

Engineered NanoMedicine Targets Intractable Cancers

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Innovation Centre of NanoMedicine (iCONM)
3-25-14 Tonomachi, Kawasaki-ku, Kawasaki, 210-0821

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on Nanomaterials (IOCN2020)
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Who Am I??



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- **Enthusiastic to work in Chemical-Biology Interface.**
- **Passionate to use chemistry for the well being of human race.**
- **Current research focus is on developing nanomedicine to solve critical issues associated with Cancers and other life-threatening diseases.**

Where do I
work?

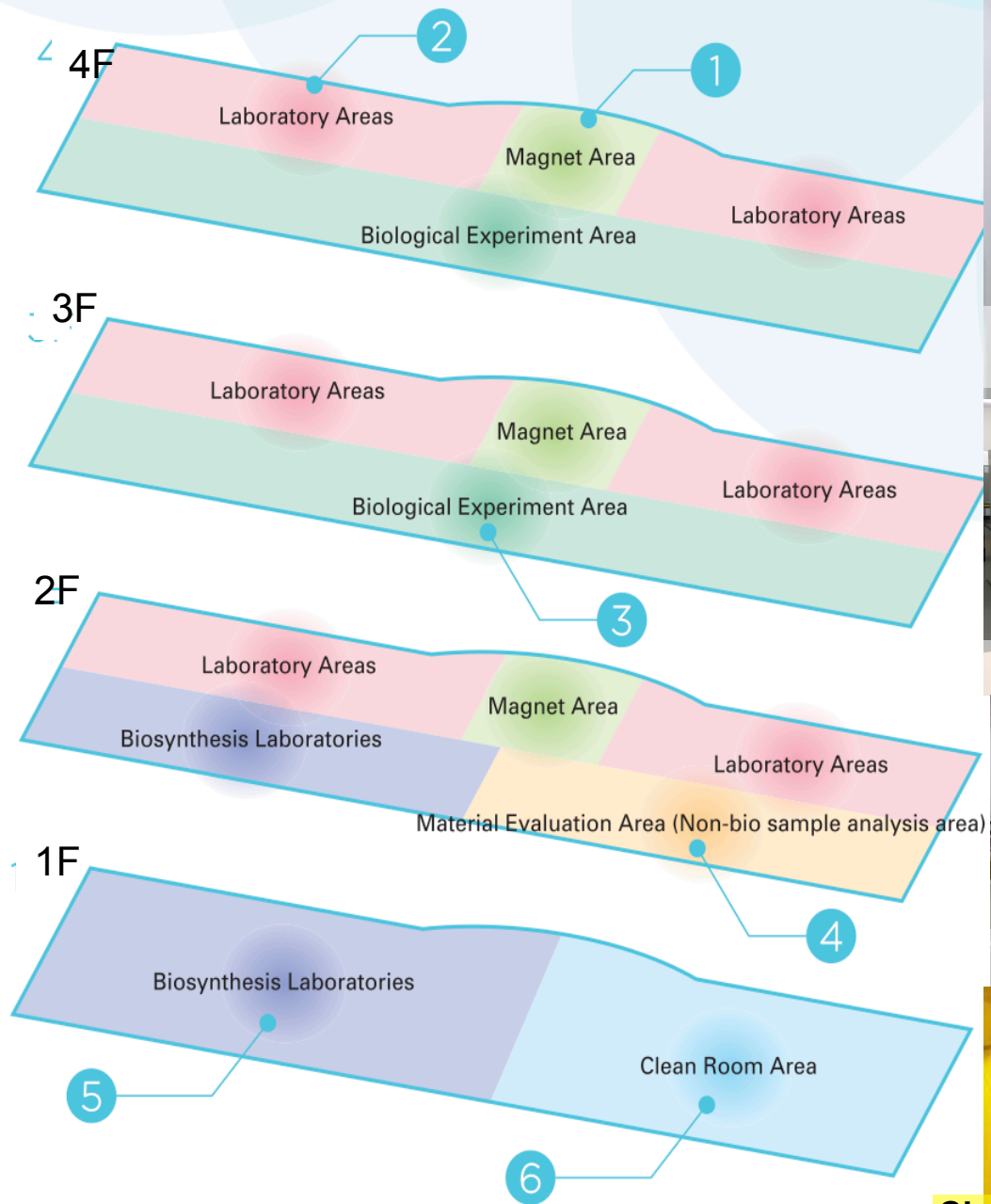
Innovation Center of NanoMedicine

The Innovation Center of NanoMedicine (**iCONM**) led by Prof. Kazunori Kataoka is the third major pillar of the Kawasaki Innovation Gateway SKYFRONT (Kawasaki, Japan), opened in 2015. iCONM is the only comprehensive research institute of nanomedicine in Japan, a pioneering facility including micro fabrication unit (incl. industrial clean room area), organic synthesis area and human diseases model laboratory.



Launched in April 2015

Under the one roof, iCONM offers facilities for material synthesis, characterization, nanomedicine formulation and preclinical evaluation of nanomedicines in small animals.



Animal testing lab (pre-clinical)



Cell Culture Facility



Chemical synthesis lab



Clean room (microprocessing)

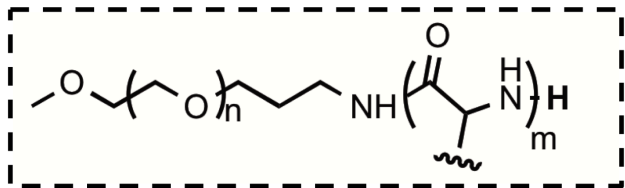
Our Technology



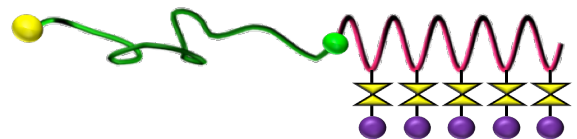
Supramolecular NANO-sized drug carrier
NanoMedicine

Polymer micelle prepared from self-assembly

Poly(ethylene glycol)-*b*-poly(amino acid)

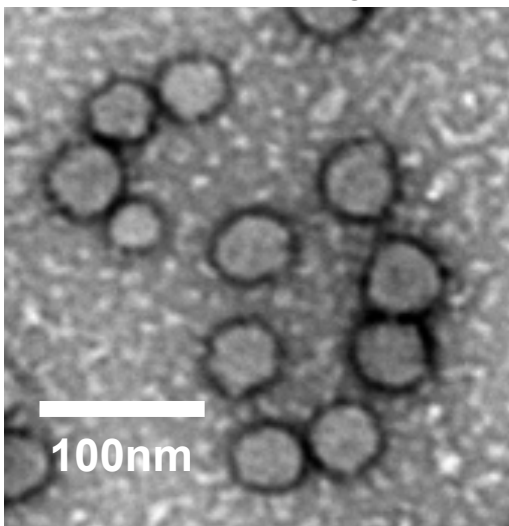


Hydrophilic Block Hydrophobic Block
Amphiphilic block copolymer

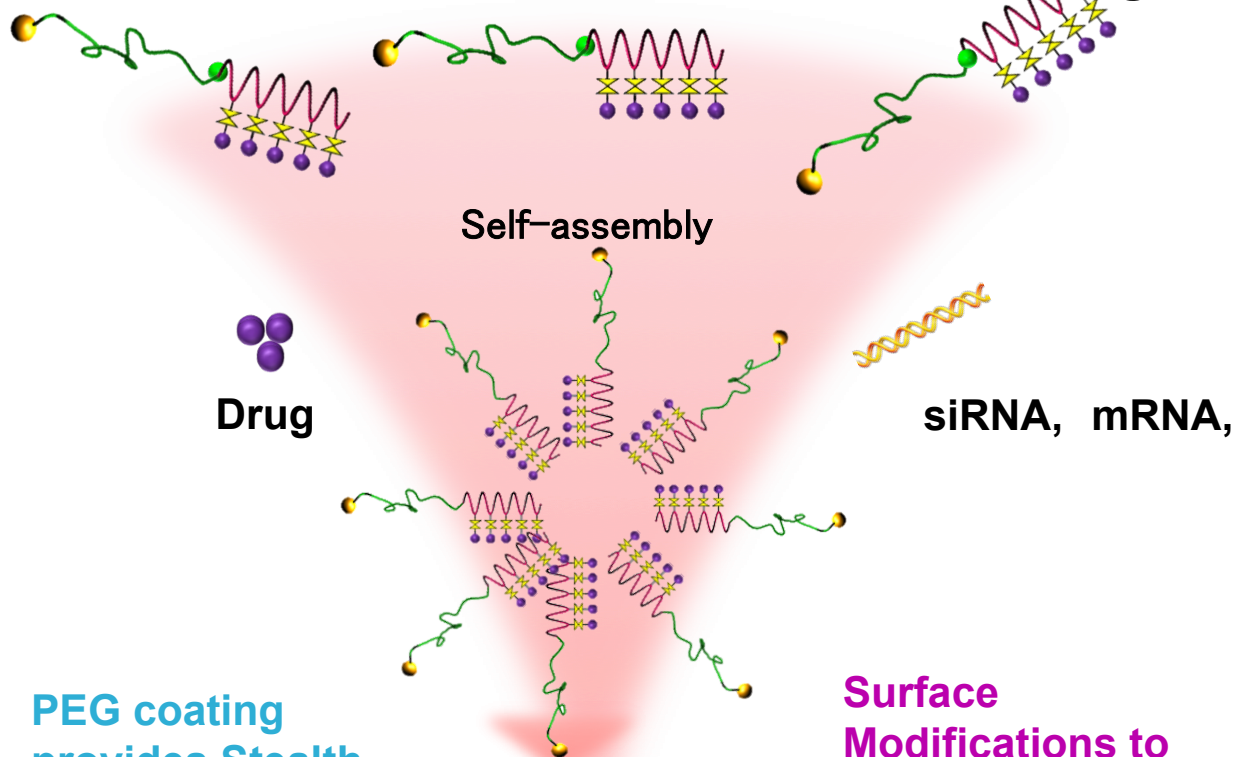


Hydrophilic
segment

Hydrophobic
segment



TEM image



Self-assembly

Drug

siRNA, mRNA,

PEG coating
provides Stealth
effect to avoid
immune
recognition

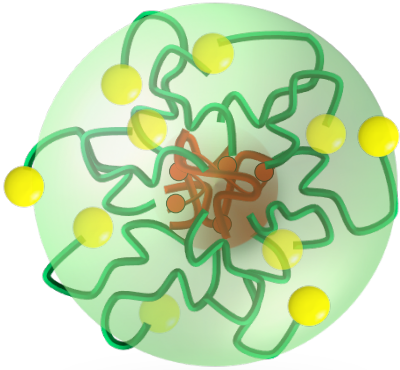
Surface
Modifications
to get recognized
by target tissue
and cells

