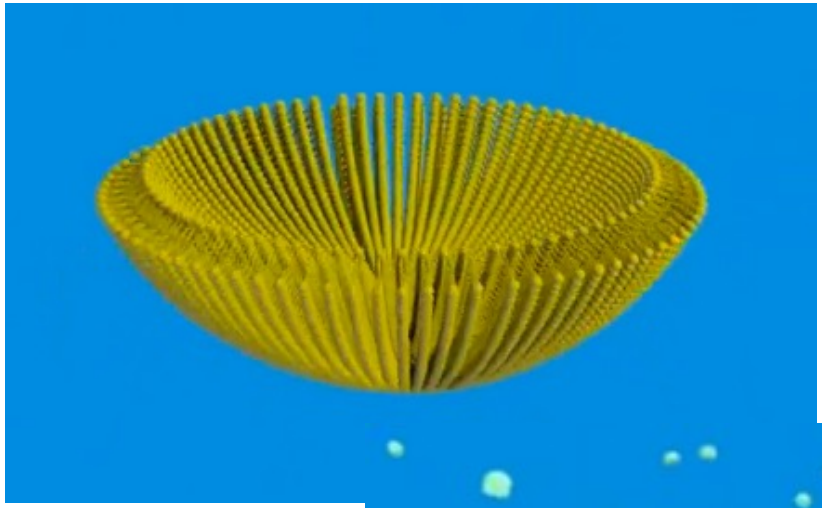
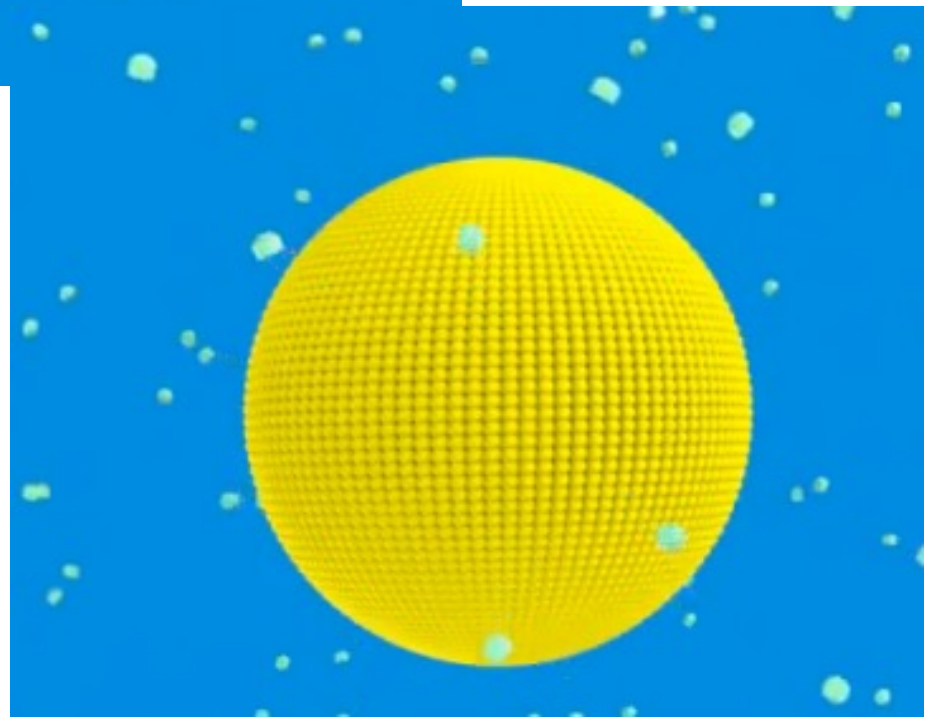
Science is about knowing; engineering is about doing.

