An Enemy within and a Cure within: Two Nanoparticles within, Ferritin and Exosomes for Cancer Immunotherapy

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The 15th Korea-US Forum on Nanotechnology, 2018. 7. 12

26 IO agents approved globally

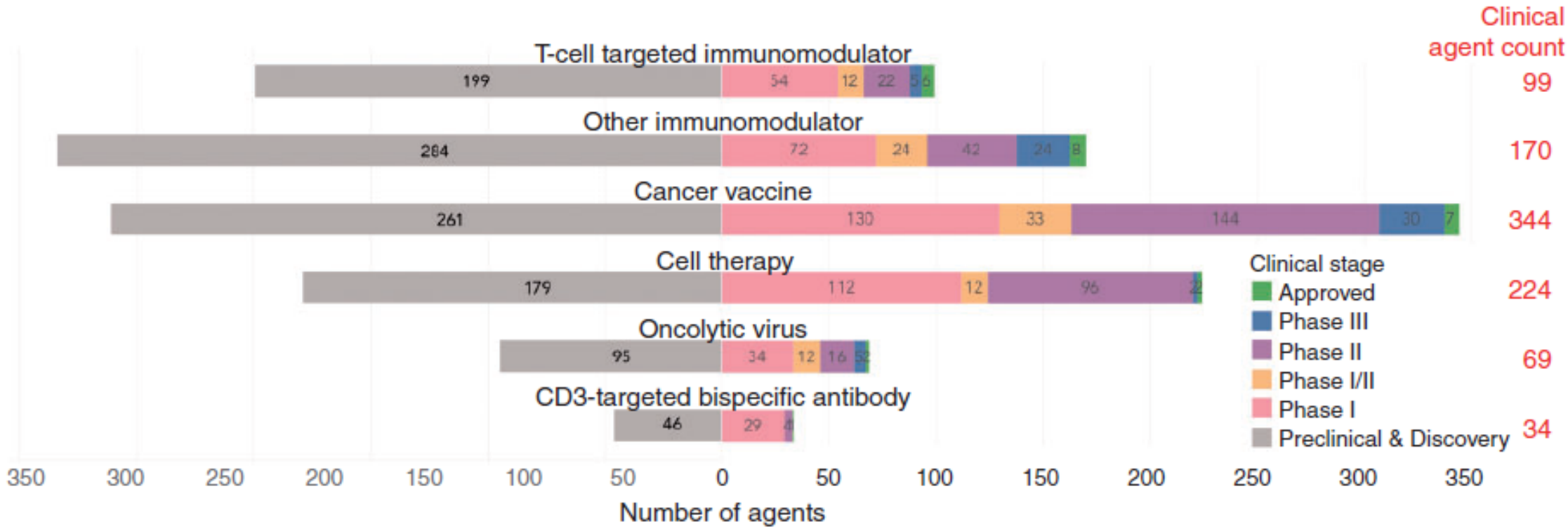
Therapy type	Therapy name	Company	Target
T-cell targeted immunomodulator (6 in total)	Ipilimumab	Bristol-Myers Squibb Co	CTLA-4
	Nivolumab	Bristol-Myers Squibb Co	PD-1
	Pembrolizumab	Merck & Co Inc	PD-1
	Atezolizumab	Roche/Genentech Ltd	PD-L1
	Avelumab	Merck KGaA	PD-L1
	Durvalumab	AstraZeneca/MedImmune LLC	PD-L1
Other immunomodulator (8 in total)	Aldesleukin	Novartis AG	IL2R
	Imiquimod	Valeant Pharmaceuticals Intl Inc	TLR7
	Interferon alfa	Sumitomo Dainippon Pharma Co Ltd	IFNAR1; IFNAR2
	Interferon alfa-1b	Shenzhen Kexing Biotech Co Ltd	IFNAR1
	Interferon alfa-2a	Cadila Healthcare Ltd	IFNAR1; IFNAR2
	Interferon alfa-2b	Merck & Co Inc	IFNAR1; IFNAR2
	Interferon beta	Toray Industries Inc	IFNAR1
	Interferon gamma-1a	Otsuka Pharmaceutical Co Ltd	IFNAR1

Therapy type	Name of Therapy	Company	Target
Cancer vaccine (7 in total)	BCG Live	Shire Plc	TLR
	ImmuCyst	Sanofi	TLR
	Immuno BCG	Ataulpho Paiva Foundation	TLR
	Mycidac-C	Cadila Pharmaceuticals Ltd	TLR2
	Sipuleucel-T	Dendreon	Unspecified TAA
	TICE BCG	Merck & Co Inc	TLR
	Uro-BCG	Medac Inc	TLR
Cell therapy (2 in total)	Tisagenlecleucel	Novartis AG	CD19
	Axicabtagene ciloleucel	Gilead	CD19
Oncolytic virus (2 in total)	Oncorine	Shanghai Sunway Biotech Co Ltd	CD40L
	Talimogene laherparepvec	Amgen Inc	GMCSFR
CD3-targeted bispecific ab	Blinatumomab	Amgen Inc	CD19 X CD3

Annals of Oncol. 2018 Tang et al.,

Ongoing revolution of IO agents

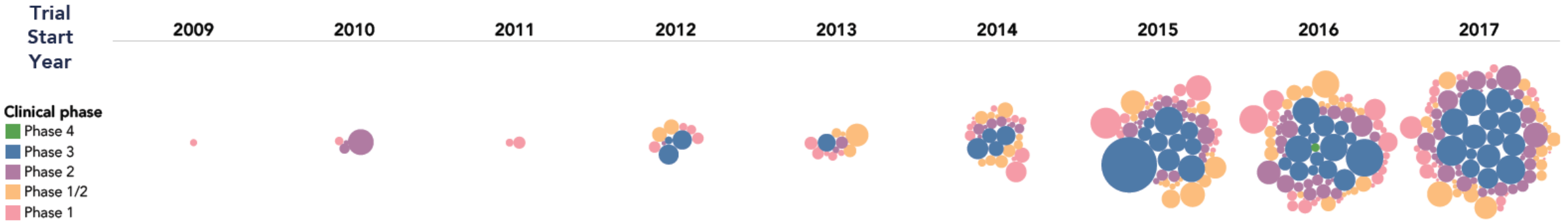
2004 new IO agents : 940 in clinical stages, 1064 in preclinical



Annals of Oncol. 2018 Tang et al.,

Number of new PD-1/L1 combo trials

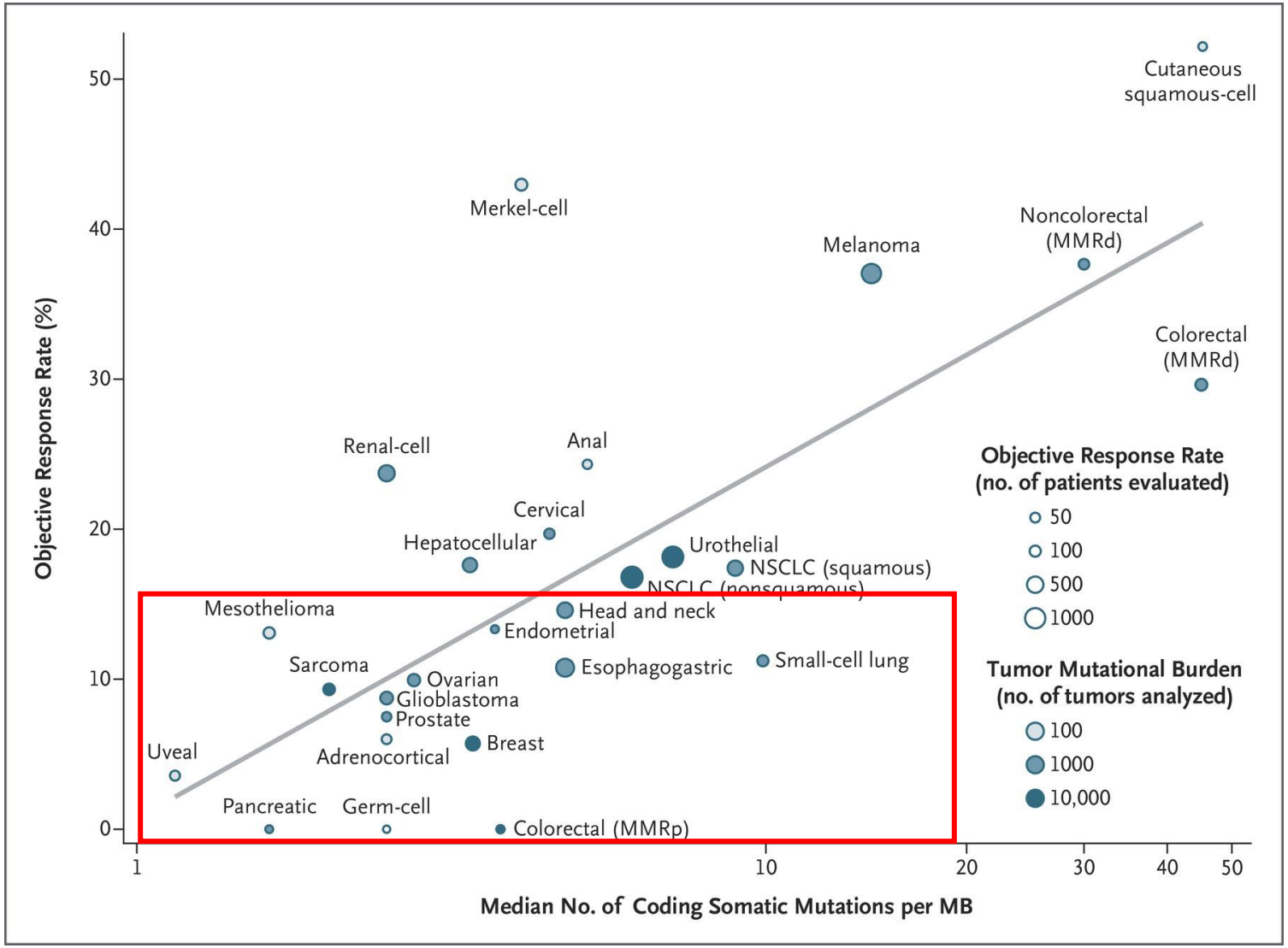
About 1100 PD-1/L1 combo in clinical trials



Trial Start Year	2009	2010	2011	2012	2013	2014	2015	2016	2017
Number of new trials	1	5	2	13	20	58	190	329	469
Planned new enrollment	136	2,473	582	4,867	5,031	11,276	39,821	46,153	52,539

Annals of Oncol. 2018 Tang et al.,

Many cancers remain unresponsive to ICBs



N Engl J Med 2017; 377:2500-2501

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Fatal Melanoma Transferred in a Donated Kidney 16 Years after Melanoma Surgery

TO THE EDITOR: We report a case of fatal melanoma that had been transferred in a donated kidney and that occurred 16 years after surgery for primary melanoma in the donor. A woman with polycystic disease received a renal transplant in May 1998. The graft functioned well. In November 1999, routine mammography showed a nodule in the left breast, and a biopsy specimen was obtained. Primary breast cancer was diagnosed. Pain and swelling then developed over the renal transplant, and two subcutaneous nodules were found. Biopsy confirmed the presence of secondary melanoma. No primary melanoma was identified. The pathological features of the breast specimen were reviewed, immunocytochemistry was performed, and secondary melanoma was diagnosed. Immunosuppression was stopped, the nodules were excised, and the patient underwent a trial of interferon, which was stopped because of toxicity. She died of metastatic melanoma in March 2000. In May 2000, a man presented with a palpable lump over a kidney, also donated in May 1998. The function of the graft had been good. Renal biopsy showed secondary melanoma, and again no primary tumor was identified.

The transplant registry showed that both of these patients had received a kidney from the same donor, who had died from a presumed subarachnoid hemorrhage. Autopsy had not been performed. The pa-

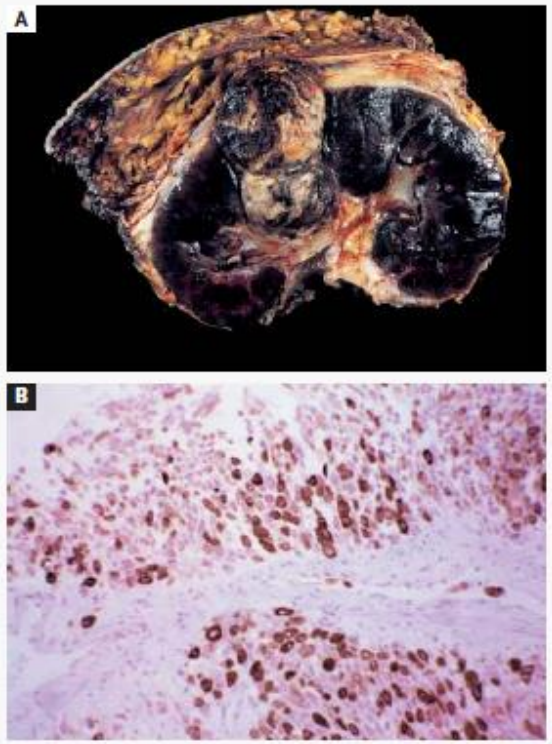
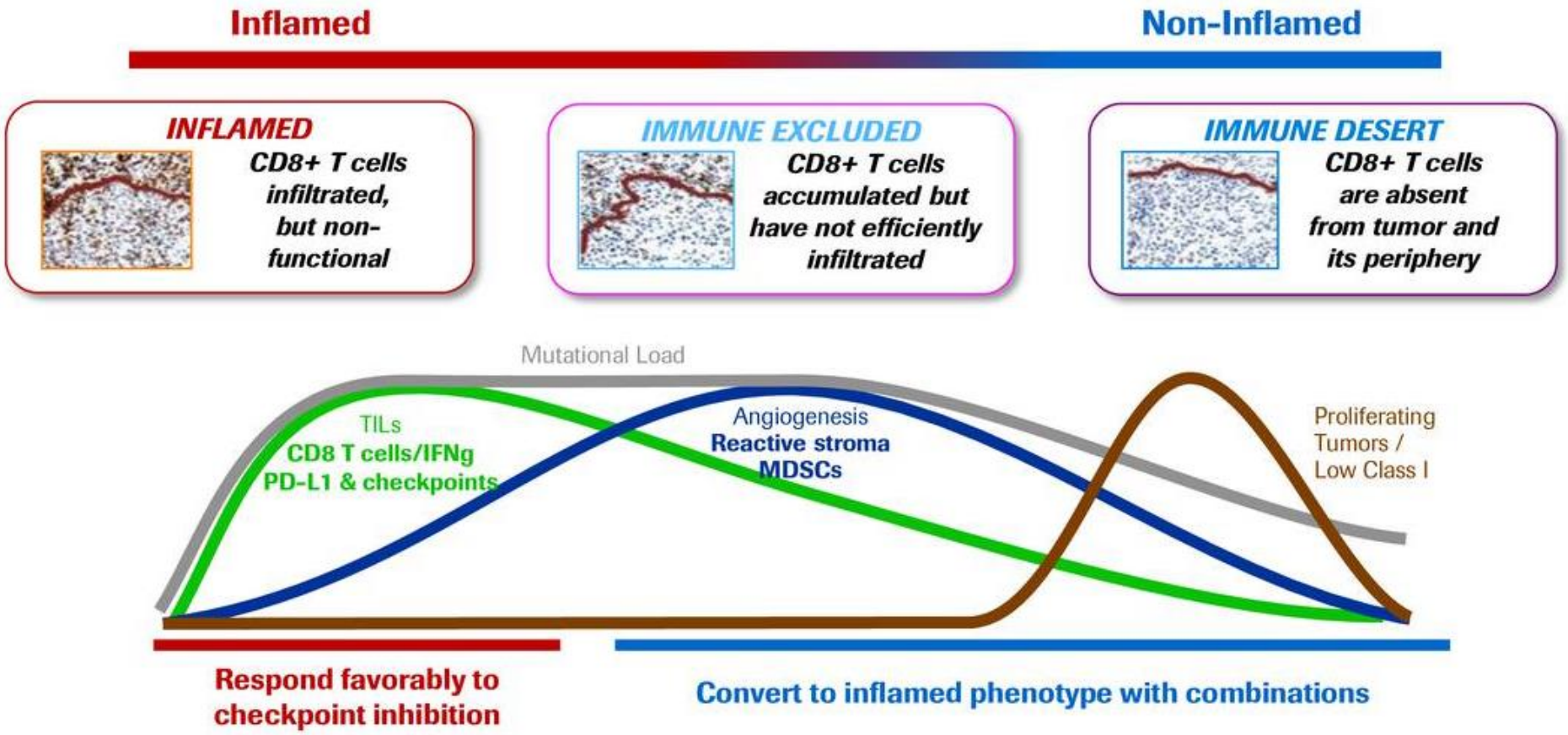