Long blood
circulation
increases site
specific
targeting

Small size and
Controlled Release
to increase target
specificity

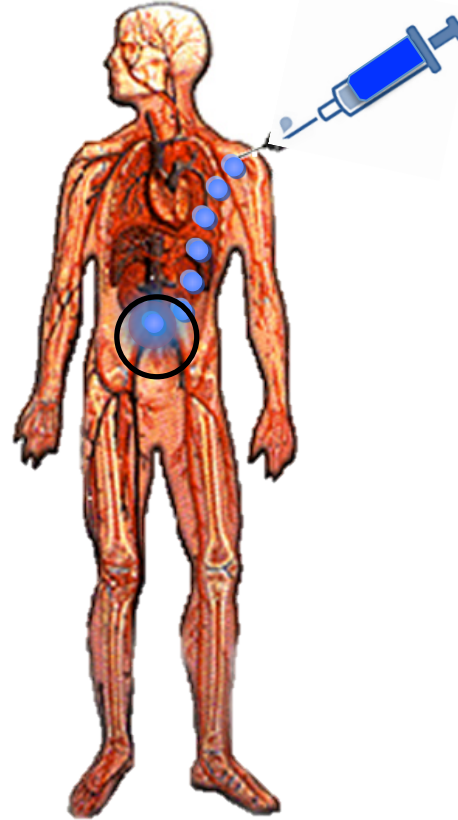
30~100 nm

NanoMedicine to target cancer



Polymer micelle prepared from self-assembly

With Our Self-assembly Technology, we sought to target Intractable Cancers



Site Specific Targeting

Long blood circulation

Target-site recognition

Tumor specific drug activation

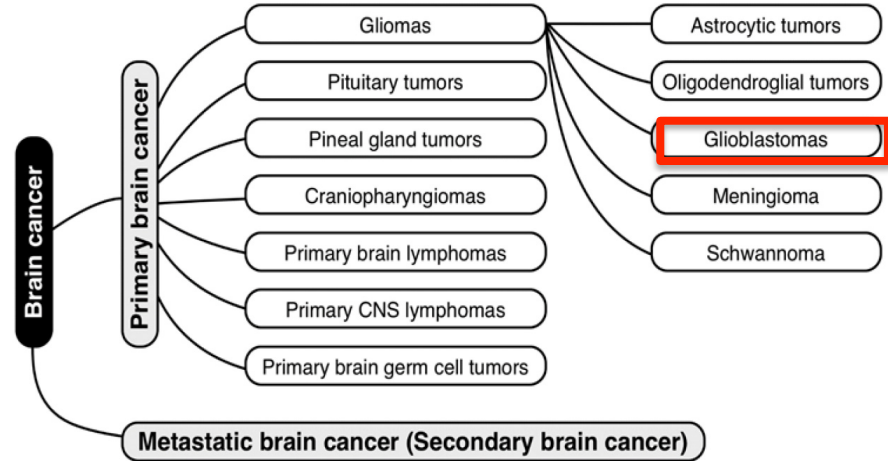
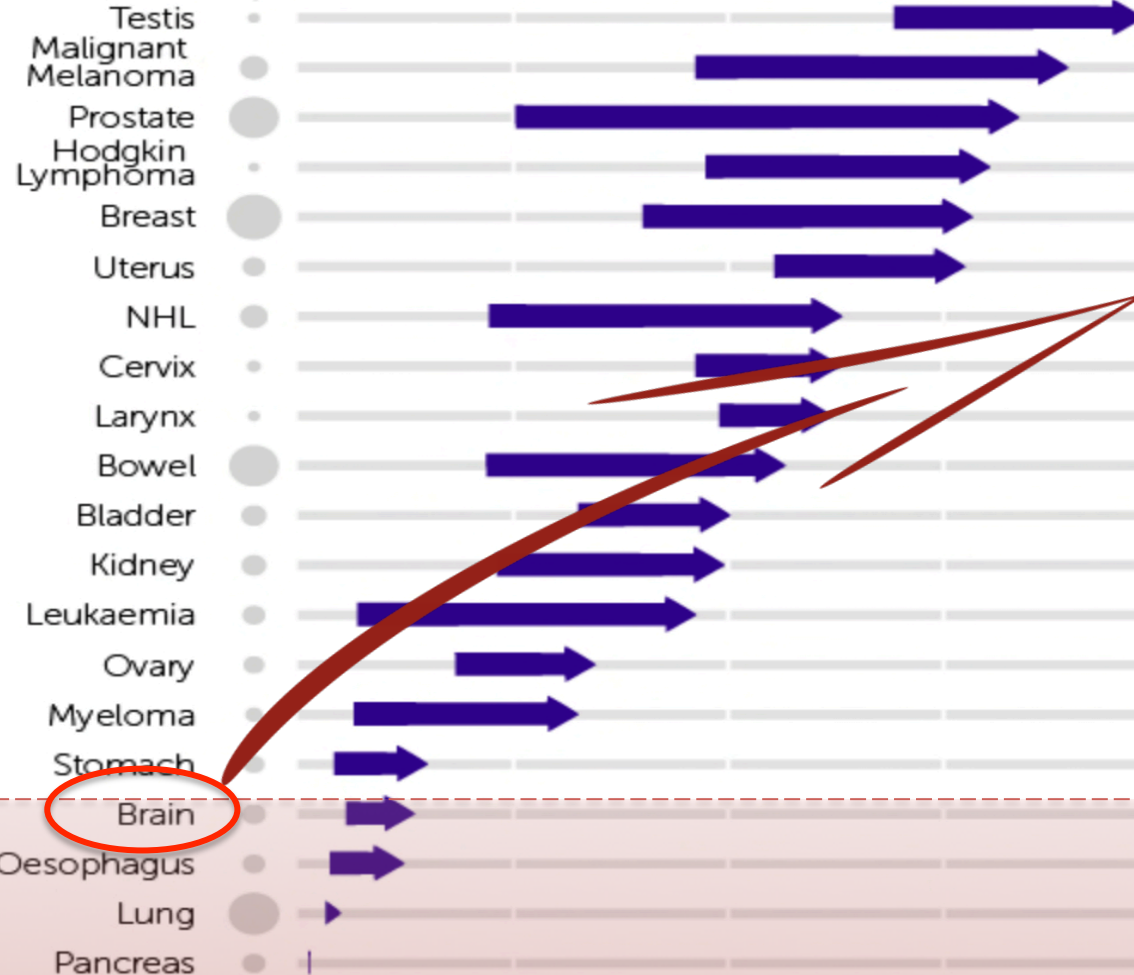
Cancer Survival rate improves with time for many cancers but not for all

changes in survival, 1971-72 to 2010-11

0% 25% 50% 75% 100%

All Cancers

incidence



Glioblastoma: Rare, deadly and incurable brain cancer.

The Washington Post



Glioblastoma Multiform (GBM) remains an unmet medical need

Multiple challenges remain in terms of successful treatment of GBM

- **Glioblastoma (GBM) is the most deadly form of human cancer.**
- **Median survival of only 10 to 14 months with only 3 to 5% of patients surviving more than three years.**
- **The best current standard of care extends overall survival to about 14 to 16 months.**

- **tumor location in a region where it is beyond the reach of local control (BBB)**
- **rapid, aggressive tumor relapse.**
- **tumor heterogeneity**

Challenge in Glioblastoma Treatment



- **tumor location in a region where it is beyond the reach of local control (BBB/BBTB)**



Solution with NanoMedicine- Example 1



cRGD peptide-installed epirubicin-loaded polymeric micelles for effective targeted therapy against Glioblastoma

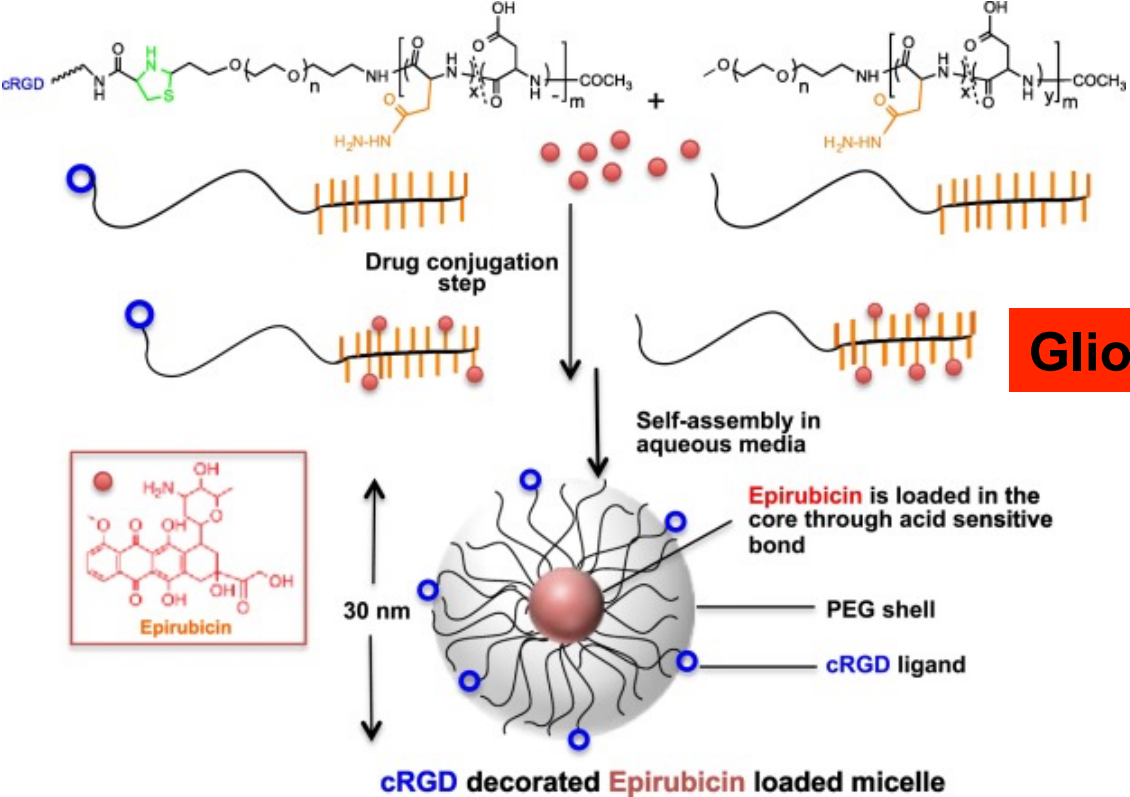
Cyclic-RGD binds favorably to the $\alpha v \beta 3$ and $\alpha v \beta 5$ integrin

Invasion

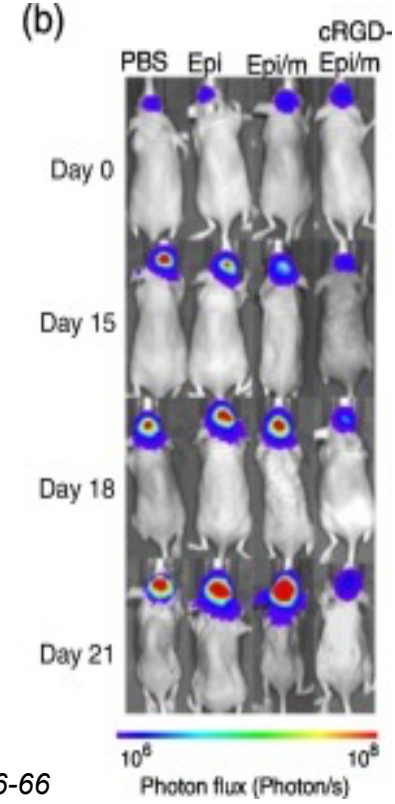
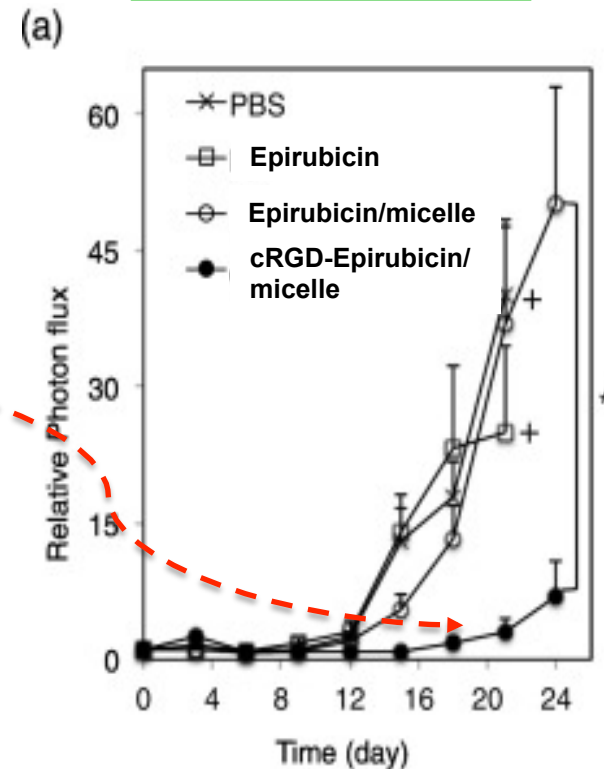
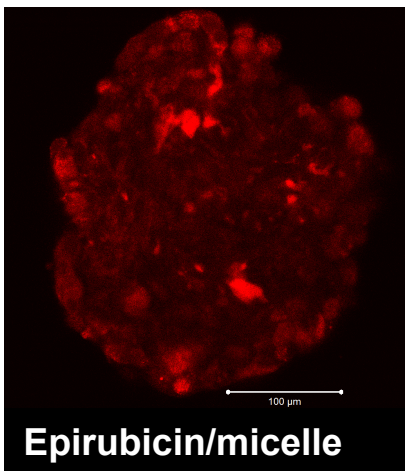
Glioblastoma

