Science is organized knowledge. Wisdom is organized life.
Immanuel Kant
BrainyQuote

Intracellular transport and plasmid biodistribution after cationic lipid mediated transfection

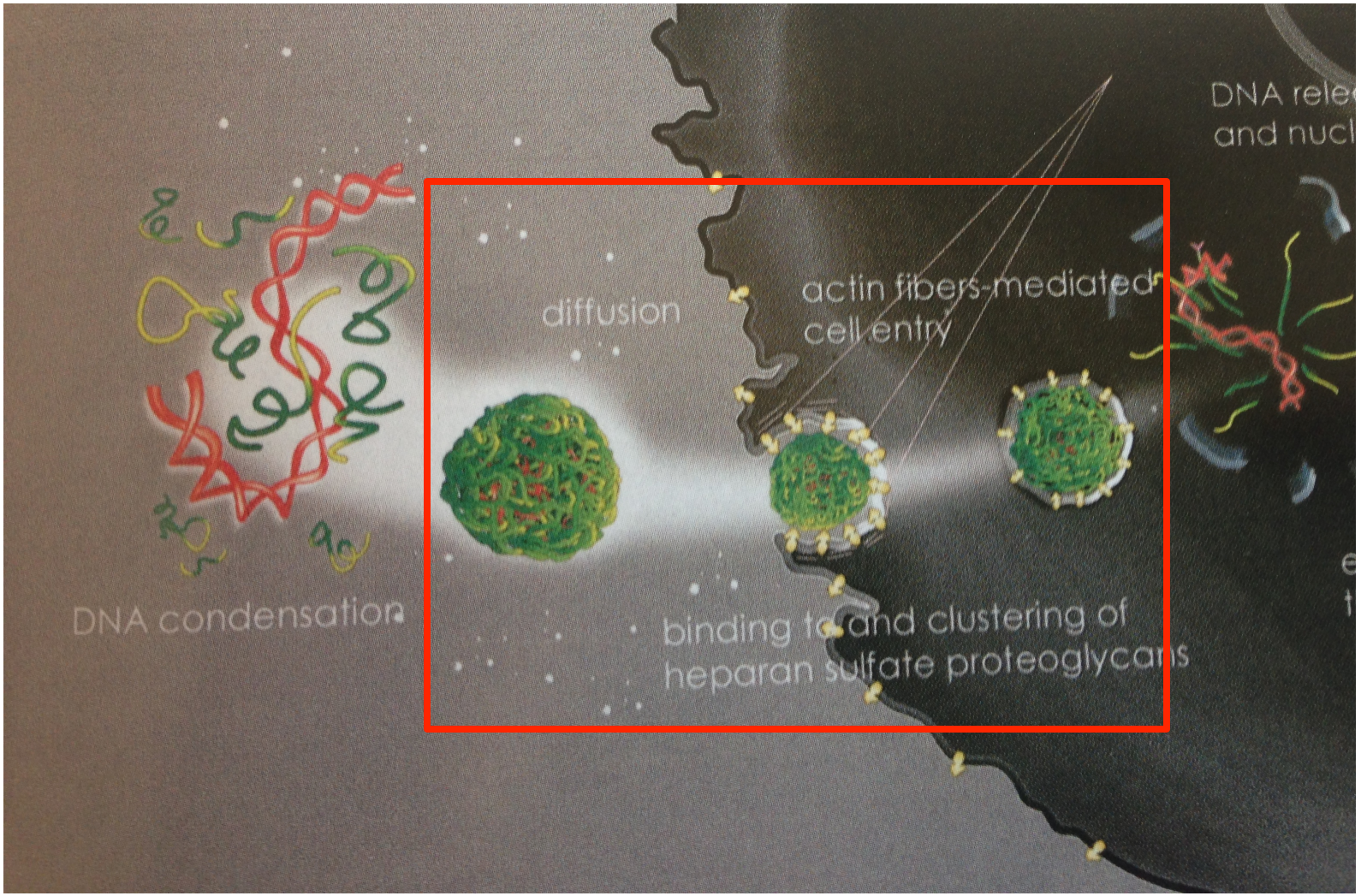
- 1.000.000 plasmids/cell transfected
- 300.000 plasmids/cell in pellet
- 50.000 plasmids/ cell intracellular
- 1.000 plasmids/cell intranuclear (1/1000)