henry_petroski

Liposomes

Nanometer size

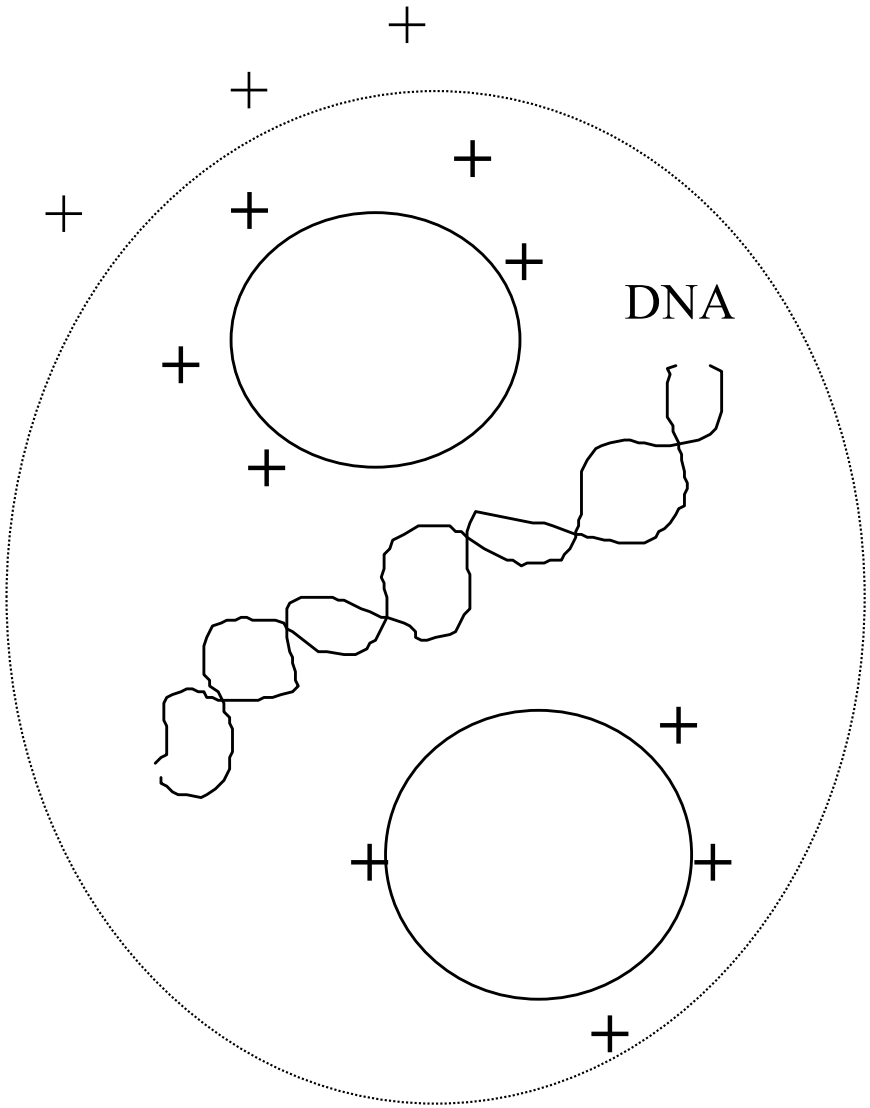




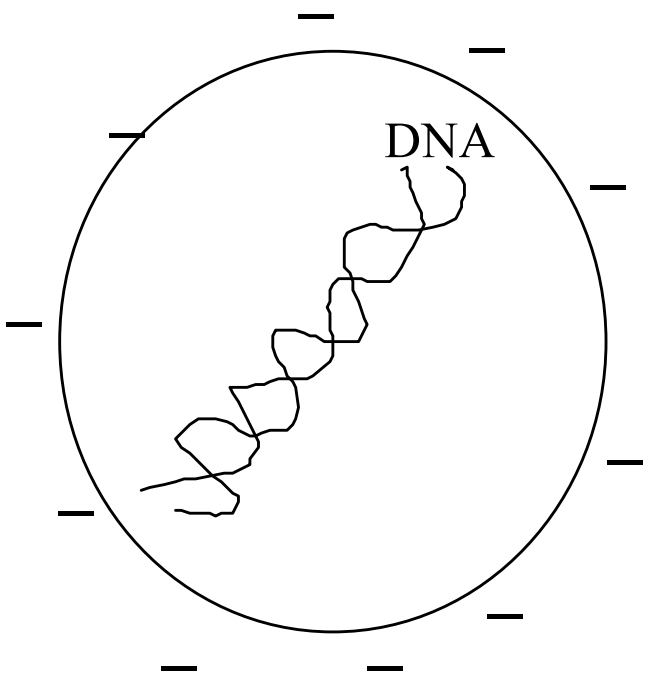
In H_2O environment

Liposomes/Lipoplexes

(12.1% clin tr.2003, 8.6% 2005,7.1% 2008; 6.5 2010)



