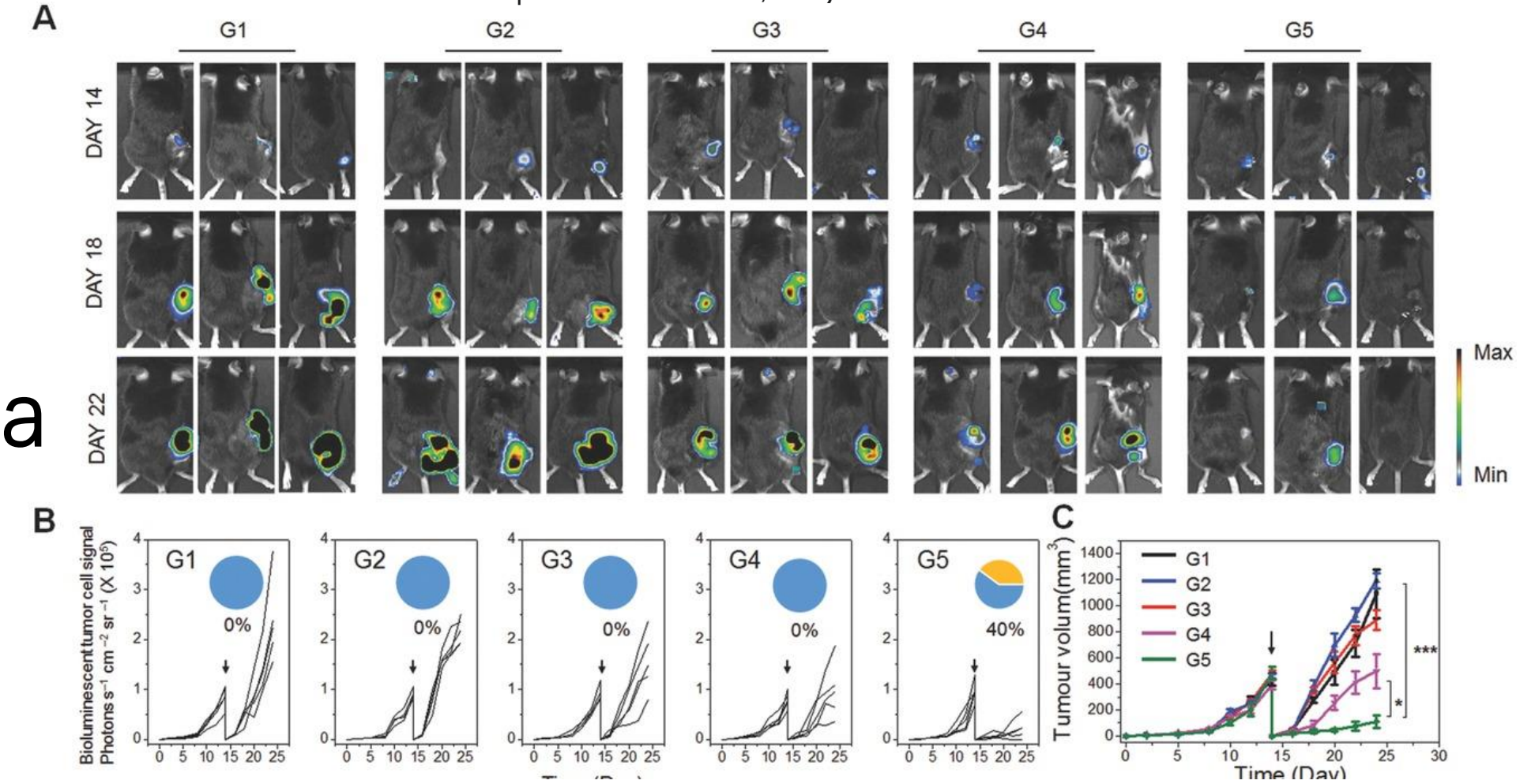
A) TEM imaging of HhaI-TGMS-DNCs-aPD1 nanocomposites (Scale bar: 500 nm). Inset: zoom-in image (Scale bar: 200 nm).
B) Dynamic light scattering characterization of HhaI-TGMS-DNCs-aPD1 nanocomposites.



- **CpG** oligodeoxynucleotides (CpG ODNs) trigger cells that express Toll-like receptor 9, including human plasmacytoid dendritic cells (pDCs), have potent immunostimulatory effects and can enhance the anti-cancer activity of a variety of cancer treatments.
- Through an enzymatic rolling circle amplification method specifically based on a template encoded with the CpG sequence, the carrier (designated as DNA “nano-cocoons,” DNCs) is assembled by a long-chain single-stranded DNA (ssDNA).

Efficacy against B16F10 mouse melanoma

G1, PBS control; G2, HhaI-TGMS-DNCs; G3, HhaI-TGMS-cDNCs-aPD1; G4, free aPD1/free CpG nucleotides; **G5, HhaI-TGMS-DNCs-aPD1**



In vivo tumor therapy to reduce postsurgical tumor relapse via CpG DNC delivery system.

A) In vivo bioluminescence imaging of the B16F10 tumors of the different groups after removal of primary tumor.

B) B) Quantified tumor signals and C) mean tumor growth of different groups of mice after various treatments indicated. Pie chart shows percent complete response rate (orange) (n = 10). The black arrow indicates the surgery time.

Systemic Efficacy?

G1, PBS control; G2, HhaI-TGMS-DNCs; G3, HhaI-TGMS-cDNCs-aPD1; G4, free aPD1/free CpG nucleotides; **G5, HhaI-TGMS-DNCs-aPD1**

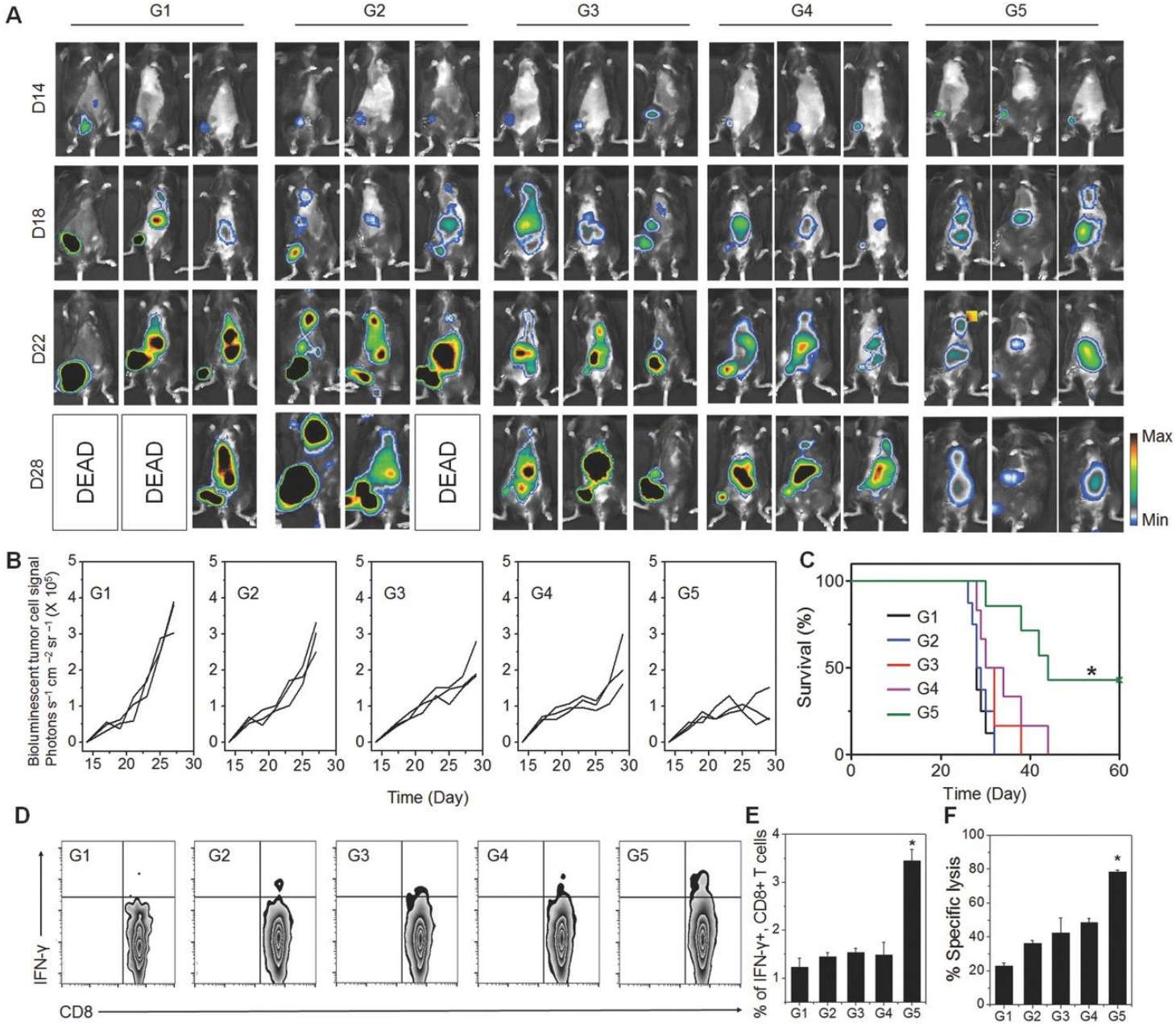
Systemic antitumor efficacy could be obtained by the local injection of DNC delivery system at the surgical site.

A) In vivo bioluminescence imaging of the B16F10 metastasis of different groups after removing of primary tumors at different time points.

B) Quantified tumor signals according to A. Every line represents one animal and each dot shows the whole animal photon count (n = 3).

C) Kaplan Meier survival curves for treated and control mice.