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

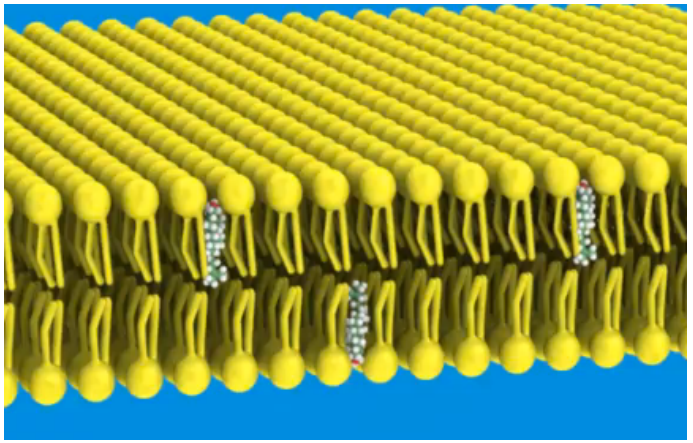
- Intracellular barriers
  Plasma membrane
  Endosome/lysosome
  Nuclear membrane

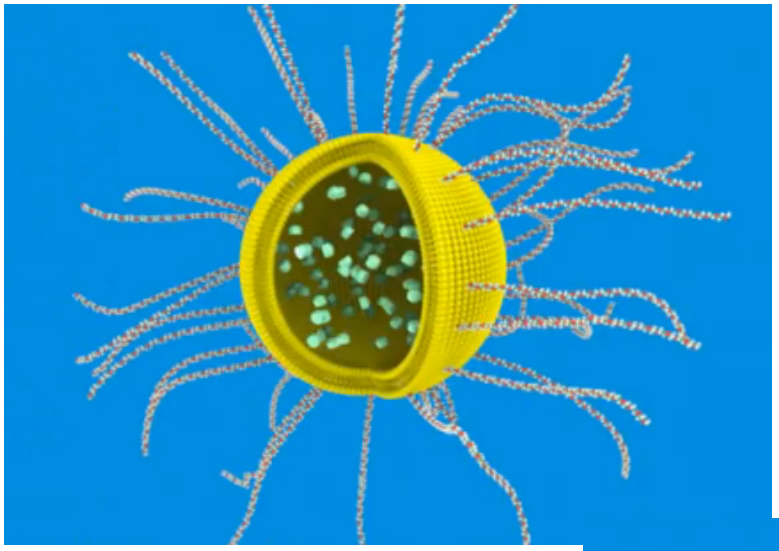
Non viral vectors: the objectives of nanotech studies applied to gene transfer