Successful



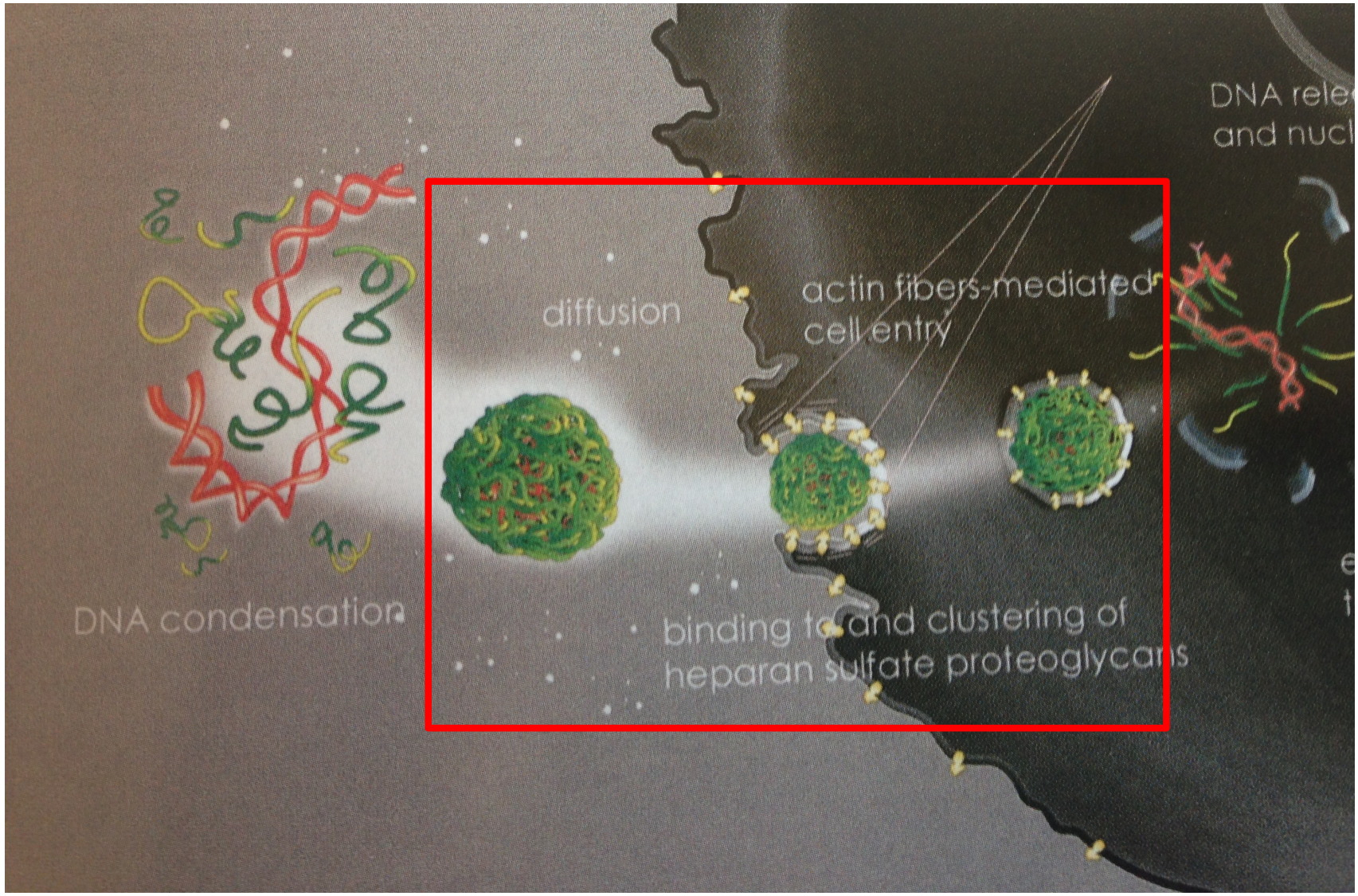
Unsuccessful, pH sensitive, DOTAP

Delivery barriers that a vector must overcome

- Extracellular barriers
  Opsonins (act as binding enhancers for the process of phagocytosis)
  Phagocytic cells
  Extra-cellular matrix
  Digestive enzymes

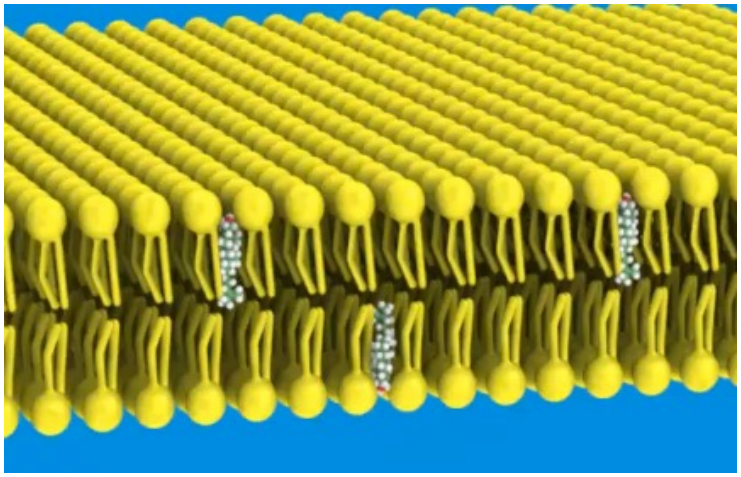
- Intracellular barriers
  Plasma membrane
  Endosome/lysosome
  Nuclear membrane

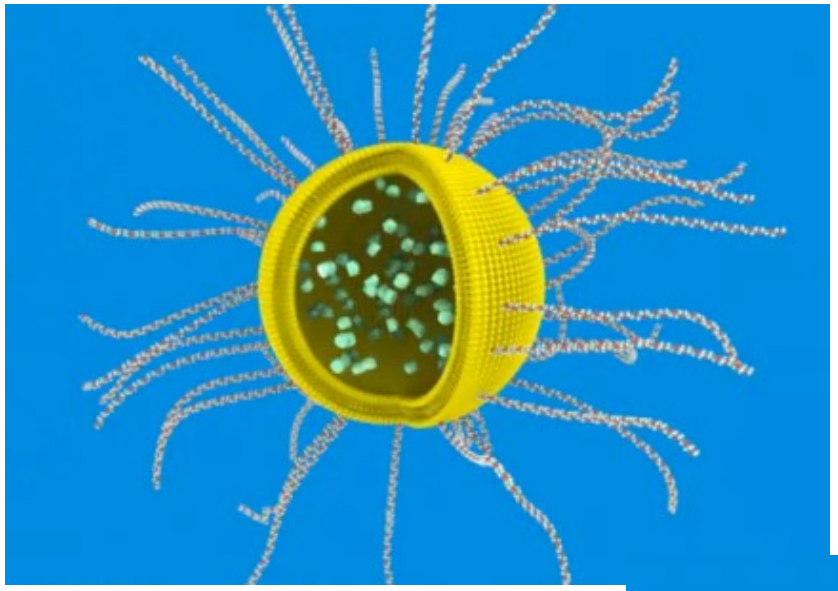
Non viral vectors: the objectives of nanotech studies applied to gene transfer



