NANOMEDICAMENTS POUR LE TRAITEMENT DU CANCER

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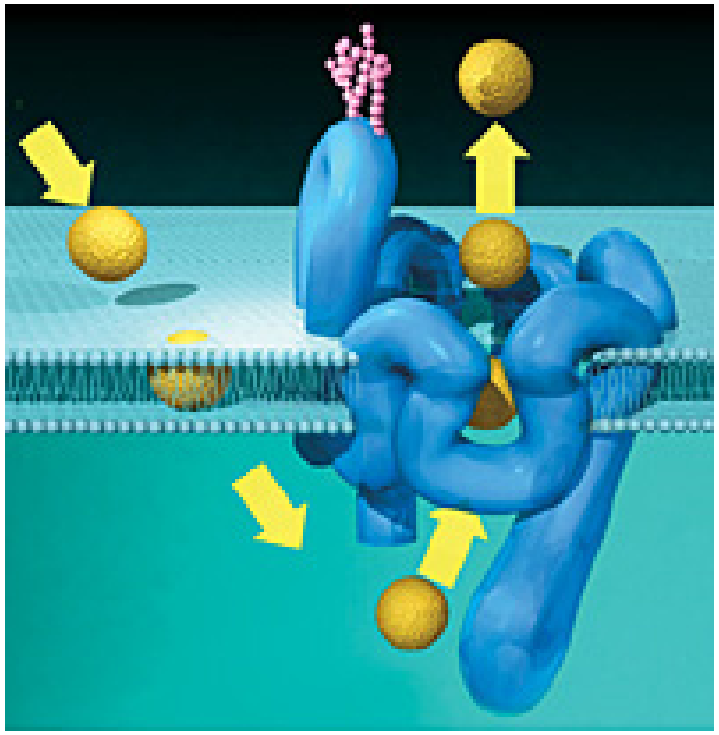
Chaire d'innovation Technologique 2009-2010

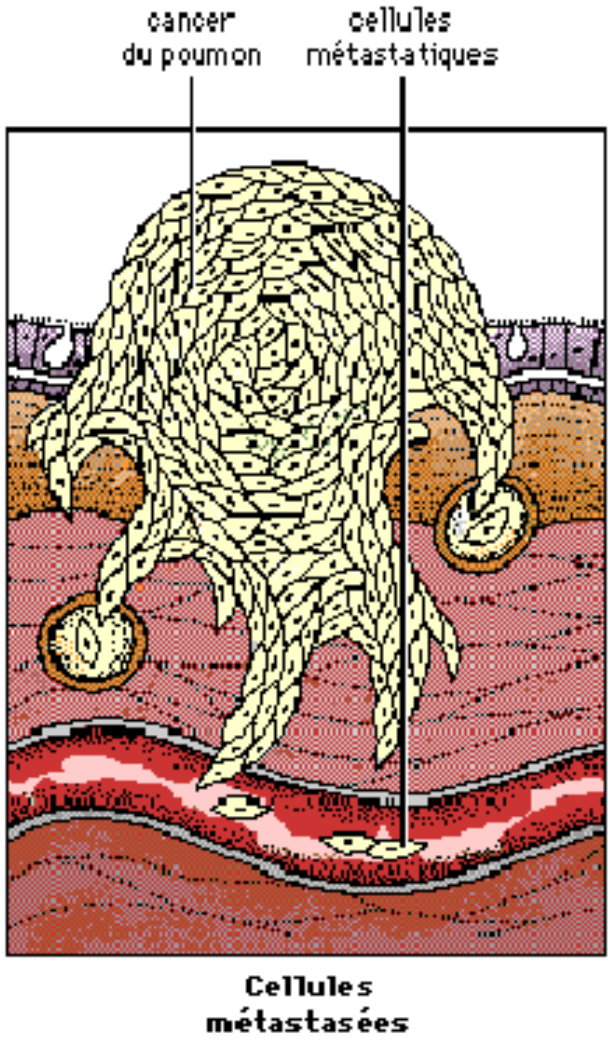
LIMITATIONS OF ANTICANCER COMPOUNDS

- Drug resistance at the cellular level (cellular based mechanisms)
  - Altered activity of specific enzymes (ie.topoisomerase or deoxycytidine kinase activity)
  - Expression or alteration of transport-based mechanisms (ie. multidrug resistance, nucleosides transporters down regulation)
  - Altered apoptosis mechanisms



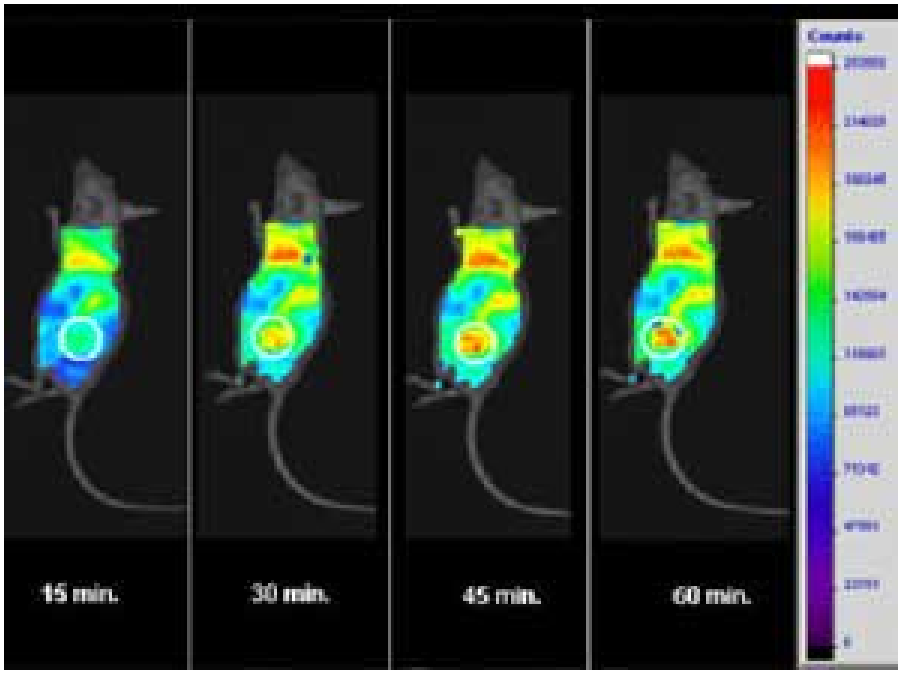
LIMITATIONS OF ANTICANCER COMPOUNDS

- Drug resistance at the tumor level due to physiological barriers (non cellular based mechanisms)
  - Vascularization (heterogeneity, nature of the endothelium)
  - high tumor interstitial pressure
  - Physico-chemical properties of the interstitium (composition, structure, charge)

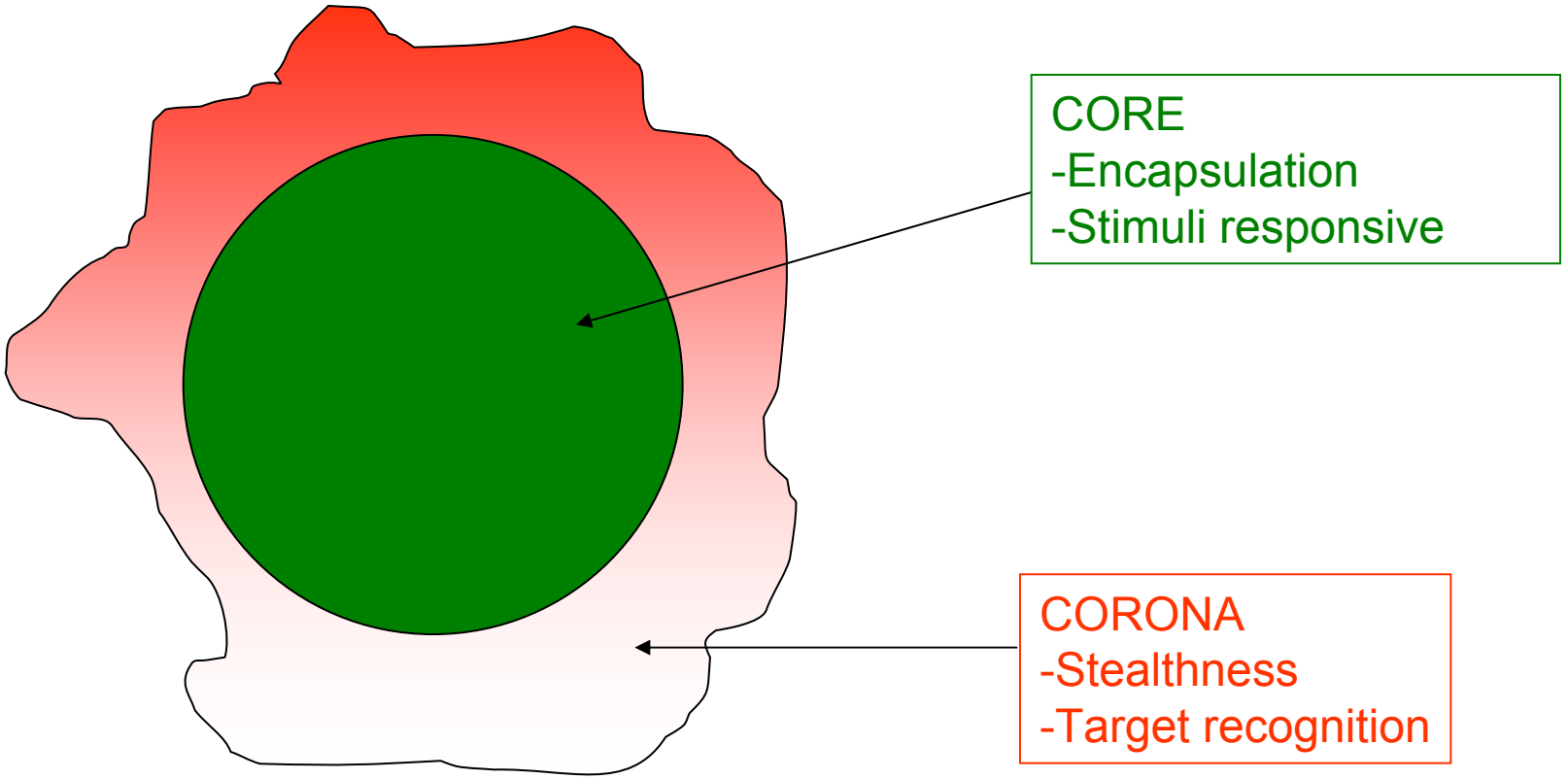


LIMITATIONS OF ANTICANCER COMPOUNDS

- Unspecific tissue/cell disposition (leading to poor activity and side- effects) and/or Rapid metabolization