Figure 1. Affected Kidney from Patient 2.
The excised kidney is necrotic and contains a large, central mass of melanoma tissue (Panel A). An S-100–stained specimen of the kidney shows striking cytologic atypia and strong S-100 positivity (Panel B).

Three basic immune profiles correlating with response



Modified from Hegde PS et al. (2016) *Clin Canc Res*

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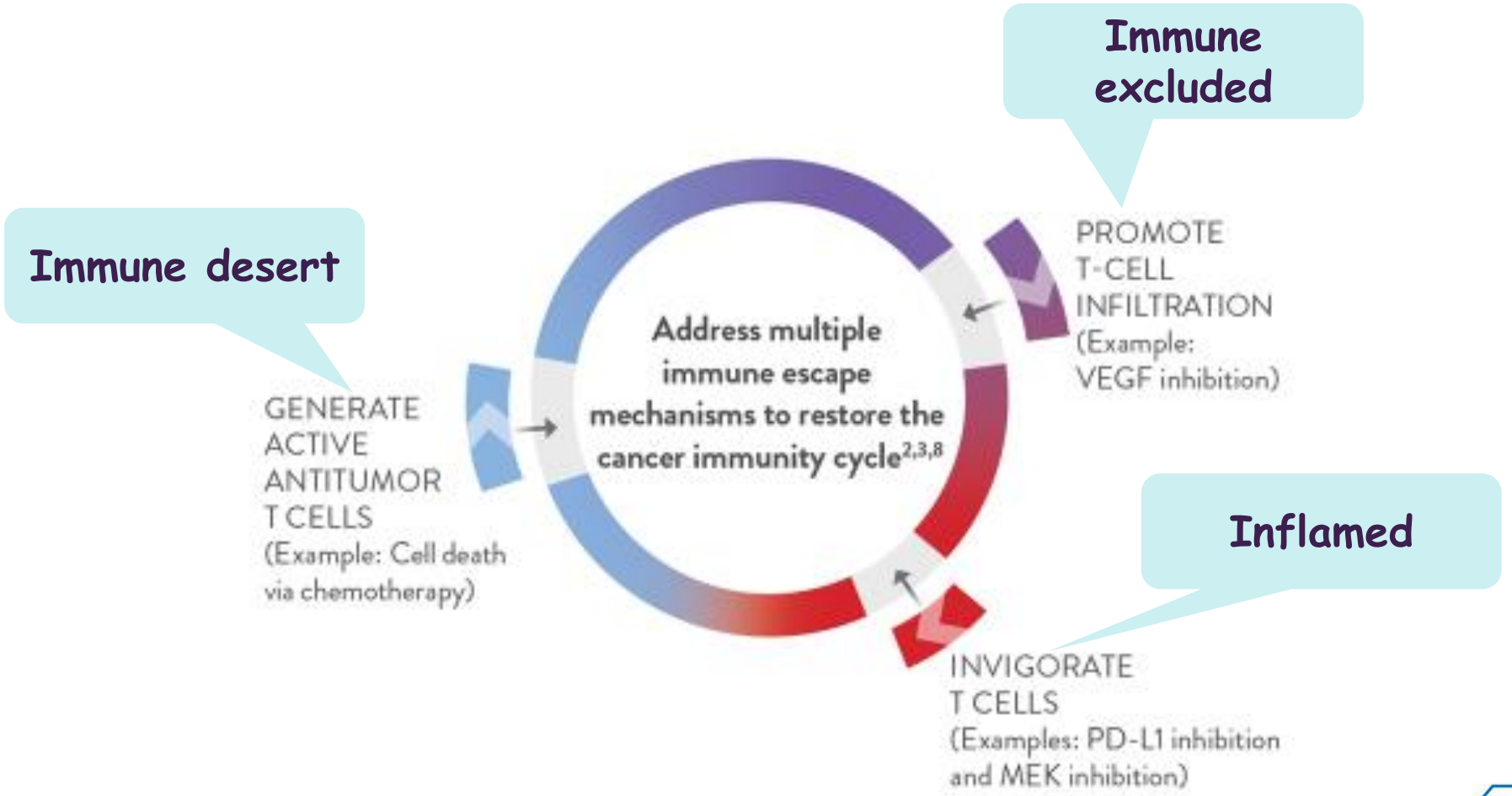
How to overcome immune desert and excluded



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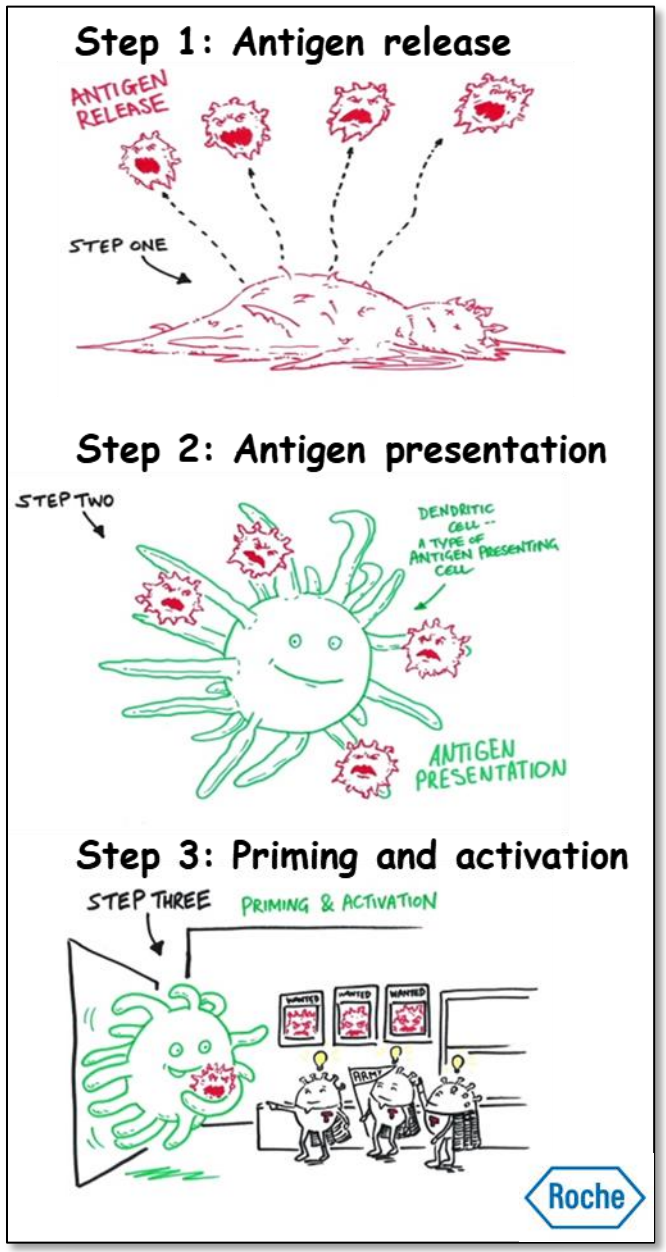
How efficiently can we generate tumor specific T cells?



Generation of tumor reactive CD8+T cells by intrinsic vaccination

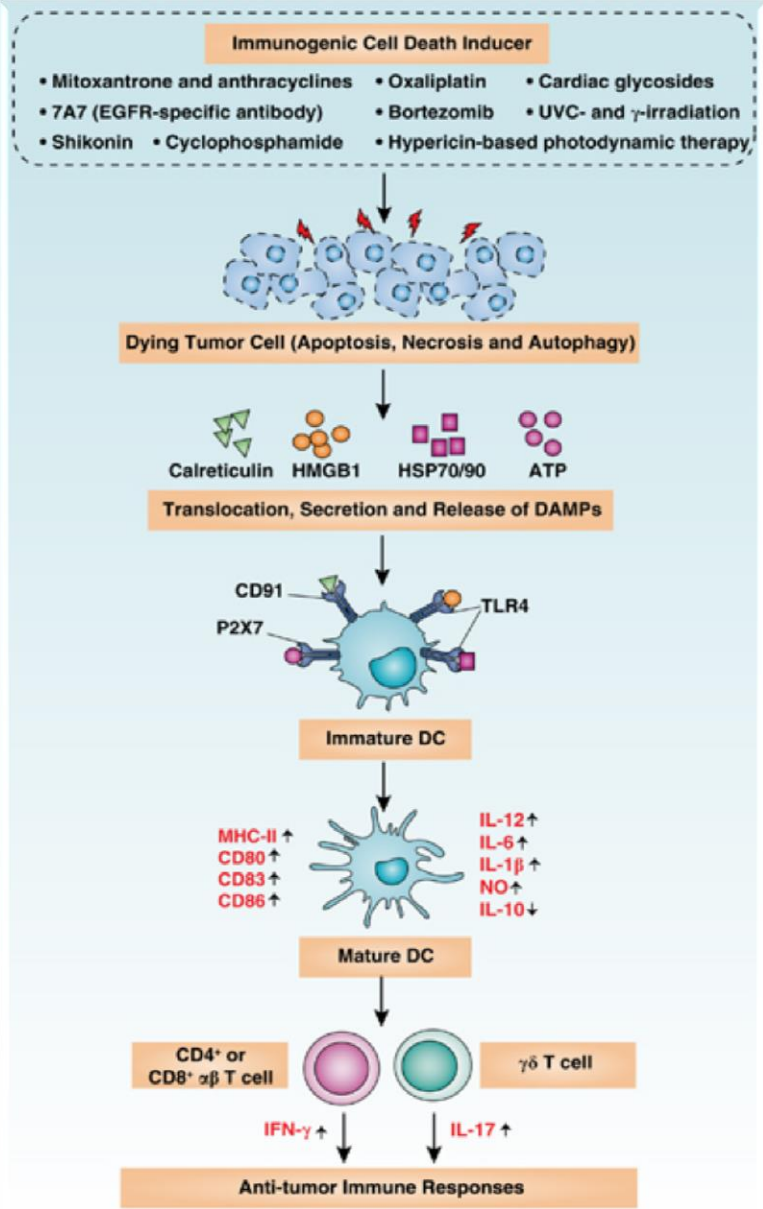
1. Release of cancer neo-antigens : Immunogenic cell death
2. Processing and X-presentation of tumor antigens : Enhanced phagocytosis
3. Priming and activation of T cells : Migratory CD103/134^{+} DC (Batf3-lineage DC)

What is "Intrinsic cancer vaccination"?

The goal of ICV is to treat cancer or to prevent it from coming back

- ICV is a therapeutic strategy to make cancer vaccines within our body harnessing our own vaccine generating system.
  - ❖ ICV induces cancer cells to release tumor specific antigens.-tumor antigens are naturally selected
  - ❖ ICV activates APCs effectively to present tumor specific antigens to the adaptive immune system.-dendritic cells and tumor-specific T cells are naturally activated
  - ❖ ICV facilitates self propagation to prime the immune system to attack the cancer cells in the body.

What is immunogenic cell death?

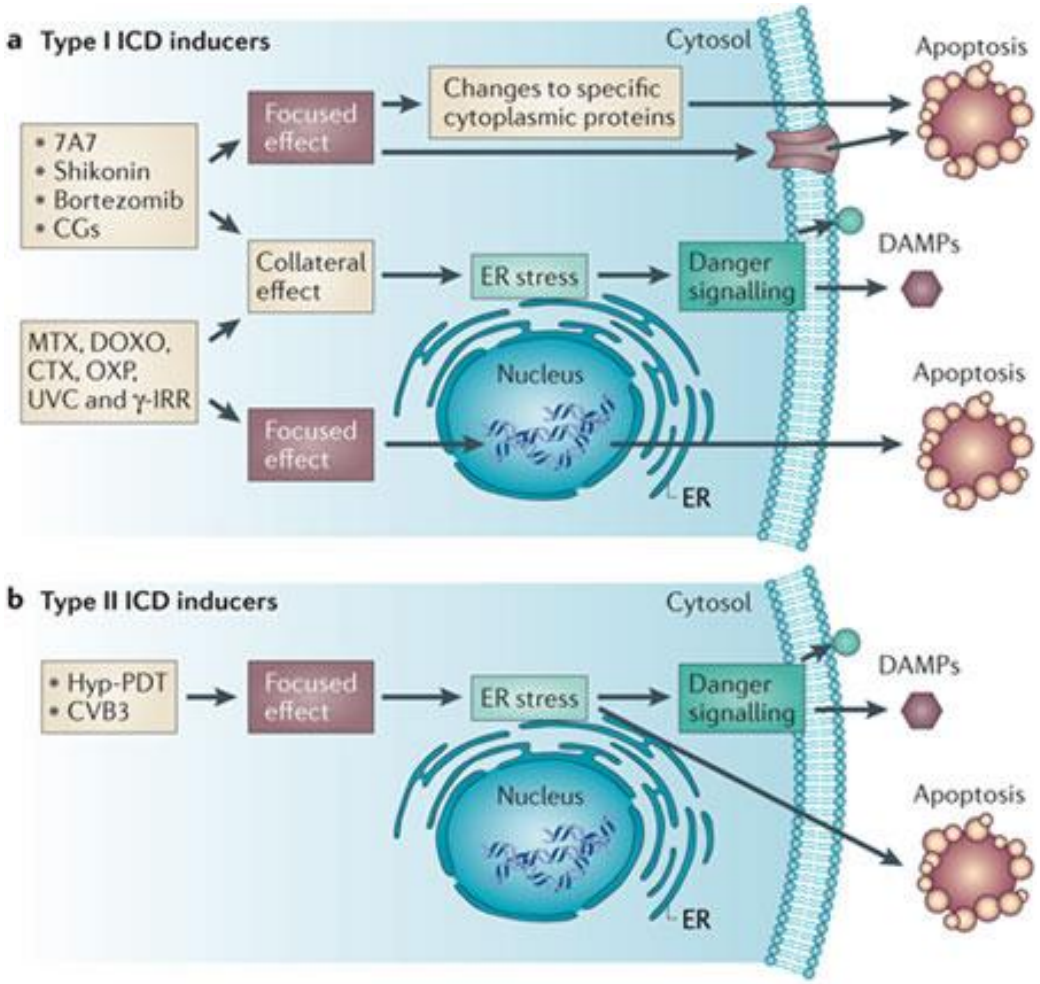


Immunogenic cell death (ICD) involves changes in the composition of the cell surface as well as the release of soluble mediators, occurring in a defined temporal sequence. Such signals operate on a series of receptors expressed by dendritic cells to stimulate the presentation of tumor antigens to T cells.