Liposomes/improvements

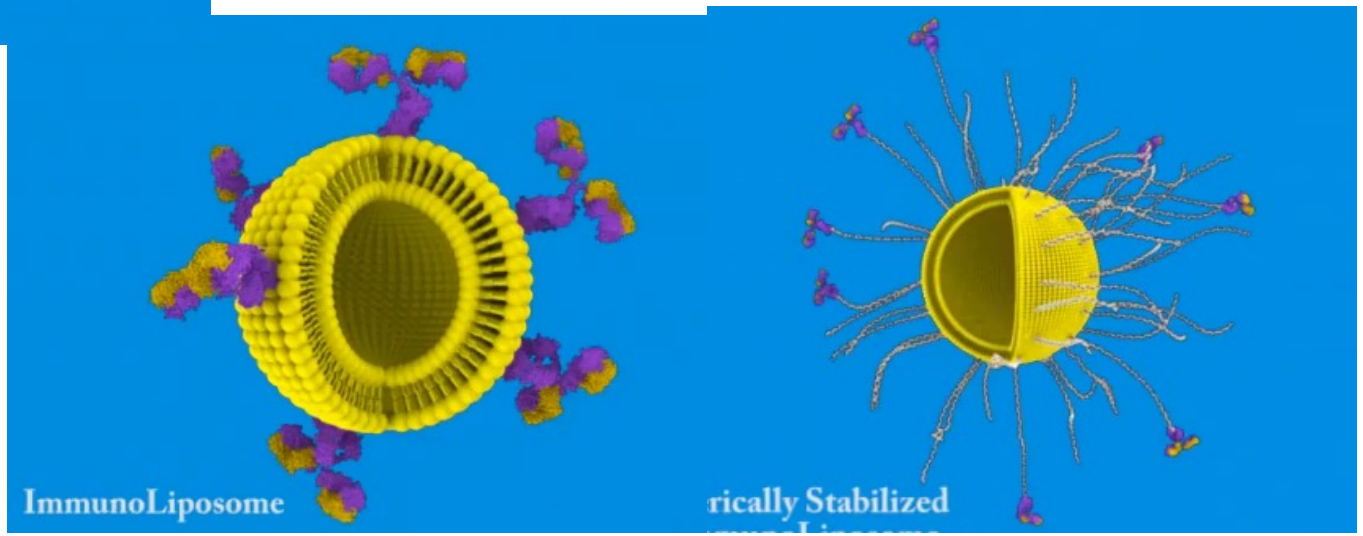
+cholesterol-increase stability in vivo



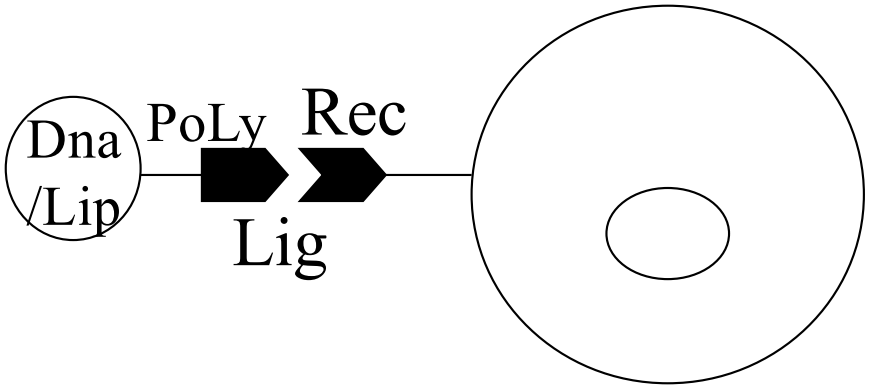


+PEG, more stable

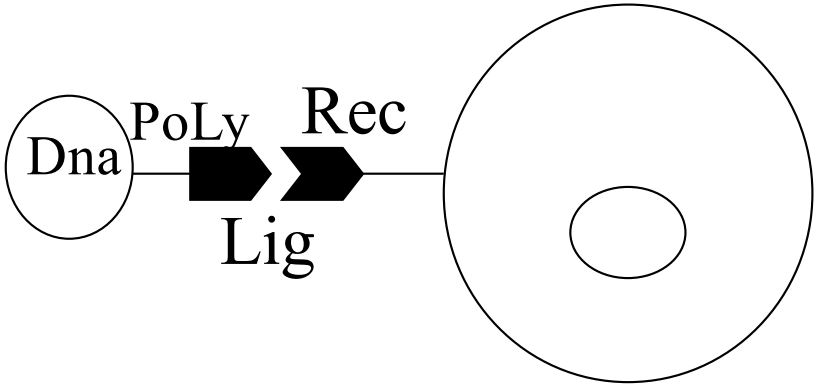
+Ab, targeting



Use of receptor ligands: to facilitate binding to cell membrane



or



Which receptor ligand?

Table 1 Ligands Used in Receptor-mediated Gene Transfer

Ligands	Refs.
Alpha2 macroglobulin	24,25
Anti-CD3	26,27
Anti-CD5	28
Anti-CD117	29
Anti-EGF	30
Anti-HER2	31
Anti-IgG	32,33
Antisecretory component Fab	34–36
Anti-Tn	37
Antithrombomodulin	38
Antibody ChCE7	39
Asialoglycoproteins	40–49
EGF	50–52
Fibroblast growth factor 2(FGF2)	9,53
Folate	54–56
Glycosylated synthetic ligands	57–69
IgG (FcR ligand)	32,70
Insulin	10,71
Invasin	72
Lectins	73–75
Malarial circumsporozoite protein	76
RGD-motif (integrin binding)	77
Steel factor (CD117 ligand)	78
Surfactant proteins A and B	79,80
Transferrin	13,81–87