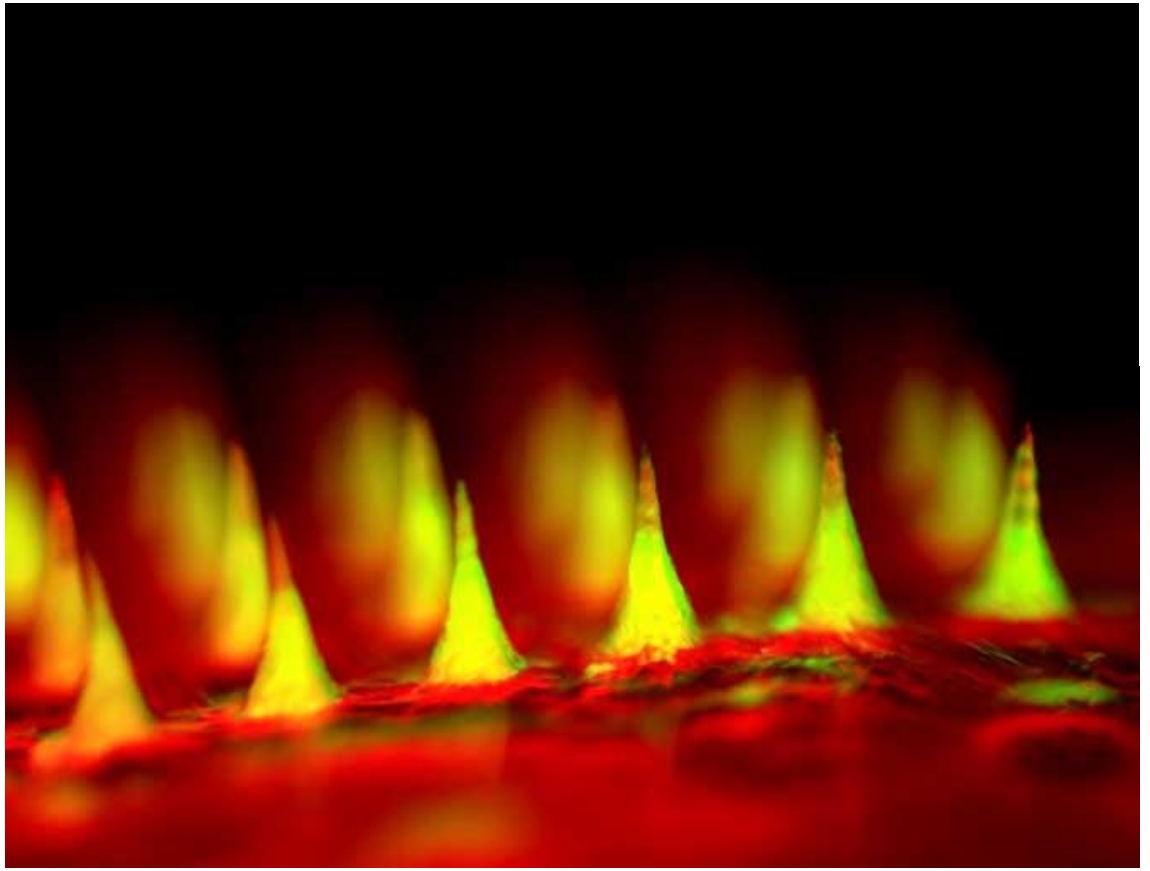
D) E) Quantified IFN-γCD8 cytotoxic T lymphocyte (CTL) T-cell in splenocytes

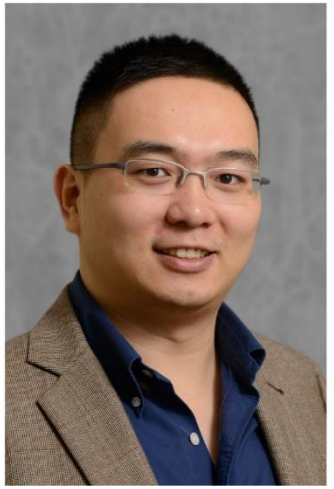


Any other technologies?

Science Top 10 Images 2015

AAAS





Prof. Zhen Gu
(UCLA/Zhejiang Univ)

These spikes may look fearsome, but they are shorter than the thickness of a credit card, **causing no more pain than a mosquito bite** when applied to the skin, according to researchers. Once there, the needles deliver insulin in response to changes in blood sugar levels. Some day, such needles—which are attached to a patch the size of a penny—may spare diabetic patients from having to inject themselves with insulin.

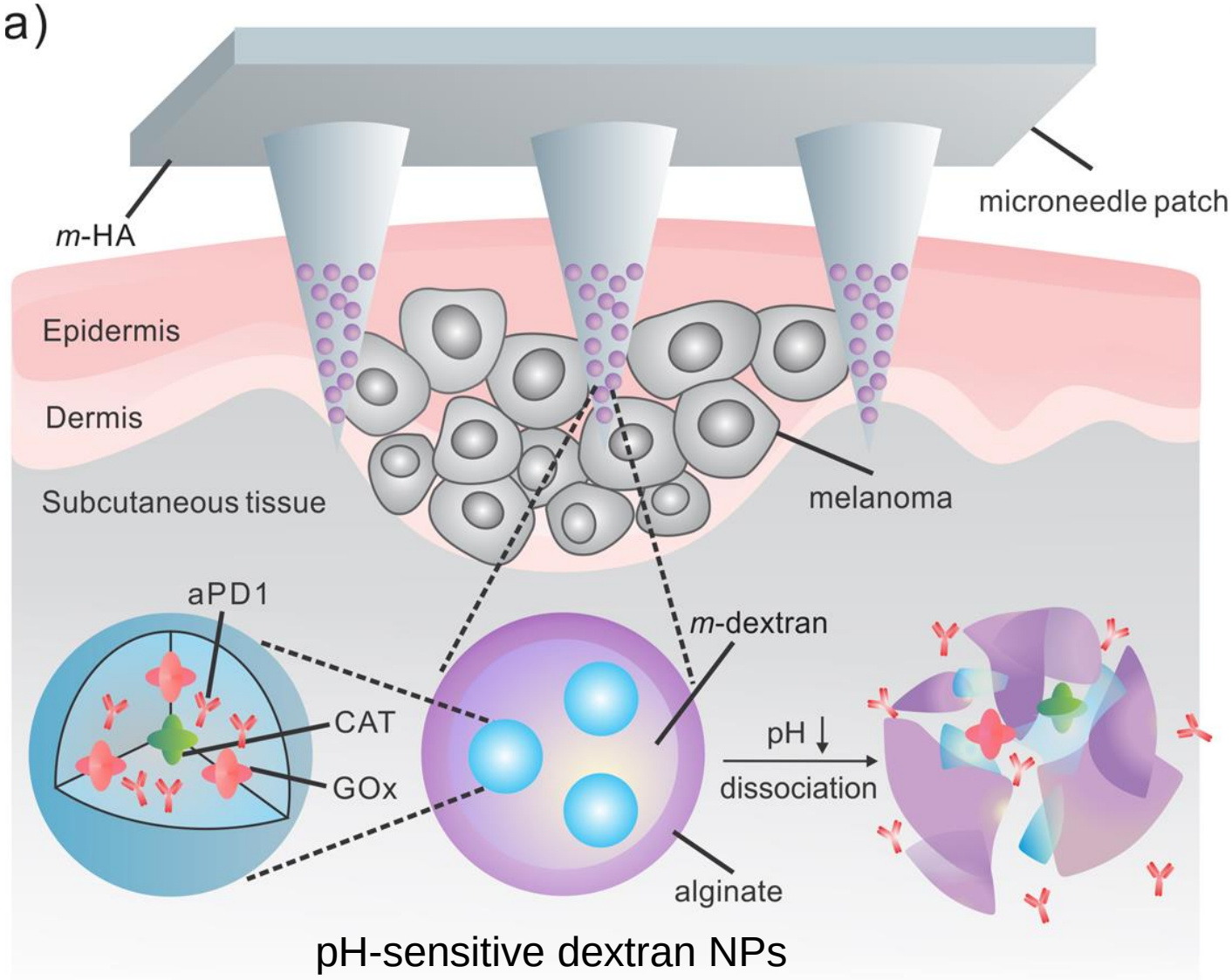
http://www.sciencemag.org/news/2015/12/top-10-images-2015

Microneedles enhance checkpoint blockade antibody therapy

- Each MN is composed of biocompatible hyaluronic acid (HA)
- MN is integrated with pH-sensitive dextran nanoparticles (NPs)
- NP encapsulate aPD1 and glucose oxidase (GOx).
- GOx is applied to convert blood glucose to gluconic acid in the presence of oxygen (O_2).
- Catalase (CAT) assists glucose oxidation by generation of O_2 and helps consume undesired hydrogen peroxide (H_2O_2)

glucose oxidase (GOx) converts blood glucose to gluconic acid

$$\text{glucose} + O_2 + H_2O \xrightarrow{\text{GOx}} \text{gluconic acid} + H_2O_2$$