Hou W et al., Cell Death Dis 2013

What can cause immunogenic cell death?

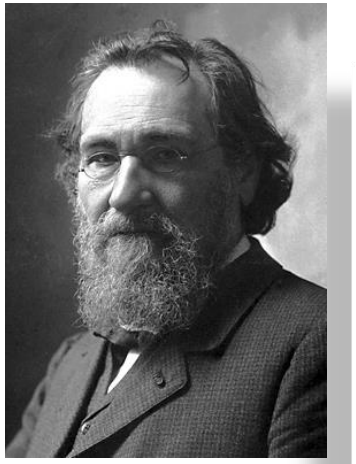
Small chemicals, Radiation, PDT, OVs…



Type I ICD inducers are modalities that induce cell death via non-ER associated targets and danger signalling via ER stress; however this split in targeting might compromise their ability to fully target the ER (site of off-target/collateral effects). On the other hand, **Type II ICD** inducers selectively target the ER to induce both cell death as well as danger signalling thereby causing ICD-associated immunogenicity in an ER-focused (on-target) manner.

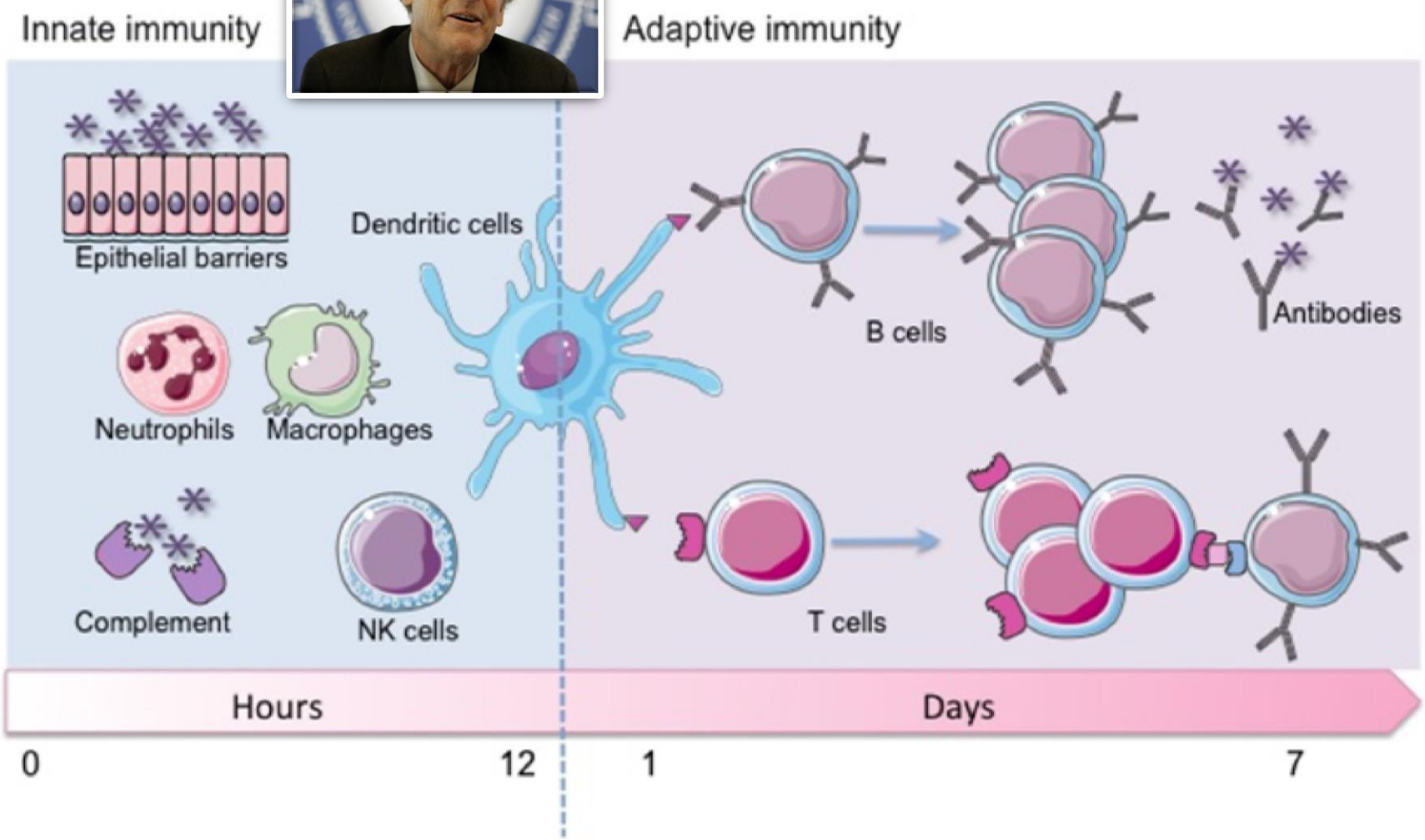
How is phagocytosis associated with caner immunity?

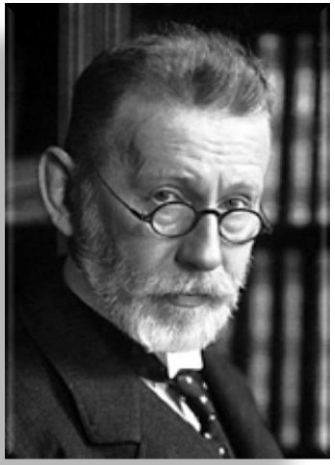
Phagocytic activity of dendritic cells links innate to adaptive immunity in cancer



Ilya Ilyich Mechnikov 1908 Nobel

RALPH STEINMAN, 2011 Nobel





Paul Ehrlich 1908 Nobel

How to enhance phagocytic activity of dendritic cells?

Modulators of "Don't eat me" signals

- Anti-CD47 antibody
- SIRPα (variant)

Modulators of Tickling signals

- ROCK inhibitor
- HMG-CoA reductase inhibitor

Inducer of phagocytic machinery

- PPARγ agonists
- LXR agonists

ICV strategy for overcoming immune desert

Nanocage-Therapeutics Prevailing Phagocytosis and Immunogenic Cell Death Awakens Immunity against Cancer

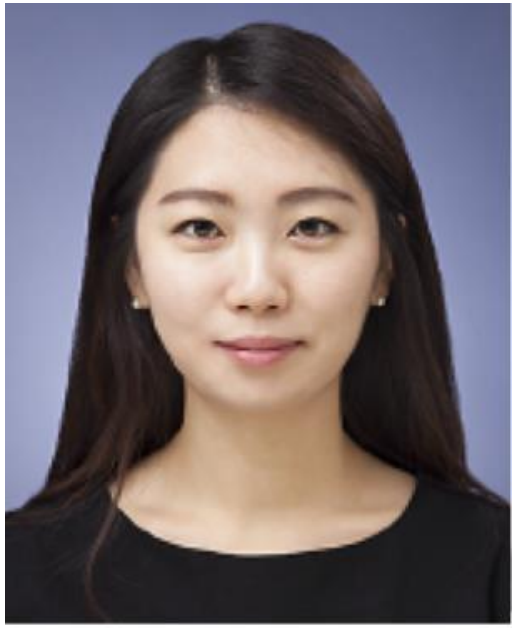
- 2018 Adv Mater

Combined Rho-kinase inhibition and immunogenic cell death triggers and propagates immunity against cancer

- 2018 Nat Commun.

Nanocage-Therapeutics Prevailing Phagocytosis and Immunogenic Cell Death Awaken Immunity against Cancer

Advanced Materials, 2018



Eun Jung Lee, Ph.D.

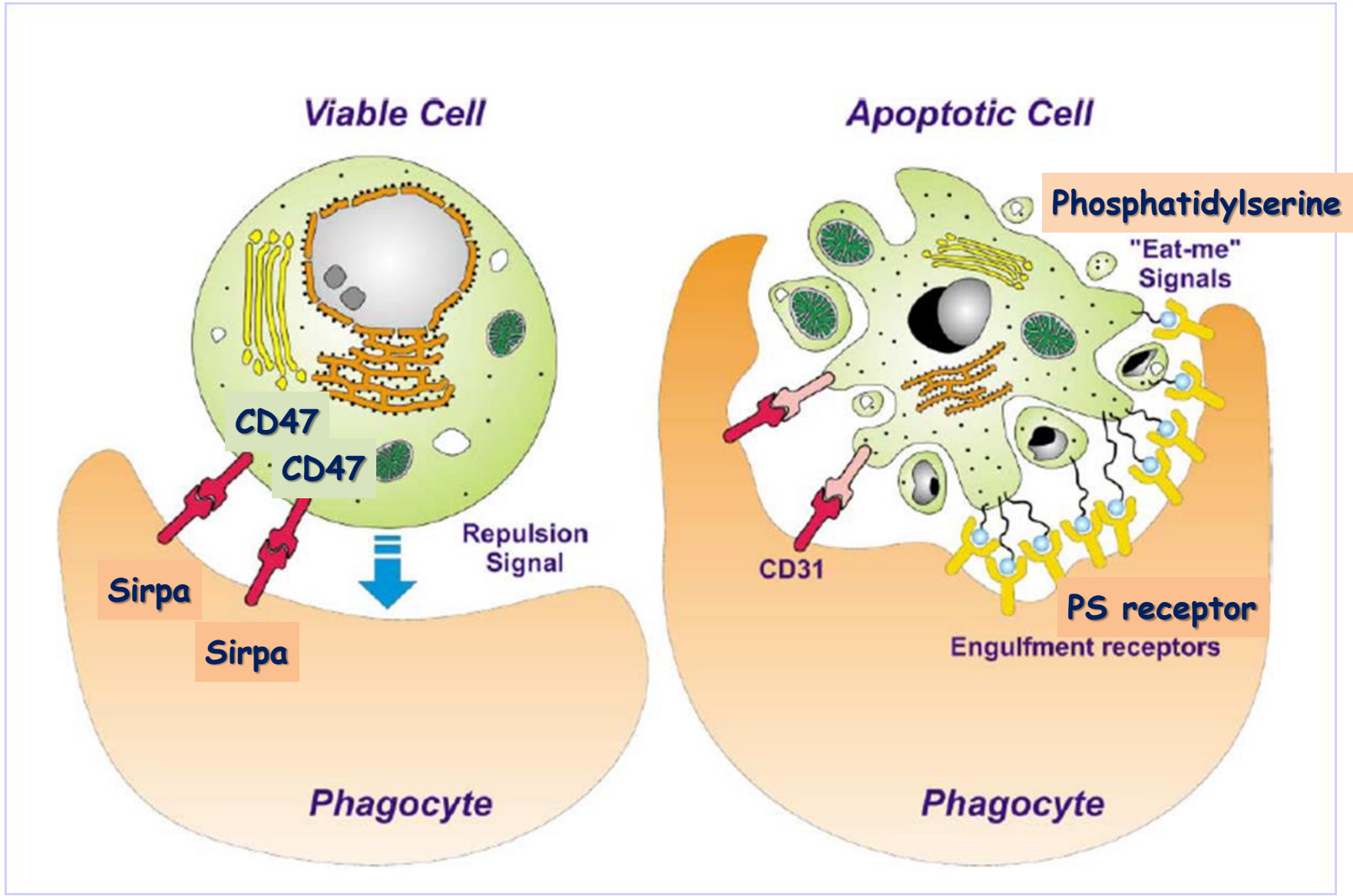
Kihoon Nam, M.D.

Doxorubicin causes immunogenic cell death.