Angiogenesis

integrins $\alpha v \beta 3$ and $\alpha v \beta 5$ + Higher expression on U87MG

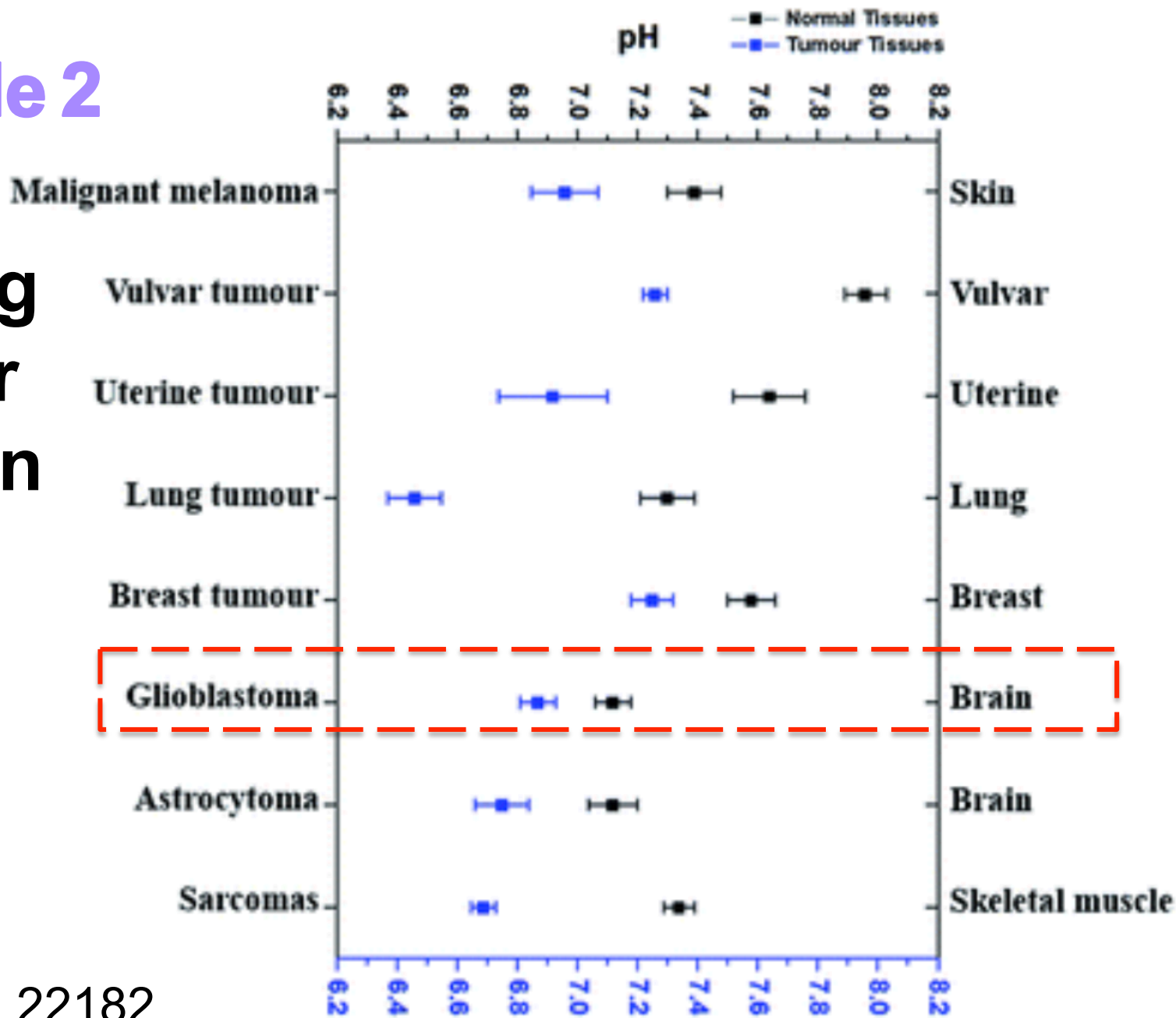


3D Cell Culture

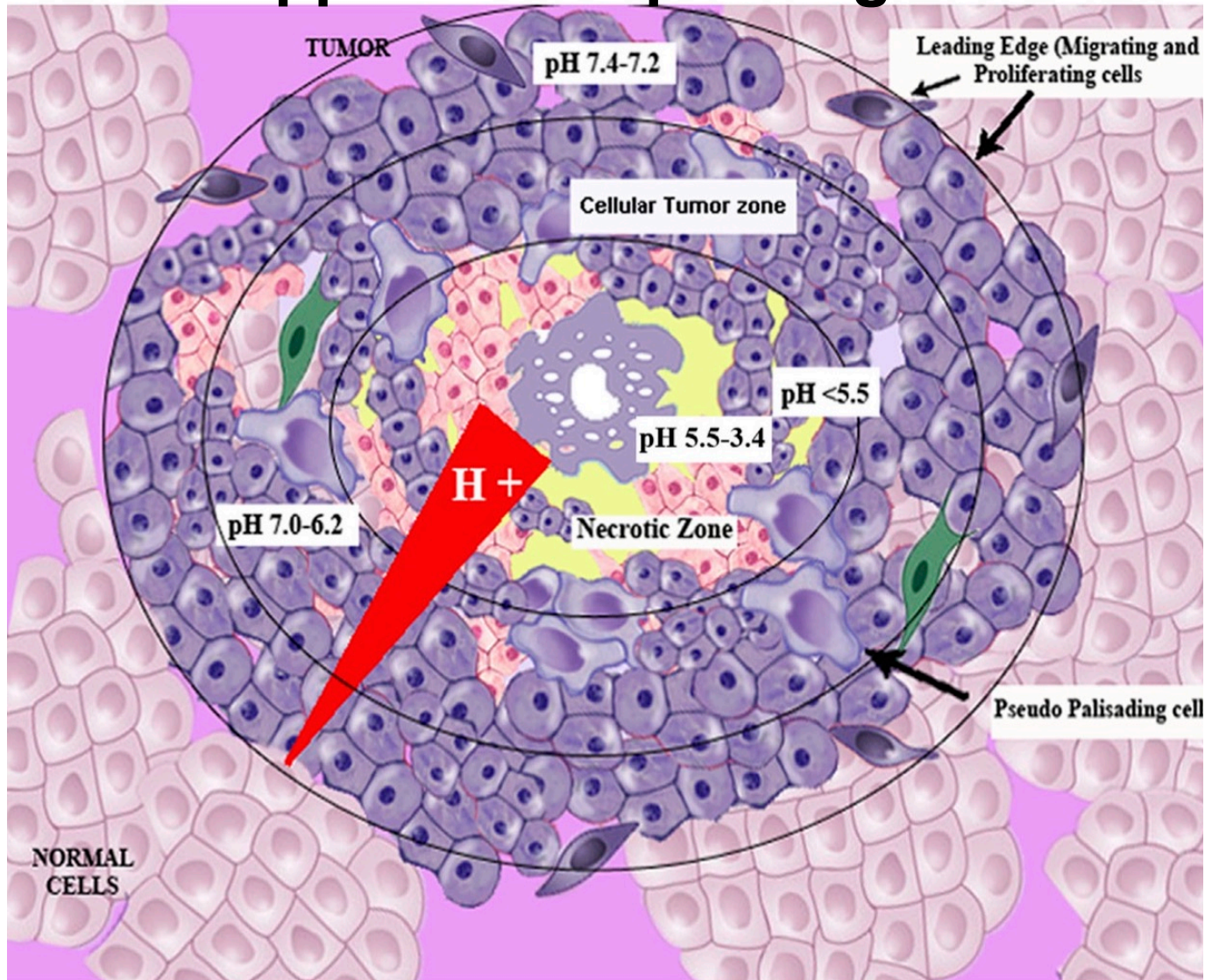


➤ Example 2

**Manipulating
extracellular
tumor pH: an
effective
target for
cancer
therapy**



Diagrammatic representation of the glioblastoma tumor zones and the approximate pH ranges associated with each zone

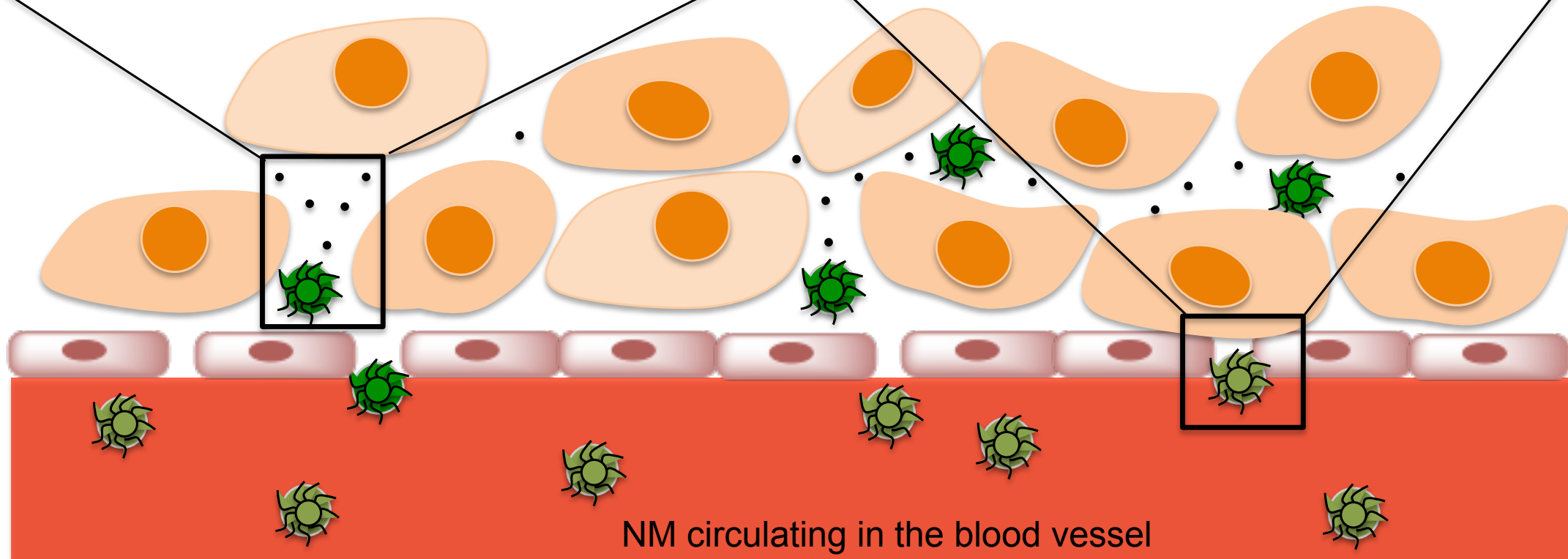


Glioblastoma acidosis is heterogeneous, with the region adjacent to blood vessels having a near-neutral pH and a hypoxic region with acidic pH.

Design of NanoMedicine relying on the Extracellular Acidosis in Glioblastoma that can sense heterogeneous GBM pH

(2) Mild acidic pH (pH 6.6-7) of T^{ex} triggers drug release from the NM at the tumor extracellular space. And released drug freely diffuse inside the tumor mass, and induce cytotoxicity to GBM cells.

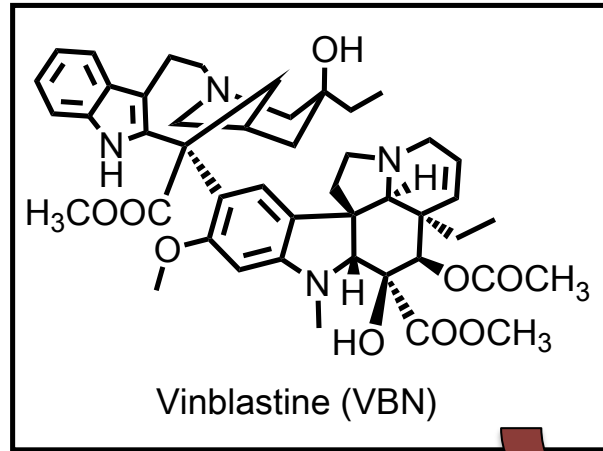
(1) Although, heterogeneous, it is well-known that invading Glioma cells disrupt BBB and causes focal breach of the BBB integrity. This assists the NM to extravasate from blood vessel to tumor site



Supramolecularly Enabled pH- triggered Drug Action at Tumor Microenvironment Potentiates Nanomedicine Efficacy against Glioblastoma.