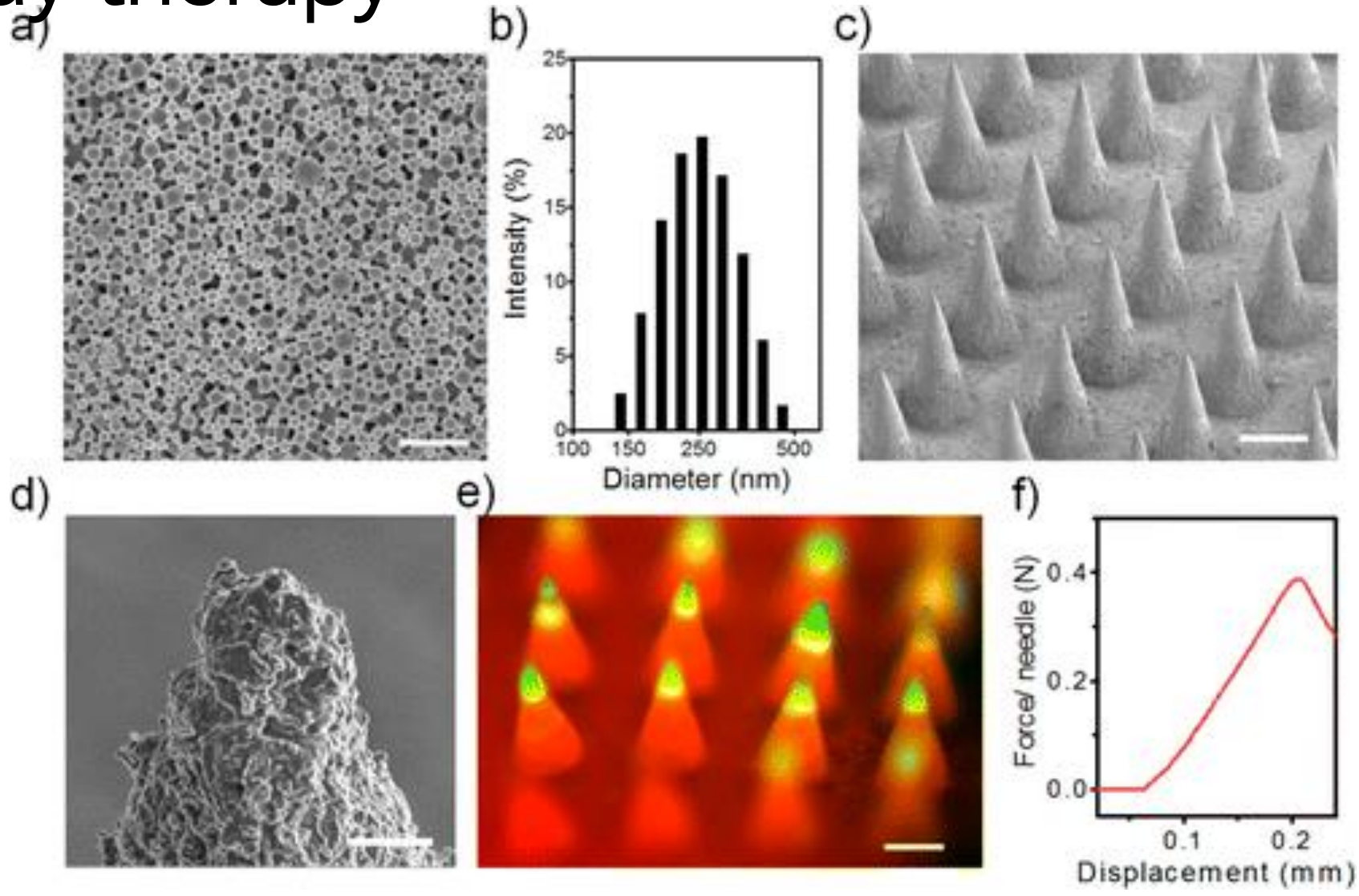


Nano Lett. 2016, 16, 2334−2340

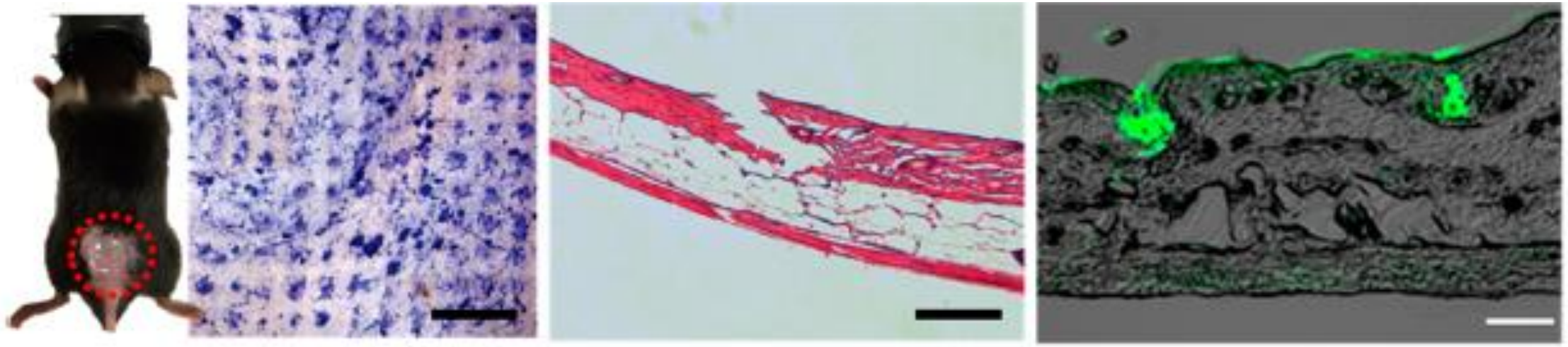
Microneedles enhance checkpoint blockade antibody therapy

Characterization of aPD1 loaded microneedles.

(a) SEM image of NPs (scale bar: 100 nm).
(b) The average hydrodynamic sizes determined by DLS. (c) SEM image of MN patch (scale bar: 200 μm).
(d) Higher magnification of SEM imaging of MN apex confirmed that the MN was loaded with NPs (scale bar: 5 μm).
(e) Fluorescence imaging of a representative MN patch that contained FITC-antibody loaded NPs (scale bar: 200 μm).
(f) Mechanical property of the MN. The failure force for desired MN was quantitatively measured as 0.38 N/needle.



Nano Lett. 2016, 16, 2334−2340

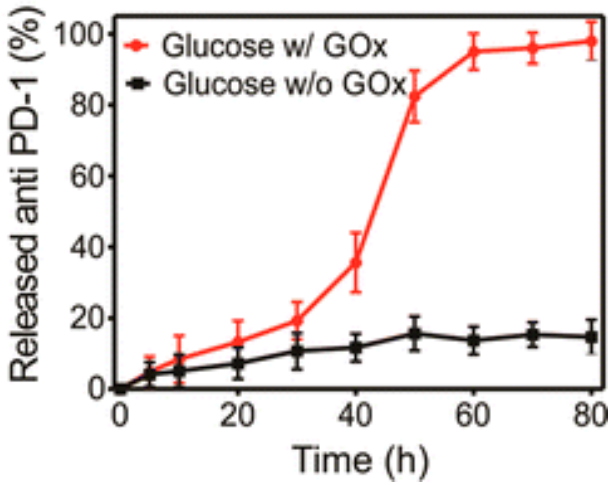


1 mm 200 µm 200 µm

In vivo anti skin cancer treatment of aPD1 delivered by MNs.

(a) Mouse dorsum and relevant skin (the area within the red dashed line) was transcutaneously treated with a MN patch (left), with the image of the trypan blue staining showing the penetration of MN patch into the mouse skin (right) (scale bar: 1 mm).
(b) H&E-stained section of cross-sectional mouse skin area penetrated by one MN (scale bar: 200 µm).
(c) Merged fluorescence and bright field image of the mouse skin penetrated by FITC-antibody loaded MNs (green: aPD1) (scale bar: 200 µm).

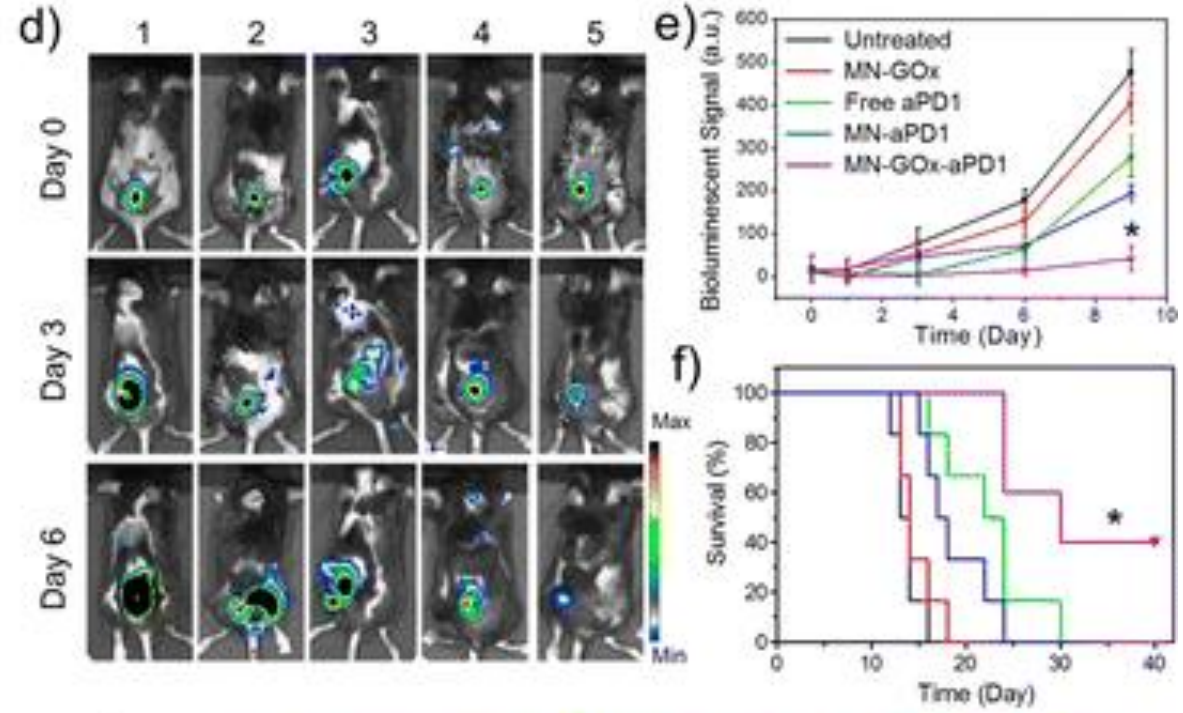
In vitro accumulated aPD1 release from the MN patches incubated in 100 mg/dL glucose solution at 37 °C over time. The error bars are based on the standard deviation (SD) of the samples (n = 3).

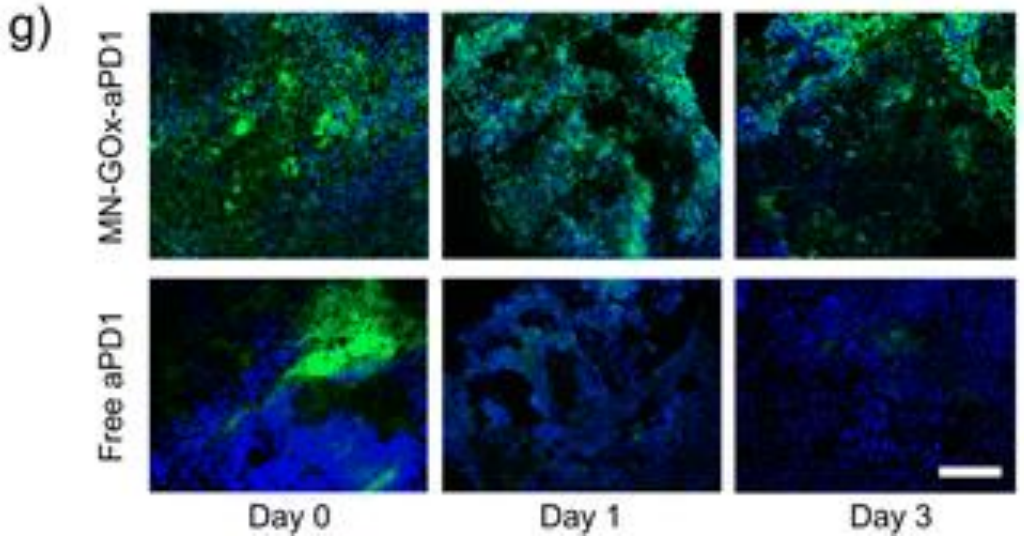


Nano Lett. 2016, 16, 2334−2340

Efficacy against B16F10 mouse melanoma

16F10-luc cancer cells were subcutaneously implanted in the rear dorsal area of female C57BL/6 mice. After the tumor sizes reached about 50–60 mm^3, **MN patch were administered by a single local administration onto the tumor site (the area of patch was larger than tumor site).**