Liposomes/improvements

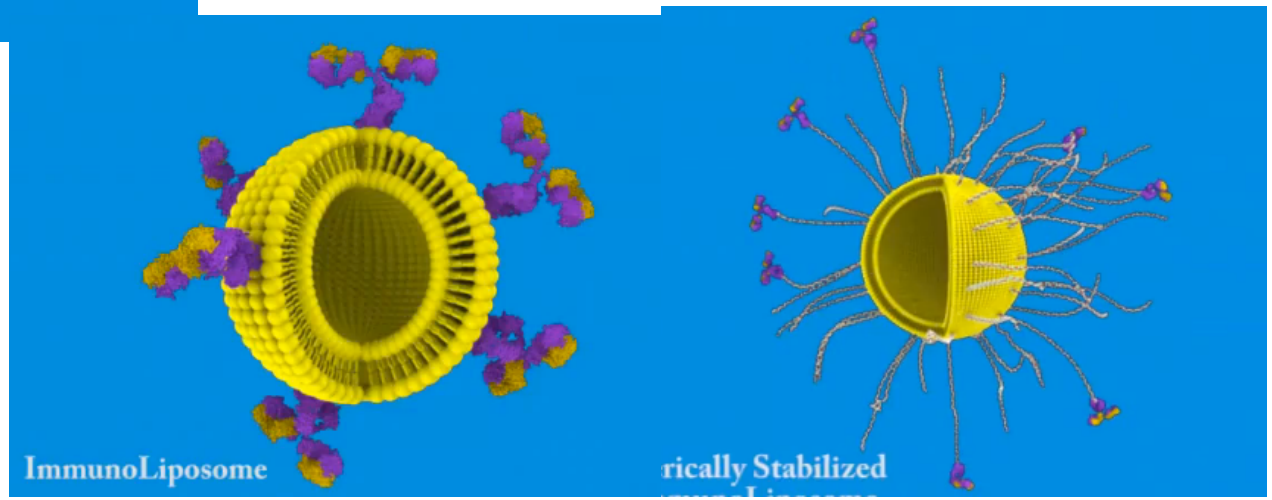
+cholesterol-increase stability in vivo



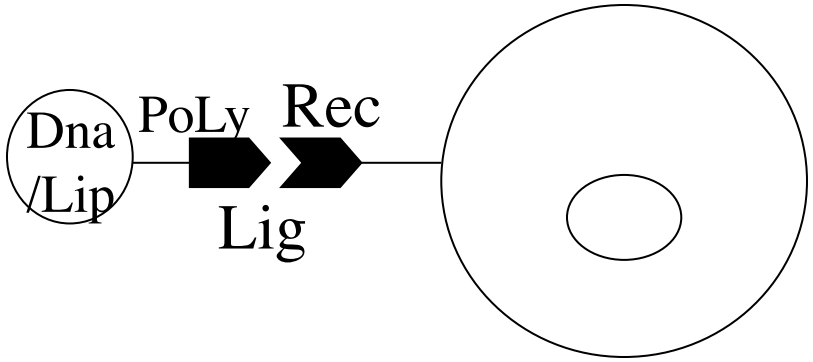


+PEG, more stable

+Ab, targeting



Use of receptor ligands: to facilitate binding to cell membrane



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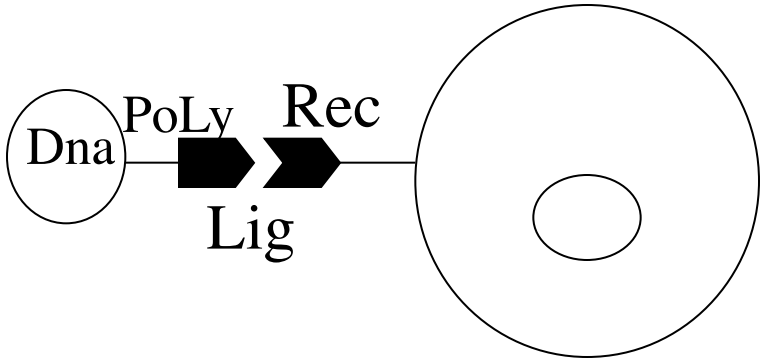


Which receptor ligand?