Small chemicals, Radiation, PDT, OVs…

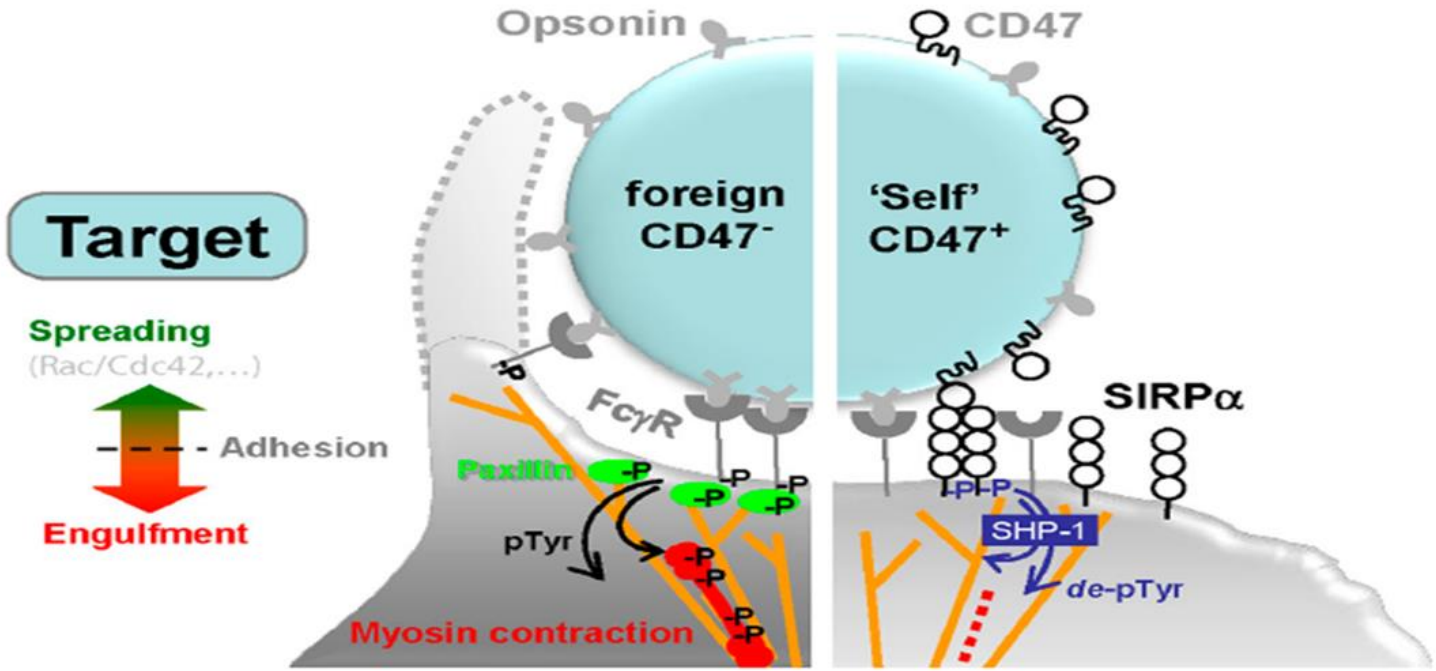
Drugs
Doxorubicin
Daunorubicin
Mitoxantrone
Oxaliplatin
Cyclophosphamide
Bortezomib
Paclitaxel
Docetaxel

"Eat-me" vs "Don't eat-me" signal



Blocking "Don't eat-me signal" (CD47-SIRPa pathway) enhances phagocytosis

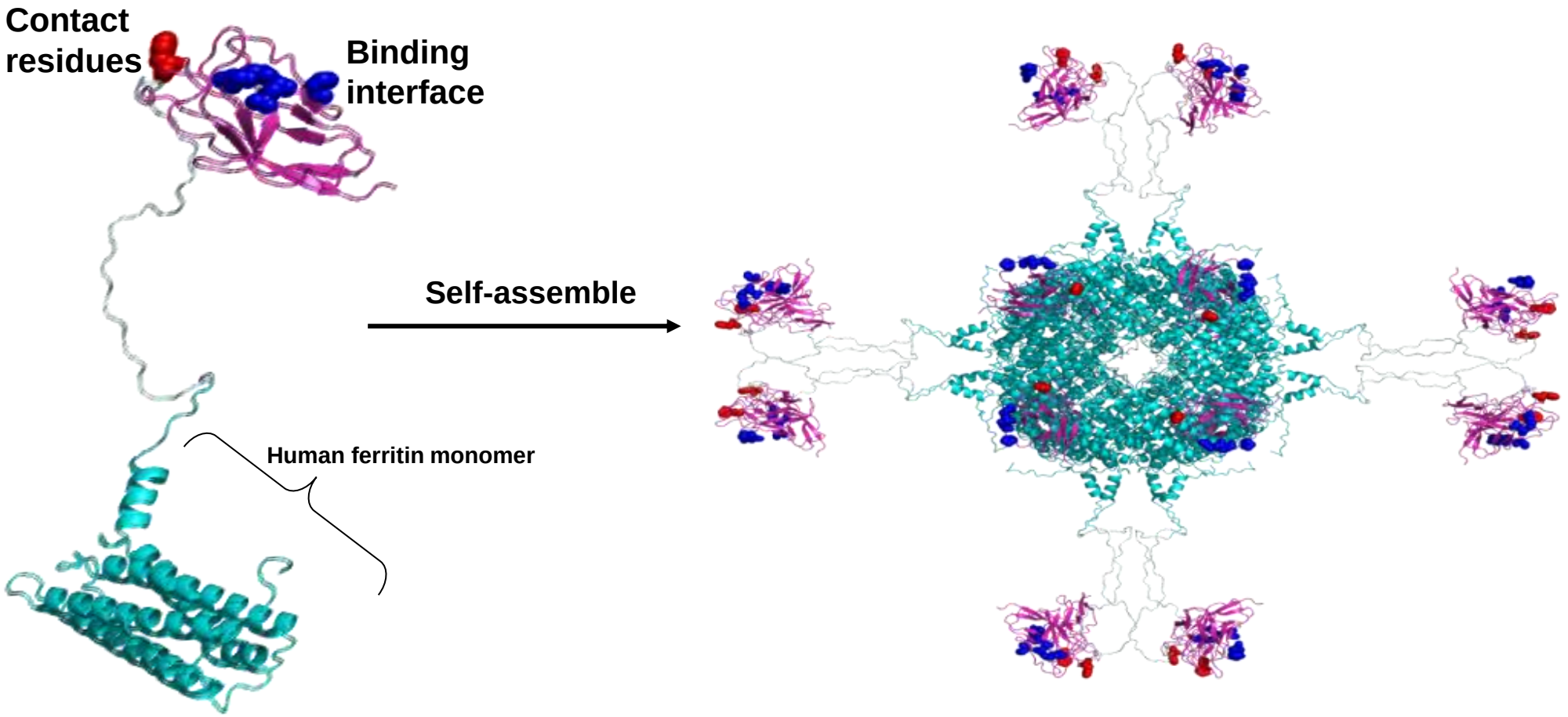
: The increased expression of CD47 on many different human tumor types, and its known function as a "don't eat me" signal



: Efforts have been made to develop therapies inhibiting the CD47-SIRPa pathway, principally through blocking monoclonal antibodies directed against CD47, but also possibly with a recombinant SIRPa protein that can also bind and block CD47

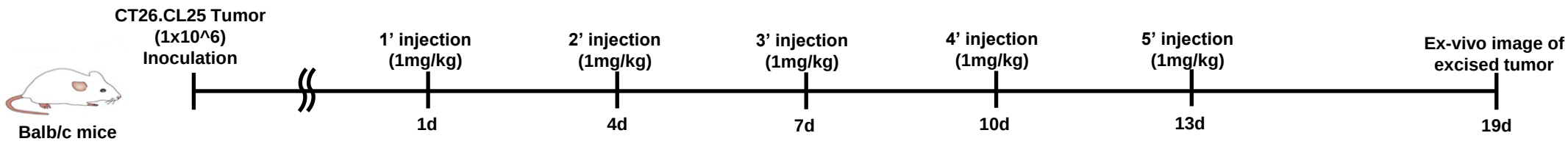
24 Sirpa fused to ferritin monomer is exposed on nanocage surface

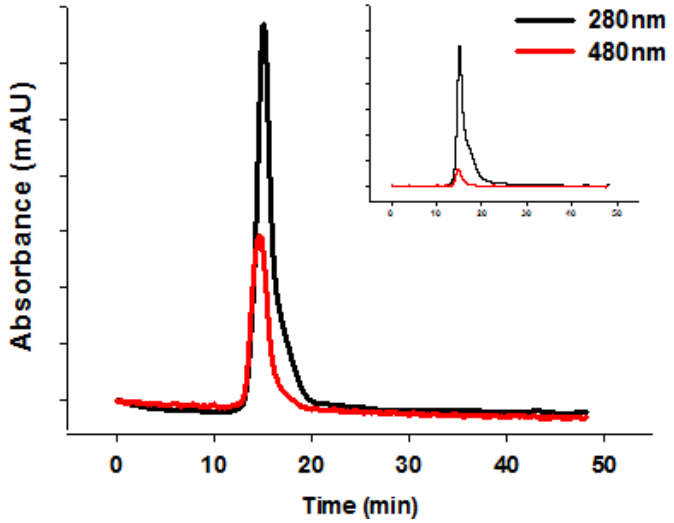
Prediction of ferritin-Sirpα structure by computer simulation

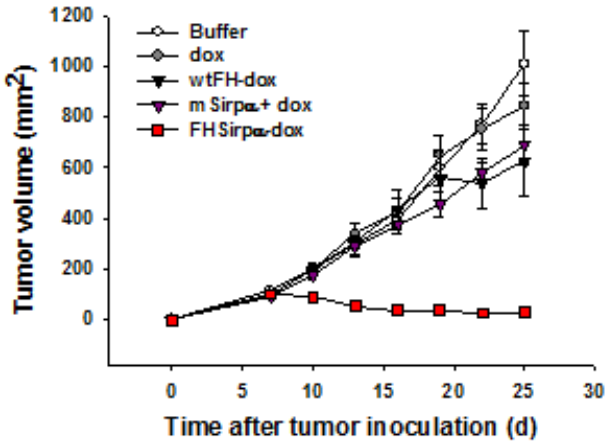


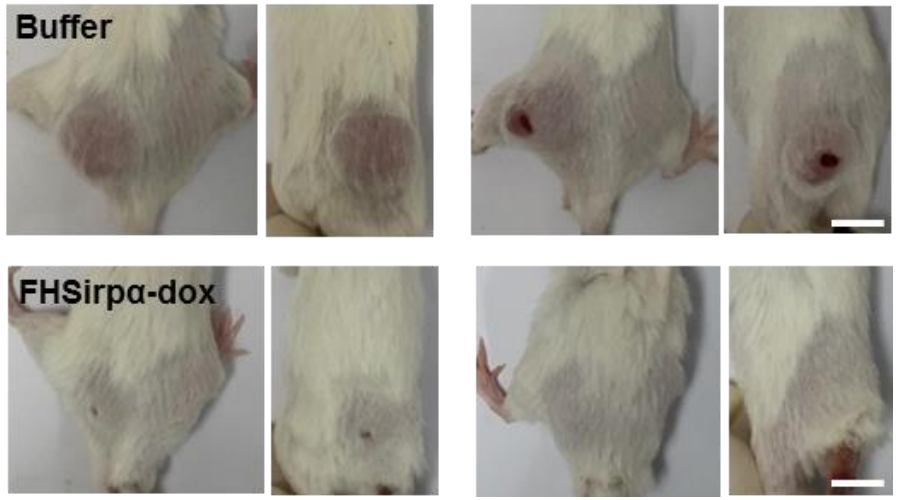
Ferritin-Sirpa-Doxo completely inhibits tumor growth

In vivo tumor growth inhibition analysis of Dox-FTH-SIRPα

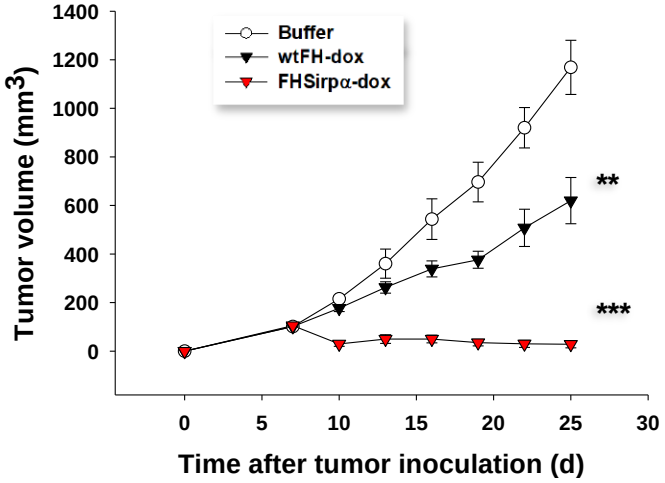




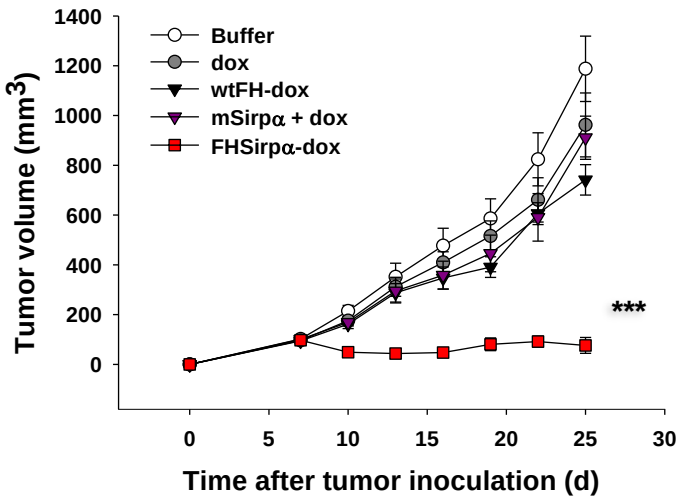




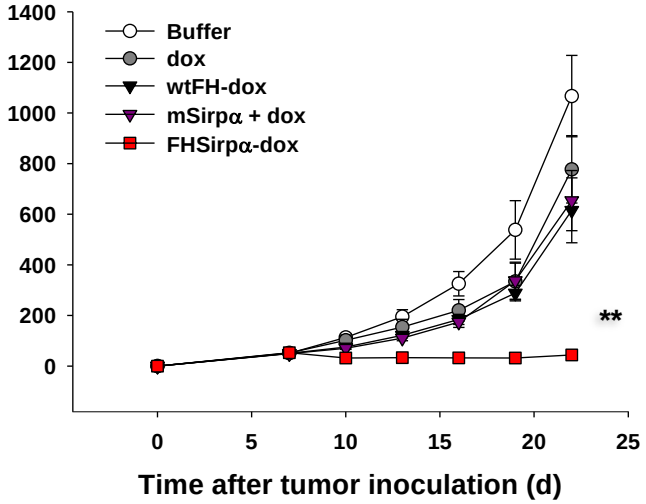
Single IT injection of Ferritin-Sirpa-Doxo completely inhibits tumor growth