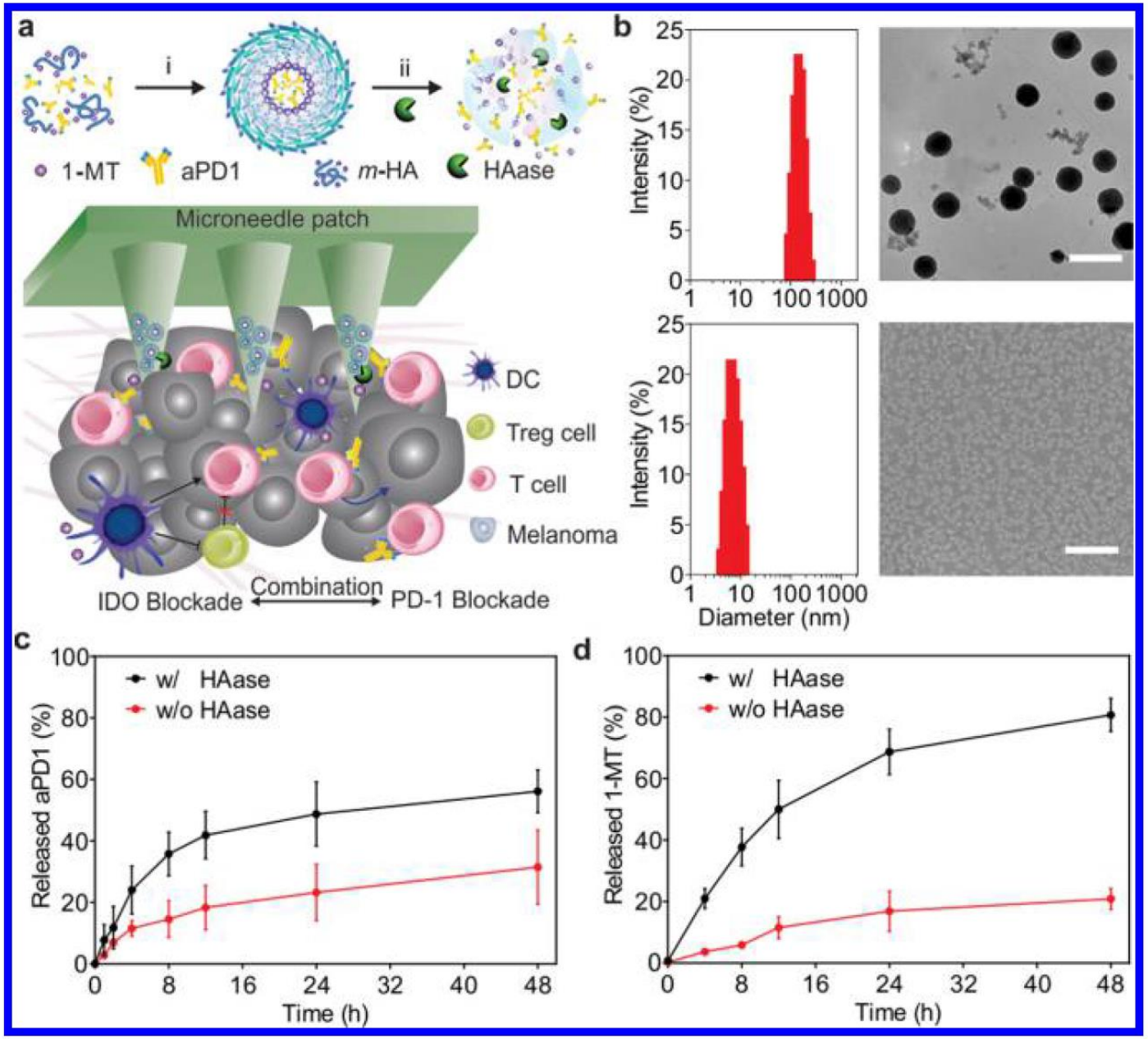


In vivo anti skin cancer treatment of aPD1 delivered by MNs.
(d) In vivo bioluminescence imaging of the B16F10 tumors of different groups indicated (1, untreated; 2, MN-GOx; 3, free aPD1; 4, MN-aPD1; **5, MN-GOx-aPD1**). The error bars are based on the standard deviation (SD) of three mice.
(e) Quantified tumor signals according to d.
(f) Kaplan–Meier survival curves for the treated and the control mice. Shown are eight mice per treatment group.
(g) Immunofluorescence staining of tumors treated with MN-GOx-aPD1 or free aPD1 at different time points (green: aPD1, blue: nucleus) (scale bar: 100 μm).

Green: released anti-PD-1 in tumor tissues

Nano Lett. 2016, 16, 2334−2340

Synergistic Transcutaneous Immunotherapy



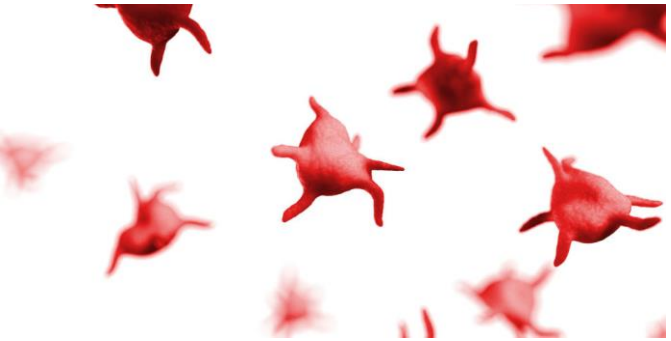
Co-delivery of **anti-PD-1** with 1-methyl-DL-tryptophan (**1-MT**), an inhibitor of immunosuppressive enzyme indoleamine 2,3-dioxygenase (**IDO**)

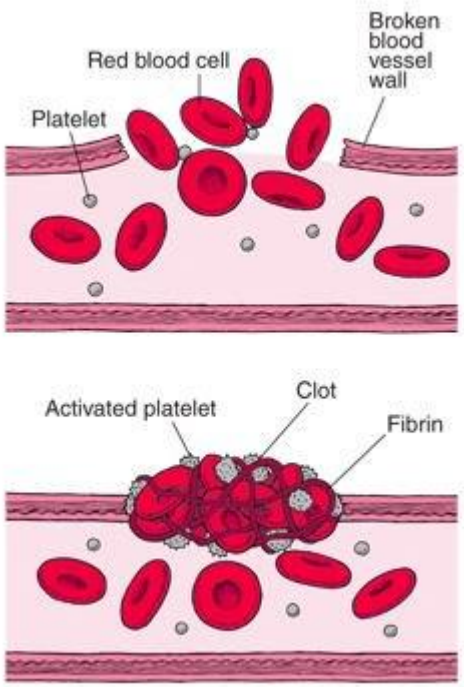
Hyaluronic acid (HA; conjugate base hyaluronate), also called hyaluronan, is an anionic, nonsulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues.

ACS Nano 10, 8956-8963 (2016).

How do we target checkpoint inhibitors to tumors systemically?

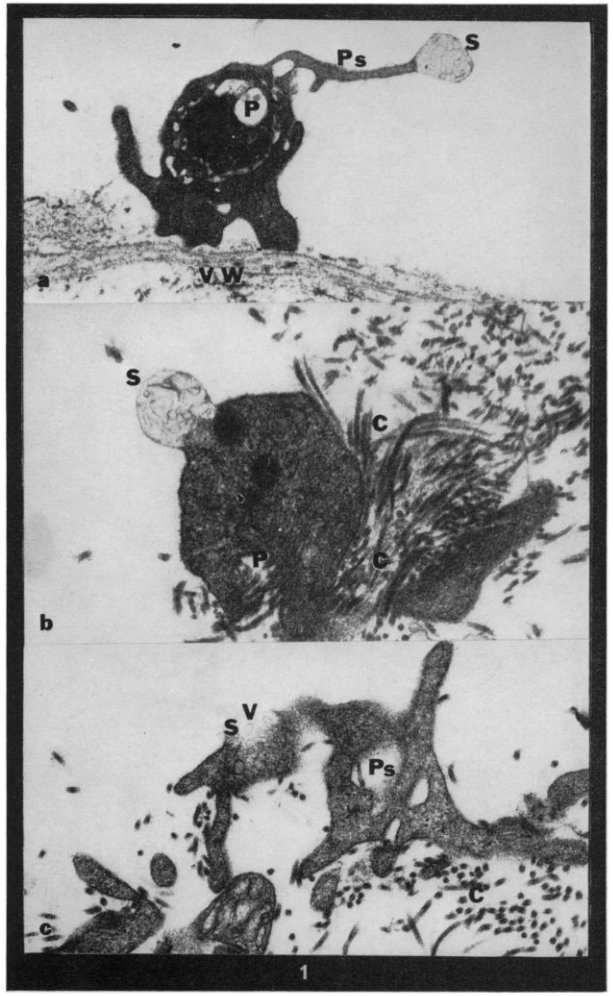
Platelets





https://www.allaboutcircuits.com/news/engineering-inspired-by-nature-janus-particles-and-self-healing-circuitry/

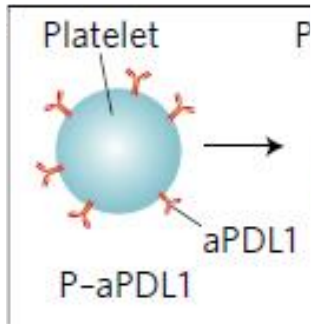
FIG. 1.-The morphology of the multivesicular membranous sac and the stage after vesicle release in human platelets attached to coronary arterial wall.

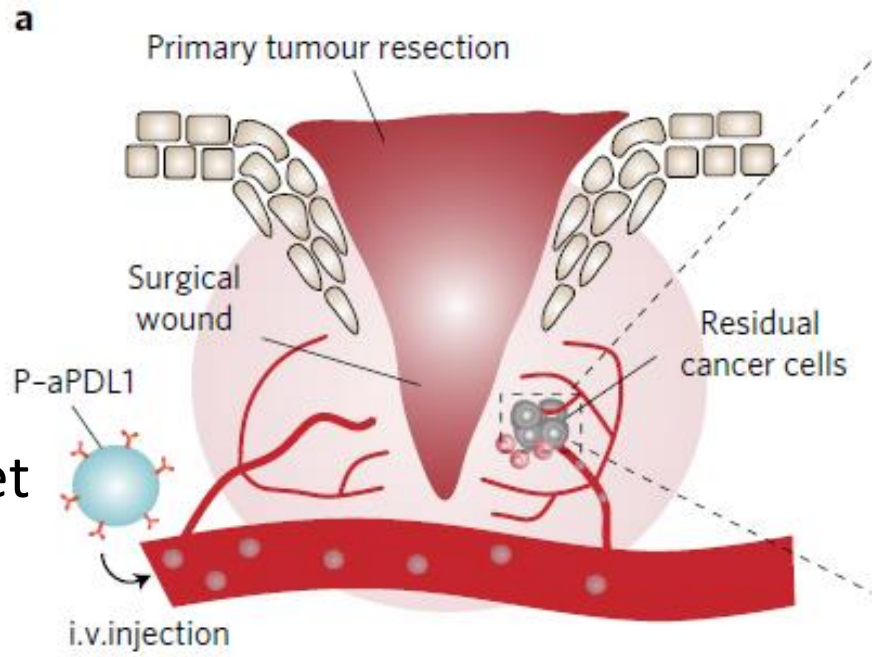


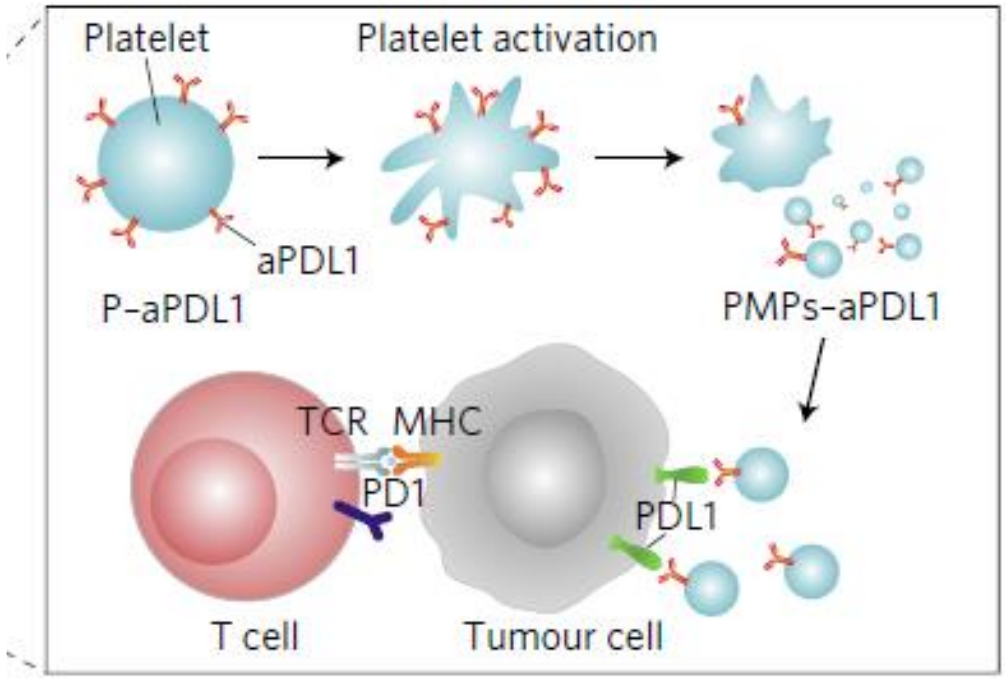
Br J Exp Pathol. 1972 Apr; 53(2): 206–215.