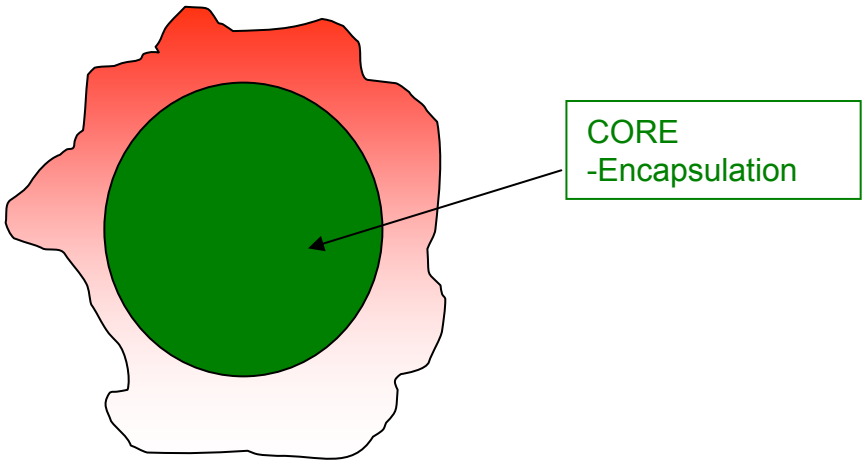


CONCEPTION OF NANOCARRIERS FOR ANTICANCER AGENTS

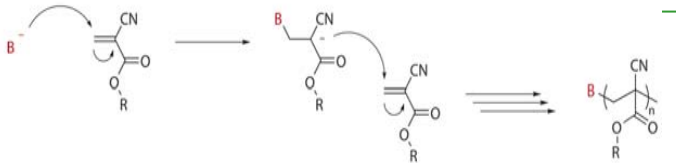


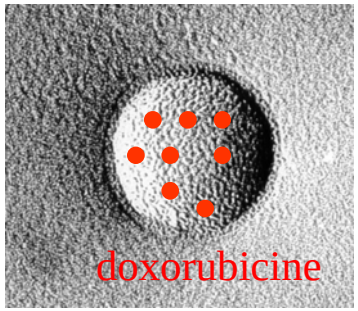
THE CORE

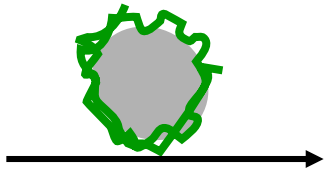
Encapsulation

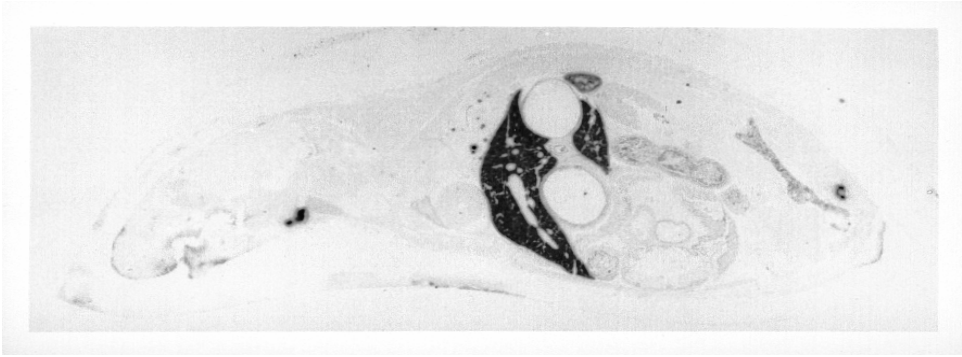


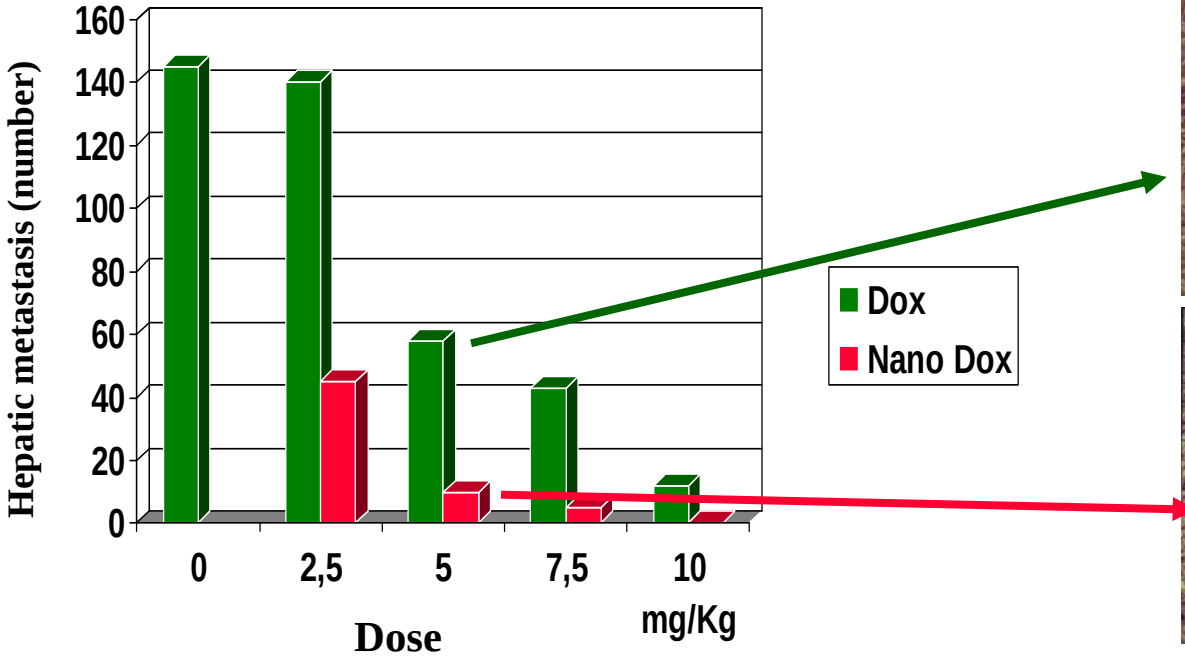
EFFICACITE ANTICANCEREUSE SUR METASTASES HEPATIQUES

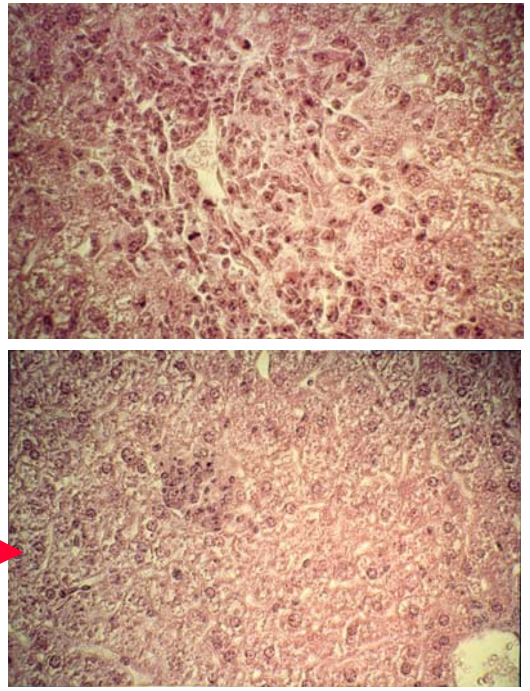






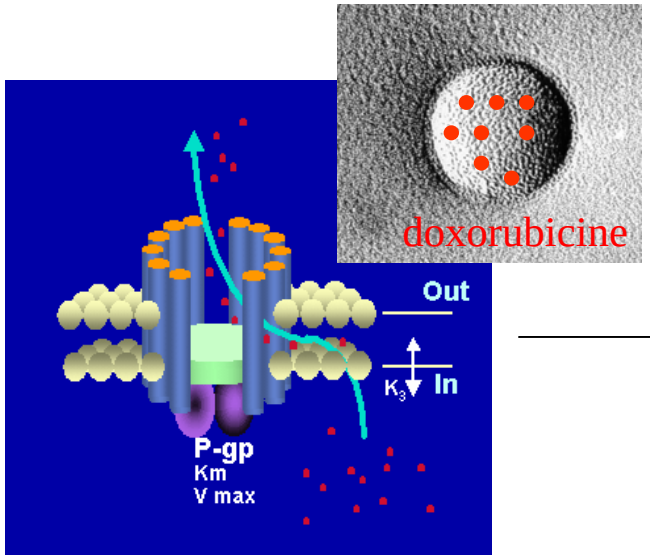


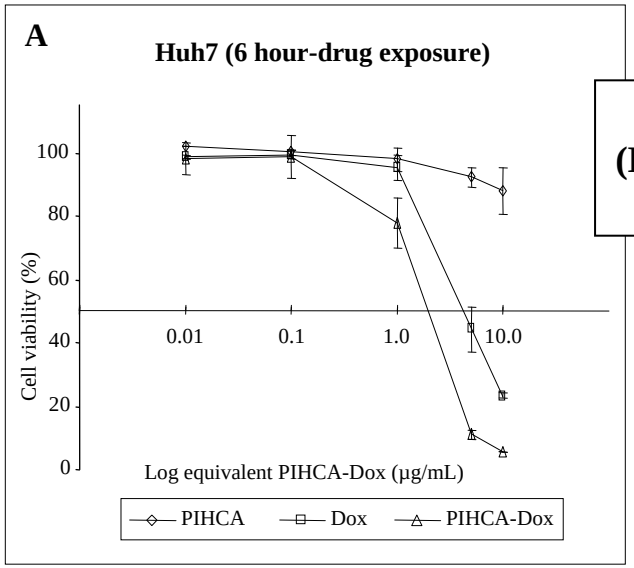




ANTICANCER ACTIVITY ON MDR HUMAN HEPATOCARCINOMA

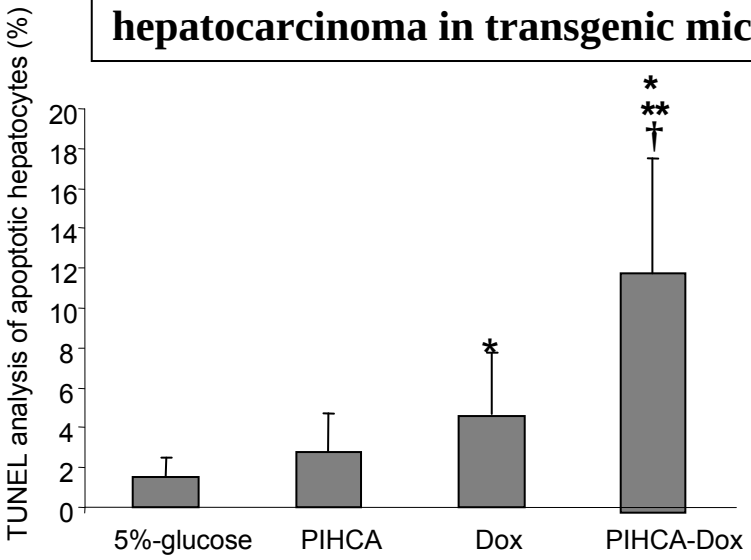
de Verdière, et al., Brit. J. Cancer, **76**, 198-205 (1997)
Barraud, et al., J. Hepathology, **42**, 736-743 (2005)

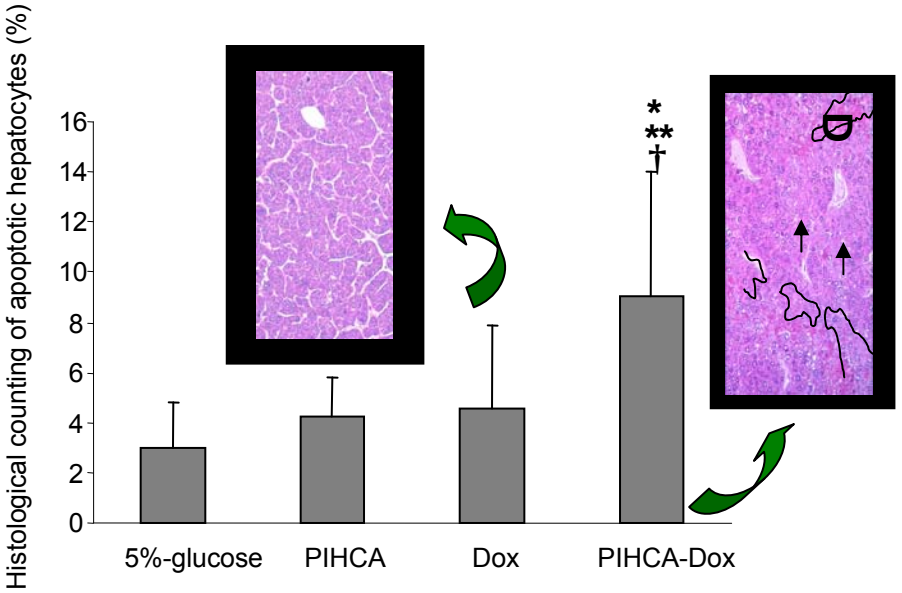




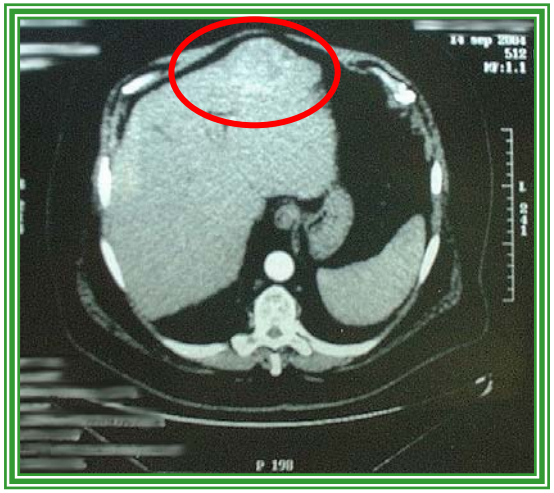
***In vitro* cytotoxicity (Human Hepatocellular Carcinoma)**

hepatocarcinoma in transgenic mice





® CLINICAL TRIAL (Bioalliance)