CT26 wild type



B16.OVA melanoma



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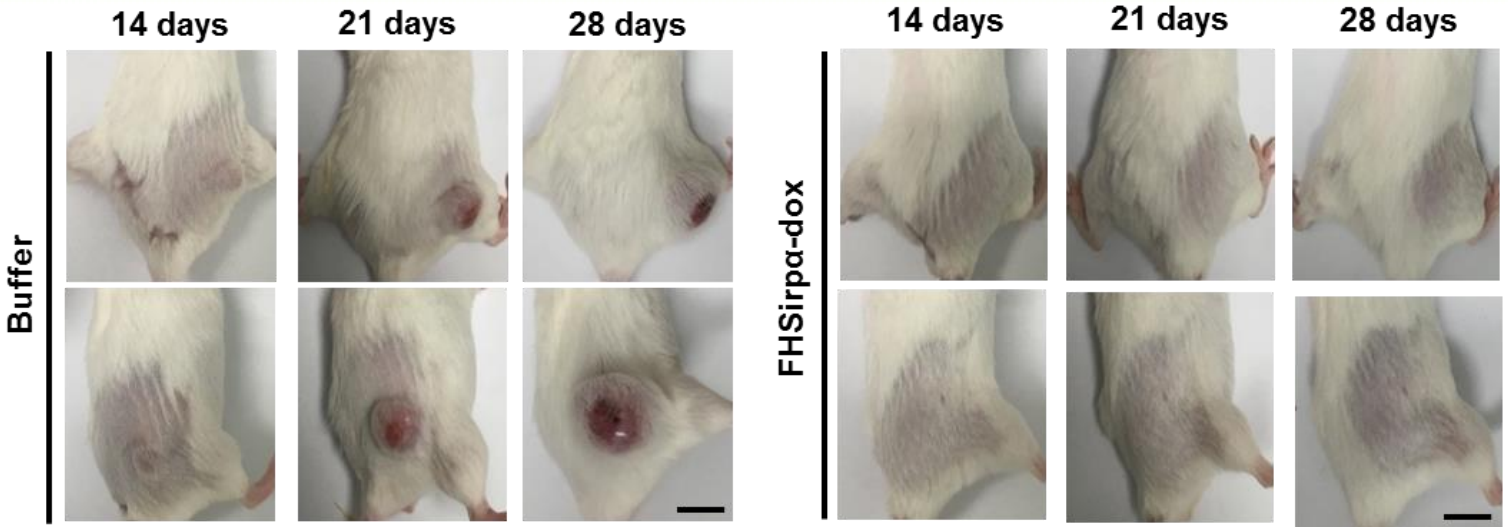
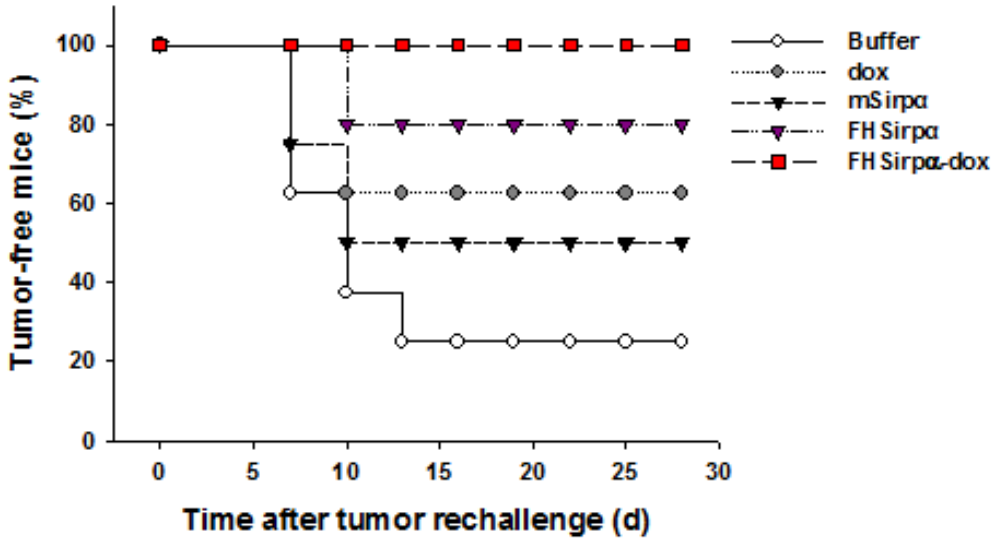
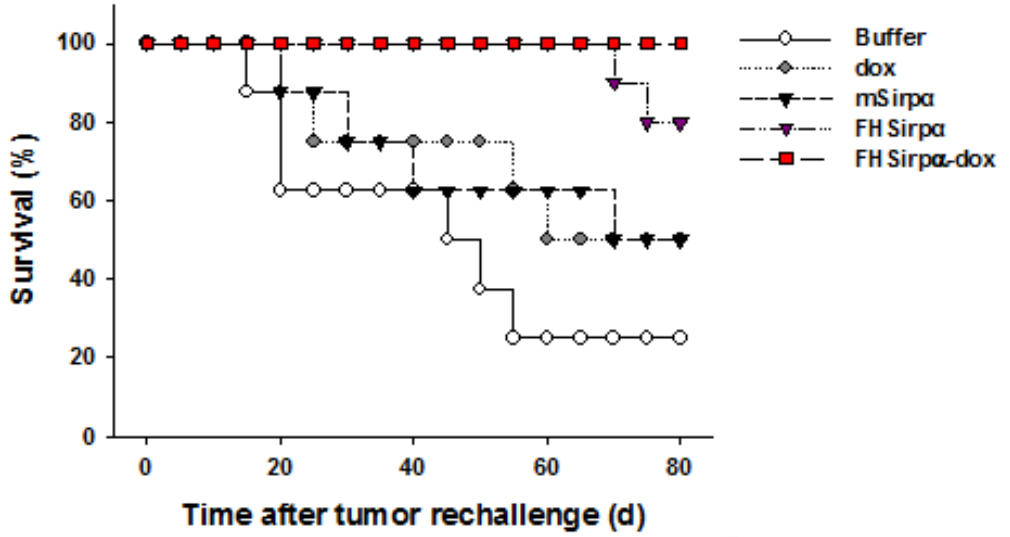
Ferritin-Sirpa-Doxo prevents regrowth of cancer



No tumor growth at all when rechallenged with the same cancer cells





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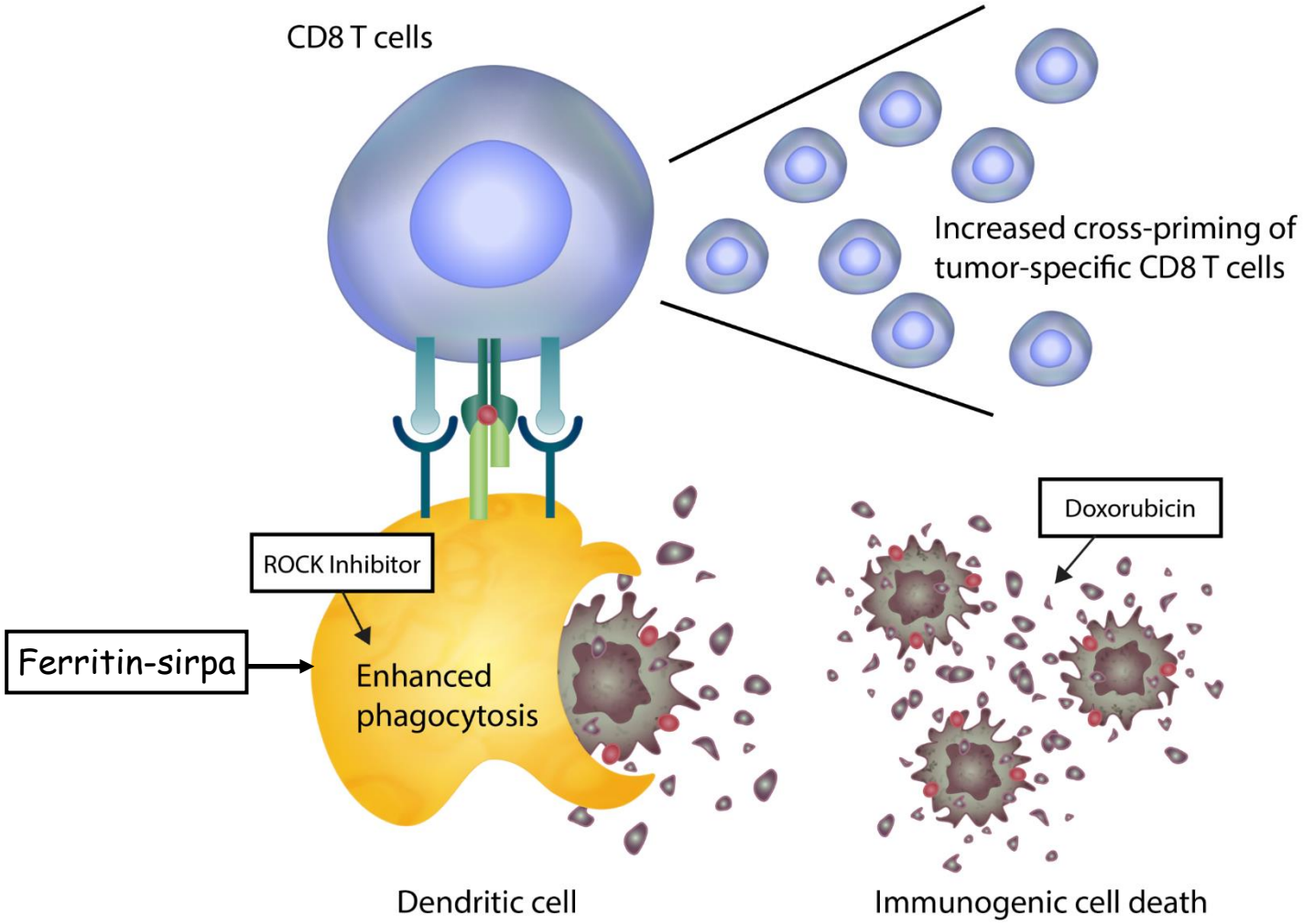
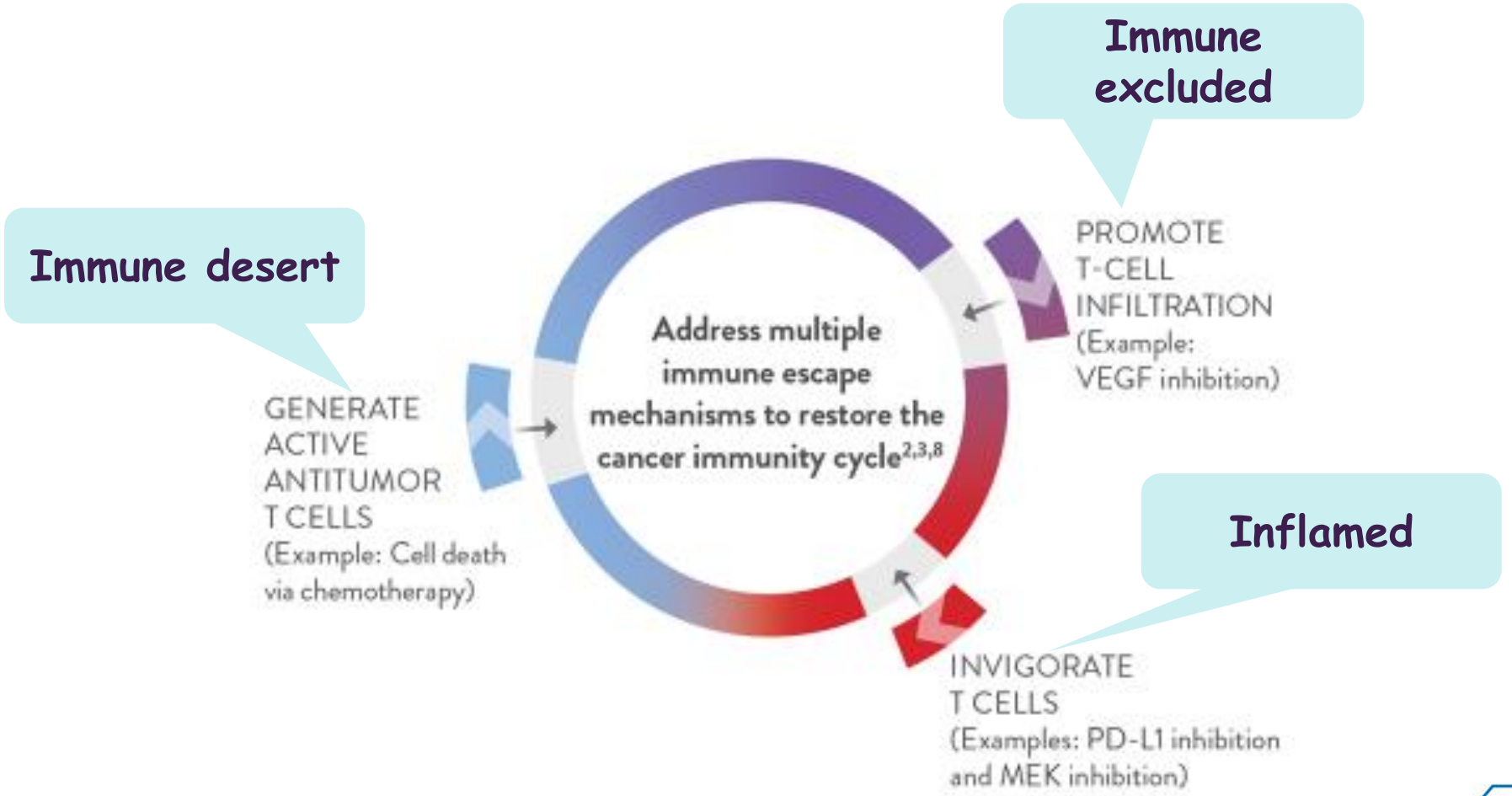
Combined phagocytosis enhancers and immunogenic cell death inducers triggers and propagates immunity against cancer



How to overcome immune desert and excluded



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Enzymatic Exosomes with Hyaluronidase PH20 for Tumor Penetration