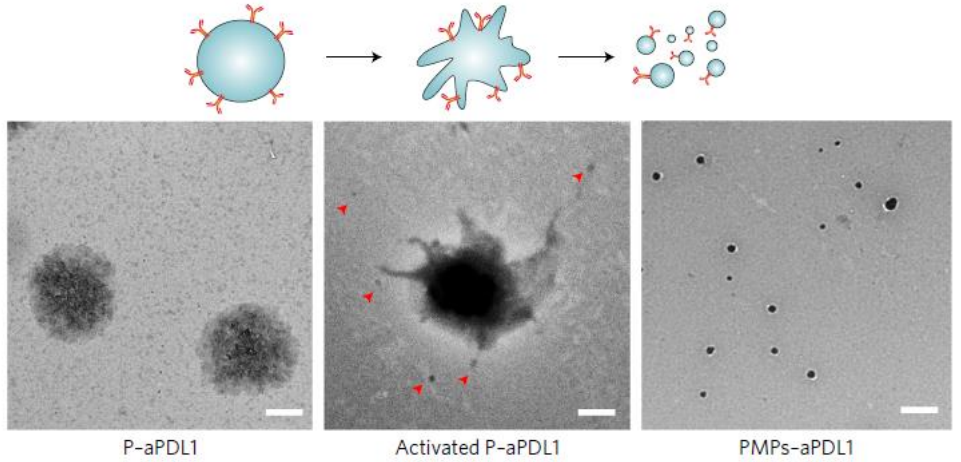
Hijacking platelets for checkpoint inhibitor delivery for post surgery

Chemically conjugate anti-PD-1 to Platelet

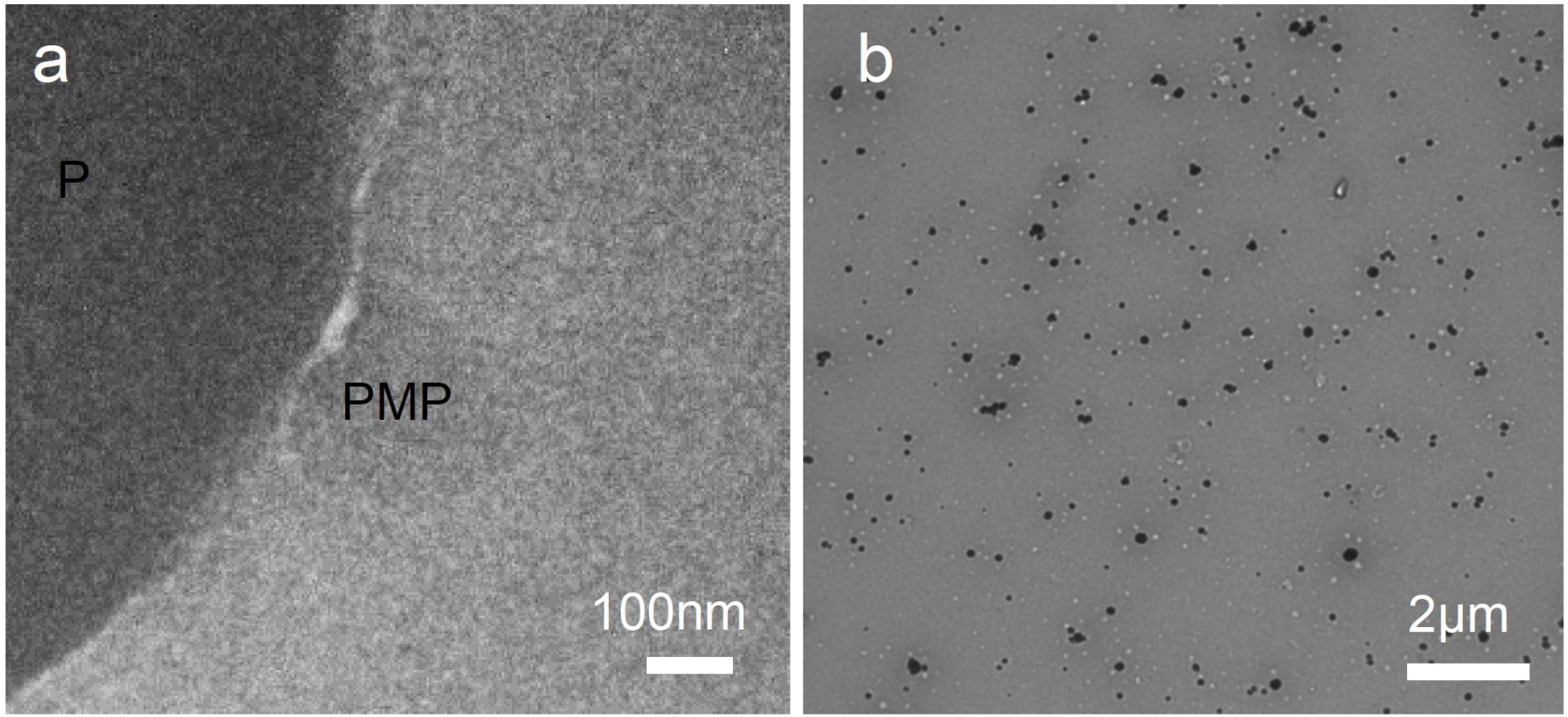




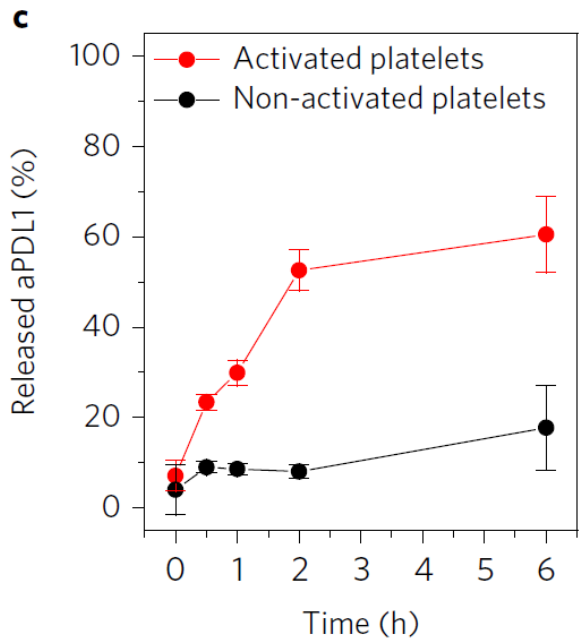




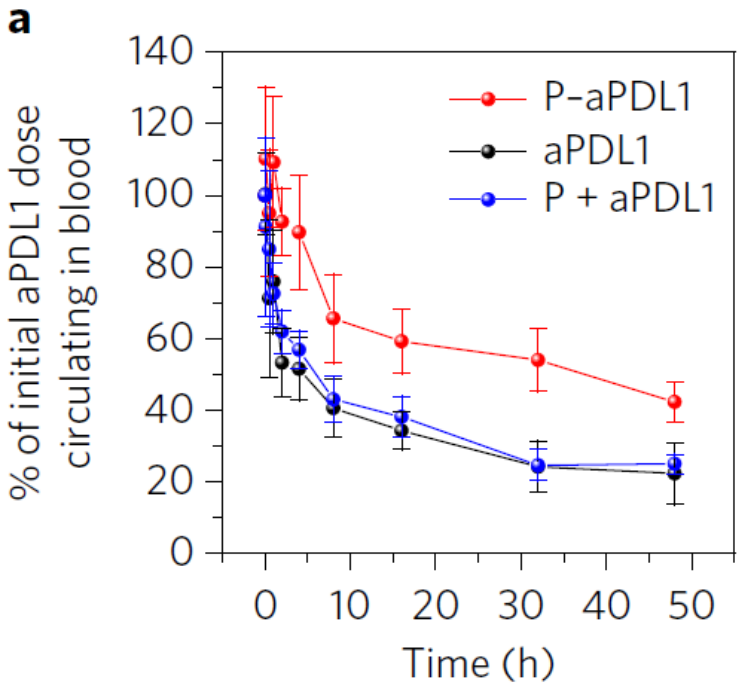
Nature Biomedical Engineering **1**, Article number: 0011 (2017), doi:10.1038/s41551-016-0011



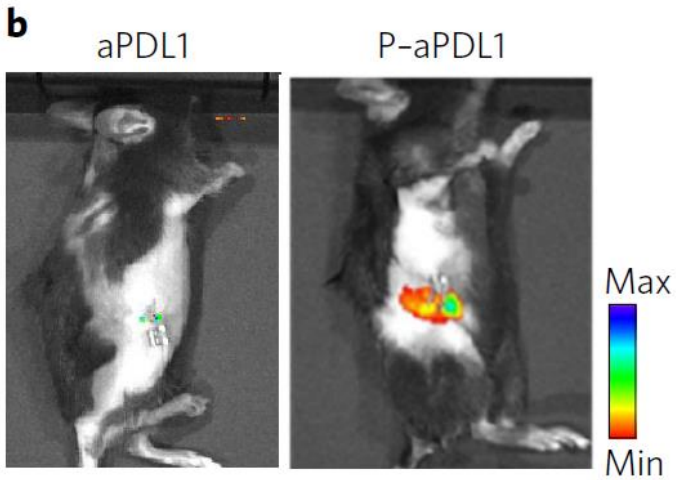
Supplementary Figure S5. Transmission electron microscopy (TEM) of P-aPDL1 after activation. (a) Platelet microparticles (PMPs) shed from activated platelets. (b) A number of PMPs can be found under TEM after P-aPDL1 activation. Size bars, 100 nm in Figure S5a and 2 μm in Figure S5b.