Baseline

4 weeks post treatment showing tumor necrosis

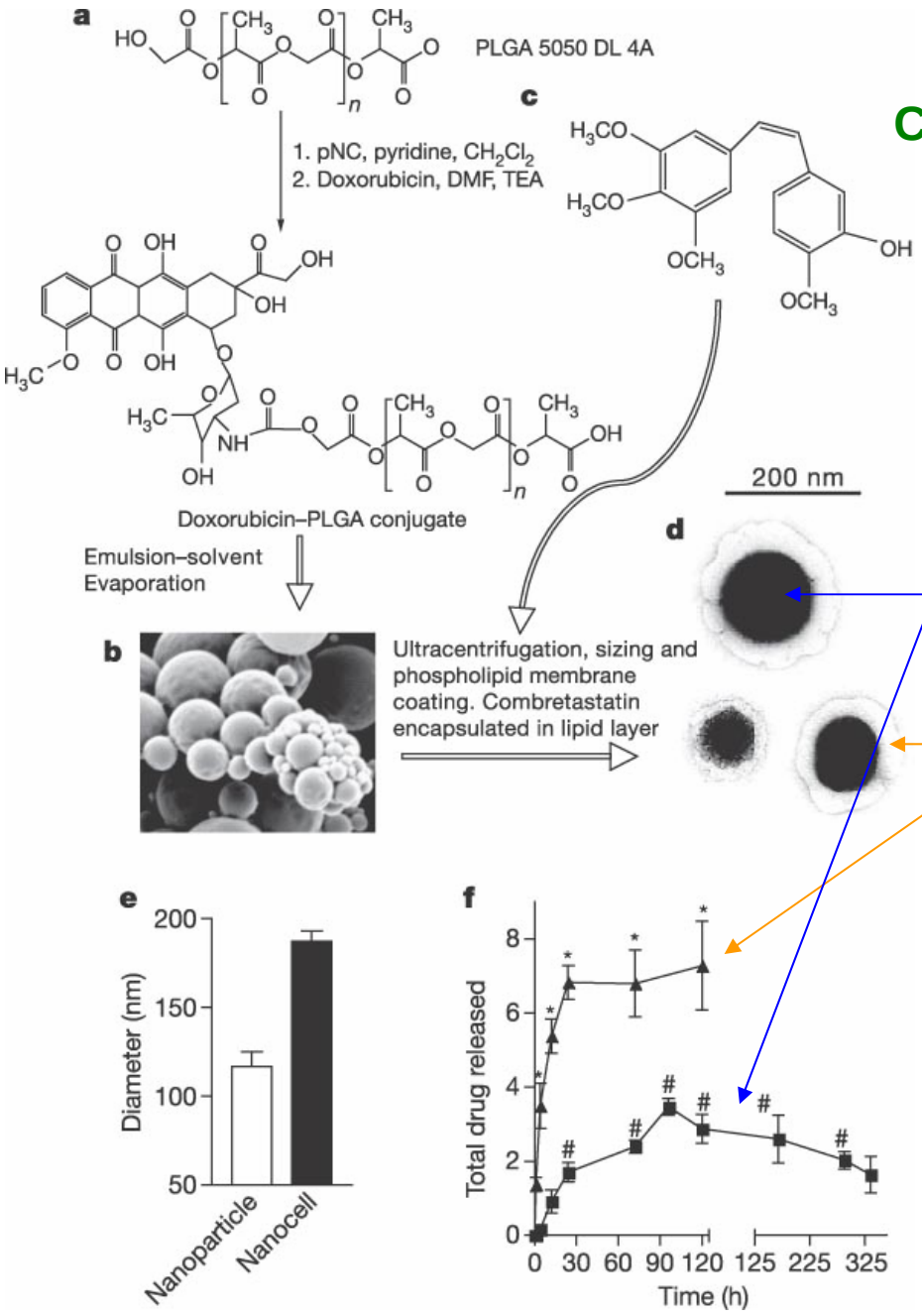
Response	Nr patients
Stable Disease	*8
Progressive Disease	5
Responders	**3
Total Patients	16

Patient #8: Presented with Single Unresectable Tumor in Segment II
Tumor measured 60 x 50 mm (3000mm^2)

After one infusion of 30 mg/m^2, tumor necrosis was evident

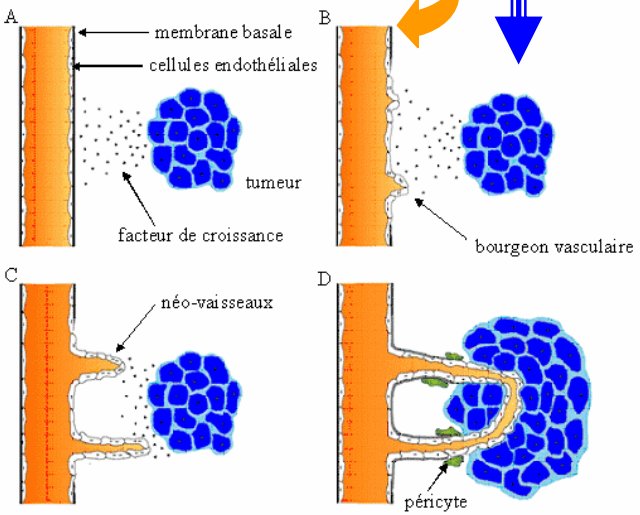
COMBRESTATIN-DOXORUBICIN NANOCELL

Sengupta S. et al, Nature, 568-572 (2005)



DOXORUBICIN (nanoparticle)

COMBRESTATIN (lipid layer)

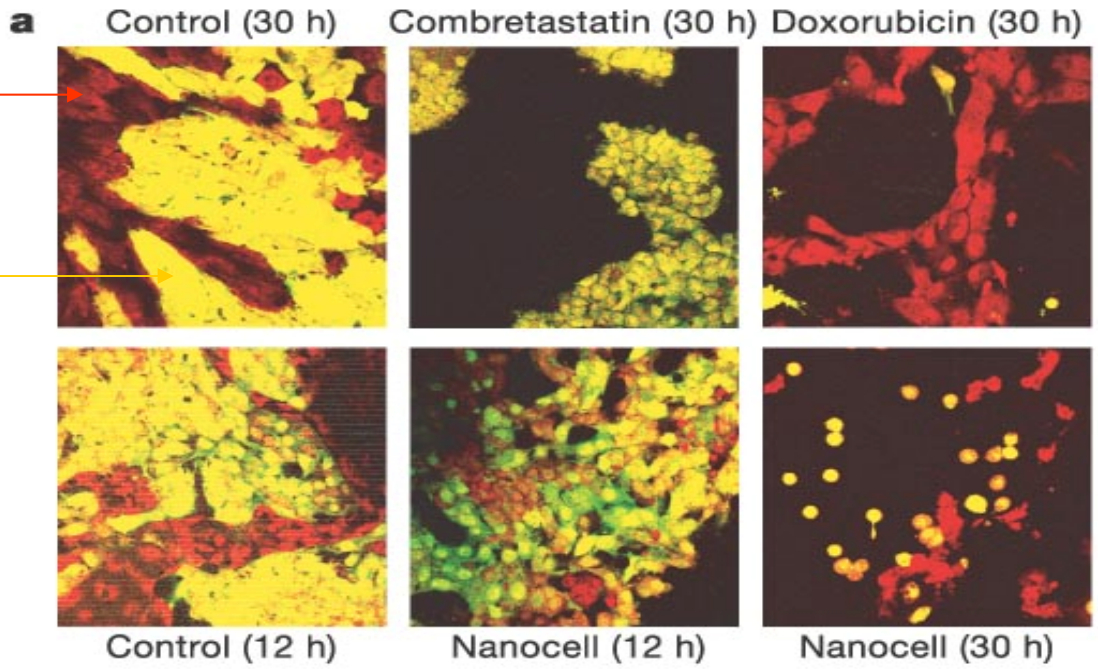


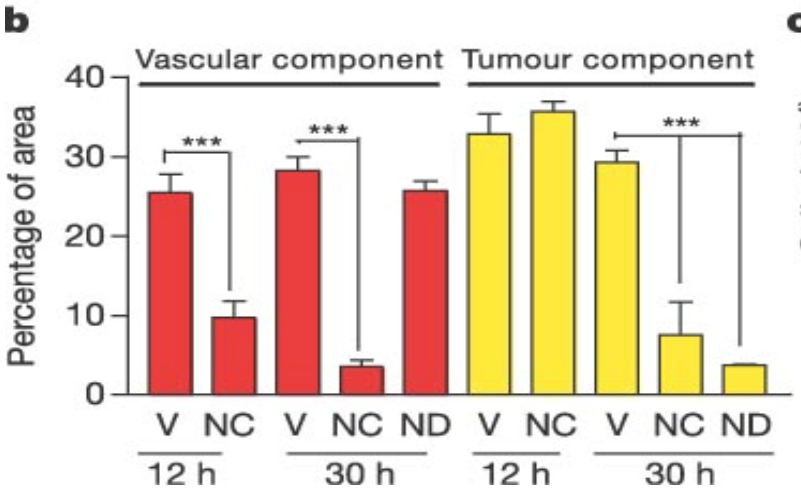
DOXORUBICINE-COMBRESTATINE NANOCELLS: EFFECT ON 3D GFP-MELANOMA-ENDOTHELIAL CELLS COCULTURE

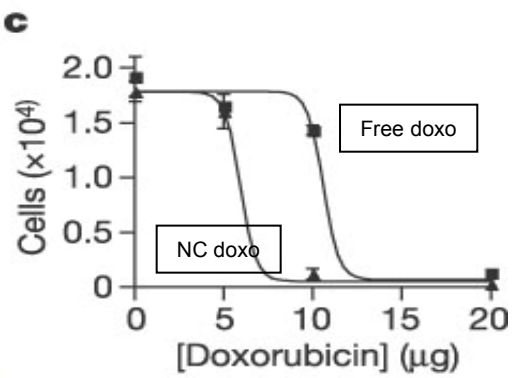
Sengupta S. et al, Nature, 568-572 (2005)

Endothelium (red)

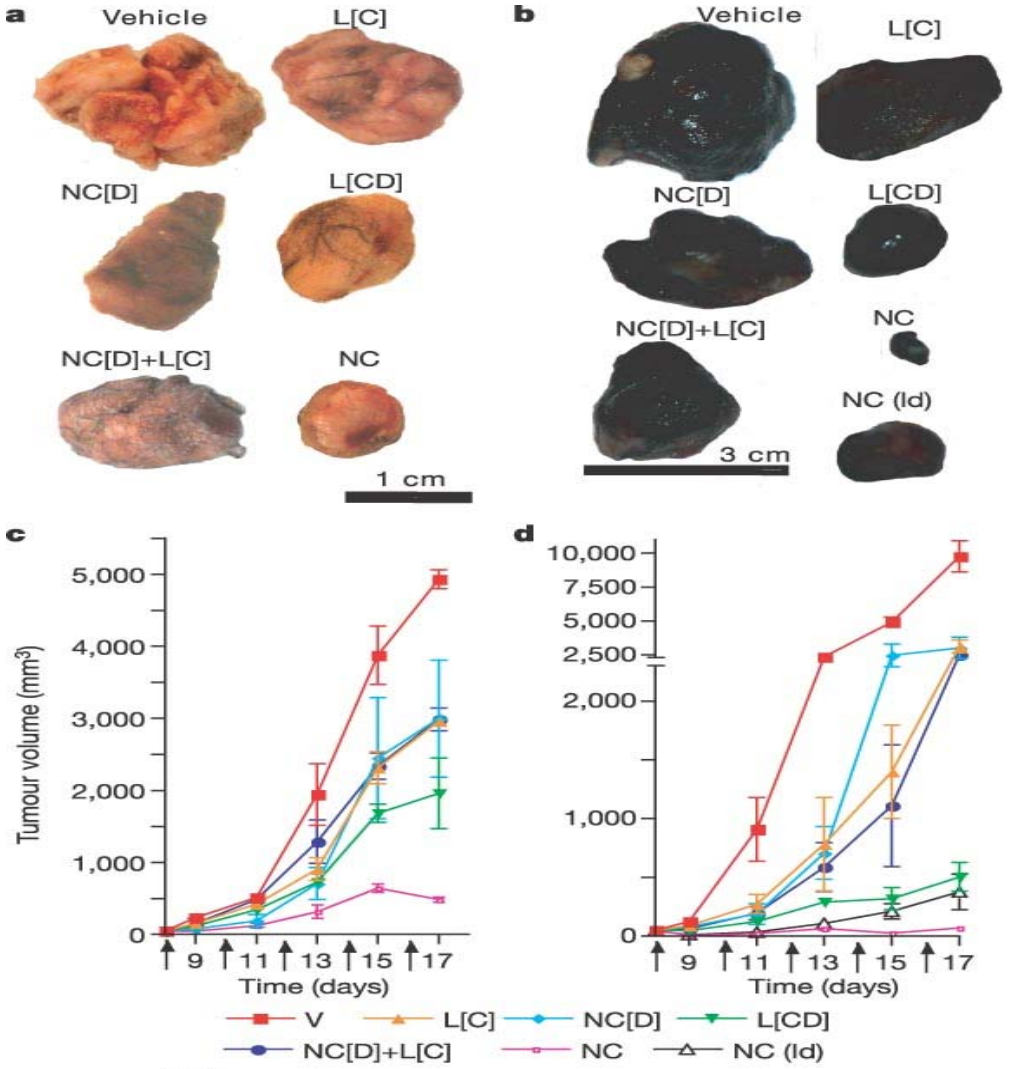
GFP melanoma (yellow)