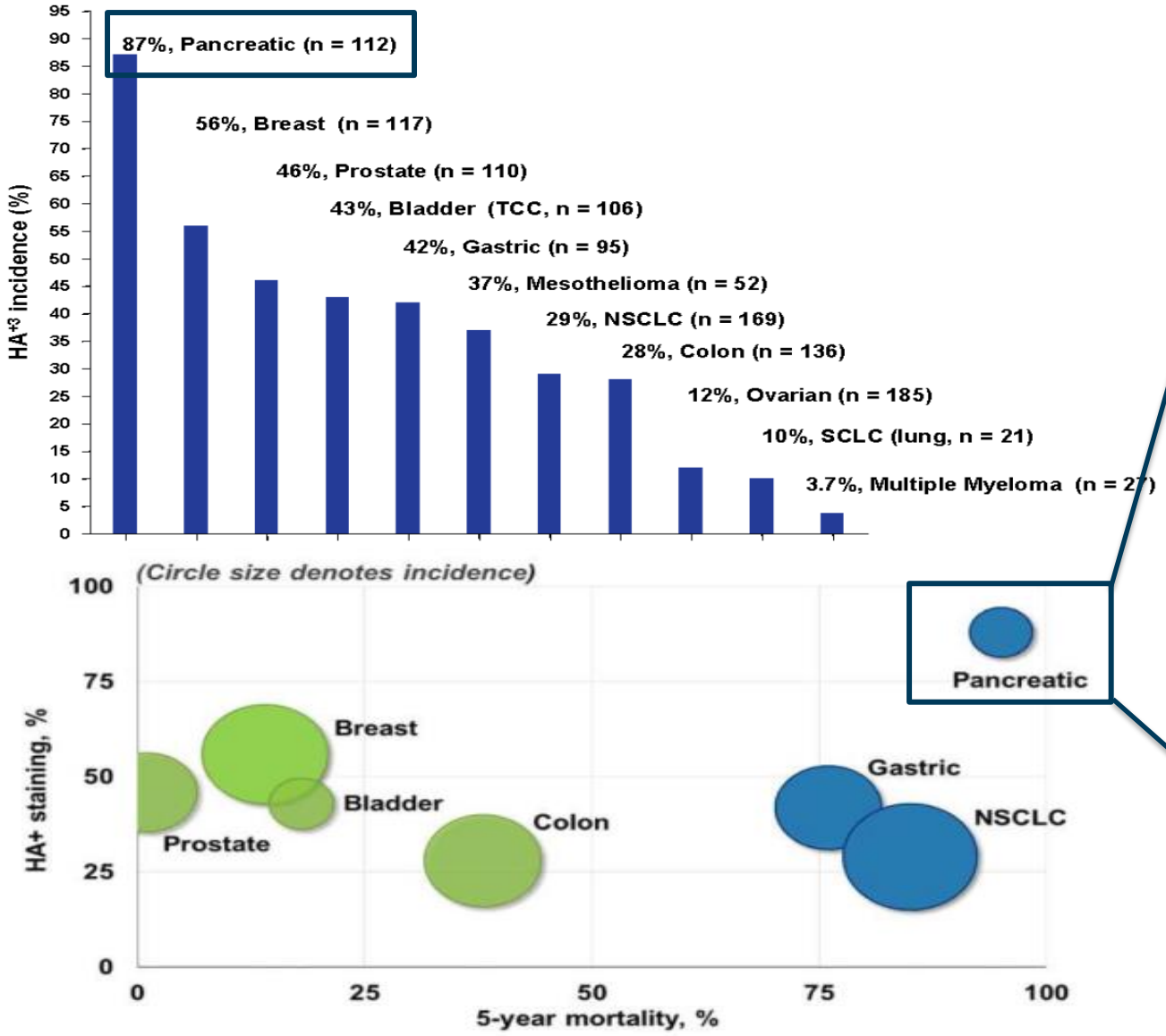
Advanced Functional Materials. 2017 & unpublished data

Hong, Yeonsun.
PhD student

Yoosoo Yang, Ph.D.
Senior Research Scientist
at KIST

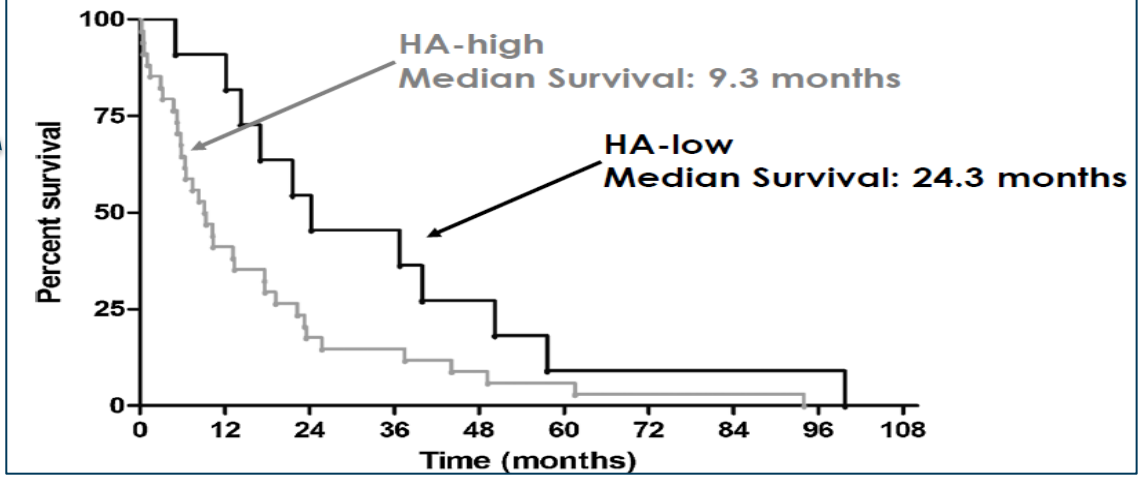
KIST
Korea Institute of Science and Technology

How abundant is HA in solid tumors?

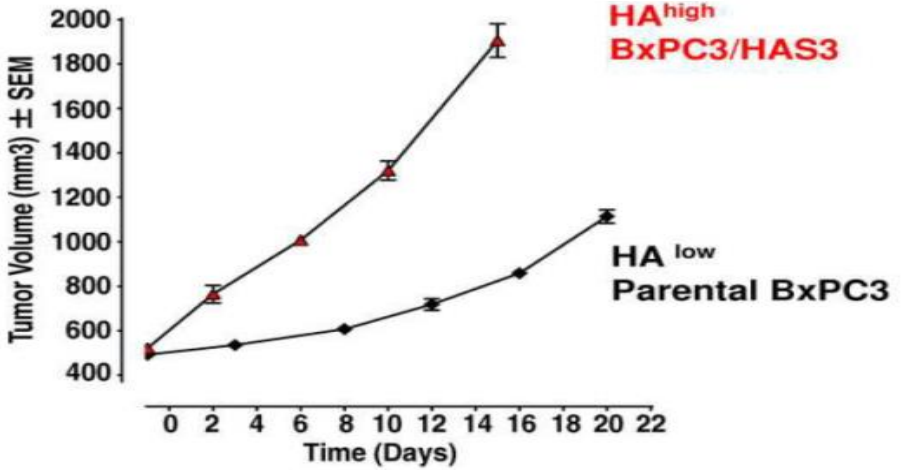


Decreased Survival

Pancreatic Ductal Adenocarcinoma[1]

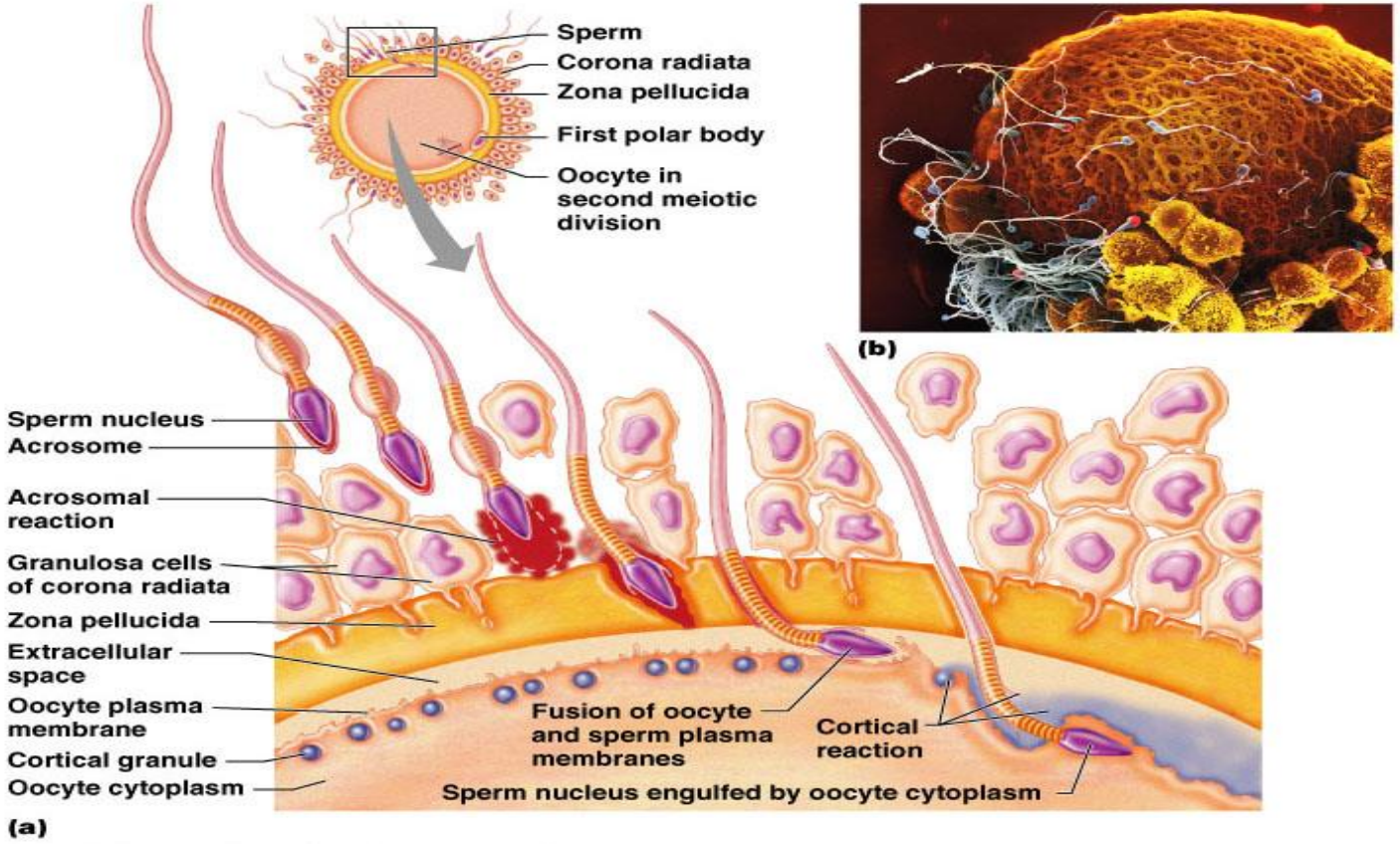


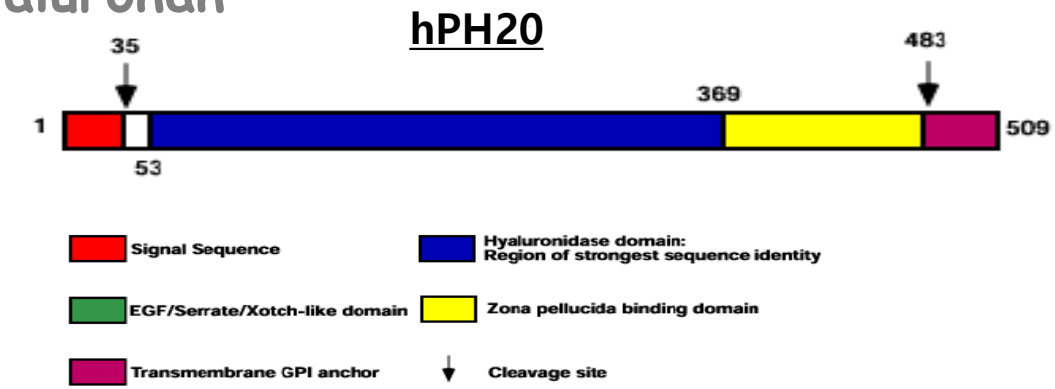
Tumor growth stimulation

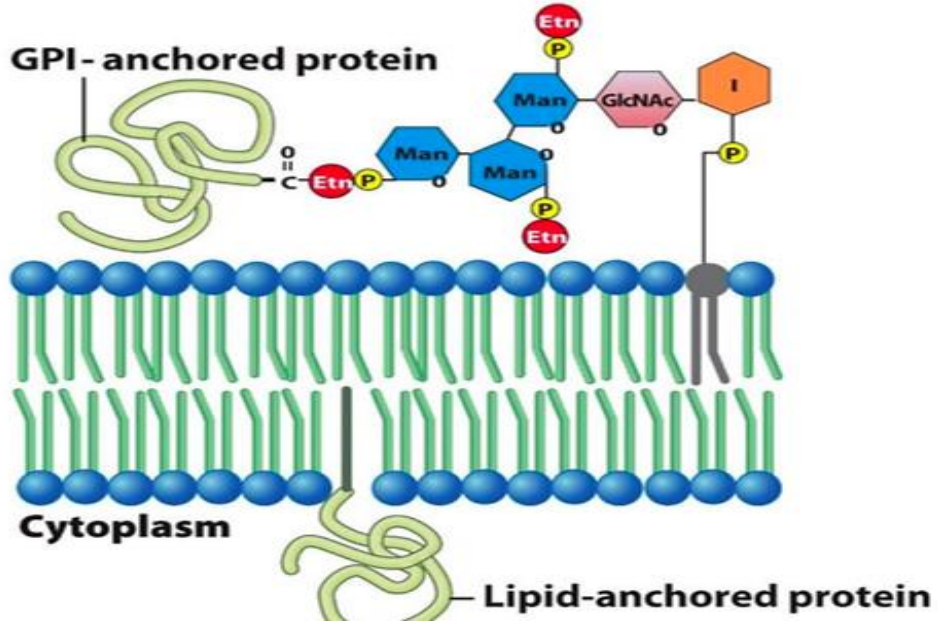


PH20 is a membrane anchored enzyme

PH20 is a hyaluronidase, GPI-anchored protein and required for sperms to penetrate oocytes, which are surrounded by hyaluronan



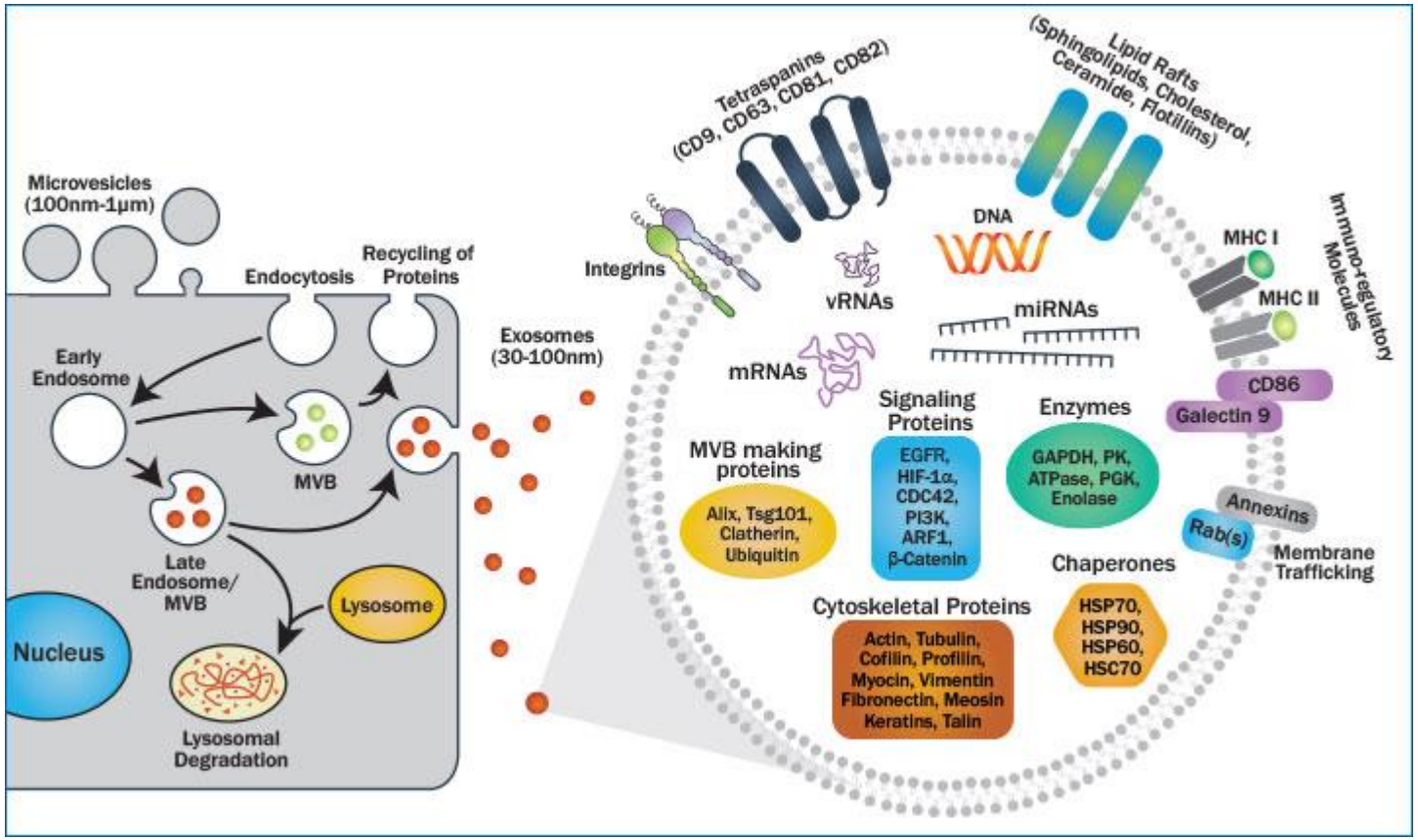




Questions?