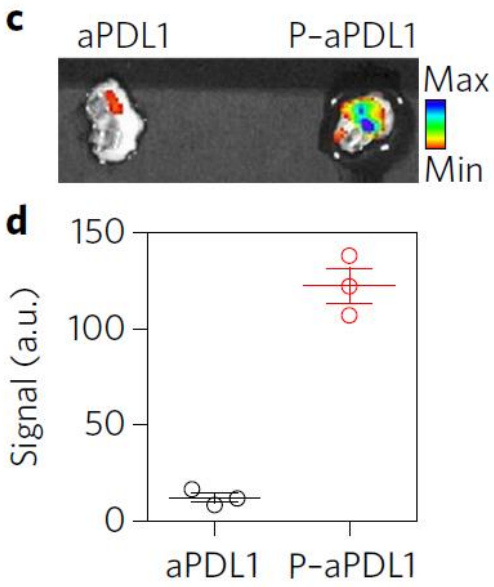


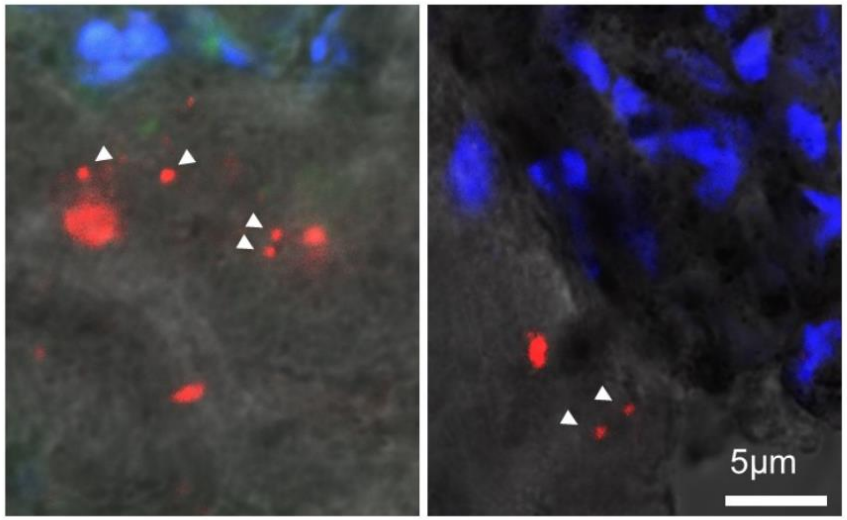
Platelet were activated by thrombin in vitro