V= Controls
NC= nanocells
ND= doxo nanopart



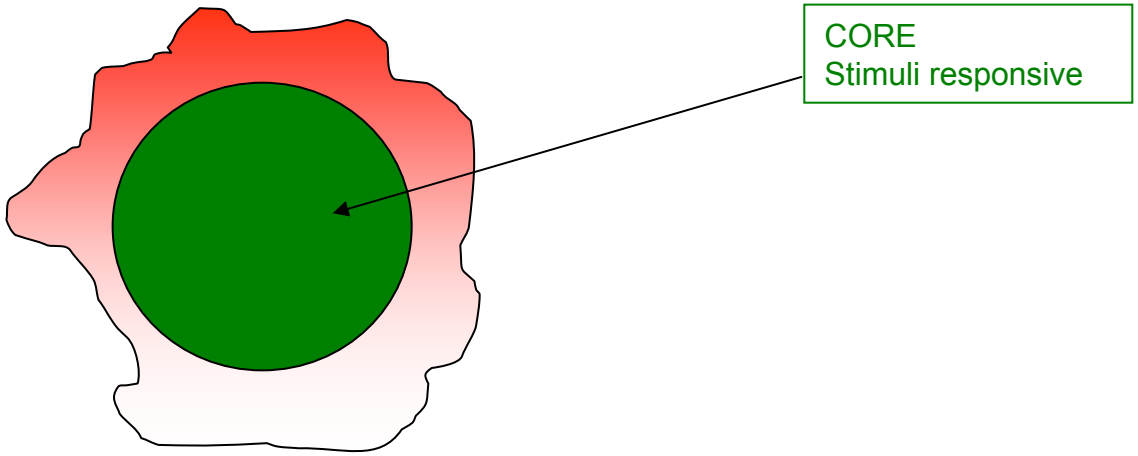
EFFECT OF NANOCELL THERAPY ON LEWIS LUNG CARCINOMA (left) AND B16/F10 MELANOMA (right)

Sengupta S. et al, Nature, 568-572 (2005)

V= untreated
NC= Nanocell loaded Doxo and Combestatine
NC[D]= Nanocell containing only Doxorubicine
L[C]= Combastatine encapsulated liposomes
L[CD]= Combastatine + doxorubicin liposomes

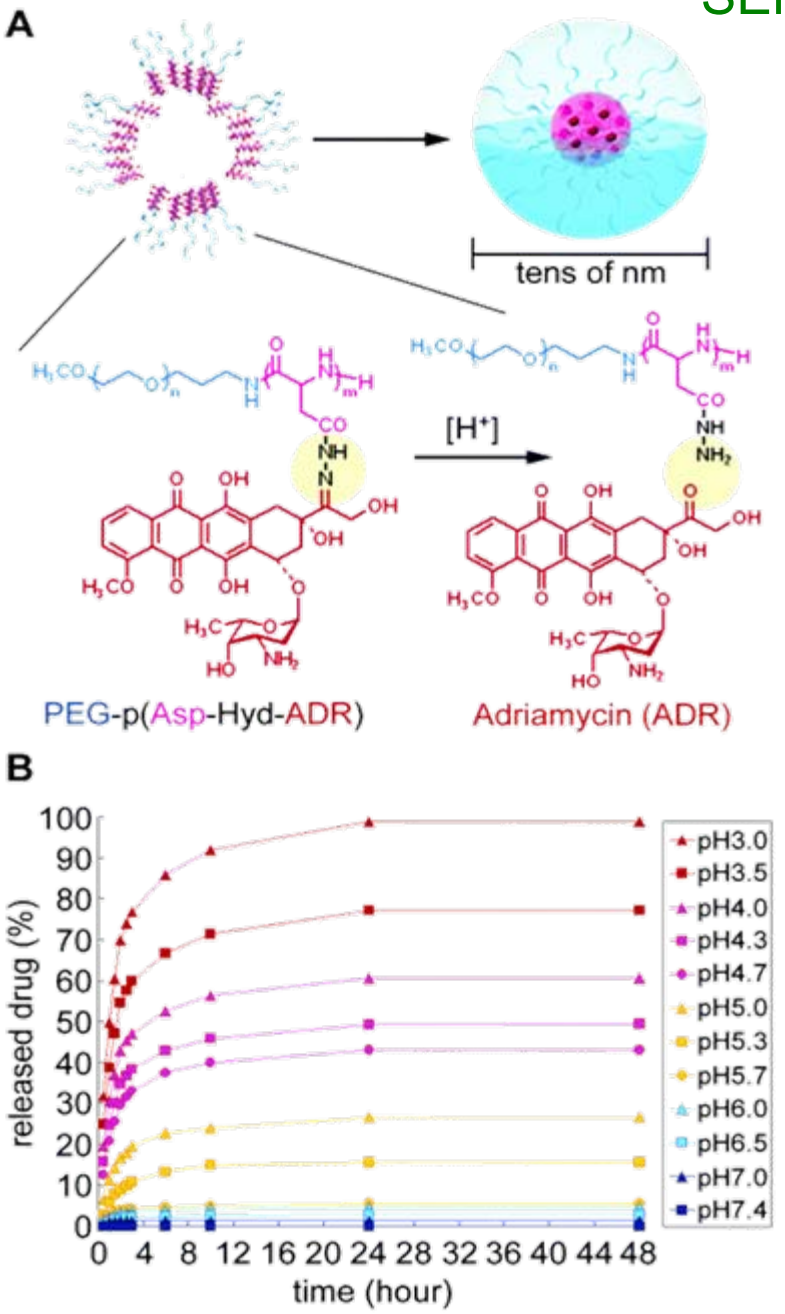
THE CORE

pH responsive



PREPARATION OF TUMOR INFILTRATING MICELLES WITH pH SENSITIVITY

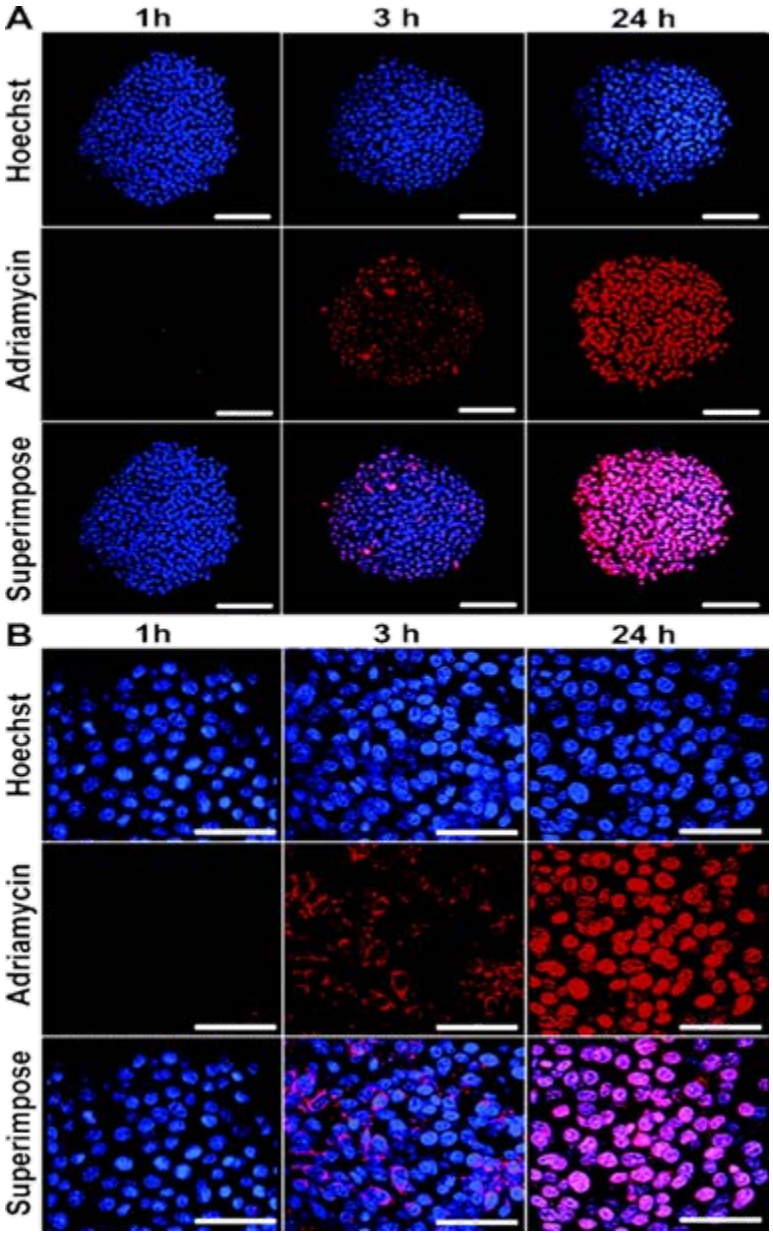
Bae Y et al.,Bioconjugate Chemistry 16, 122-130, 2005



(A) Micelles of 10 nm from self-assembling amphiphilic block copolymers, PEG-poly(Aspartate-Hydrazone-ADR) in which the anticancer drug, adriamycin (ADR) is conjugated through acid-sensitive hydrazone linkers. (B) The micelles released the loaded drugs under acidic conditions below pH 6.0 corresponding to intracellular space, but remained stable under the conditions of vascular and extracellular space (pH 7.4−7.0).

TUMOR PERMEABILITY AND INTRACELLULAR DRUG RELEASE

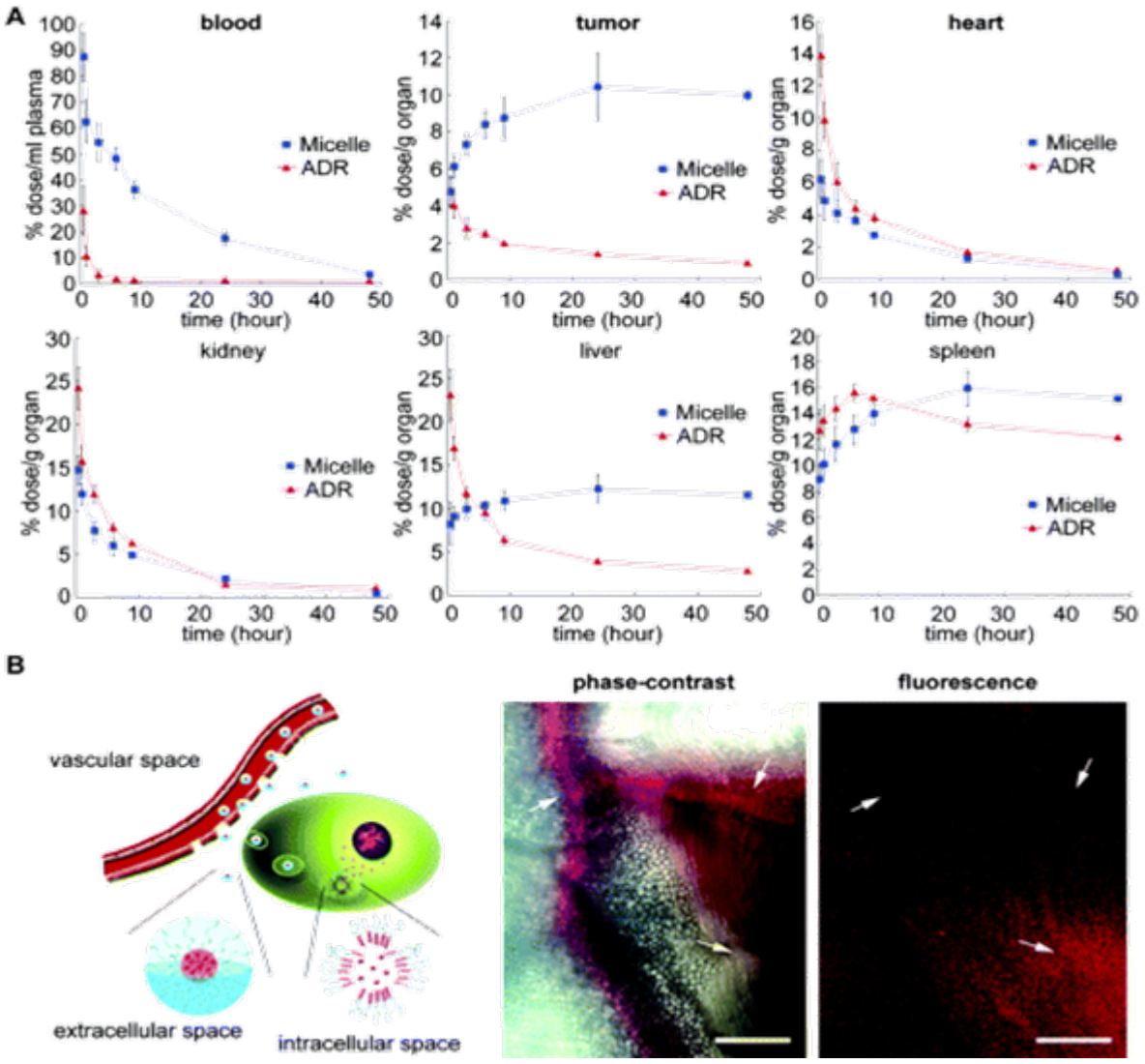
Bae Y et al.,Bioconjugate Chemistry 16, 122-130, 2005



- (A) Confocal Laser Scanning Microscope observations showed the time-dependent change in the fluorescence intensities of ADR in the micelle system in multicellular tumor spheroid (MCTS). The images showed that the micelles can access the inside of the MCTS and release the loaded drugs (bar = 100 μm).
- (B) The intracellular drug release and localization of the micelles in each cell of MCTS were observed in detail using a high-magnification 63× objective. The images clearly demonstrated that the micelles internalized into the cells and released drugs, and that the released drugs eventually accumulated in the cell nuclei (bar = 50 μm).