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

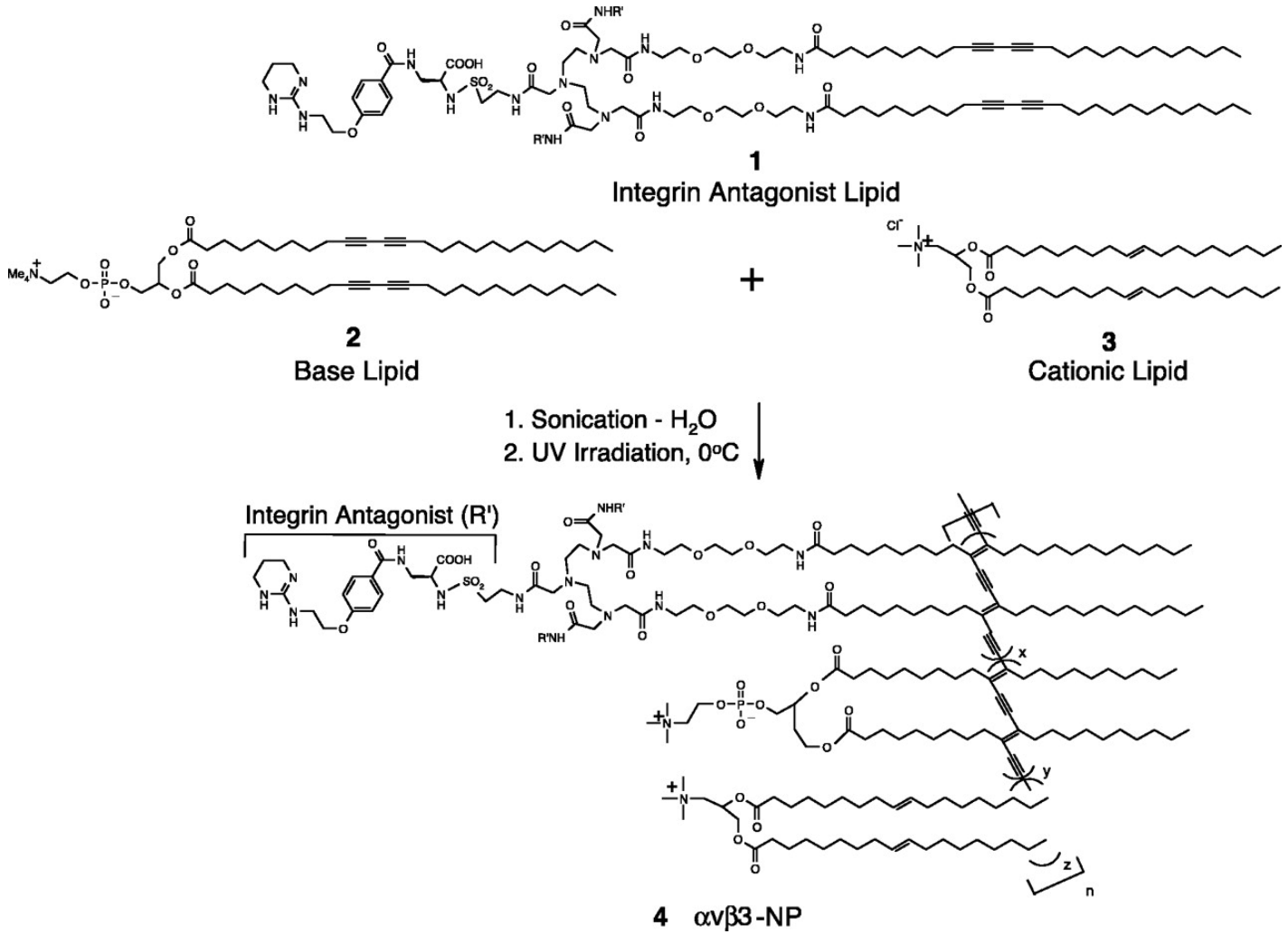
Tumor Regression by Targeted Gene Delivery to the Neovasculature

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 $\alpha_v\beta_3$."