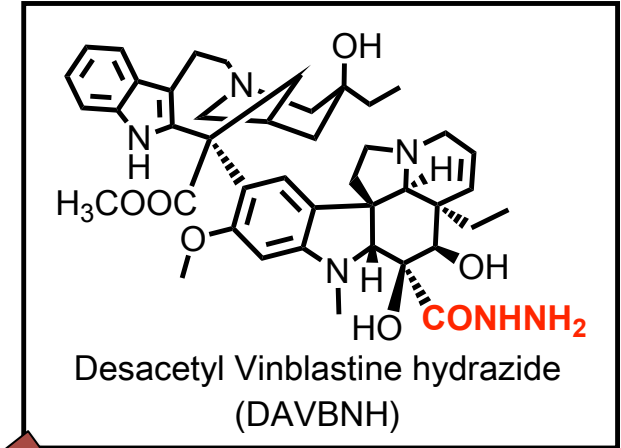
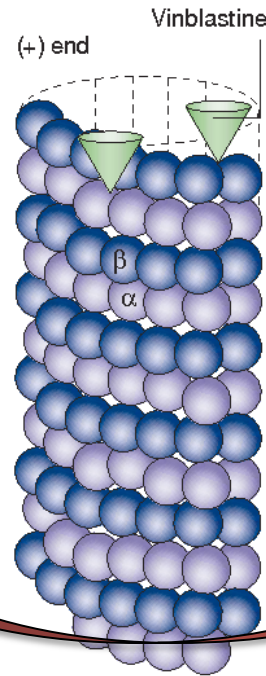
Highlight of this paper- In this paper, we reported the development process of a supramolecularly enabled tumor-extracellular (T^{ex}) pH-triggered nanomedicine that can progressively release drug in the tumor by rightly sensing heterogeneous tumor-pH. Desacetylvinblastine hydrazide (DAVBNH), a derivative of potent anticancer drug vinblastine, was conjugated to an aliphatic ketone-functionalized poly(ethylene glycol)–b-poly(amino acid) copolymer and the hydrolytic stability of the derived hydrazone bond was efficiently tailored by exploiting the compartmentalized structure of polymer micelle. We confirmed an effective and safe therapeutic application of T^{ex} pH-sensitive DAVBNH-loaded micelle (T^{ex} -micelle) in orthotopic glioblastoma (GBM) models, extending median survival to 1.4 times in GBM xenograft and 2.6 times in GBM syngeneic model, compared to that of the free DAVBNH.

Vinblastine, a Potent Tubulin binding, agent for Glioblastoma treatment



Parent Drug

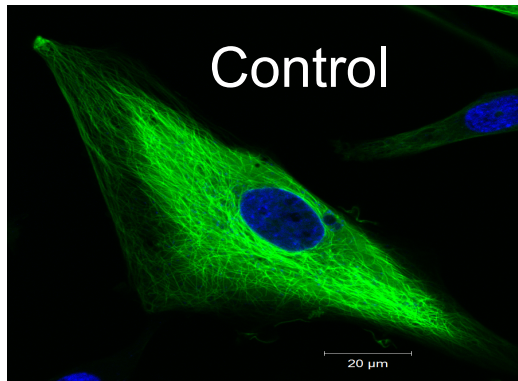
IC₅₀ against U87MG- 12 nM



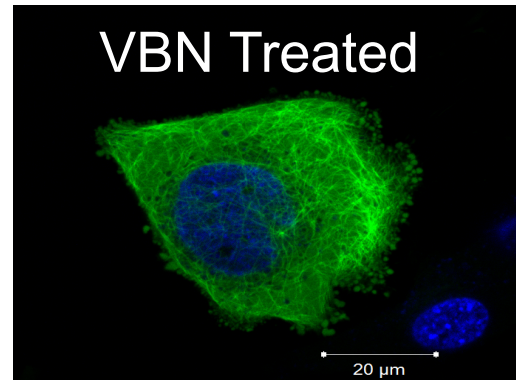
Synthetic Analogue

IC₅₀ against U87MG- 2 nM

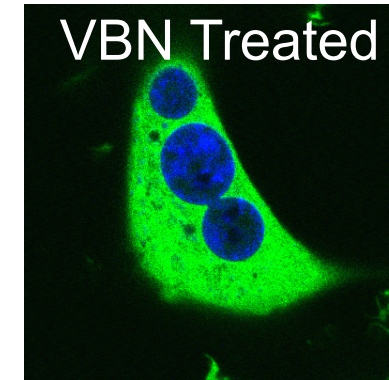
GBM
Cell-line
U87MG



Control



VBN Treated

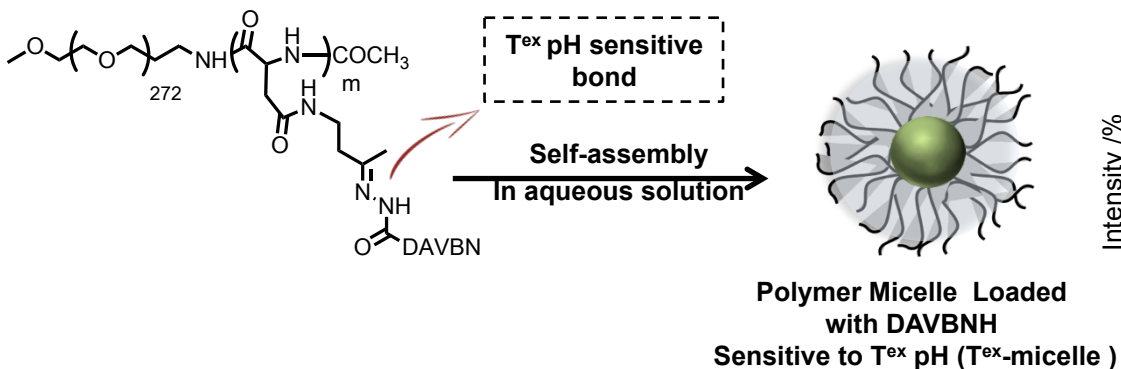


VBN Treated

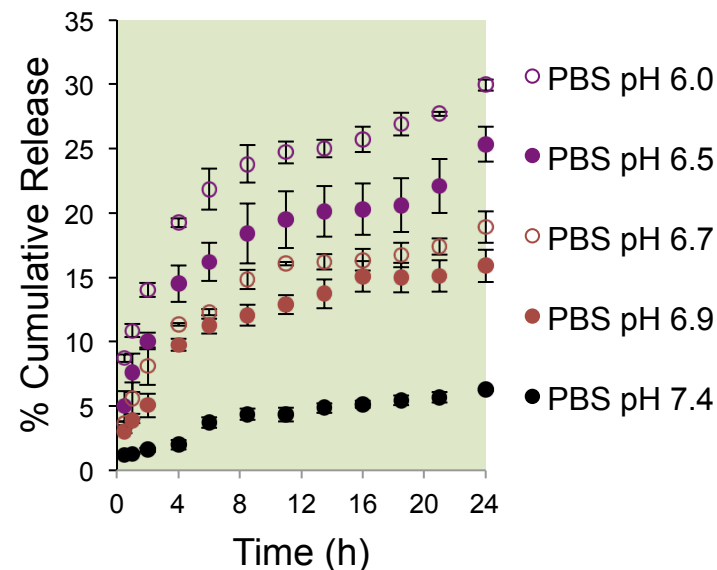
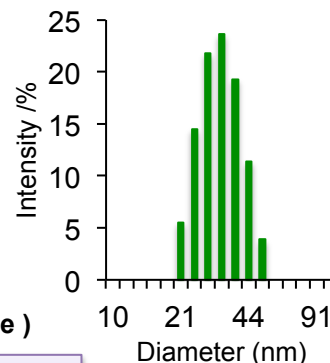
Collapse of tubulin
after VBN treatment

Multinucleated cells
after VBN treatment

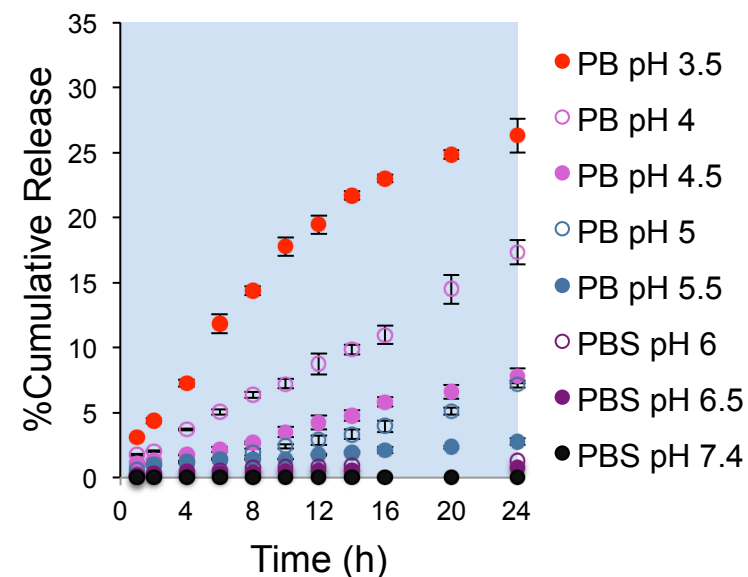
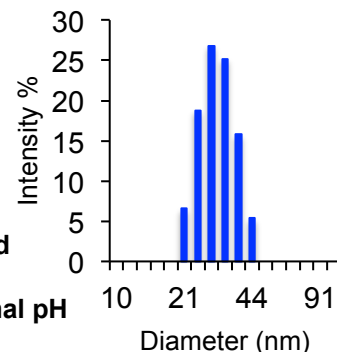
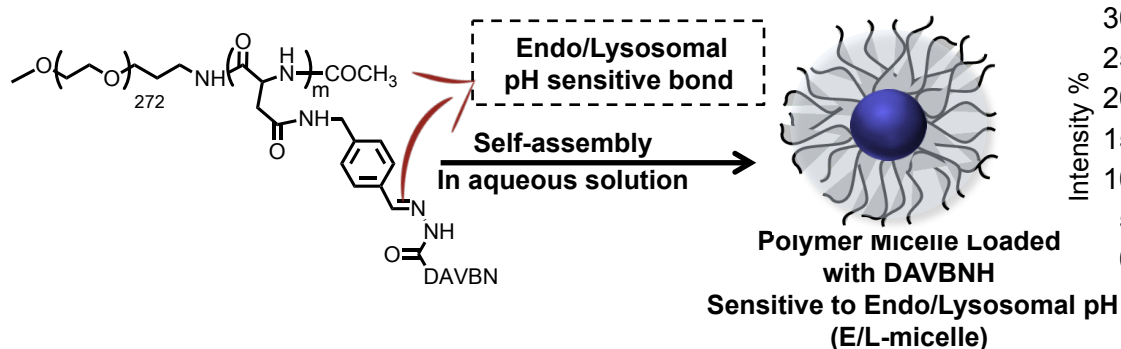
Vinblastine Analogue DAVBNH Loaded Tumor Extracellular pH sensitive Polymer Micelle



DAVBNH was conjugated to an aliphatic ketone-functionalized PEG-PAA block copolymer, and the hydrolytic stability of the derived hydrazone bond was effectively tailored by taking advantage of the compartmentalized micellar structure.

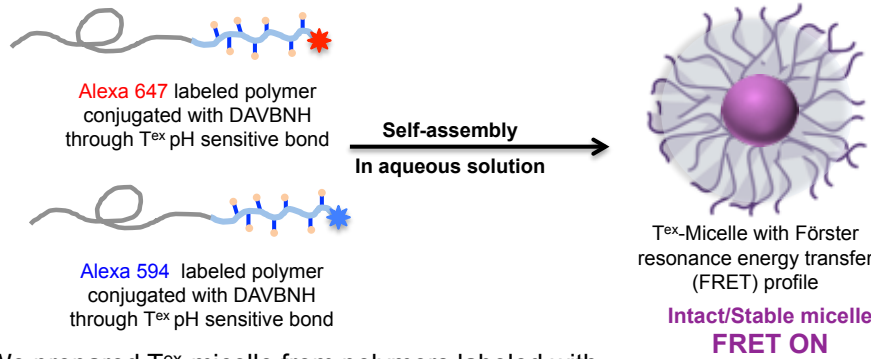


Vinblastine Analogue DAVBNH Loaded Tumor Intracellular pH sensitive Polymer Micelle



Another type of DAVBNH-loaded PM system was designed that releases drug only at very low pH condition, typically observed in the endo-lysosomal compartments (pH 3.5-5.5). For this purpose, an aromatic aldehyde-functionalized PEG-PAA block copolymer was used as the base polymer for drug conjugation.