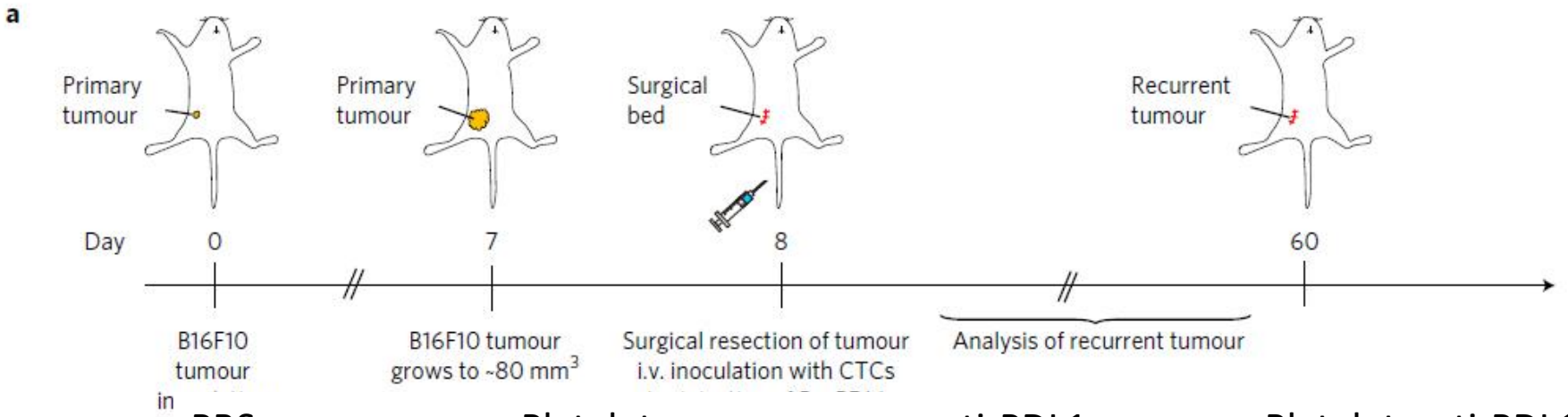
aPDL1 released from platelets in tumors

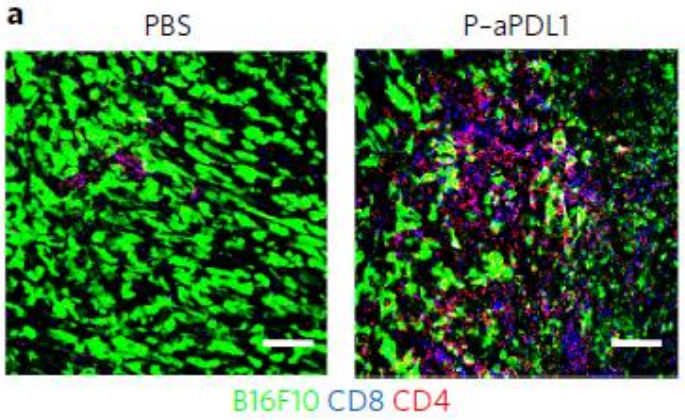


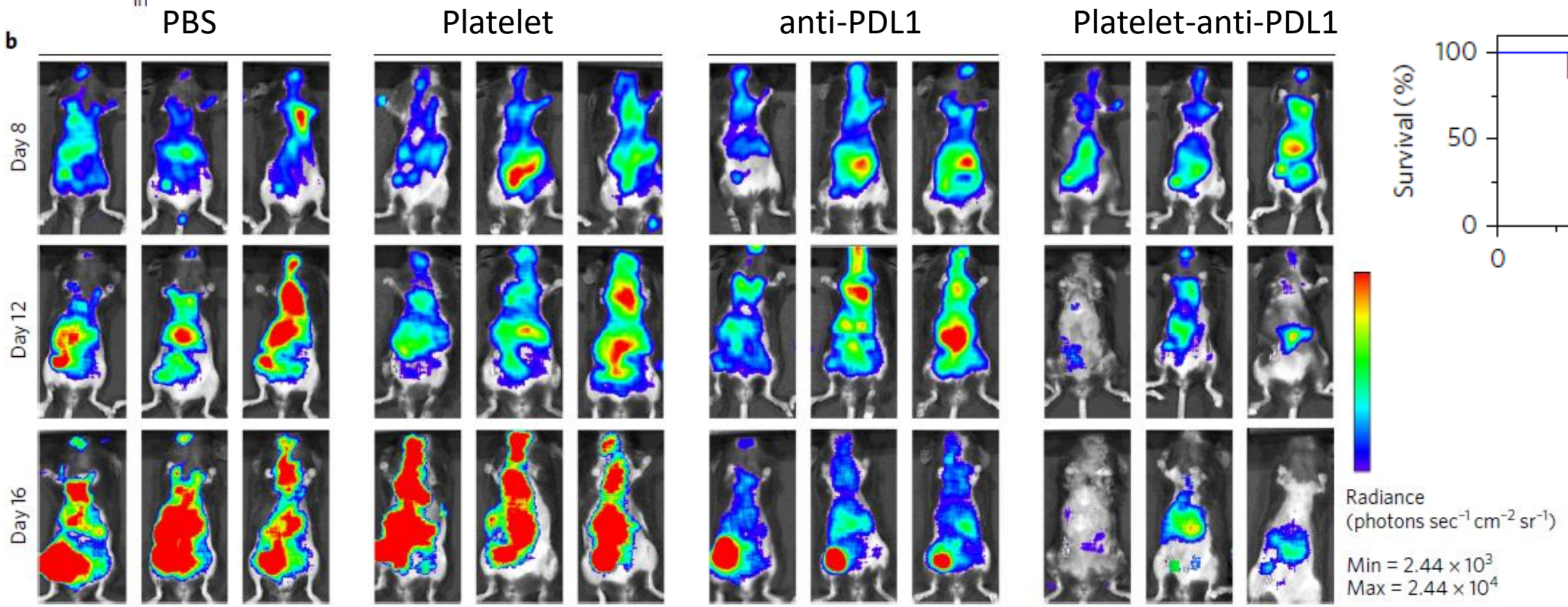


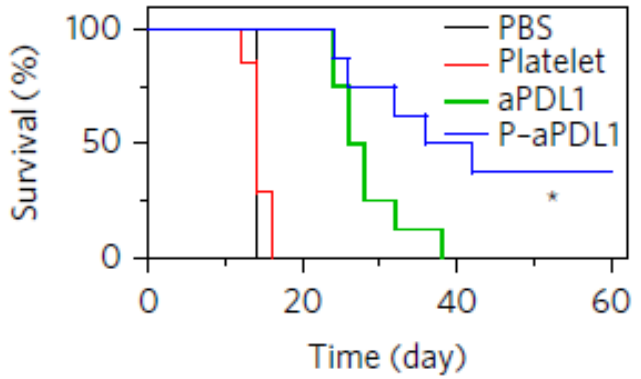


blue and red represent nucleus and aPDL1







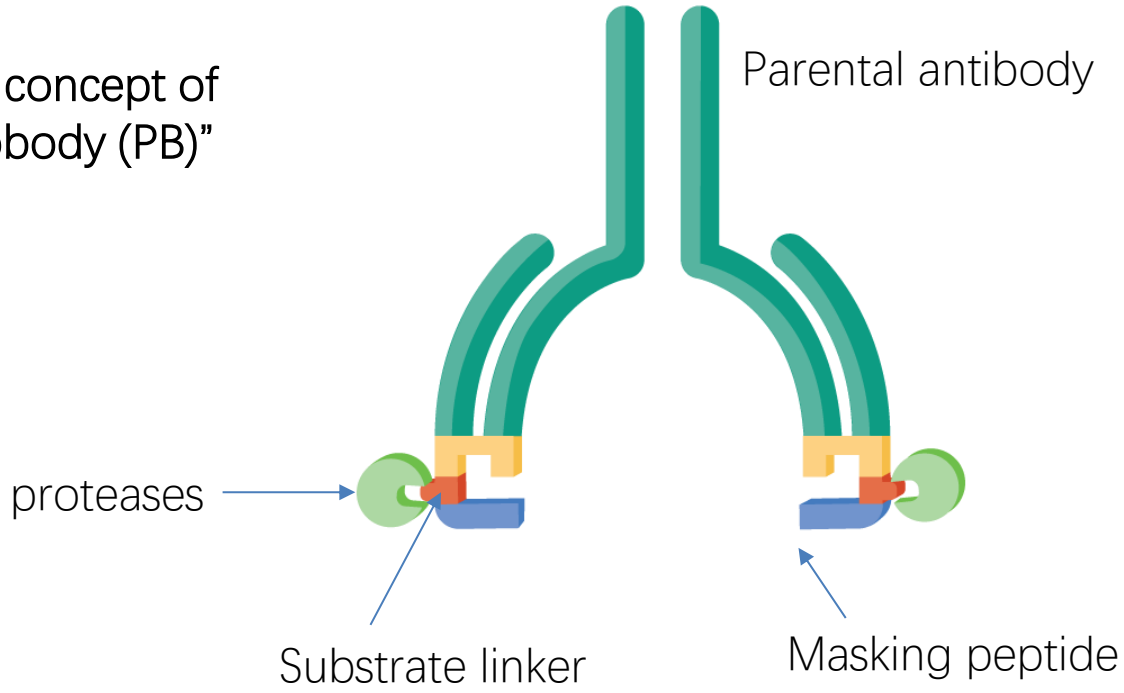


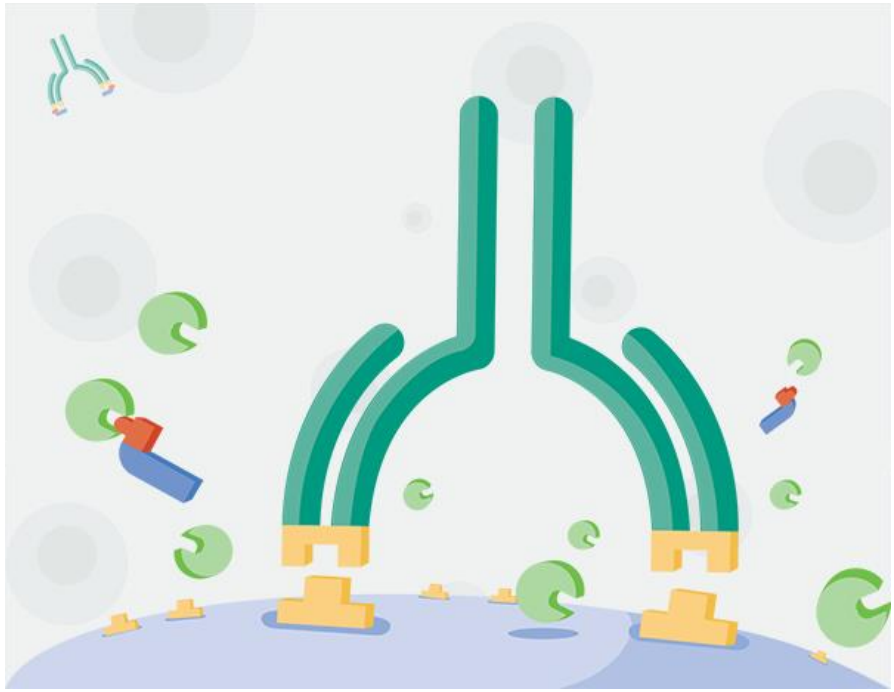
bioluminescence signal from tumors

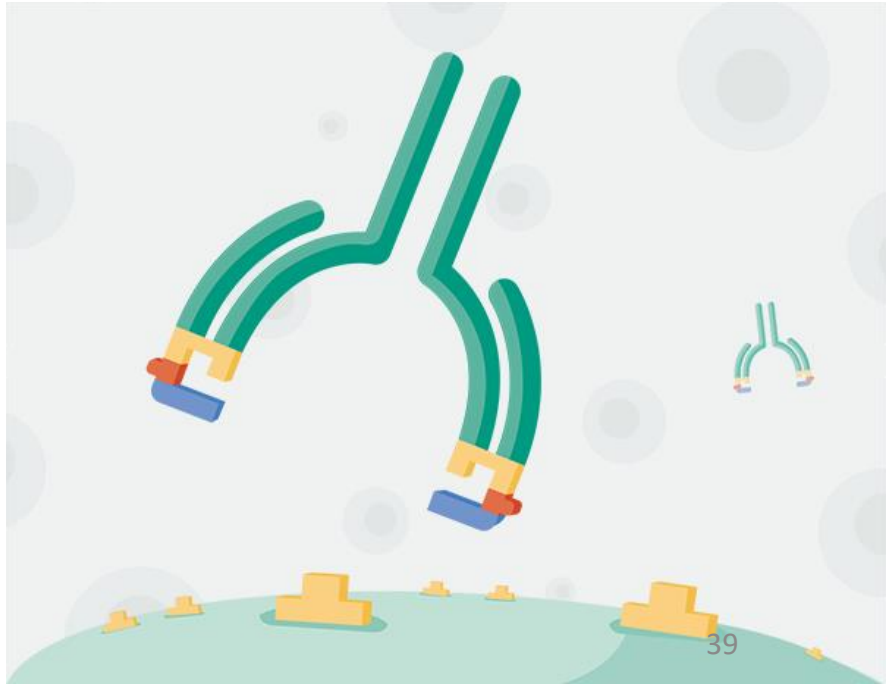
Nature Biomedical Engineering Article number: 0011 (2017), doi:10.1038/s41551-016-0011

‘Smart’ antibodies?

The concept of “probody (PB)”

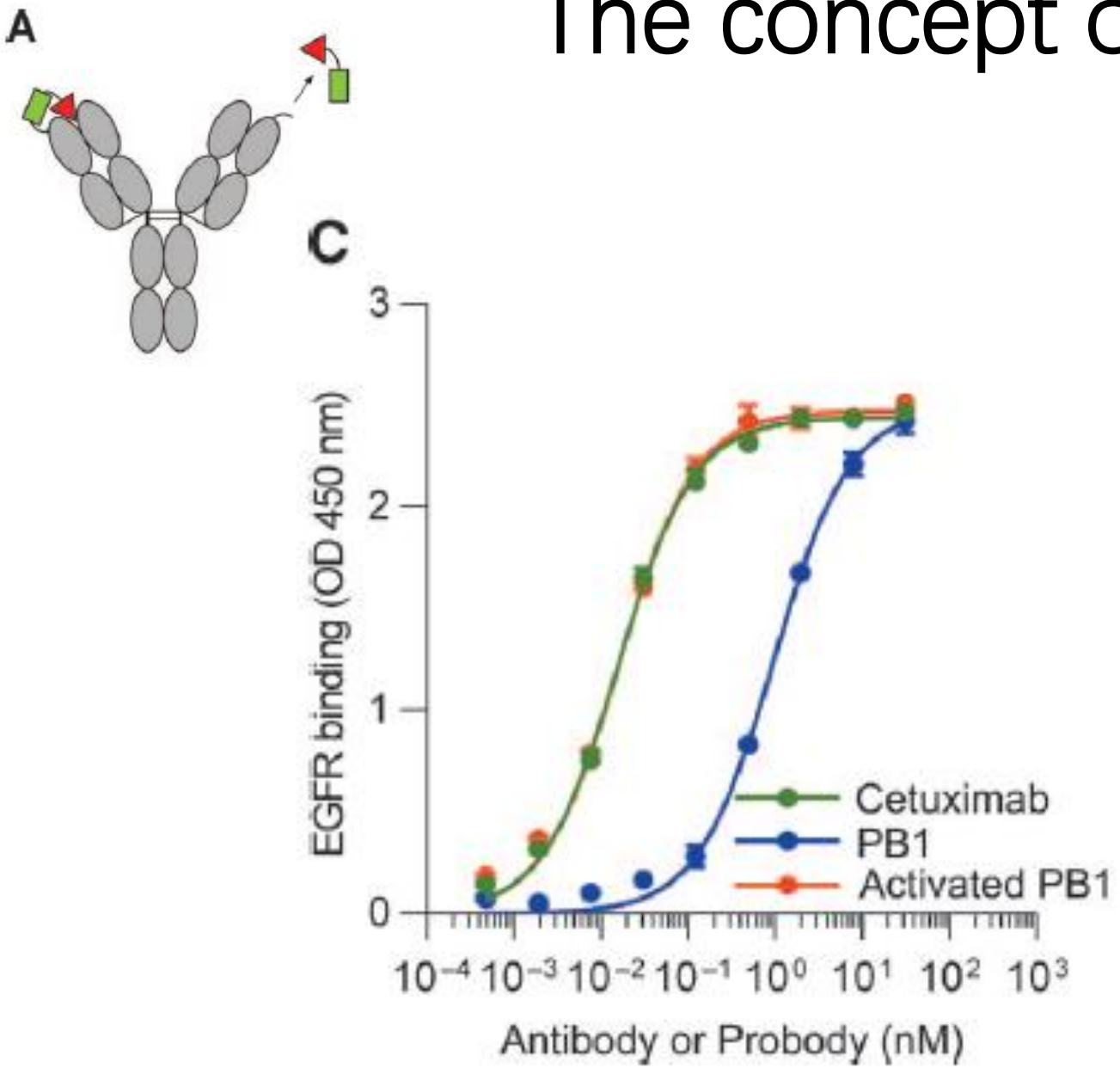






http://cytomx.com/probody-therapeutics/

The concept of “probody (PB)”



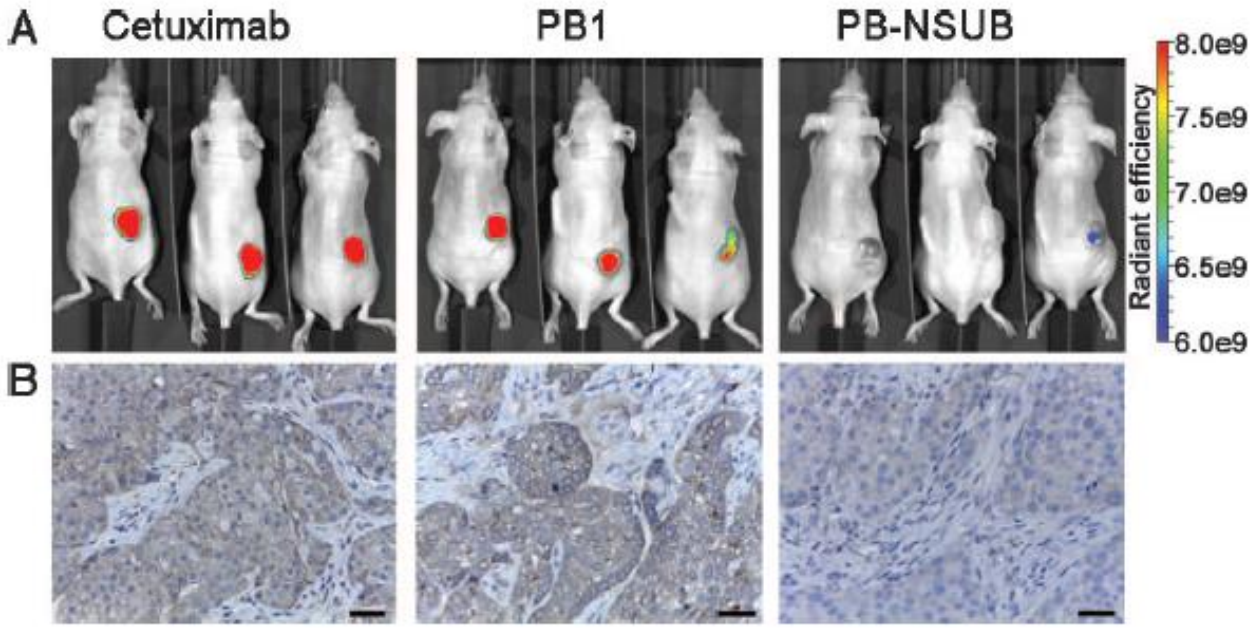
(**A**) Cartoon model of a Probody showing the masking peptide (red), substrate linker (green), flexible peptide linkers (gray lines), and IgG (gray ellipses). The left Fab arm represents the intact Probody form with the masking peptide tethered and bound in the antigen-combining site, whereas the right Fab arm represents an activated Probody from which the masking peptide has dissociated.

(**C**) Intact PB1 shows decreased binding to immobilized EGFR by ELISA, whereas digestion of PB1 with uPA protease (Activated PB1) restores binding comparable to cetuximab.

Sci. Transl. Med. 5, 207ra144 (2013).