Literature classic

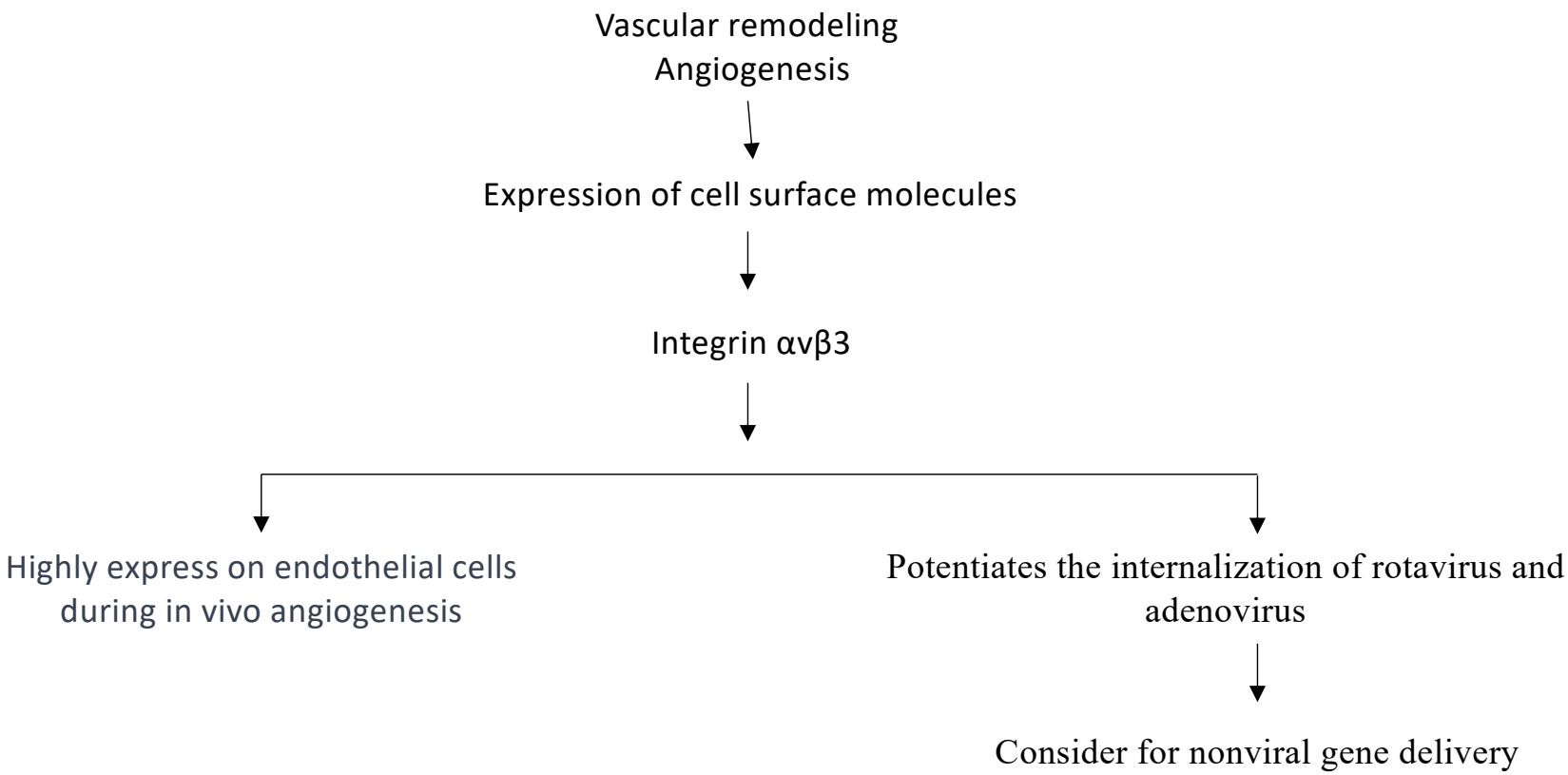
Tumor Regression by Targeted Gene Delivery to the Neovasculature

John D. Hood,1 Mark Bednarski,2 Ricardo Frausto,1 Samira Guccione,2 Ralph A. Reisfeld,1 Rong Xiang,1 David A Ceresh

Scripps, Science 2002

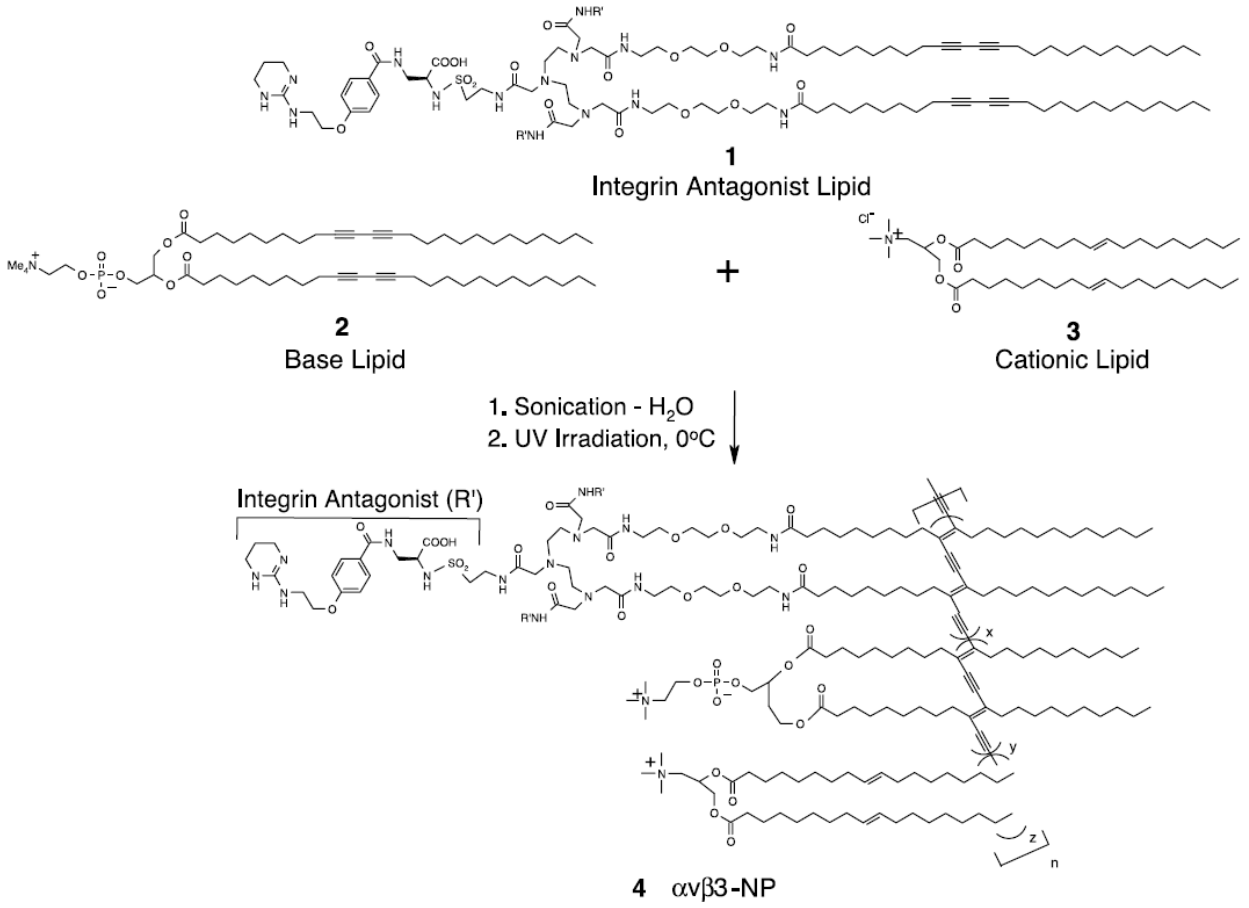
"The goal is to make a smart bomb for the tumor endothelium, and to increase the efficacy of agents by directing them straight to $\alpha_V\beta_3$."