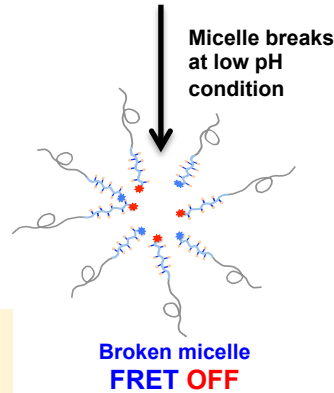
Preparation of Micelle with FRET character



We prepared T^{ex}-micelle from polymers labeled with Alexa 594 and Alexa 647 dyes; this micelle showed FRET character due to the nearness of the FRET paired dyes within the micelle core

We administered this T^{ex}-FRET-micelle intravenously to a healthy mouse and followed the FRET activity inside the blood vessels within the earlobe area using the intravital confocal laser scanning microvideography (IVCLSM) technique.

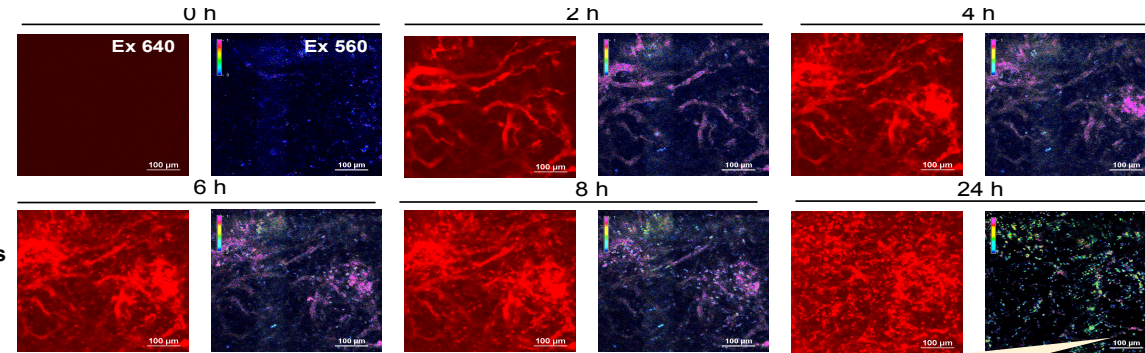
Stable micelle with FRET ON inside the blood vessel for more than 12 hours



Stability of DAVBNH loaded micelles during the systemic circulation, and their extravasation and distribution in the tumor site. Using FRET Technique.

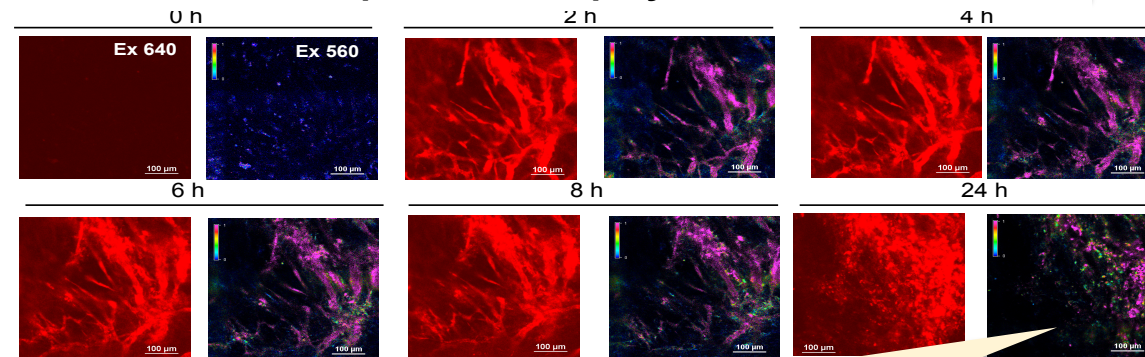


Tumor extracellular pH sensitive polymer micelle



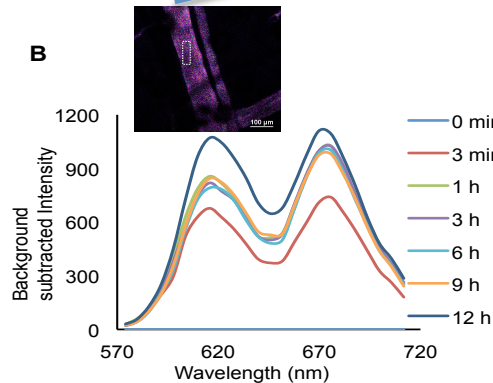
Tumor extracellular pH sensitive polymer micelle disintegrate inside tumor - FRET OFF

Tumor Intracellular pH sensitive polymer micelle

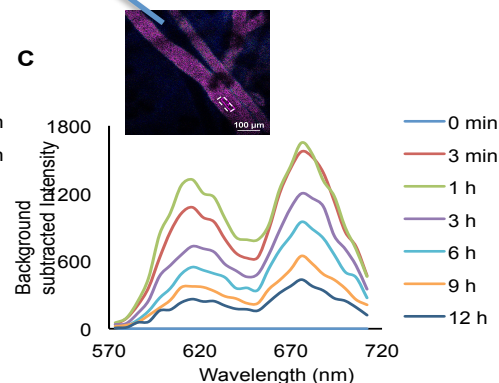


Tumor Intracellular pH sensitive polymer micelle stays intact inside tumor - FRET ON

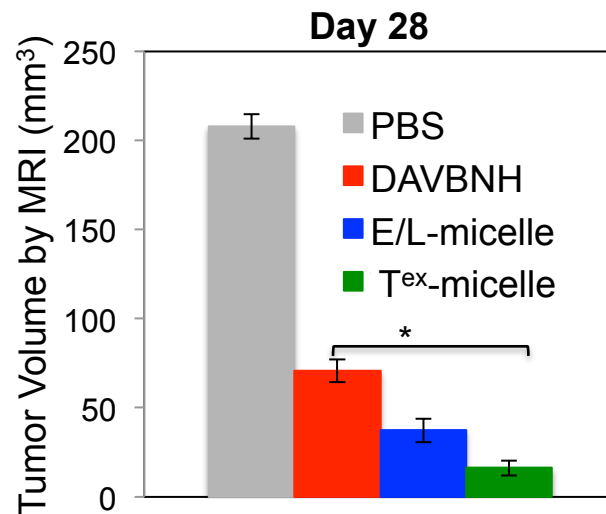
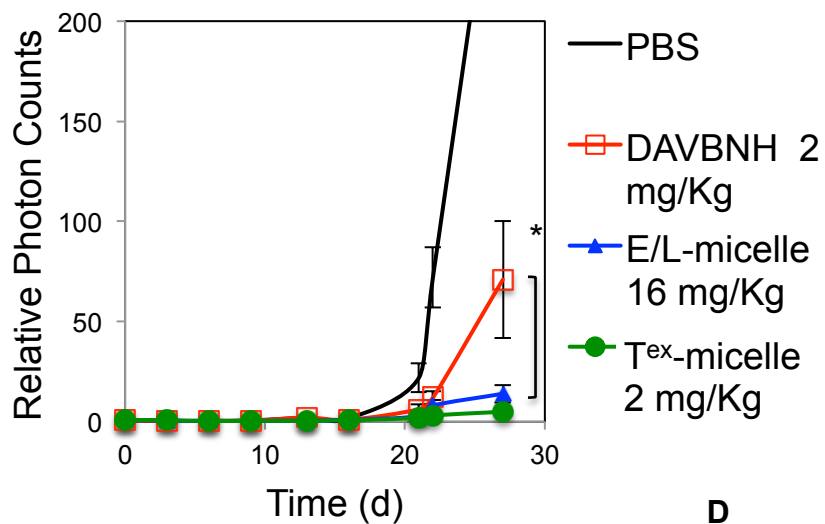
B



C



Antitumor activity and survival study against orthotopic brain tumor

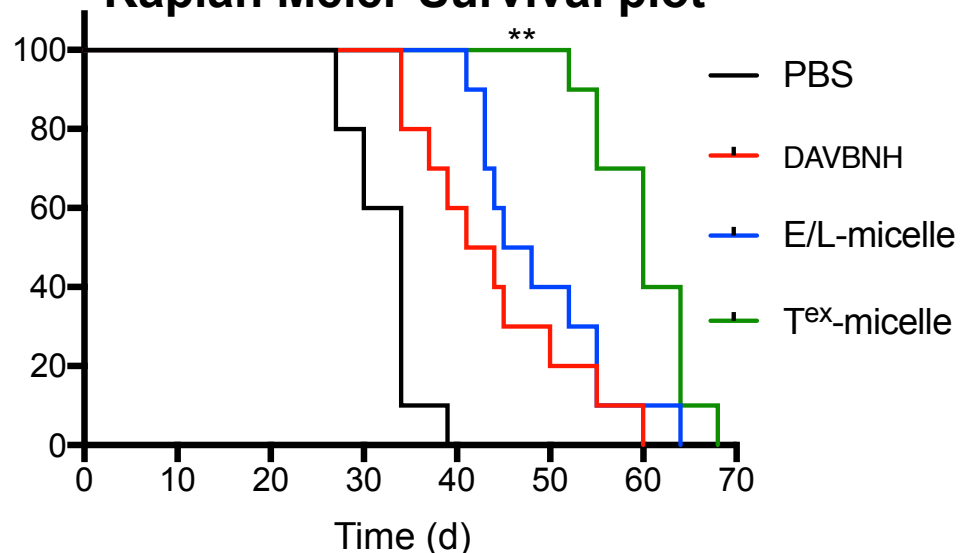


U87MG-LUC cells were implanted in the intracranial space of immunodeficient mouse



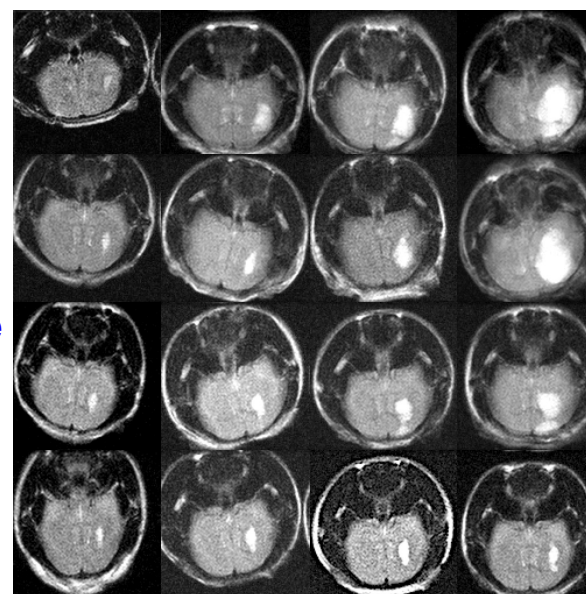
D

Kaplan Meier-Survival plot

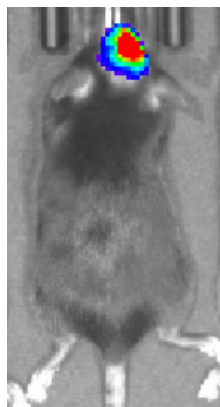
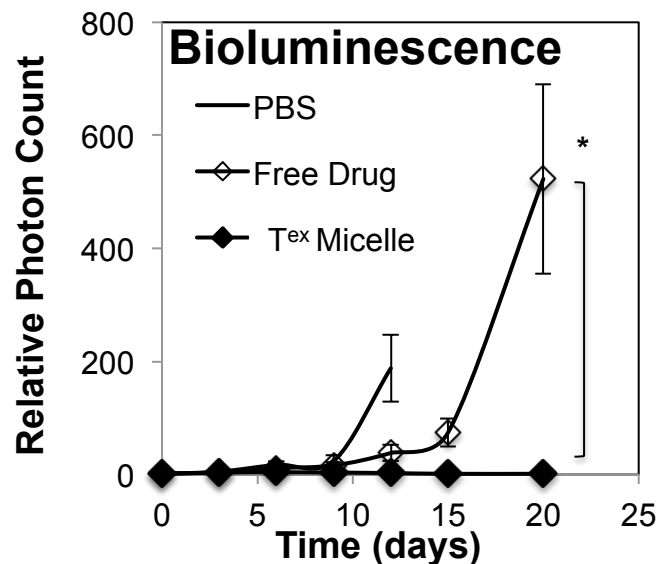