IN VIVO TUMOR SPECIFIC ACCUMULATION AND DRUG RELEASE

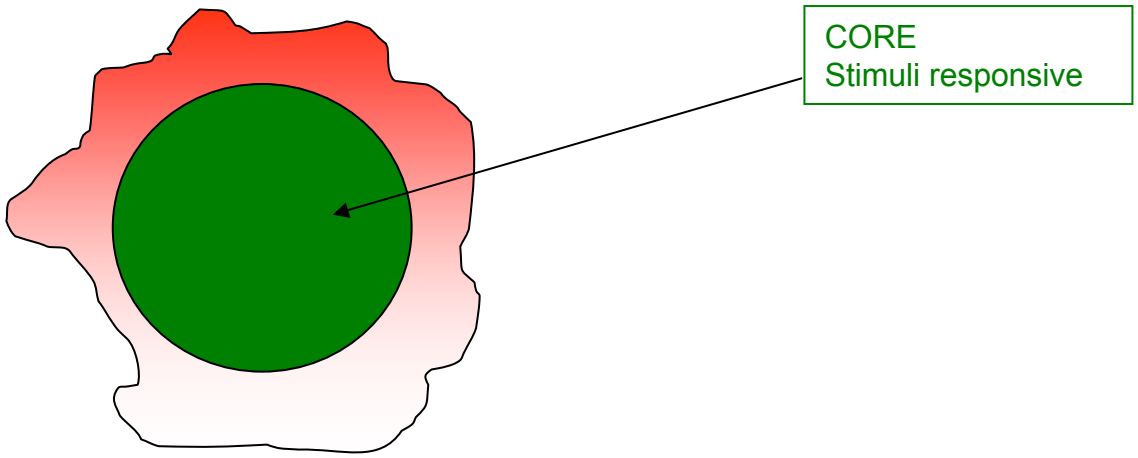
Bae Y et al.,Bioconjugate Chemistry 16, 122-130, 2005

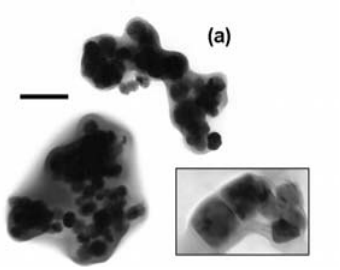


- (A) Biodistribution study revealed the prolonged circulation in the blood and tumor-specific accumulation of the micelles. (B) Fluorescence microscopic observations of the solid tumor and its peripheral regions at 24 h after micelle injection
- → MICELLES LEAK FROM THE TUMOR VASCULATURE (ARROW LEFT) AND CAN INFILTRATE THE CORE OF THE TUMOR TISSUE

THE CORE

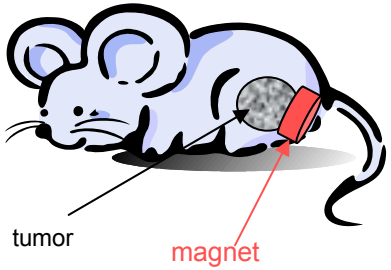
Magnetically responsive

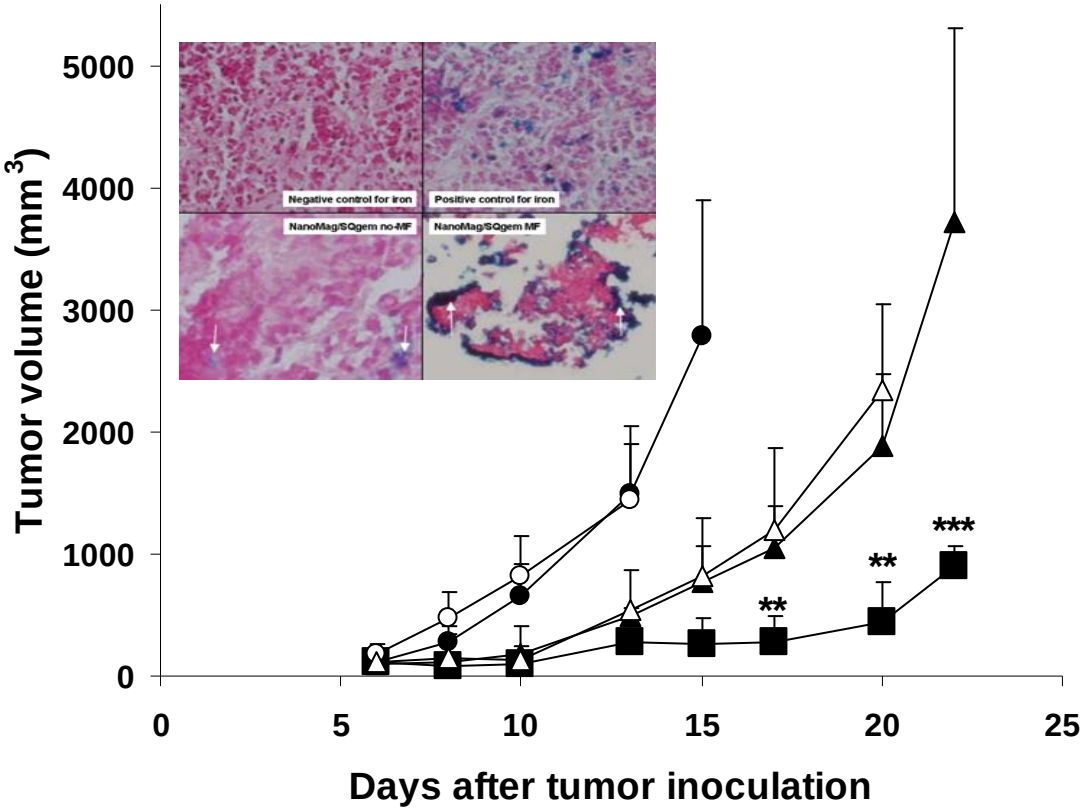


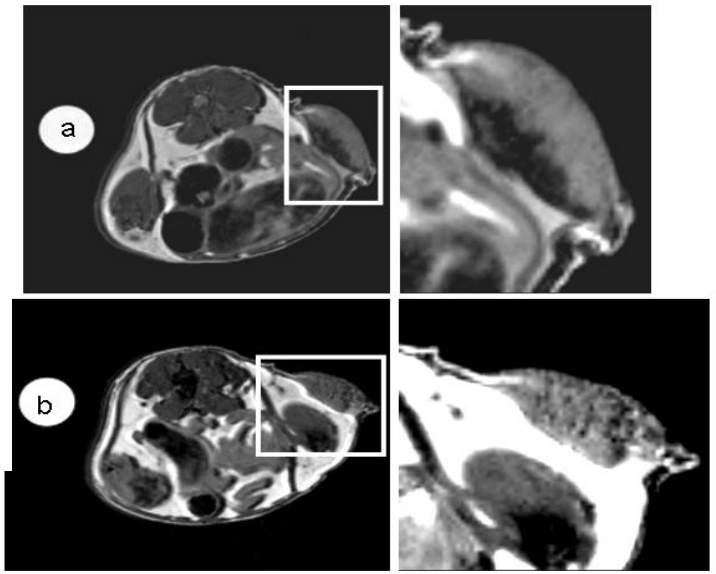


MAGNETICALLY GUIDED NANOPARTICLES (L1210 Leukemia sc/iv)

Arias et al., Submitted, 2009



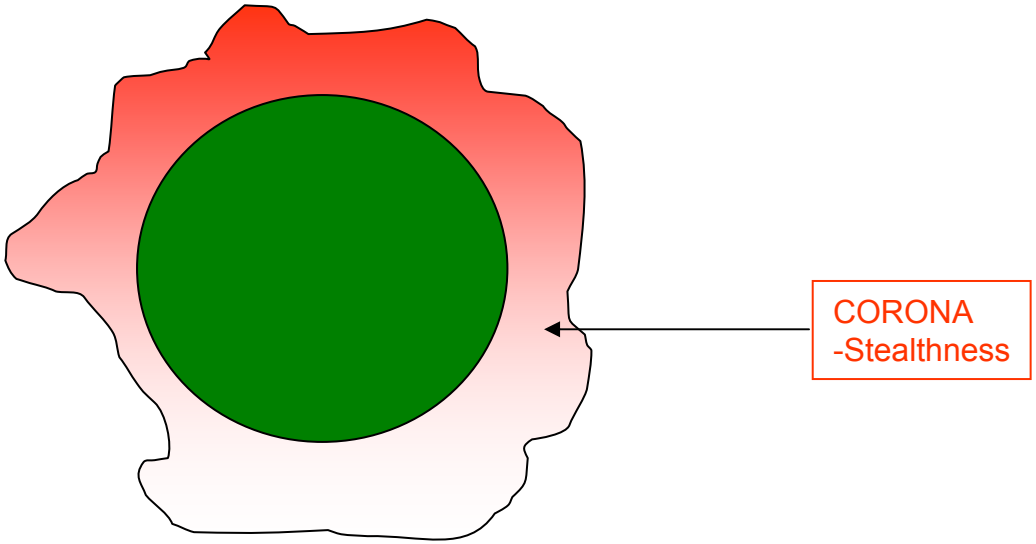




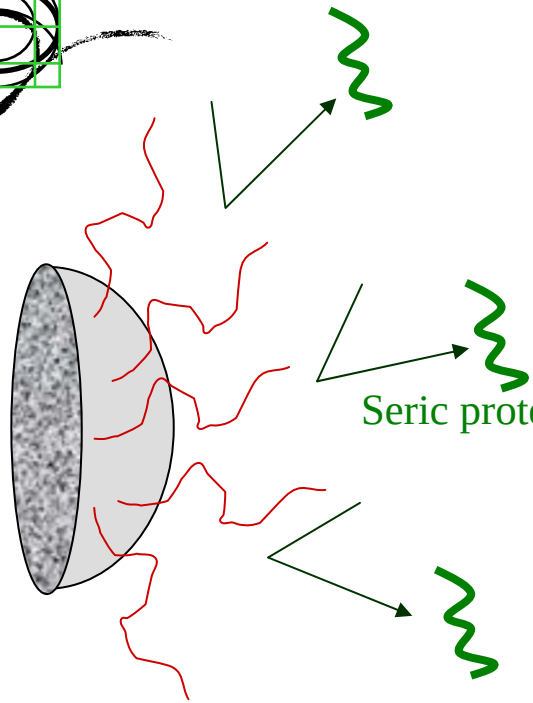
Untreated (●)
gemcitabine (○)
SQgem nanoassemblies (▲)
Mag-SQgem composite nanoassemblies (without magnet) (△)
Mag-SQgem composite nanoassemblies (■)

THE CORONA

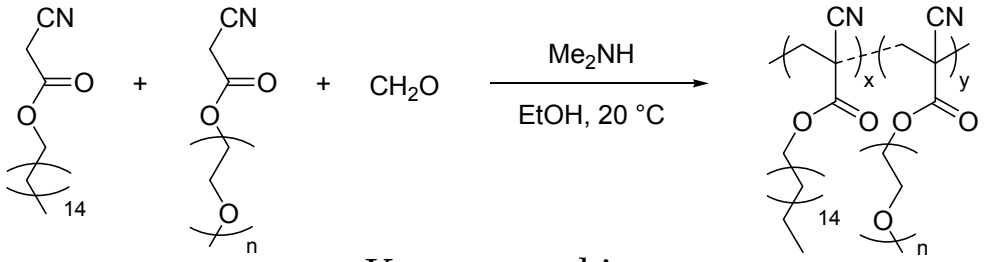
Stealthness



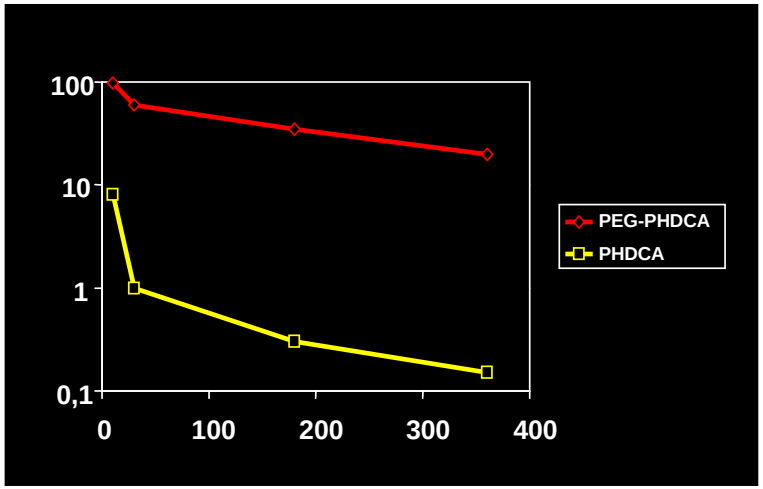
SURFACE FUNCTIONNALIZATION

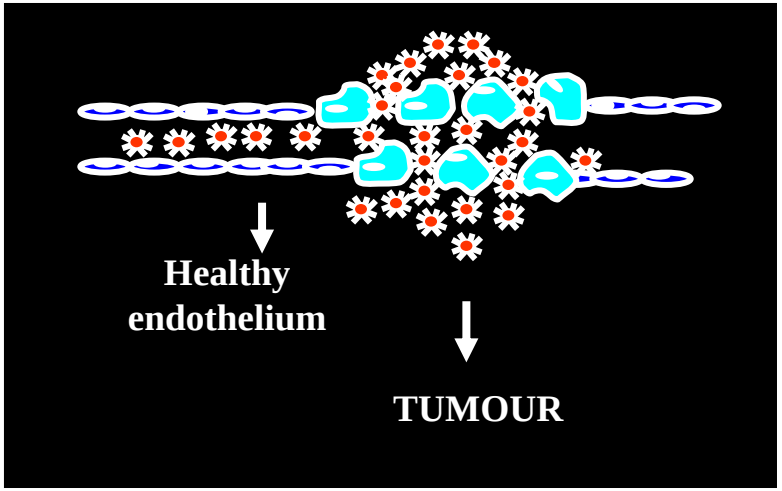


Perrachia et al. Macromolecules, **30**, 846-851 (1997)