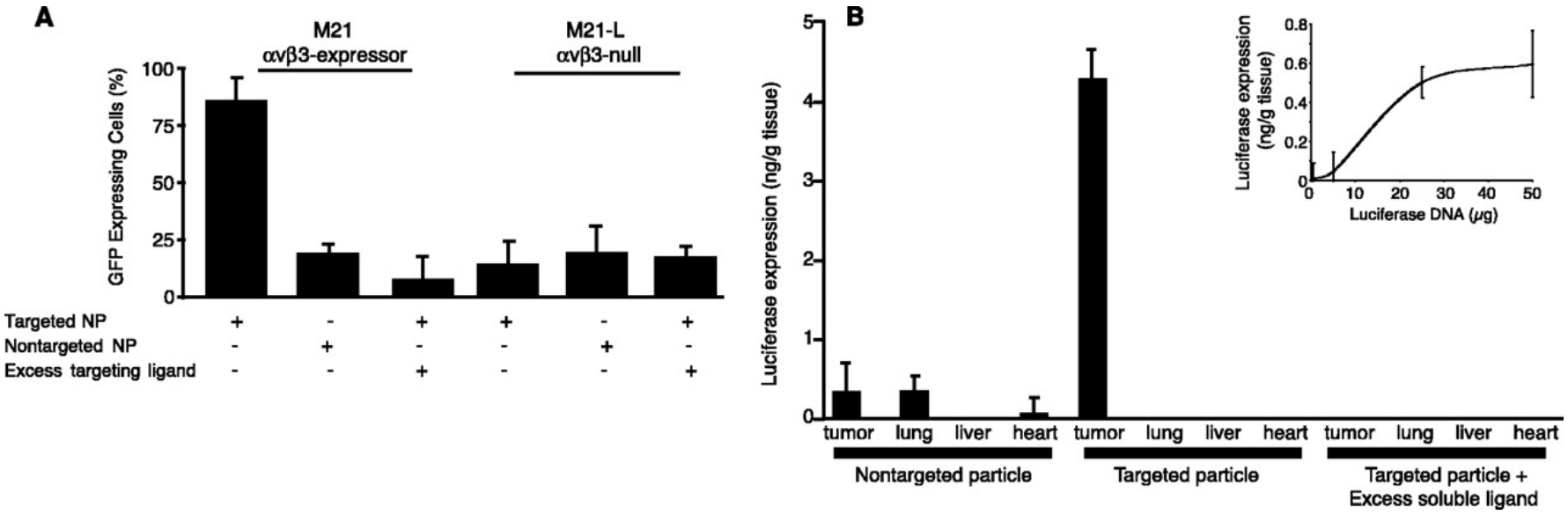
Fig. 1. Schematic diagram outlining the formation of the NPs by self-assembly and polymerization of the appropriate lipids.



αvβ3 integrin over-expressed in neo-vascularised tissue & tumor

Fig. 2. alphav3-NP-mediated gene delivery to alphav3-bearing cells in vitro and in vivo - melanoma model

25ug DNA



In vitro

24h after inj in tail vein

The transgene: Raf mutant

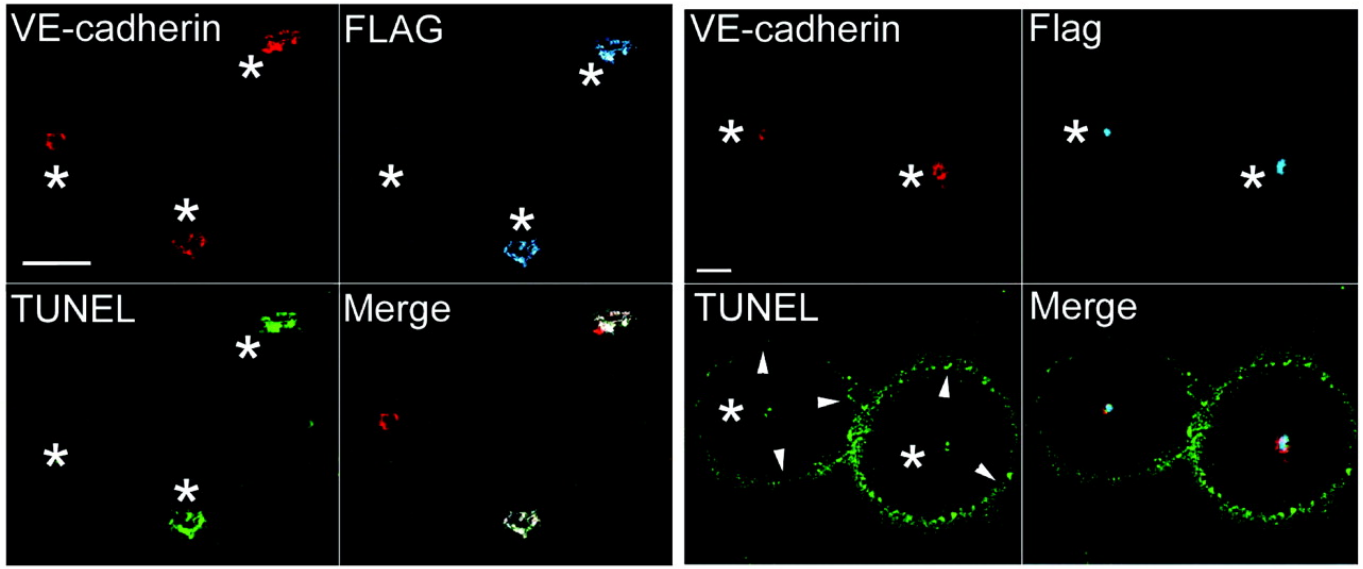
Components of the Ras-Raf-MEK-ERK pathway appear to play an important role in *neovascularization*, because blockage of this pathway suppresses angiogenesis in vivo.