Tumor Regression by Targeted Gene Delivery to the Neovasculature



students analysis

How we use integrin αvβ3 to gene delivery in endothelial cells?



Vary the surface charge

students analysis

Does integrin va3b bind selectively to avb3-NP?

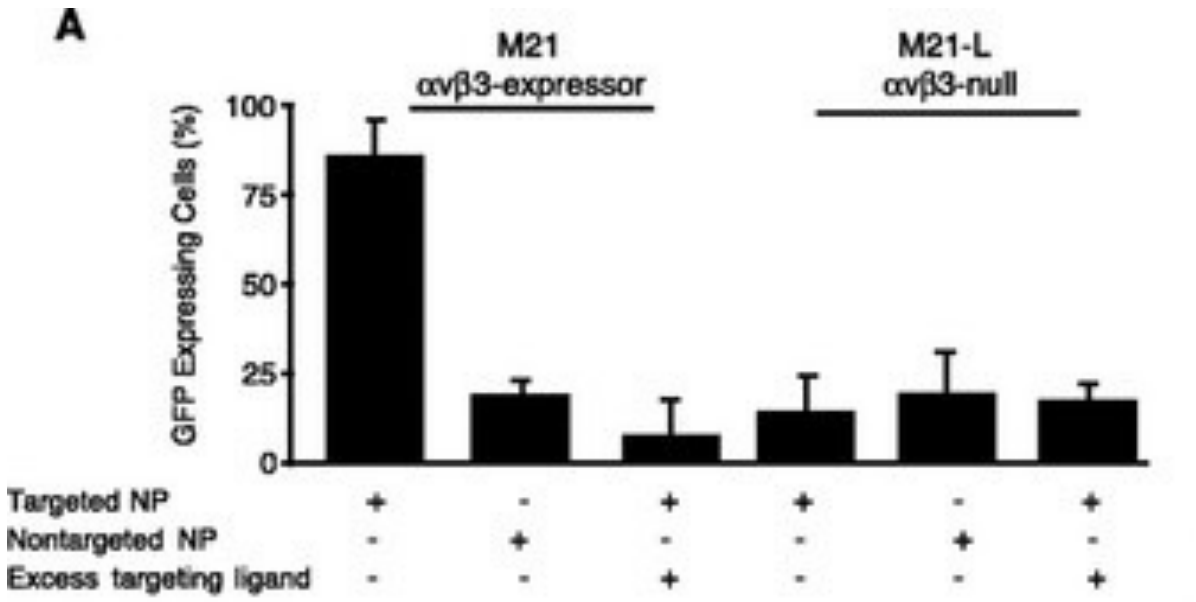


Fig. 2A: GFP gene transfer mediated by avb3-NP to M21 or M21-L human melanoma cells was evaluated.

The selective delivery of the gene is integrin avb3-dependent.

students analysis

Is integrin avb3 linked with angiogenesis?