Knoevenagel inverse



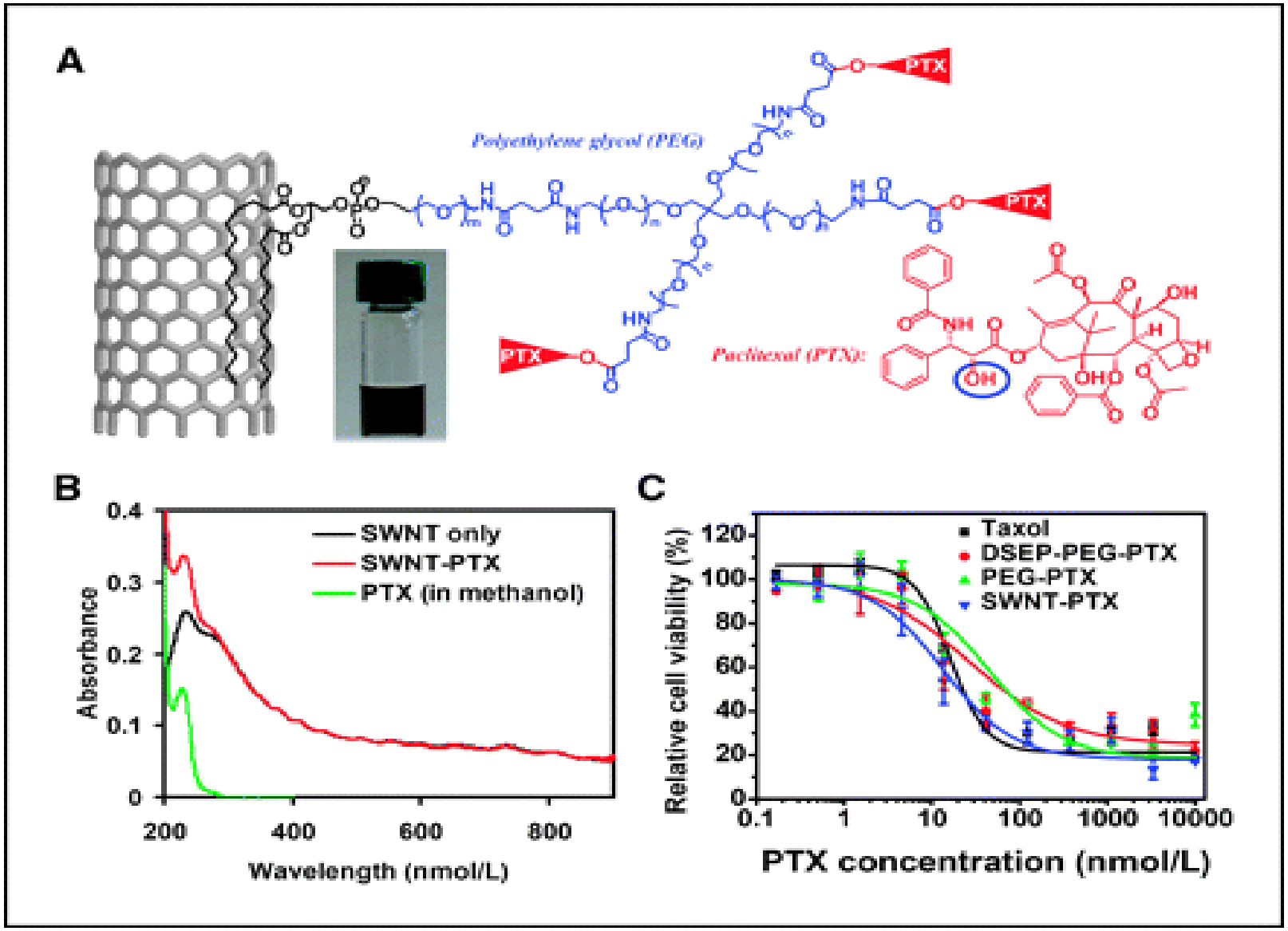


Perrachia et al., J. Control. Rel, **60**, 121-128 (1999)
Perrachia et al., Biomaterials, **20**, 1269-1275 (1999)

« EPR » effect

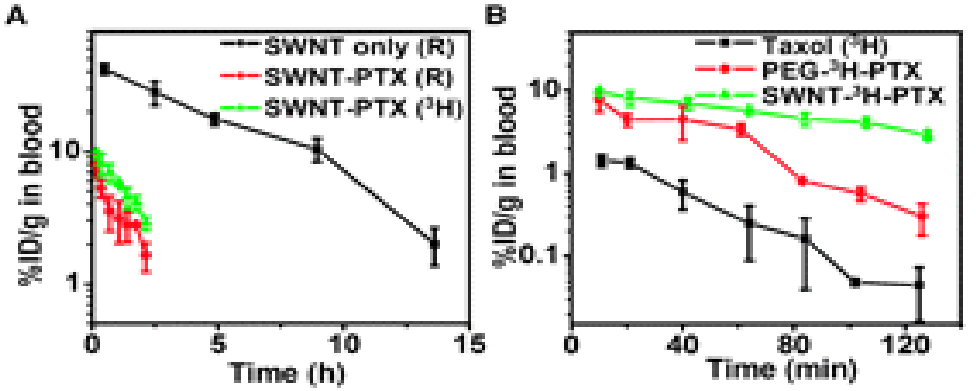
DESIGN OF PACLITAXEL PEGylated CARBON NANOTUBES

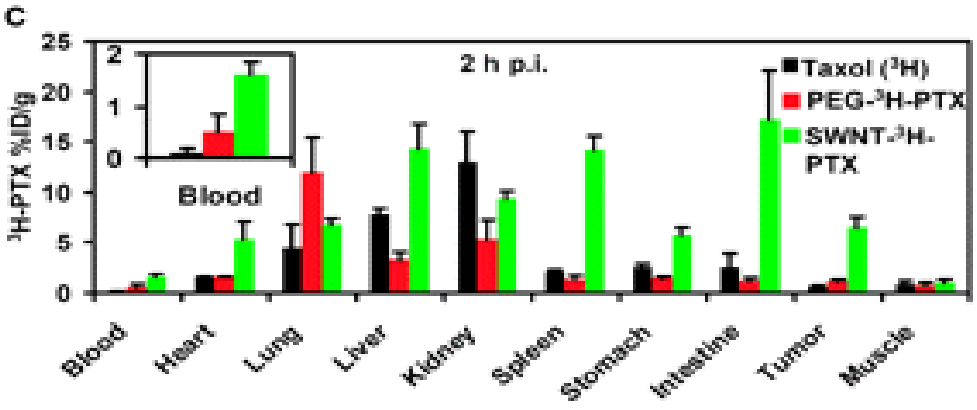
Liu Z et al., Cancer Research, 16, 6652-6660, 2008

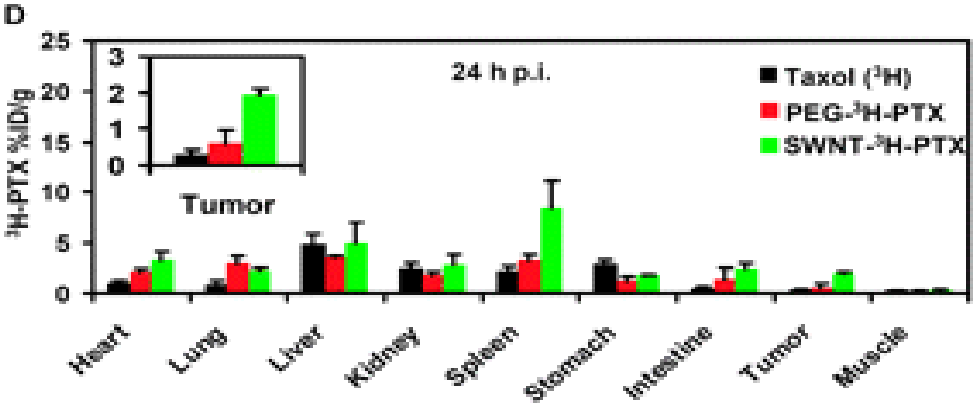


PACLITAXEL PEGylated CARBON NANOTUBES BIODISTRIBUTION

Liu Z et al., Cancer Research, 16, 6652-6660, 2008





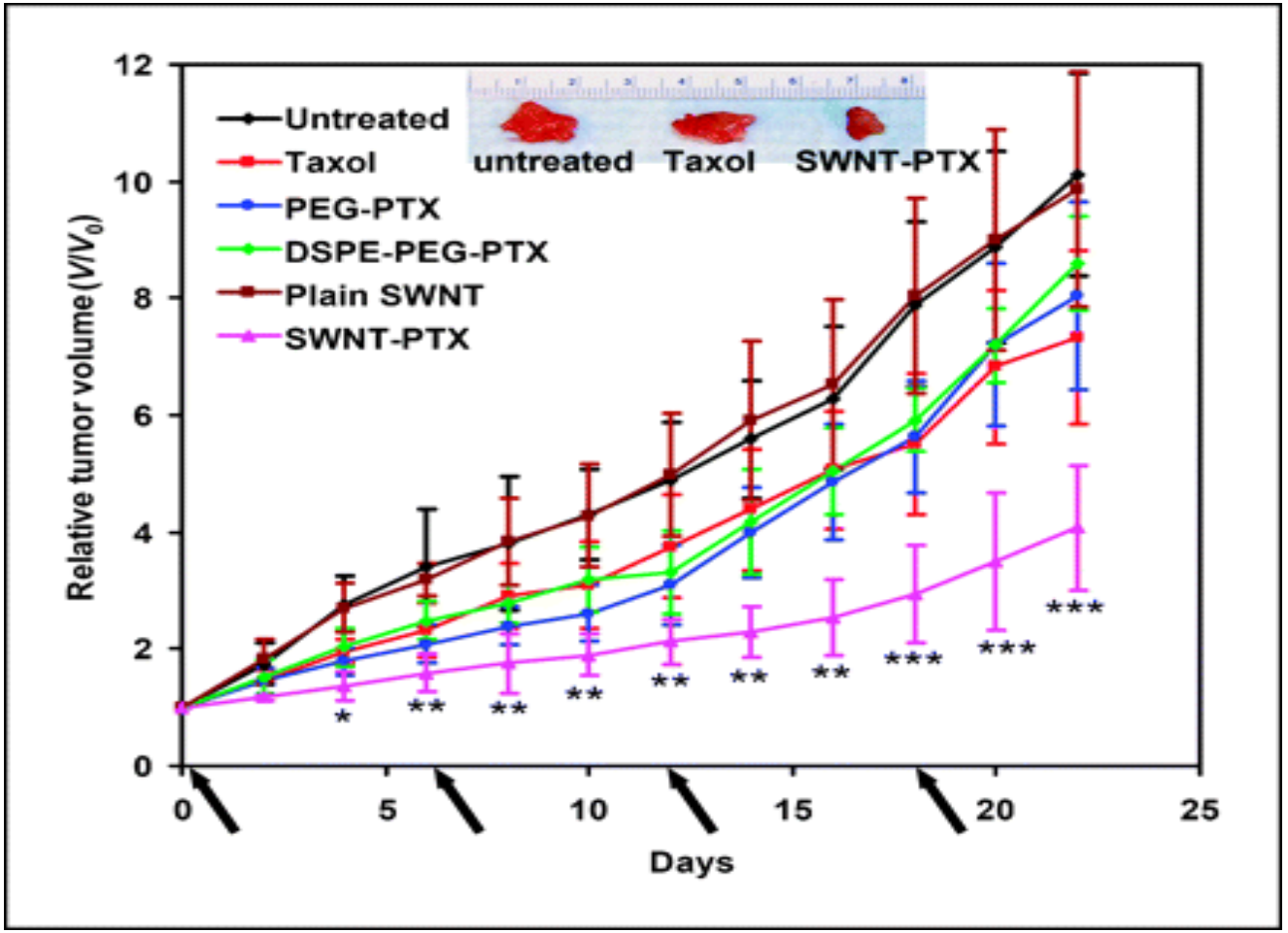


- *B* blood circulation data of 3H-labeled Taxol, PEG-PTX, and SWNT-PTX →SWNT-PTX exhibited significantly prolonged circulation half-life than Taxol and PEG-PTX

- *C* and *D,* 3H-PTX biodistribution in 4T1 tumor-bearing mice injected intravenously with 3H-labeled Taxol, PEG-PTX, and SWNT-PTX at (*C*) 2 h after intravenous injection (*p.i.*) and (*D*) 24 h after iv injection.