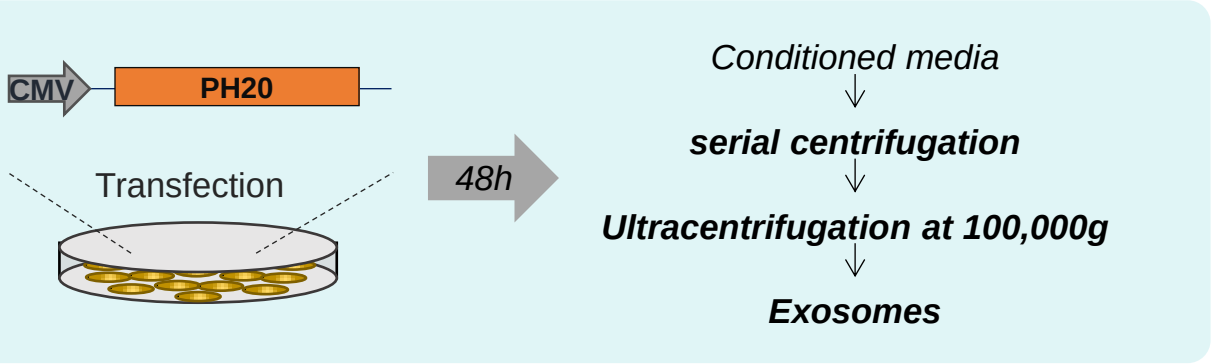
- Can we reduce dose of PH20 and deliver it selectively to tumor?
- Degradation products of HA act as an immune stimulator?

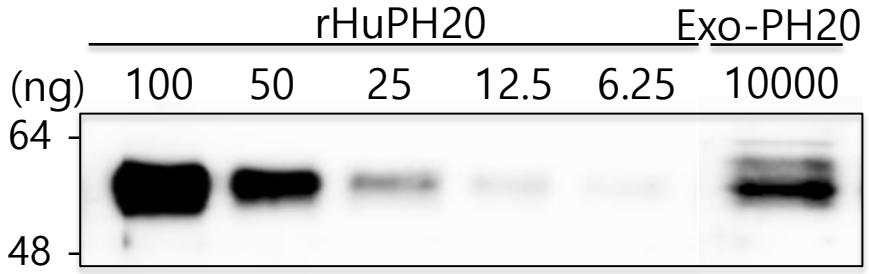
Exosomes are perfect nanovesicles for membrane protein delivery



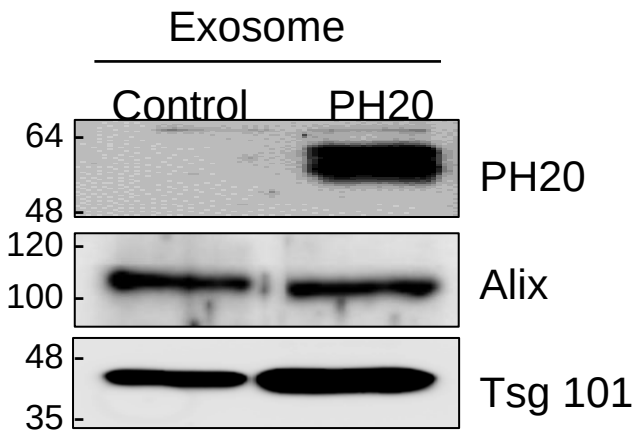
Results

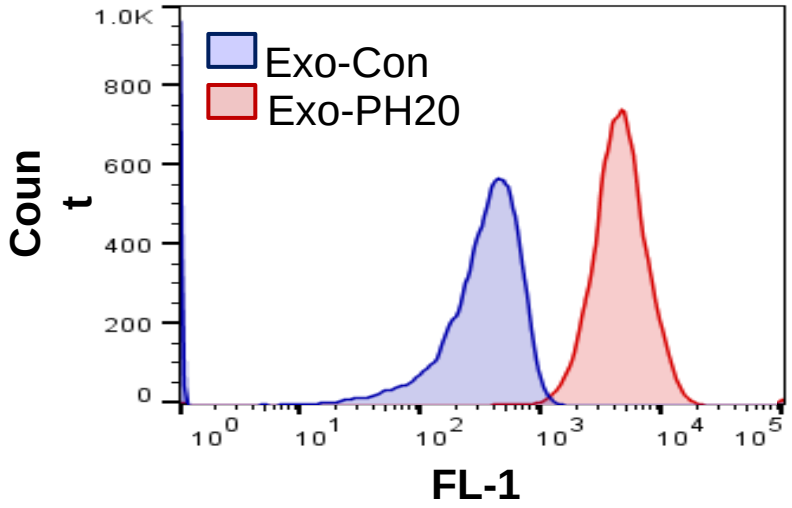
Preparation of exosome harboring PH20 proteins from HEK293T cell lines

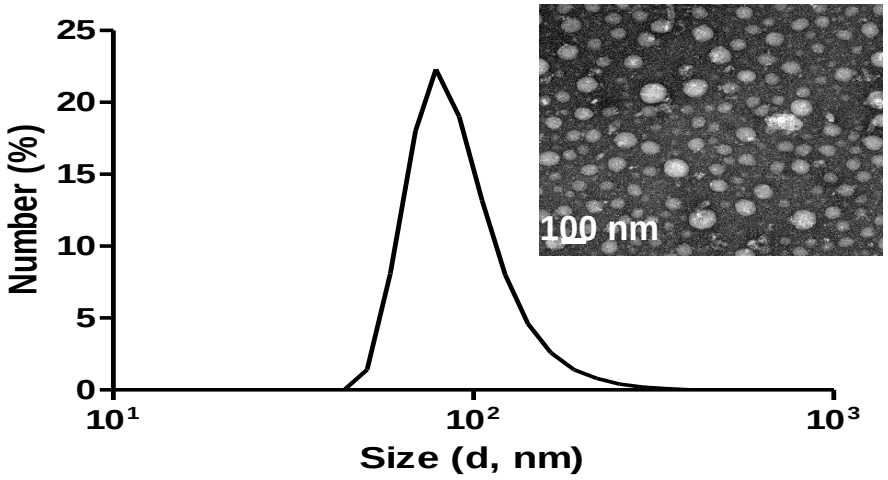




- Amount of PH20: 57 ng PH20 in 10 μg exosomes







- The purified Exo-PH20 contained exosomal marker proteins (Alix and Tsg101) and PH20 on their surface membranes.
- TEM, DLS : Exosomes have round in shape with an average size of 100 nm