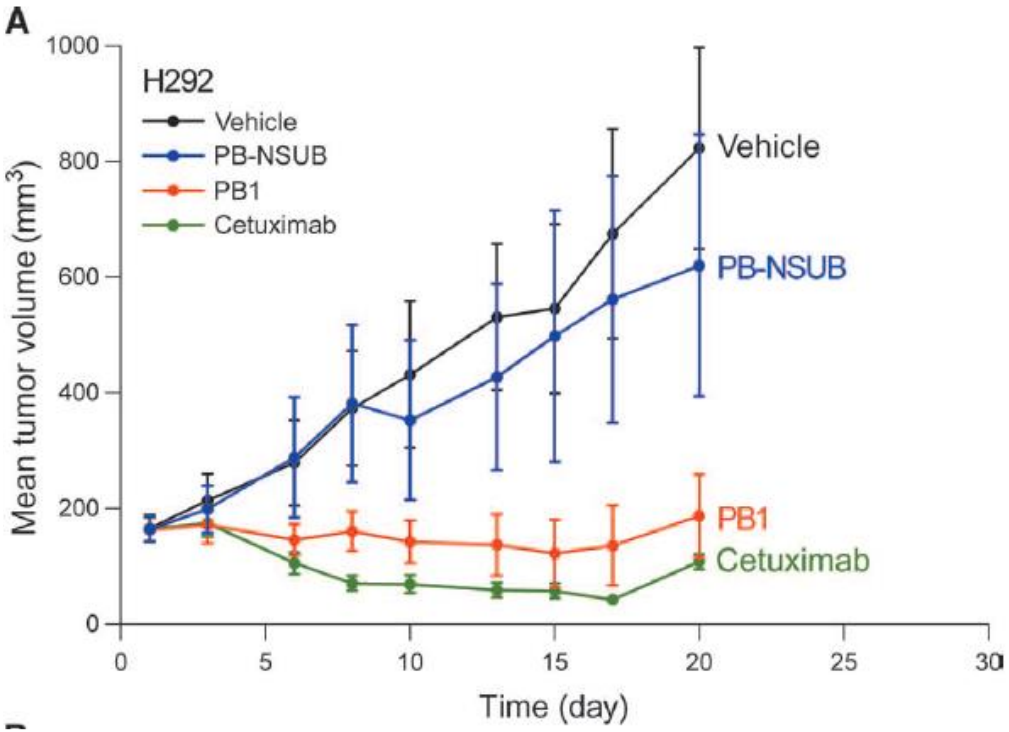
The efficacy of “probody (PB)”

This slide is not required.

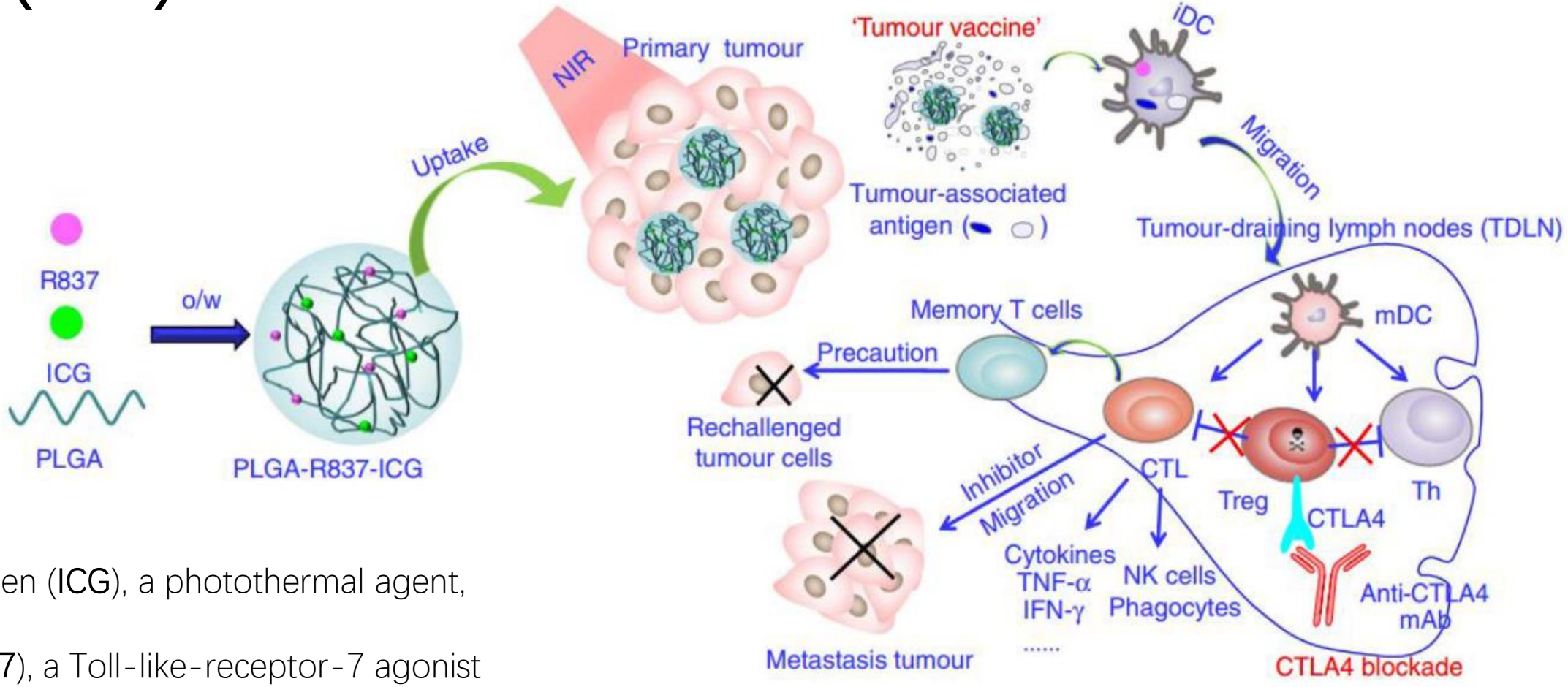


Optical imaging of H292 xenograft tumor–bearing mice 48 hours after intraperitoneal administration of **Alexa750-conjugated PB1, cetuximab, and noncleavable Probody (PB-NSUB).** n = 3 mice per group were injected with fluorescent conjugate. A high-intensity fluorescent signal was detected only in the tumors of mice dosed with PB1 or cetuximab, suggesting that PB1 was activated and accumulated in the tumor through EGFR binding.

PB1 inhibits tumor growth in xenograft models.



Immunotherapy in combination with photothermal therapy (PTT)



Indocyanine green (ICG), a photothermal agent,

imiquimod (R837), a Toll-like-receptor-7 agonist

co-encapsulated by poly(lactic-co-glycolic) acid (PLGA) to form the nanoparticle

Nature Communications **volume7**, Article number: 13193 (2016)

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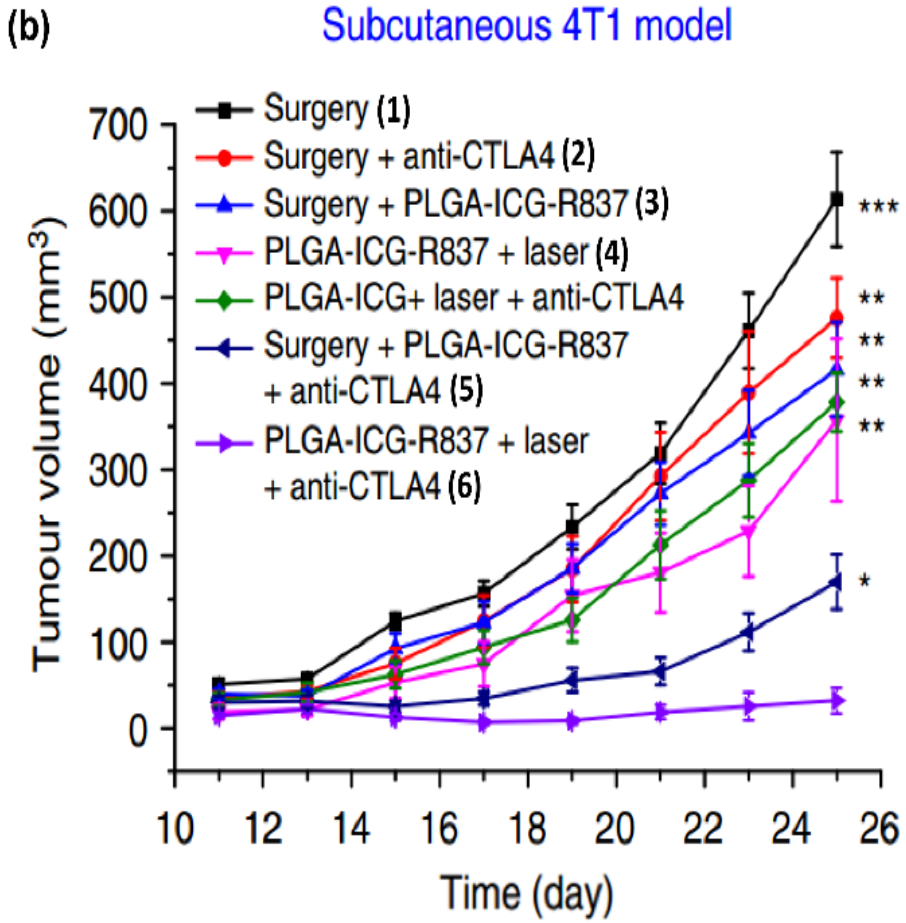

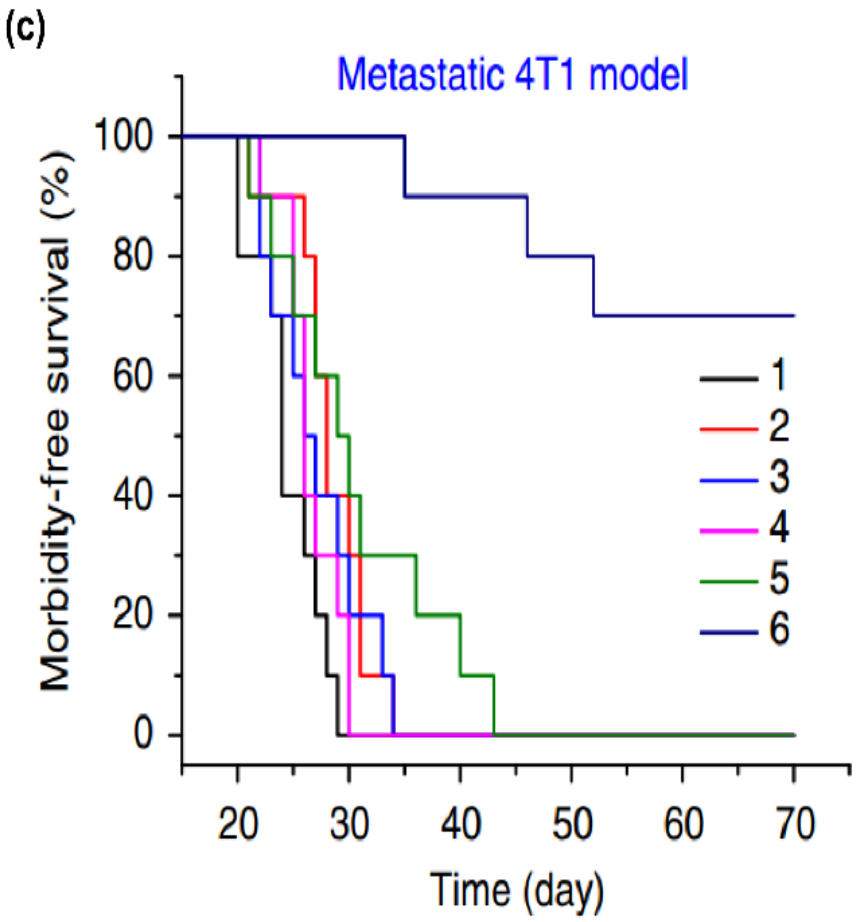

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