ANTICANCER ACTIVITY OF PACLITAXEL PEGylated CARBON NANOTUBES

Liu Z et al., Cancer Research, 16, 6652-6660, 2008



- Nanotube PTX delivery suppresses tumor growth of 4T1 breast cancer mice model. Dose: 5 mg/kg

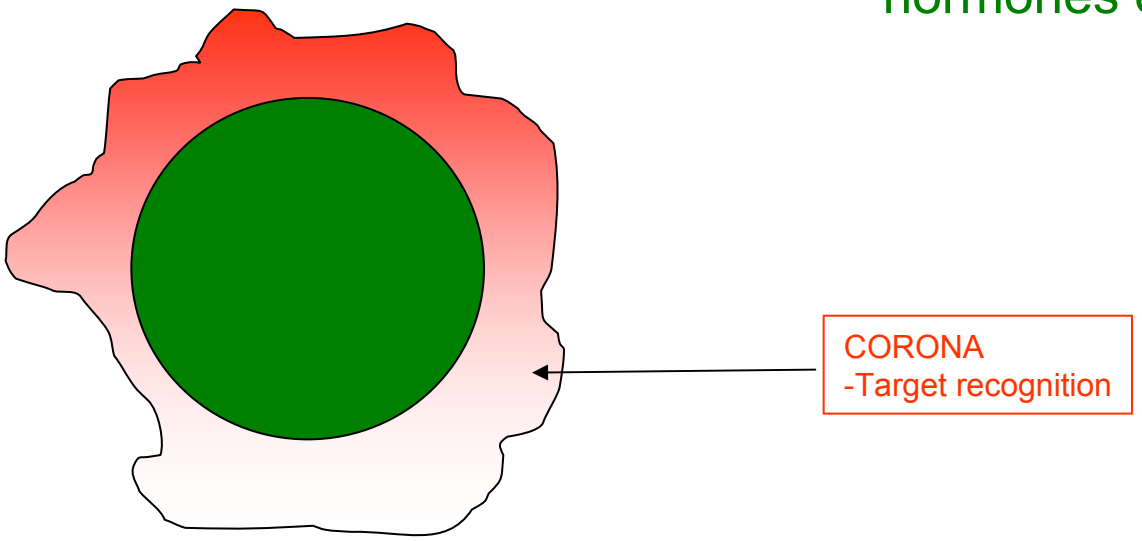
THE CORONA

Target recognition

-antibodies

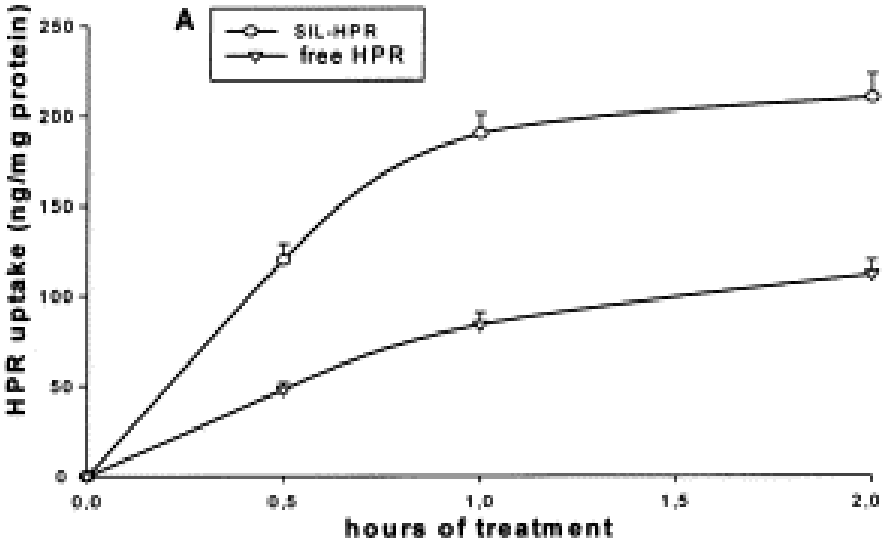
-peptides

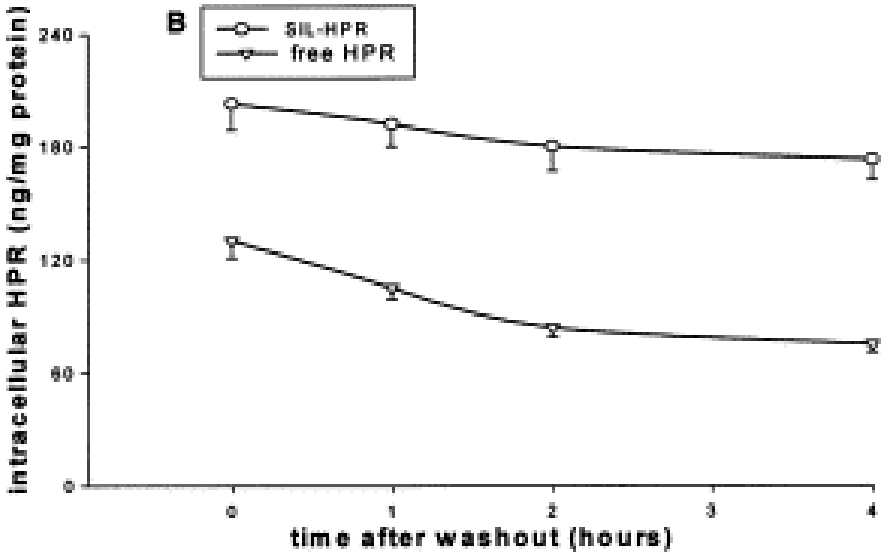
-small molecules (vitamines, hormones etc.)



NEUROBLASTOMA CELL UPTAKE AND RELEASE OF FENRETINIDE RETINOID LOADED ONTO ANTI-GD2 IMMUNOLIPOSOMES

Raffaghello et al.; Cancer Letters, 197, 151-155 (2003)

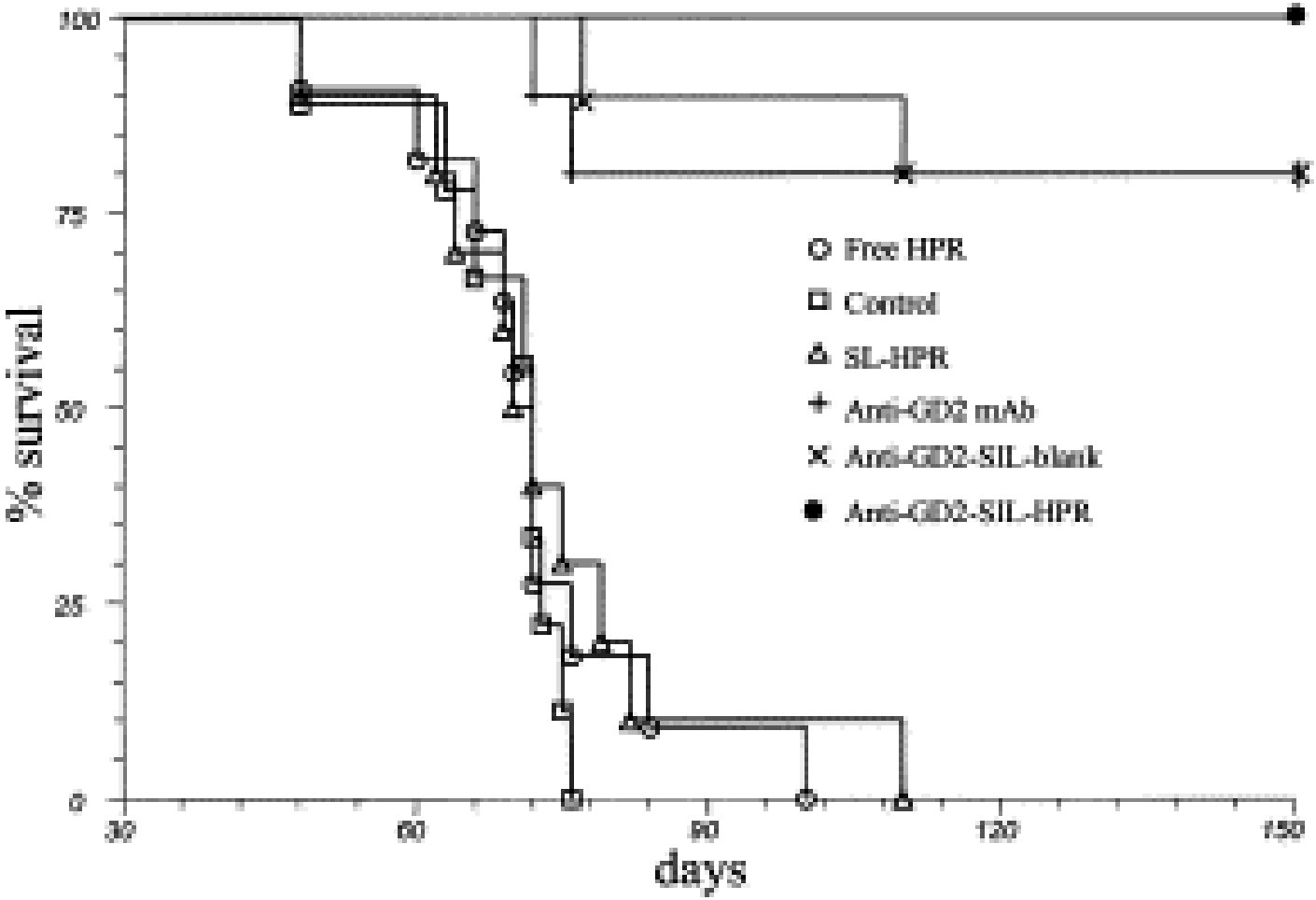




GD2= disialoganglioside
expressed in neuroectoderma tumours

ANTICANCER EFFECT OF FENRETINIDE RETINOID LOADED ONTO ANTI-GD2 IMMUNOLIPOSOMES

Raffaghello et al.; Cancer Letters, 197, 151-155 (2003)

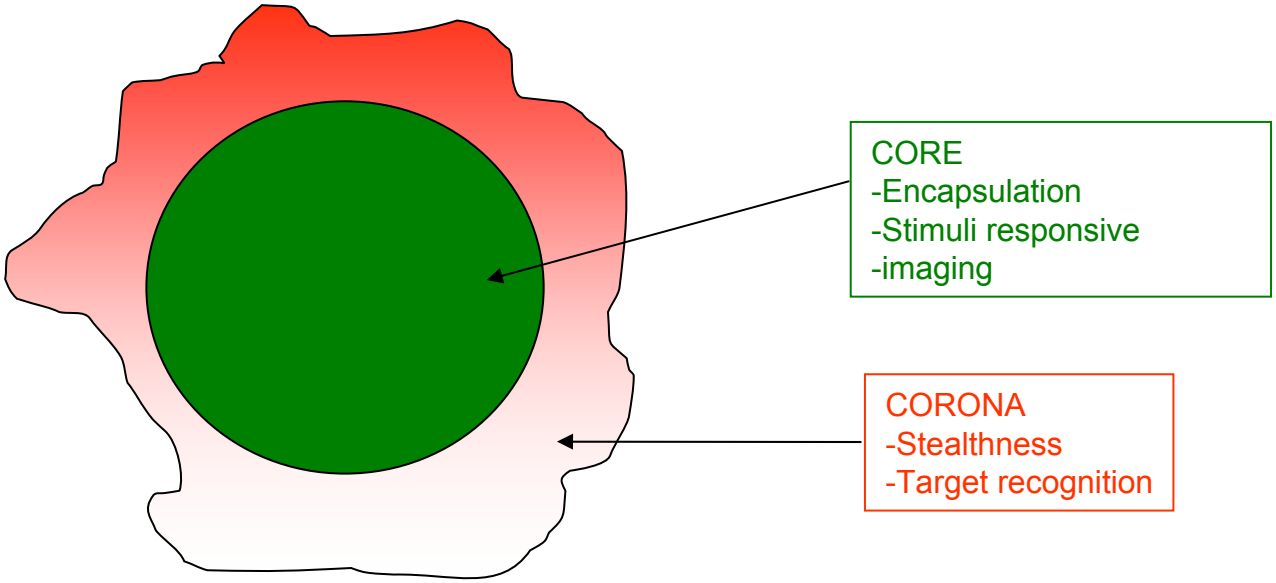


HUMAN EXPERIMENTAL METASTATIC NEUROBLASTOMA MODEL

CORE + CORONA

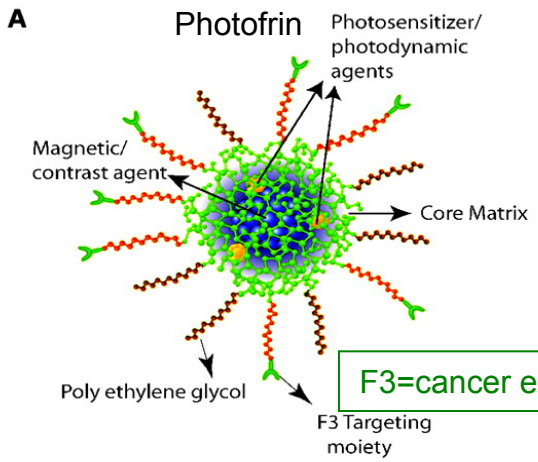
Multifunctional nanocarriers

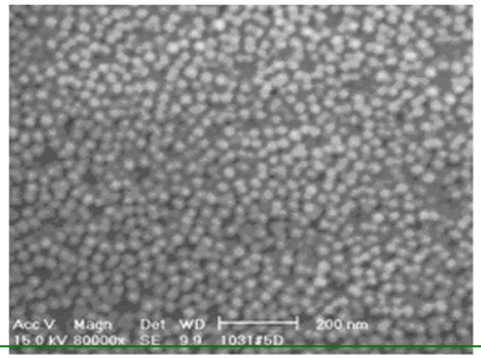
« Theranostics »