IV injection of Exo-PH20 enhances liposome accumulation in tumor

I.V injection

Exo-PH20 200 ug

Exo-control 200 ug

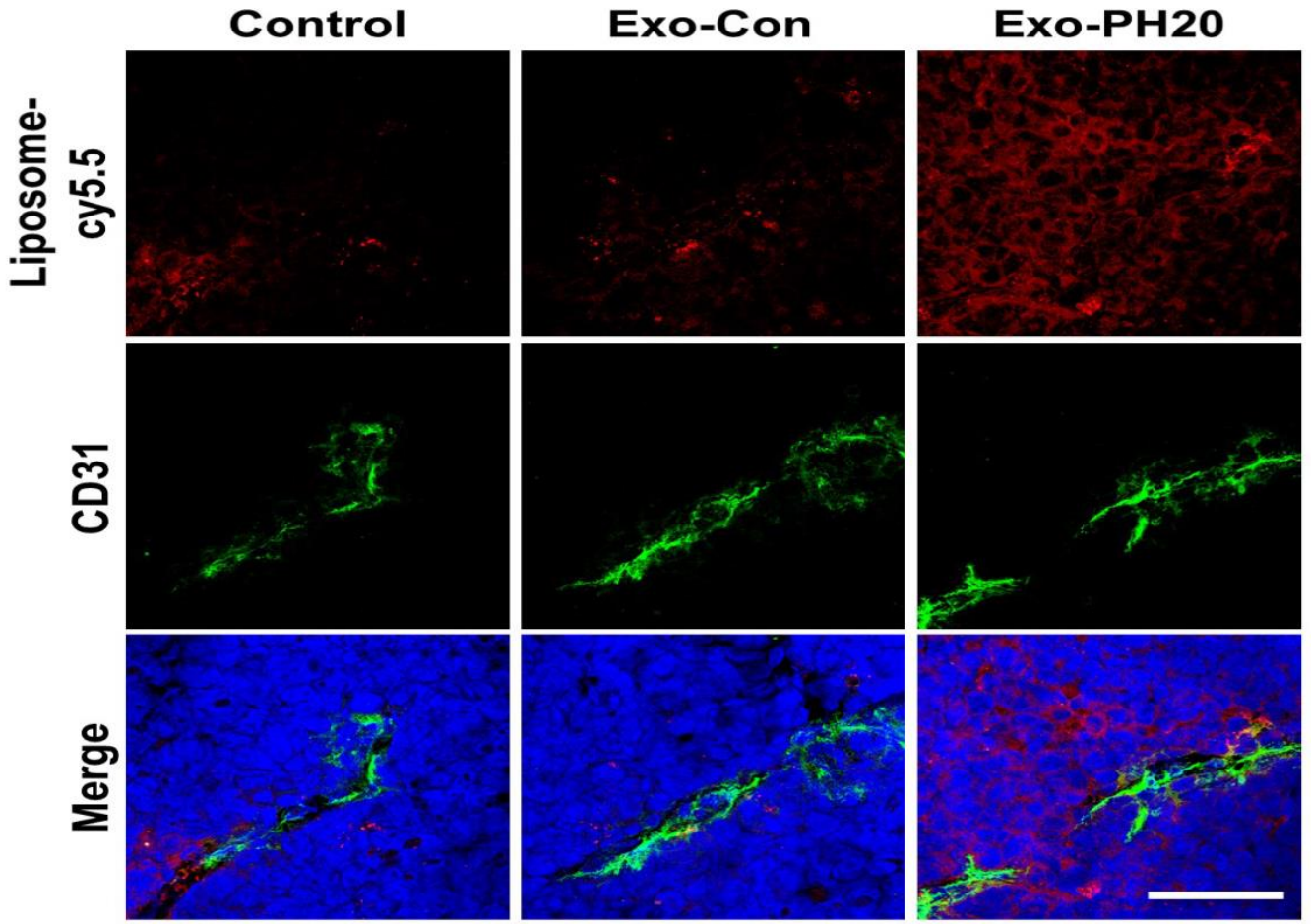
3h →

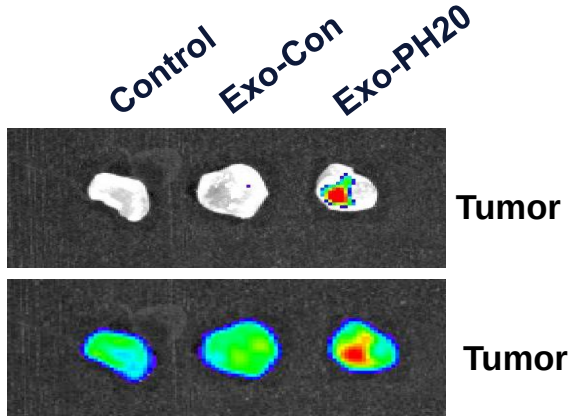
I.V injection

[Liposome-cy 5.5] 4 mg/kg

7h →

Tumor extraction





Intravenous injection of Exo-PH20 increases blood flow

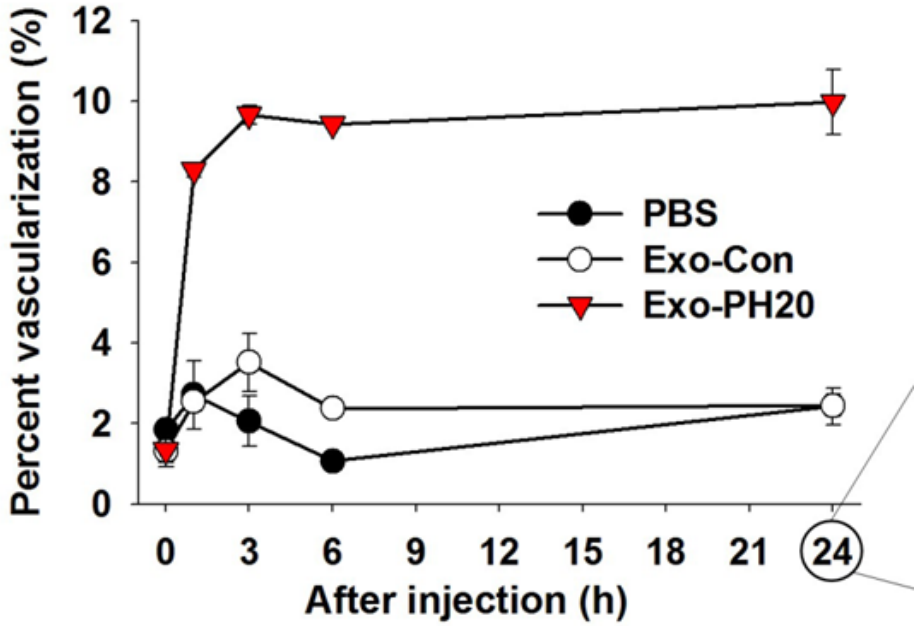
I.V injection

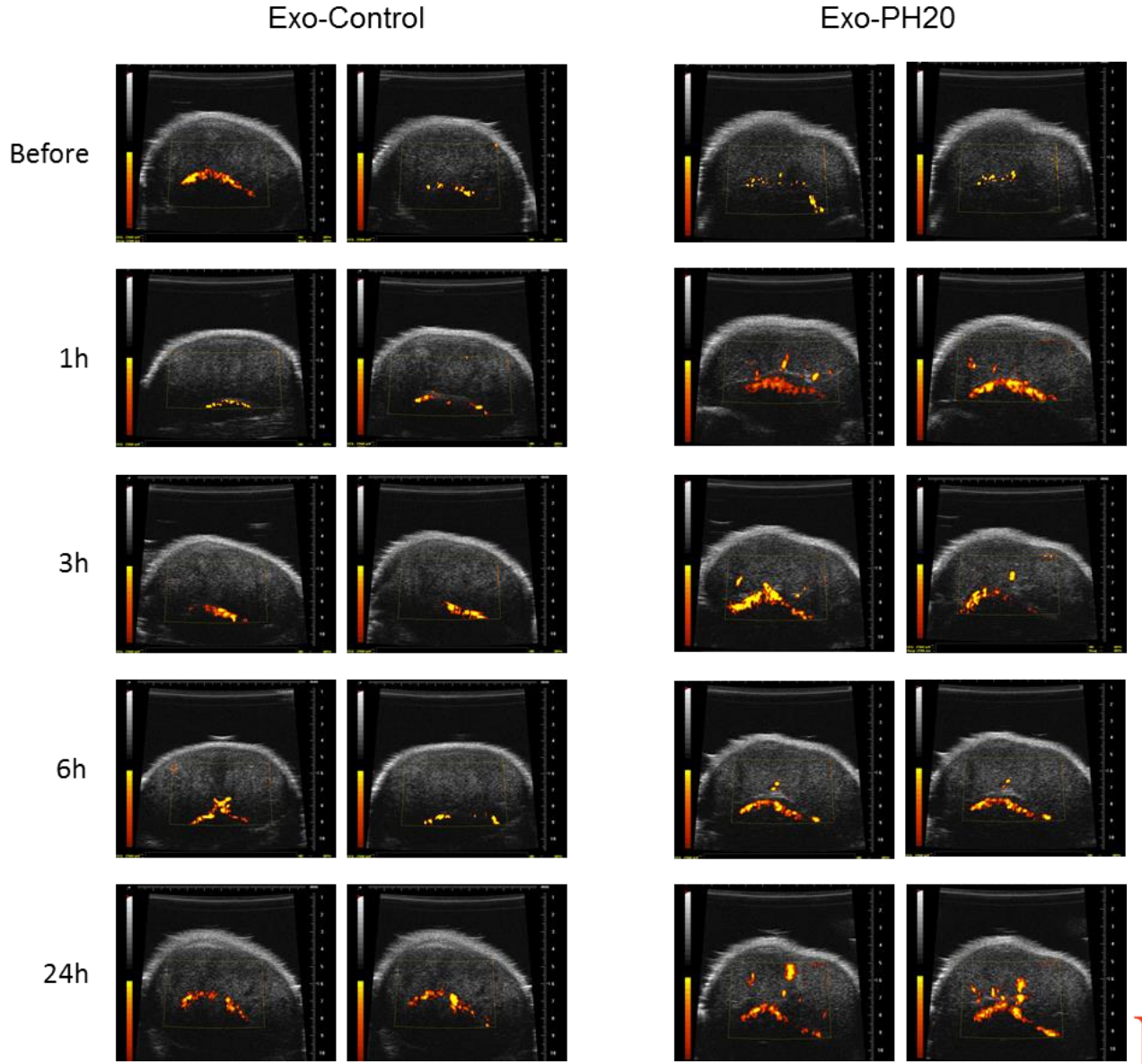
Exo-PH20 10 mg/kg

Exo-Con 10 mg/kg

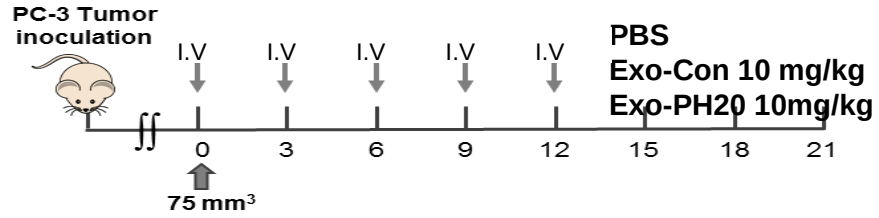
Doppler detection

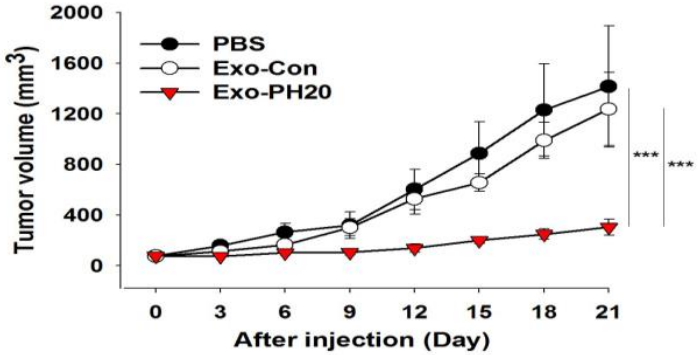
After 0h, 1h, 3h, 6h, 24h

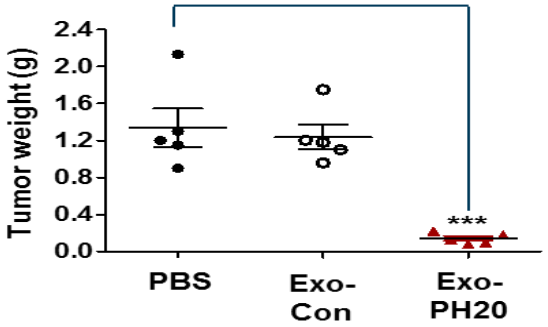


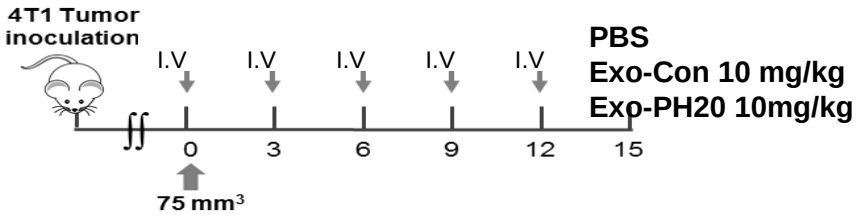


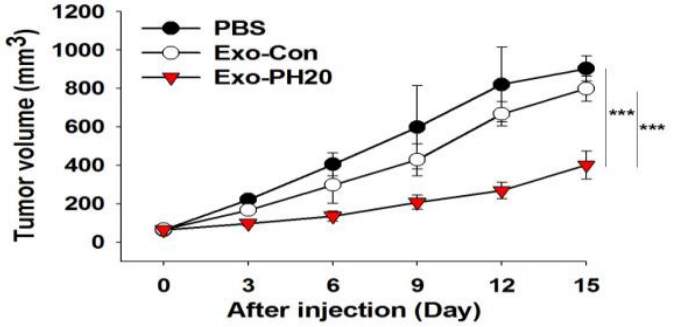
IV injection of Exo-PH20 inhibits tumor growth and increases CD8+ cells in tumor

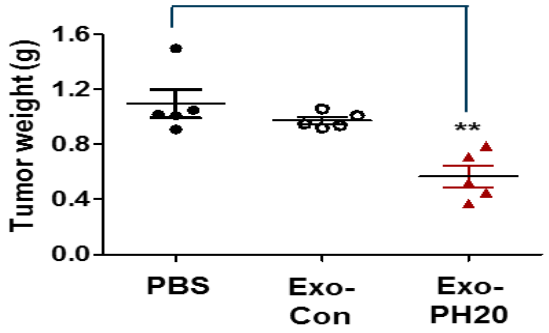


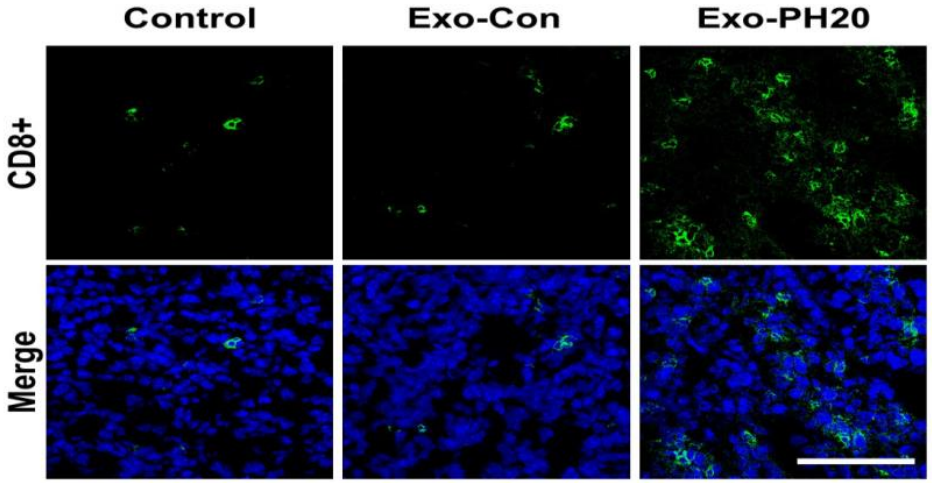












Exo-PH20^{Dox} deliver more Doxo and inhibit tumor growth

KIMS Intermembrane Signaling Lab

Dox delivery by Exo-PH20 into tumor foci

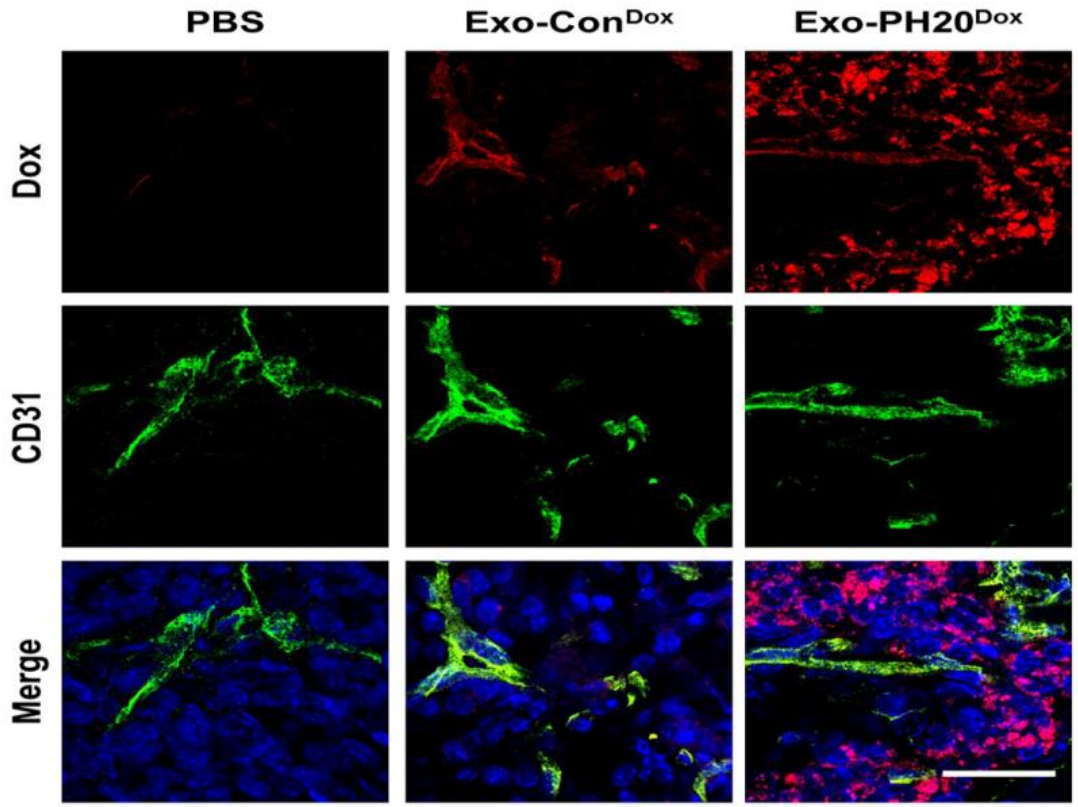
I.V injection

Exo-PH20^{Dox} 300 ug

Exo-Con^{Dox} 300 ug

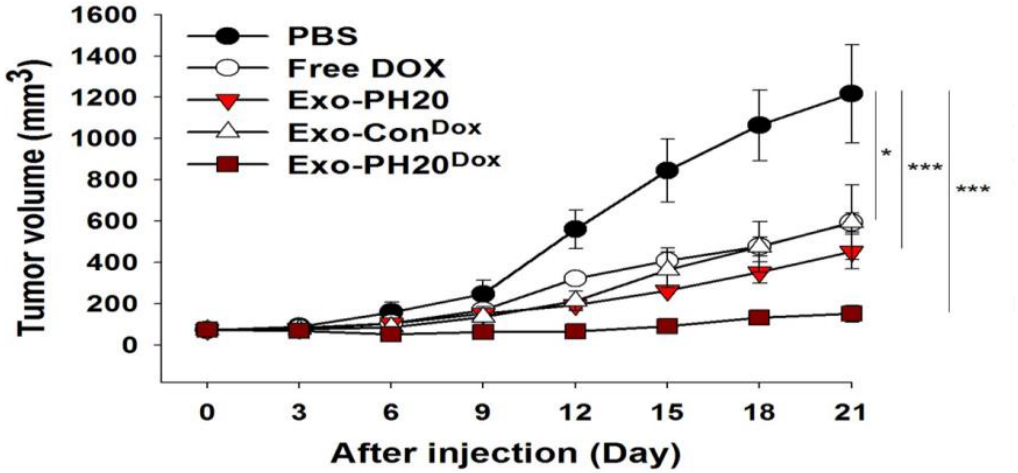
24h →

Tumor extraction



Tumor growth inhibition by Exo-PH20^{Dox}

Dox 1mg/kg
Exo-PH20 10mg/kg



Korea Institute of Science and Technology

Hippocrates said

"Natural forces within us are the true healers of disease"

Thanks for your attention

CANCER

Complex
Adaptive
Therapeutic
Strategy