Day 0 Day 7 Day 14 Day 28

PBS
DAVBNH
E/L-micelle
Tex-micelle

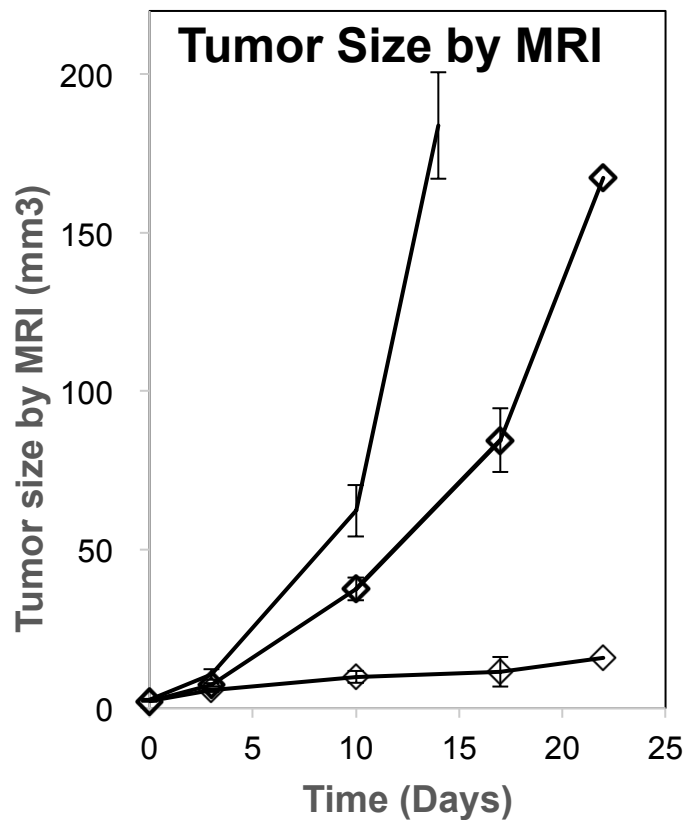
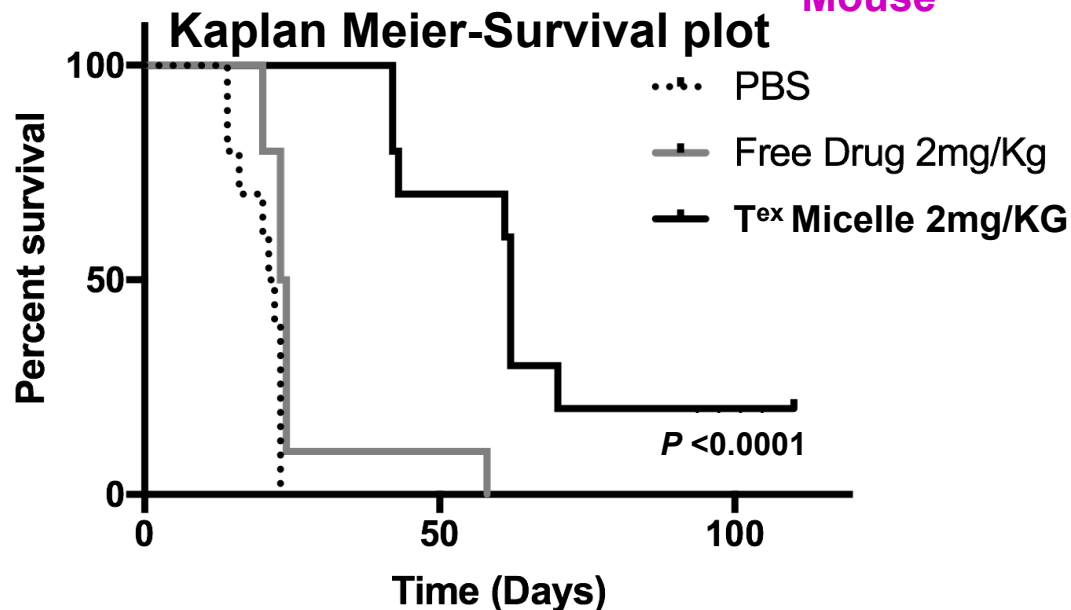


Antitumor activity and survival study against orthotopic brain tumor Syngeneic Model (GL261LUC-C57BL6J)

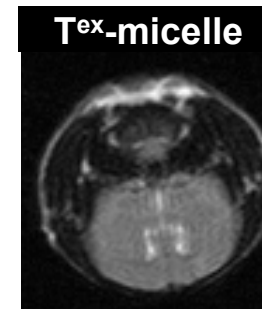
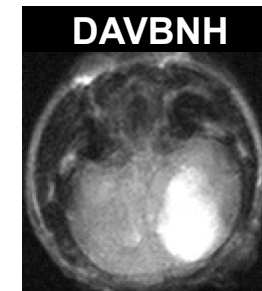
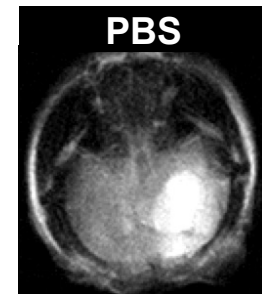


GL261-LUC cells in Immunocompetent Mouse

- Closely mimic GBM phenotypes
- Carry point mutations in the K-ras and p53 genes
- High expression of c-myc



Representative MRI Image



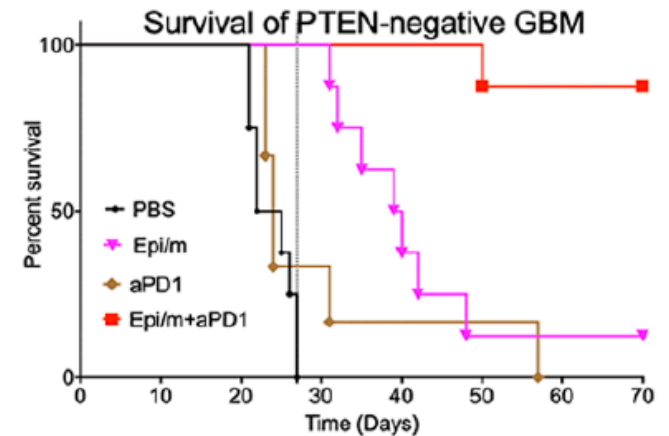
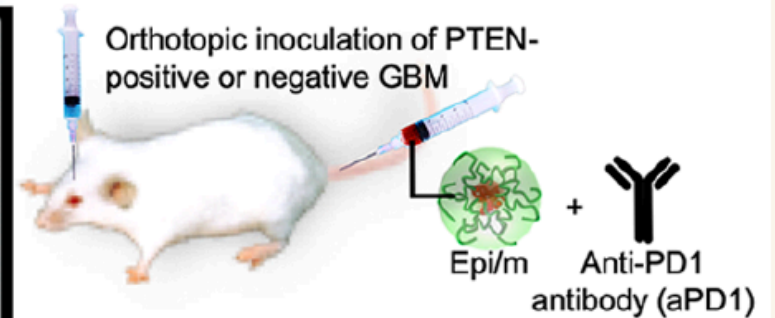
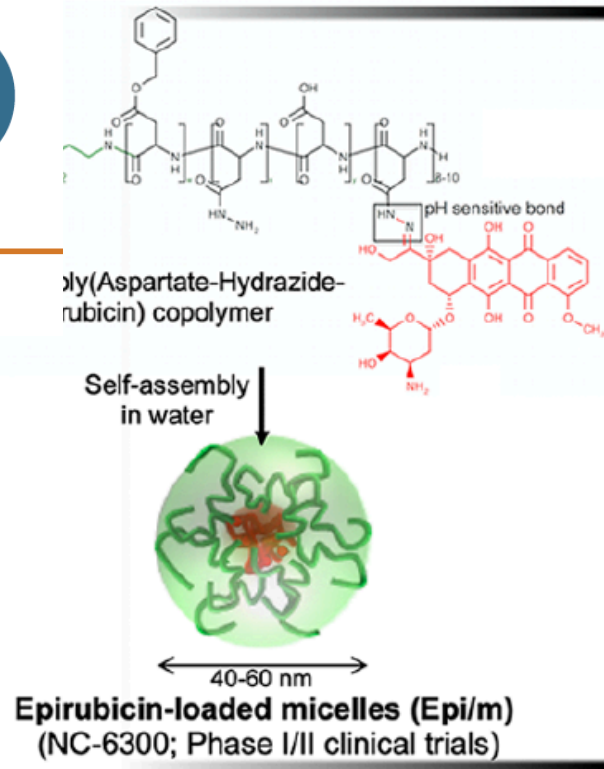
Translational Nanomedicine Boosts Anti-PD1 Therapy to Eradicate Orthotopic PTEN-Negative Glioblastoma

Hiroaki Kinoh, Sabina Quader, Hitoshi Shibasaki, Xueying Liu, Amit Maity, Tatsuya Yamasoba, Horacio Cabral,* and Kazunori Kataoka*

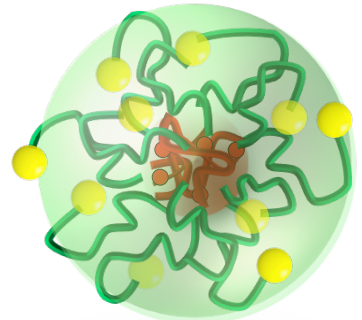
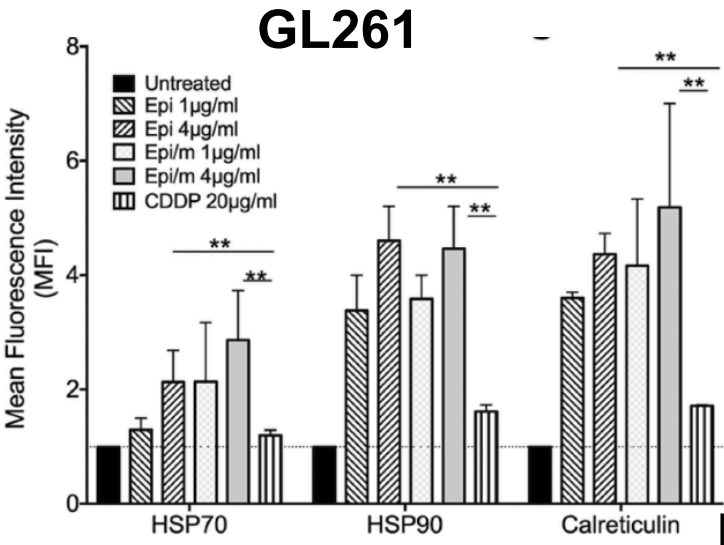
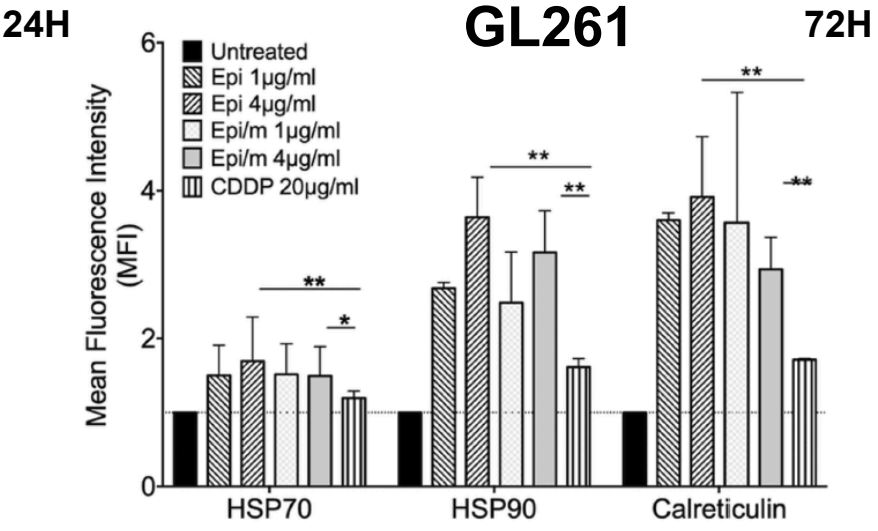
ACS NANO