Mutant form of Raf that fails to bind ATP blocks endothelial cell Raf activity and angiogenesis.

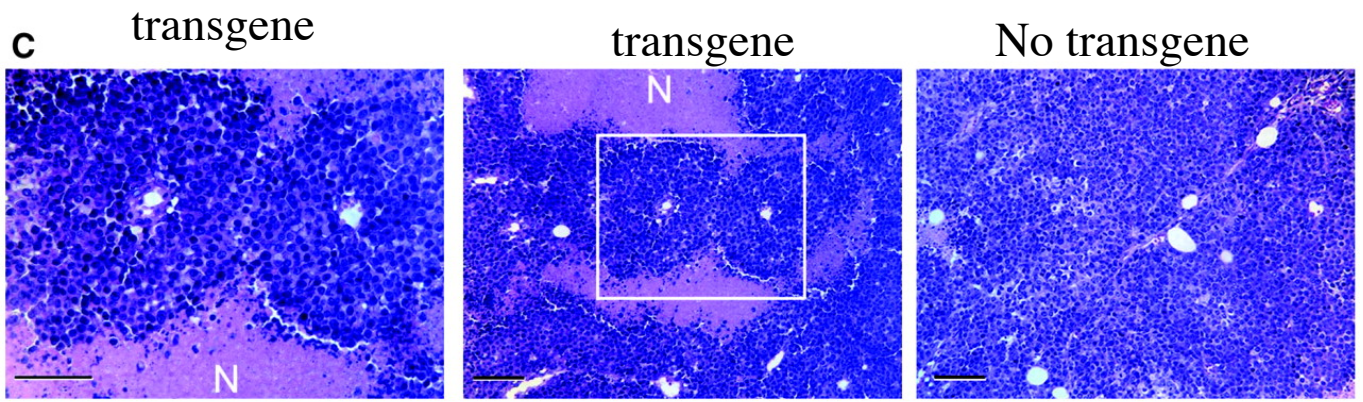
Fig. 3. Delivery of ATPμ-Raf to tumor-associated blood vessels causes endothelial and tumor cell apoptosis.