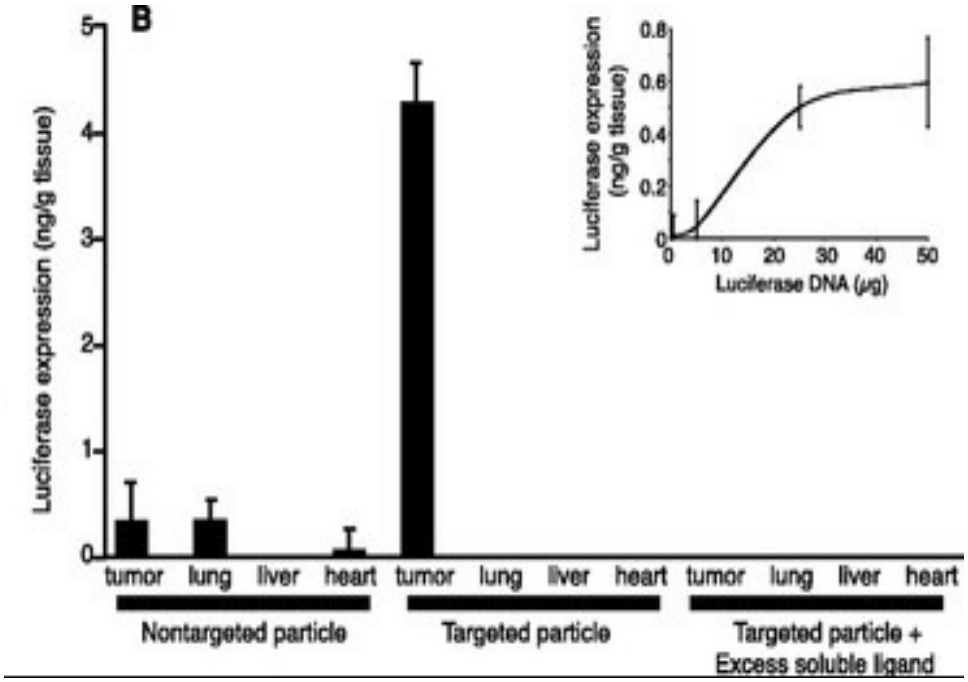
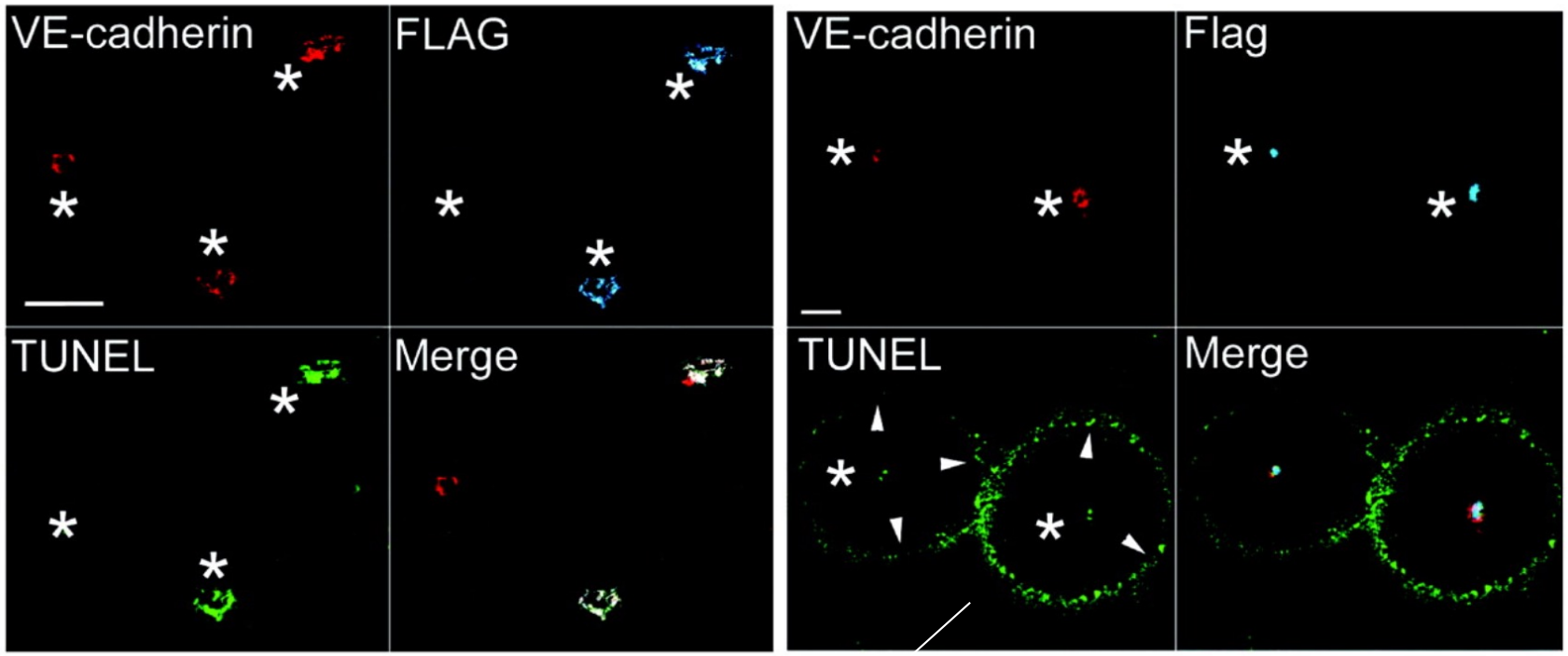


Fig. 2B: Athymic WEHI mice were subcutaneously injected with M21-L cells (5 106), and tumors were allowed to grow to 100 mm3. Mice were then injected i.v. with 450 nmoles of NP electrostatically coupled to 25 g (*13*) of plasmid expressing firefly luciferase. One group also received a coinjection of 20-fold molar excess of the soluble avb3-targeting ligand. After 24 hours, mice were killed, tissues were surgically removed, and luciferase activity was quantified.

students analysis

Does a mutated form of Raf-1 block angiogenesis and induce apoptosis?



24 hours after tumor removal

Rings of apoptosis

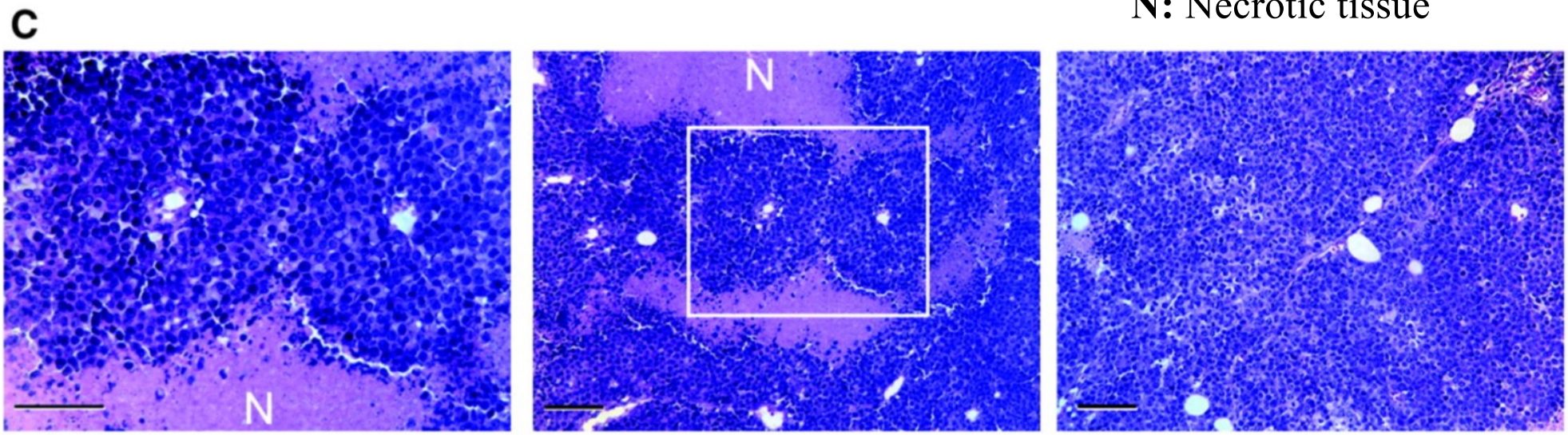
72 hours after tumor removal

VE cadherin: Blood vessels identification
FLAG: αvβ3-NP/Raf(–) gene expression detection
TUNEL: Apoptotic cell marker