www.acsnano.org

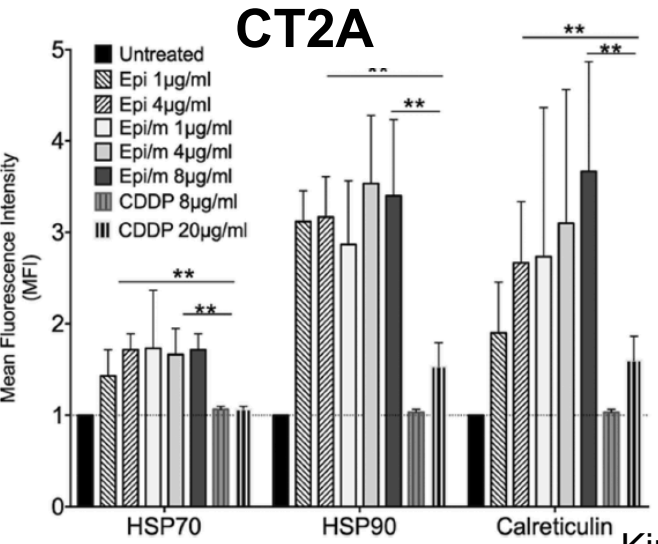
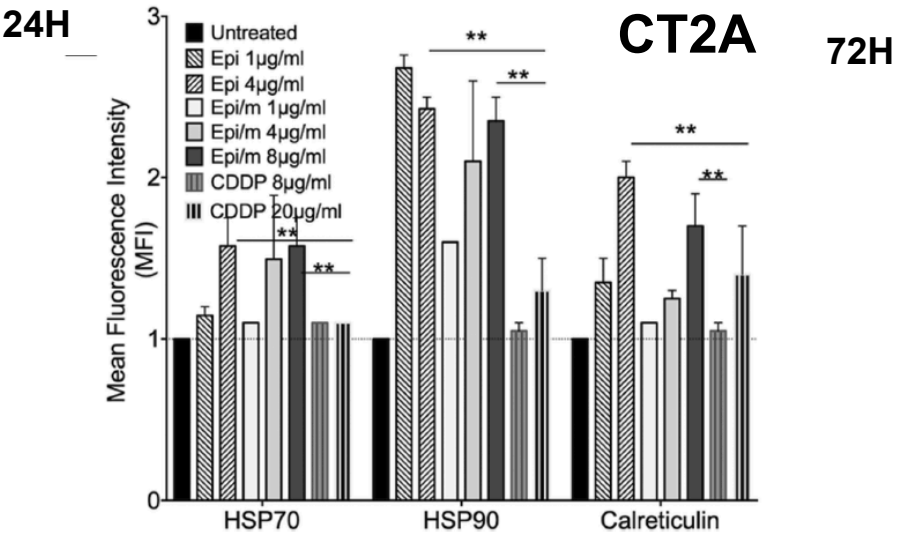
➤ Example 3



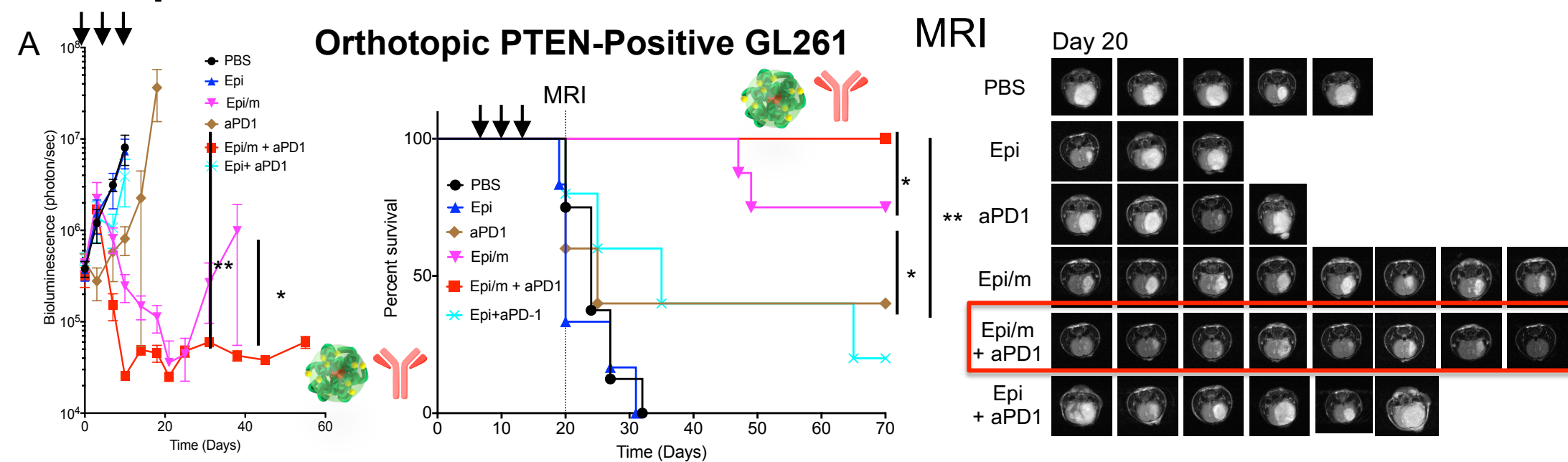
Chemotherapy induced Immunogenic Cell Death (ICD) effect in PTEN +ve GL261 and PTEN -ve CT2A mouse GBM cells



Micelle loaded with ICD drug Epirubicin Epi/m
NC6300
Phase II



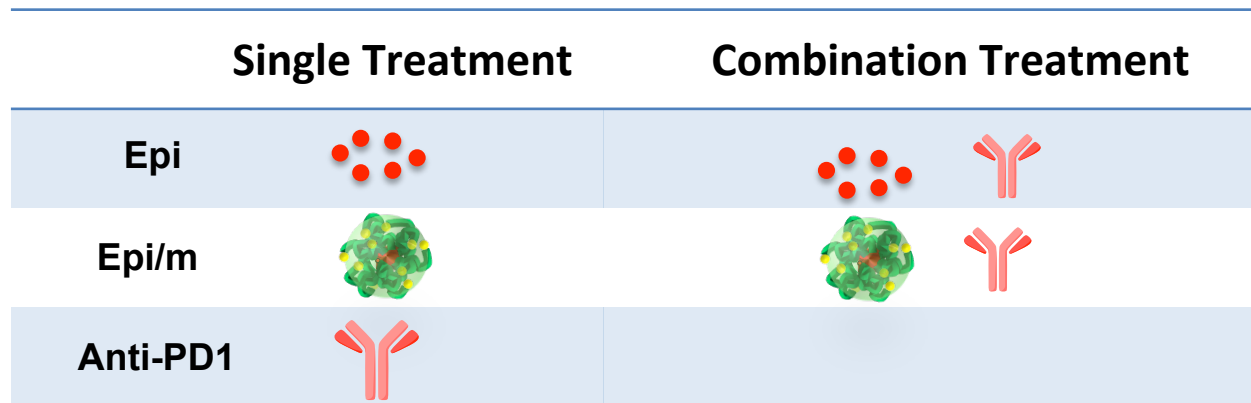
Nanomedicine Boosts Anti-PD1 Therapy to Eradicate Orthotopic PTEN-positive Glioblastoma



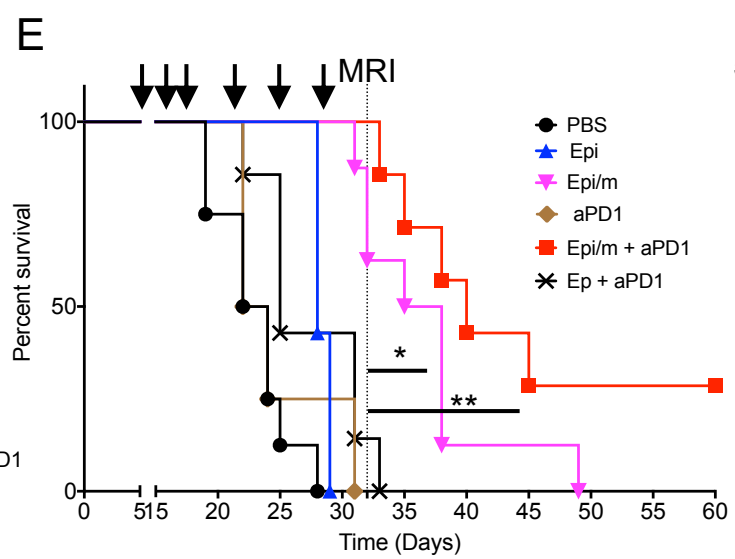
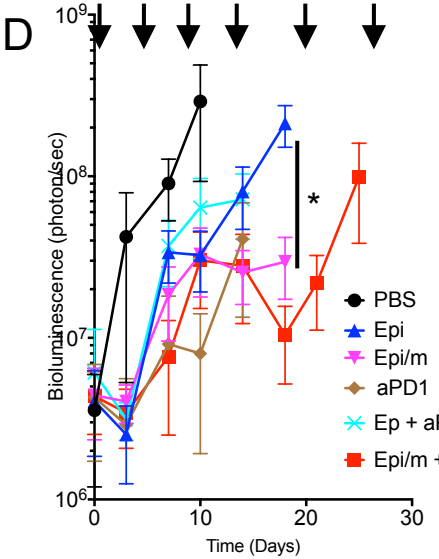
Treatment groups



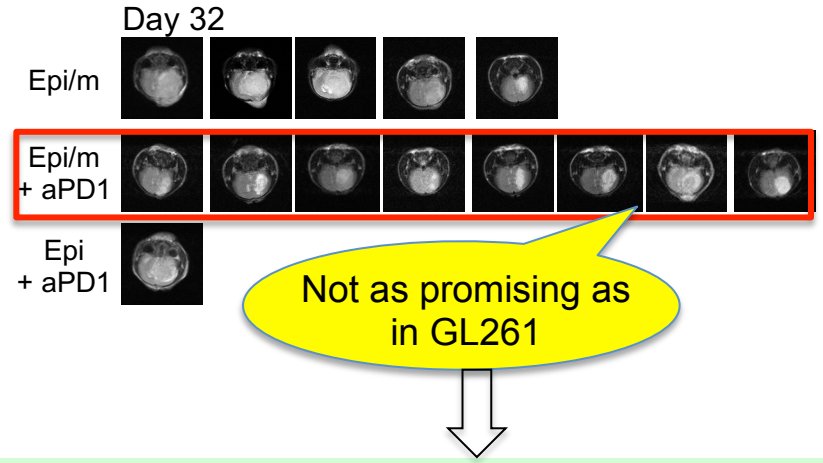
**5 mg/kg single agent
or combination**



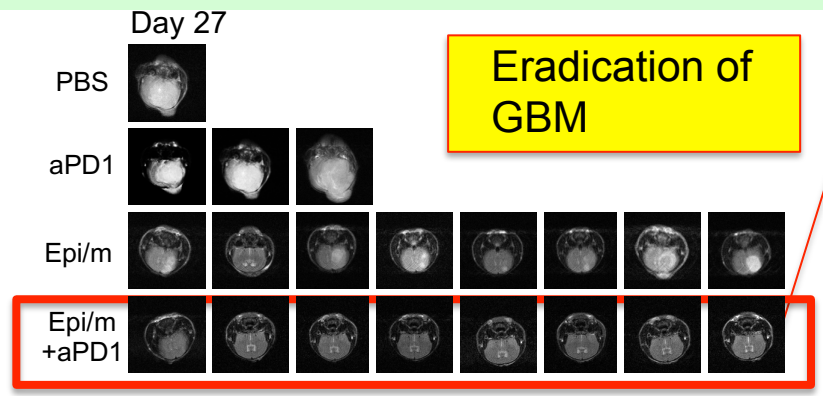
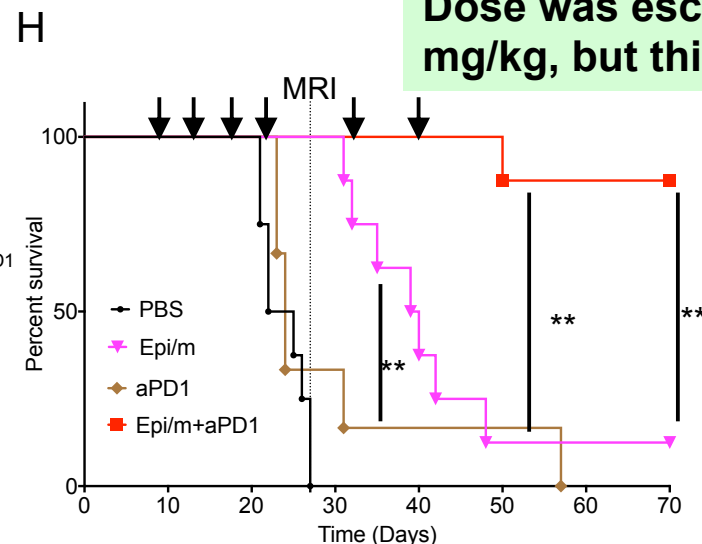
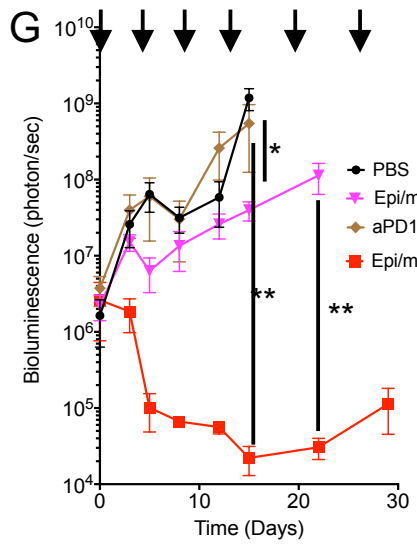
Nanomedicine Boosts Anti-PD1 Therapy to Eradicate Orthotopic PTEN-Negative Glioblastoma