M21 derived melanomas. 9 days after subcutaneous implants of M21 ells -> 400mme3 tumors. Tail vein inj of Raf

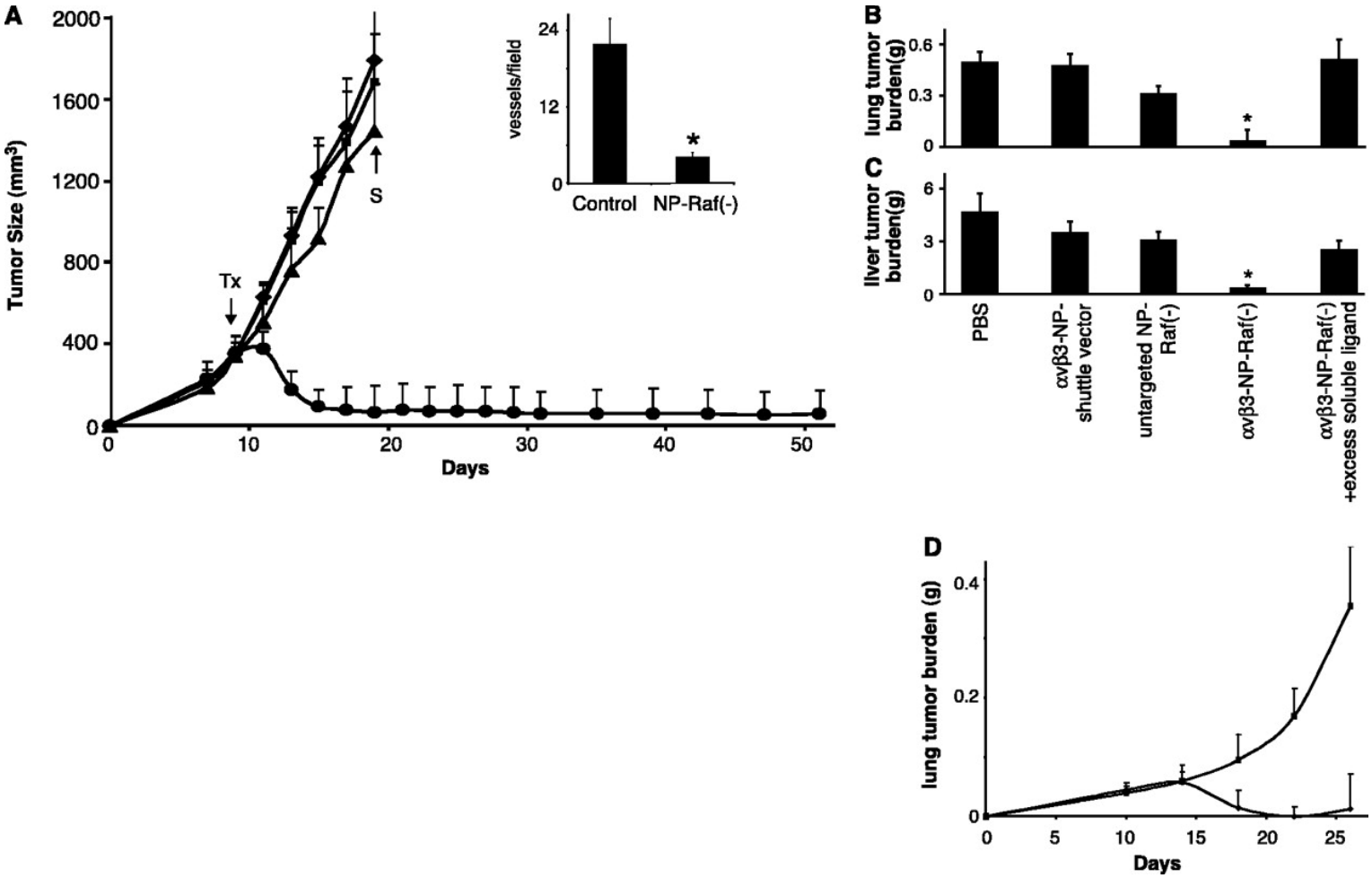
Tumor **24h after** treatment Apoptotic vessel cells



Tumor 72h after treatment, apoptotic tumor cells around the treated vessel



Tumor 72h after treatment, tumor necrotic tissue (N) around the treated vessel

Fig. 4. Delivery of mutant *Raf* to tumor vessels inhibits angiogenesis, causing regression of **established tumors**.

Single tail vein injection of Raf