students analysis

Tumoral staining 100x after 72h control vs treatment

Staining: Hematoxylin and eosin
N: Necrotic tissue



Treatment

Control

Mutated form of Raf-1 induces apoptosis and blocks angiogenesis, being a great antitumoral option

students analysis

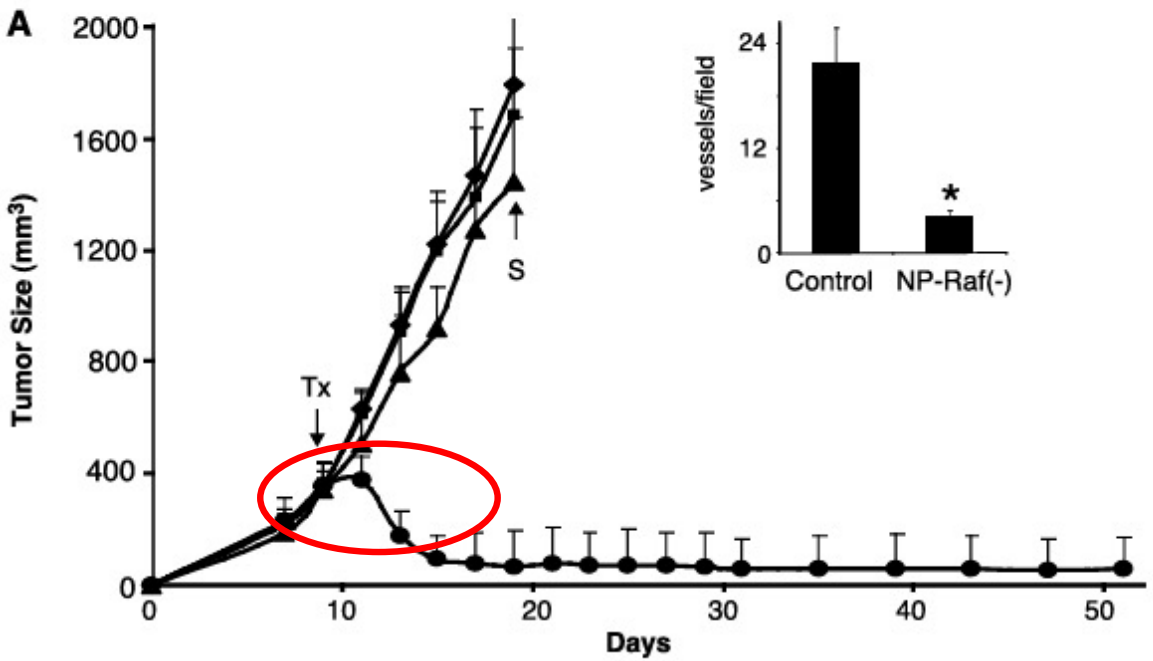
Does αvβ3-NP/Raf(-) work in the inhibition of tumor-induced angiogenesis?

(◆) PBS control

(■) αvβ3-NP-shuttle- vector;,

● αvβ3-NP/Raf(-)

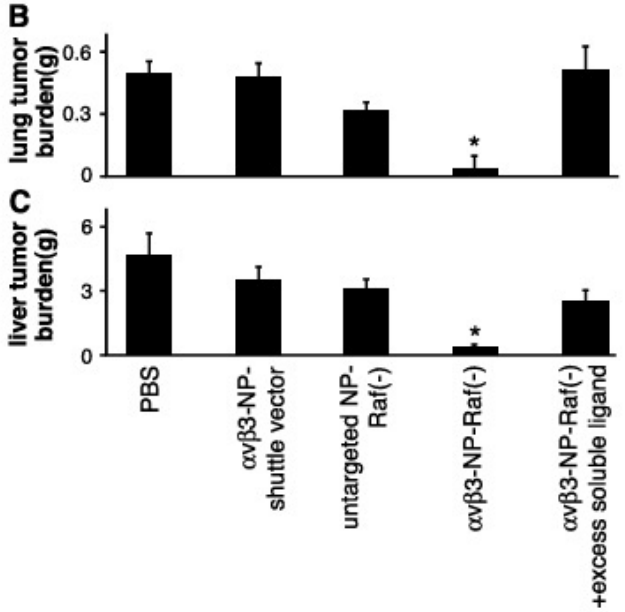
▲ αvβ3-NP/Raf(-) plus excess soluble v 3 ligand

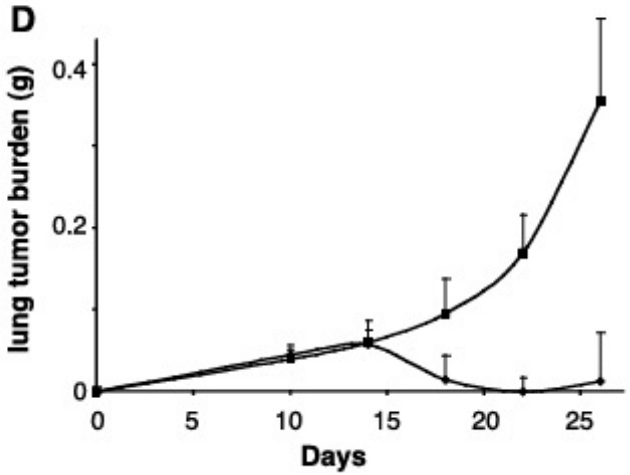


αvβ3-NP/Raf(-) inhibits angiogenesis causing tumor regression in size

students analysis

Is this therapy effective against pulmonary and hepatic metastases of colon carcinoma?





αvβ3-NP/Raf(-) shows effectiveness in reducing tumor metastasis

students analysis