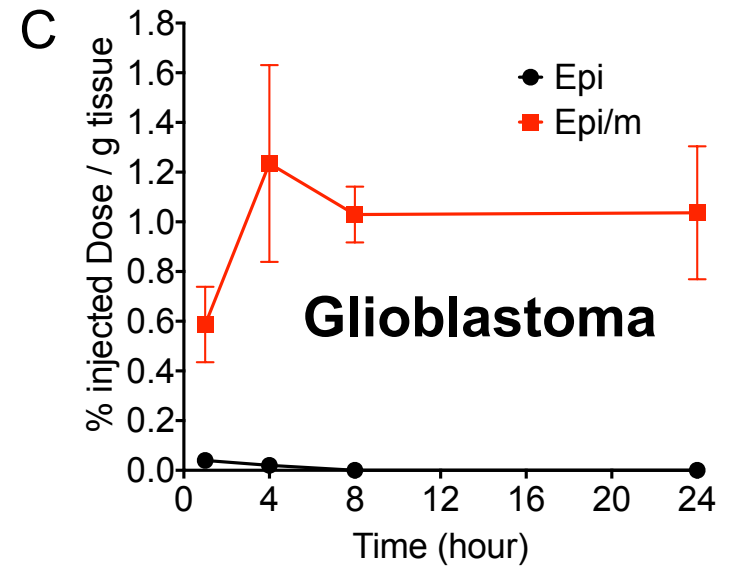
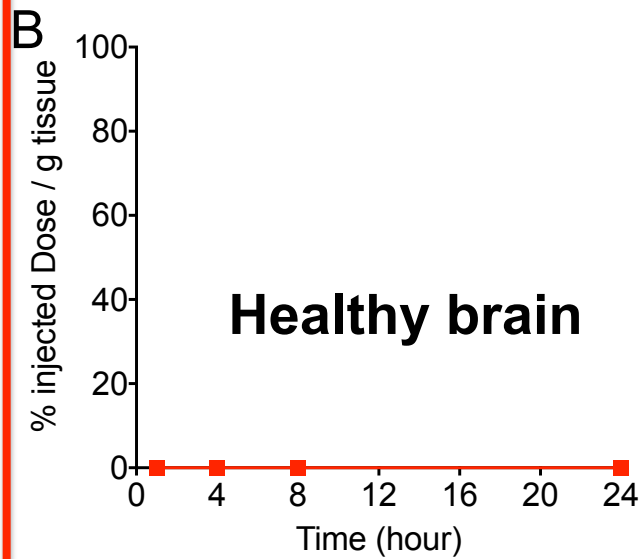
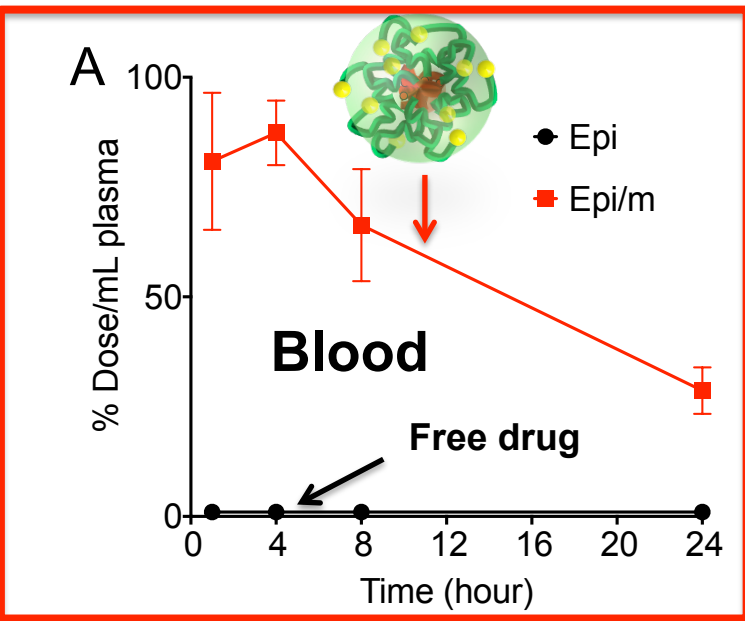
PTEN-Negative CT2A
5 mg/kg single agent or combination



Dose was escalated for micelle group from 5mg/kg to 15 mg/kg, but this dose was not tolerable for free drug.



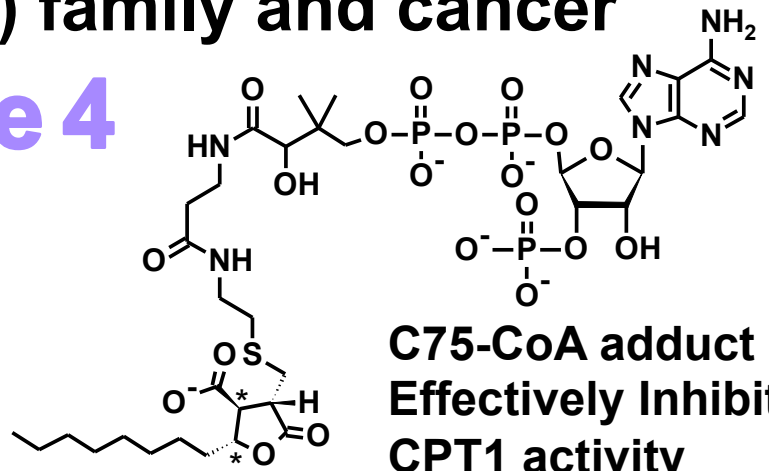
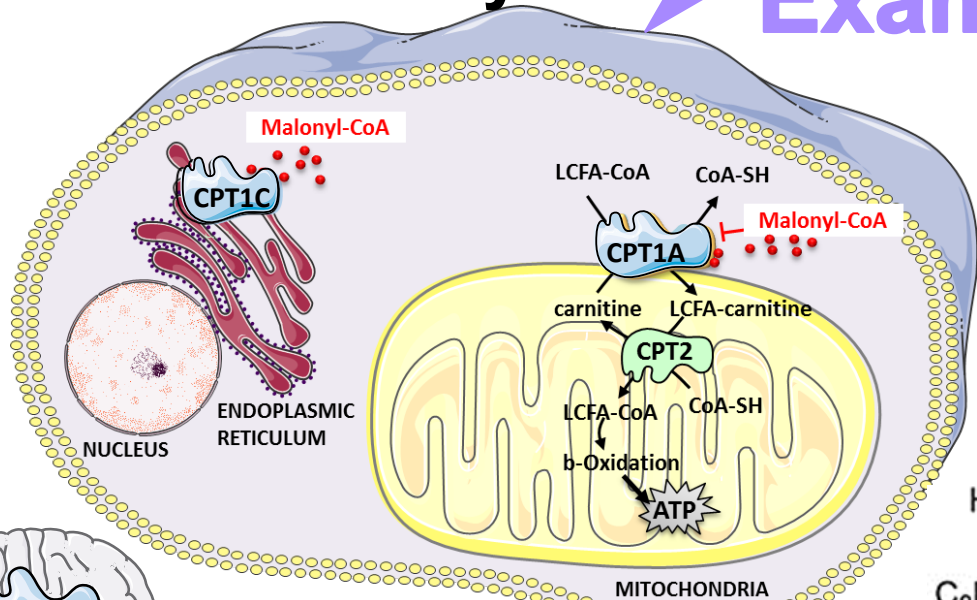
Nanomedicine (Epi/m) can target glioblastoma significantly better than free drug (Epi)



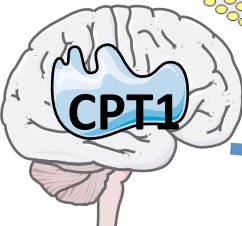
| | AUC (%injected dose/g of tissue × h) ±S.D. | | | |
|-------|--|---------------|--------------|------------------|
| Drug | Plasma | Healthy brain | Tumor | |
| Epi | - | - | 0.15 ± 0.01 | 165 times higher |
| Epi/m | 1704.00 ±136.16 | 0.37 ±0.06 | 24.77 ± 8.96 | |

Carnitine palmitoyl transferase (CPT1) family and cancer metabolic Plasticity

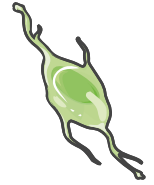
Example 4



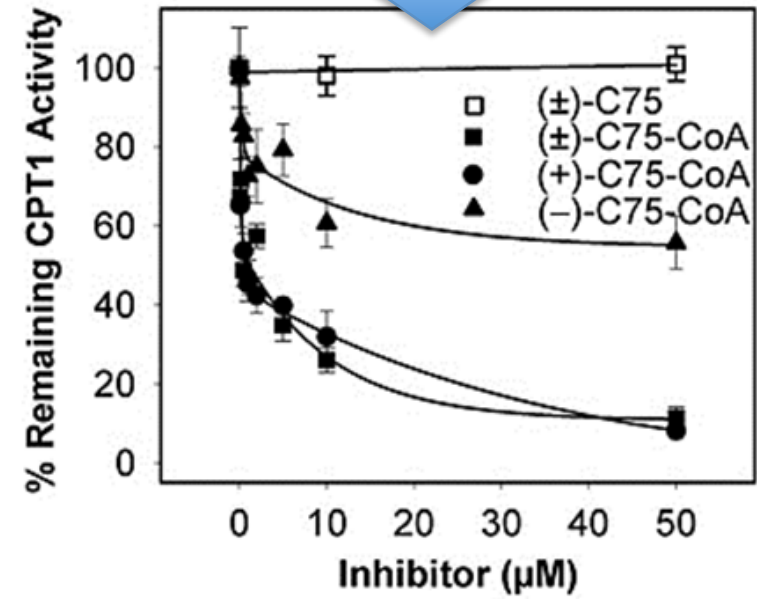
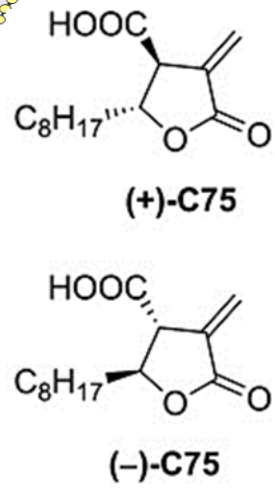
C75-CoA adduct Effectively Inhibits CPT1 activity



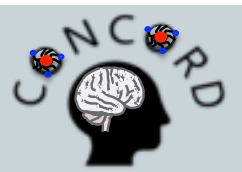
Overexpress in Cancer: Glioblastoma



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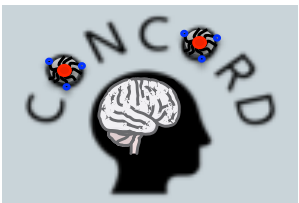
Makowski...Serra et al. Chirality (2013)



Check out our poster

Title: A new nanomedicine platform to deliver a carnitine palmitoyl-transferase 1 (CPT1) inhibitor into glioma cells and neurons

Authors: West Kristian Dizon Paraiso, Jesús García Chica, Xavier Ariza Piquer, Jordi García Gómez, Kazunori Kataoka, Rosalía Rodríguez Rodríguez, Sabina Quader



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Concluding Remarks

- ❑ In this presentation, we have demonstrated four different examples of nanomedicine based approaches for targeting one of the most lethal human cancers, Glioblastoma.
- ❑ So far, our research approaches provided promising outcome in pre-clinical GBM mouse models with great potential for fast clinical translation.

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A traditional Japanese garden scene. In the foreground, two workers wearing white long-sleeved shirts, blue pants, and conical straw hats are kneeling on a mossy ground. One worker is holding a woven basket. In the background, there is a stone lantern, a stream, and various trees and rocks. The text "Thank you very much for your attention" is overlaid in white on the left side of the image.

**Thank you very
much for your
attention**