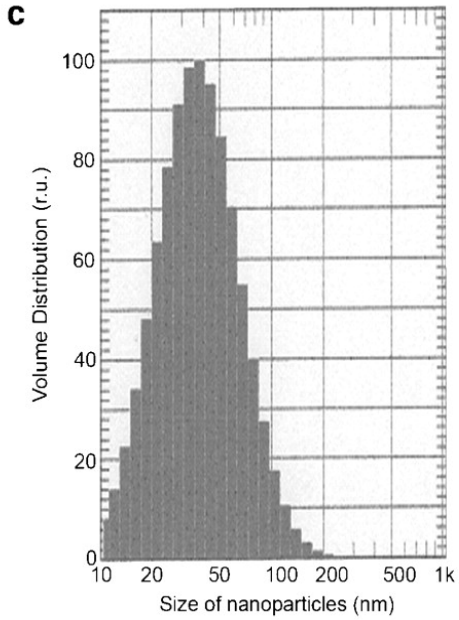
MULTIFUNCTIONAL IRON OXIDE NANOPARTICLES FOR BRAIN TUMOR TREATMENT

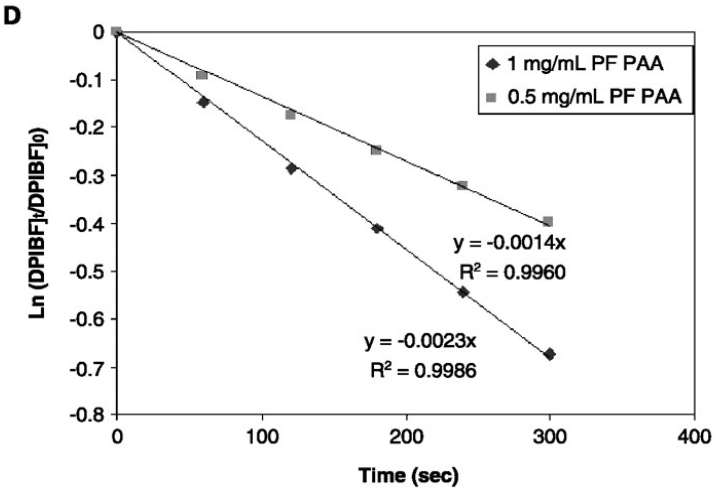
Reddy R. et al.,Clin Cancer Research, 22, 6677-6686, 2006





F3=cancer endothelial cell recognition peptide of 31aa





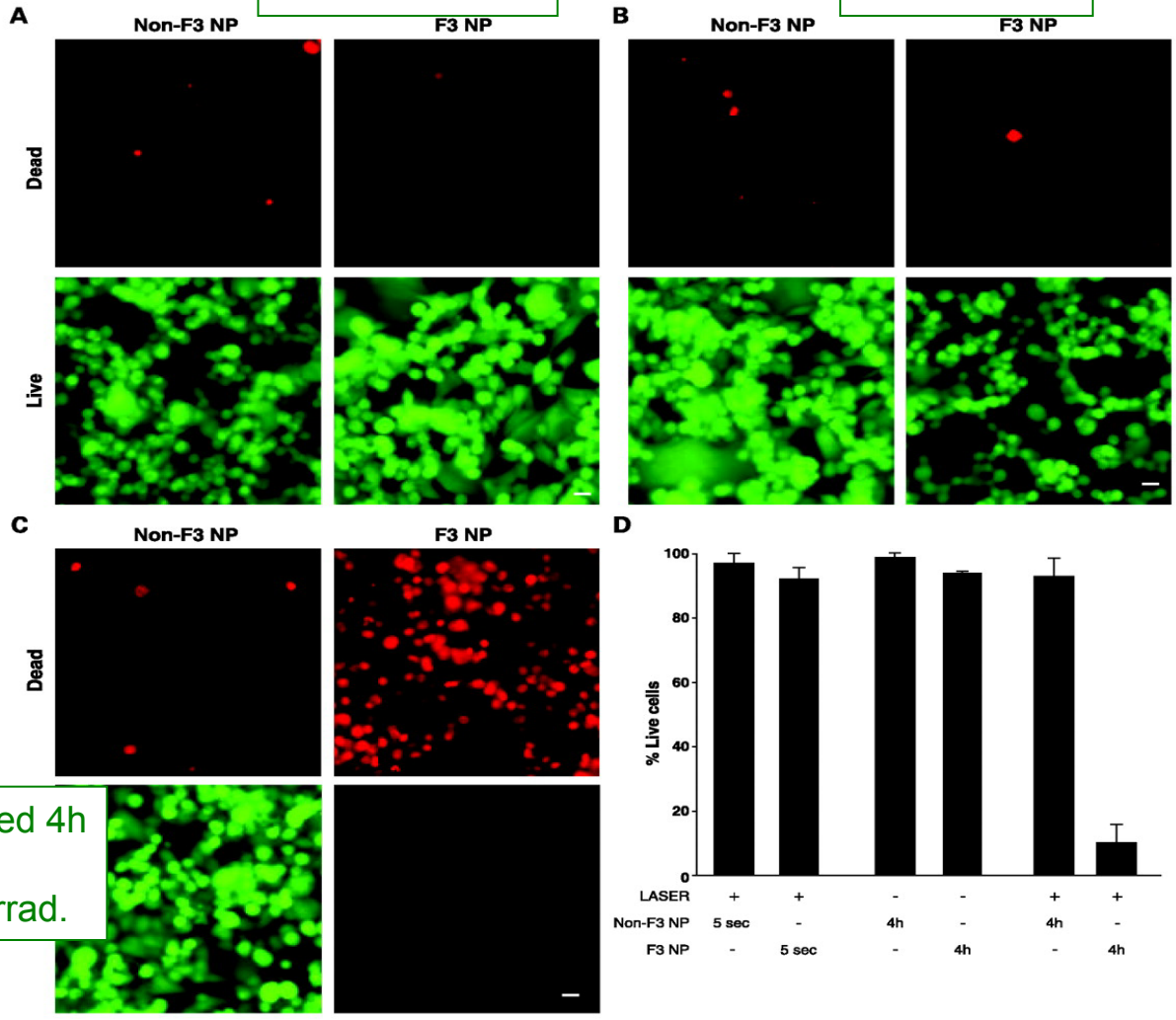
Production of singlet $^1O_2$ by laser irradiation

CYTOTOXICITY OF F3 TAGGED PHOTOFRIN IRON OXIDE NANOPARTICLES

Reddy R. et al.,Clin Cancer Research, 22, 6677-6686, 2006

Incubated 5 sec

Incubated 4h



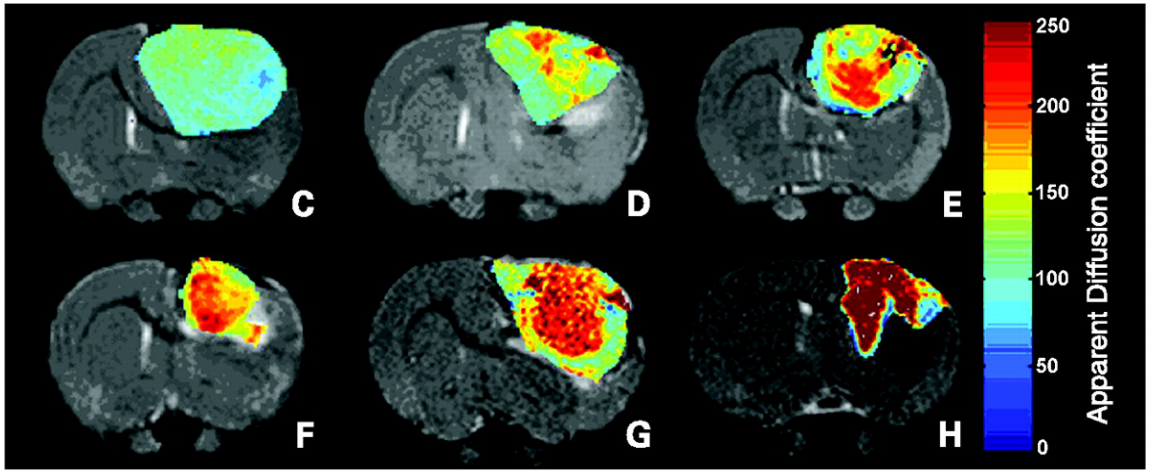
Incubated 4h
+
Laser irrad.

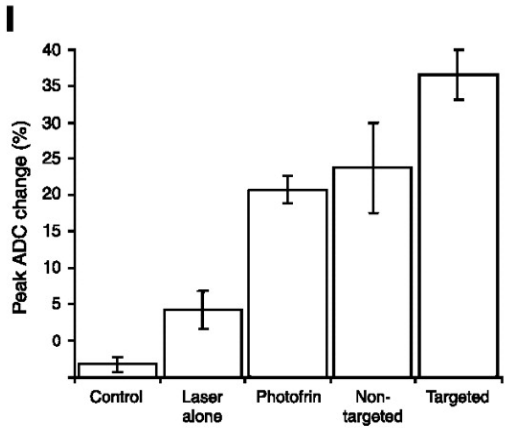
Green are calcein labelled living cells

Red are propidium iodide labelled dead cells

F3 TAGGED PHOTOFRIN IRON OXIDE NANOPARTICLES FOR 9L GLIOMA TARGETING

Reddy R. et al.,Clin Cancer Research, 22, 6677-6686, 2006





APPARENT DIFFUSION COEFFICIENT OF TUMOR IS CORRELATED WITH INCEASED SURVIVAL

(*C*) control i.c. 9L tumor and tumors treated with:

(*D*) laser light only

(*E*) i.v. administration of Photofrin plus laser light

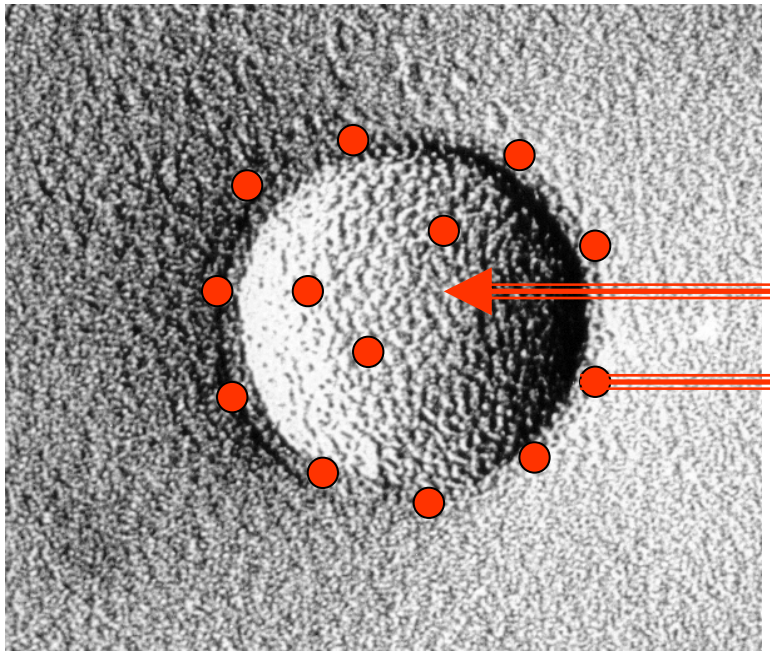
(*F*) nontargeted nanoparticles containing Photofrin plus laser light

(*G*) targeted nanoparticles containing Photofrin plus laser light.

(*H*) is from the same tumor shown in (*G*), which was treated with the F3-targeted nanoparticle but at day 40 after treatment. The color diffusion maps overlaid on top of T2-weighted images represent the apparent diffusion coefficient (*ADC*) distribution in each tumor slice shown. *I,* columns, mean peak percentage change in tumor apparent diffusion coefficient values for each of the experimental groups; bars, SE.

BIOMIMETIC APPROACHES

De grands progrès ont été faits mais d'importants verrous technologiques demeurent...



Taux de charge

« Burst release » non contrôlé par La cible pharmacologique

La quantité de médicament administré est insuffisant

Ou

Il est nécessaire d'administrer de grandes quantités de matériel vecteur

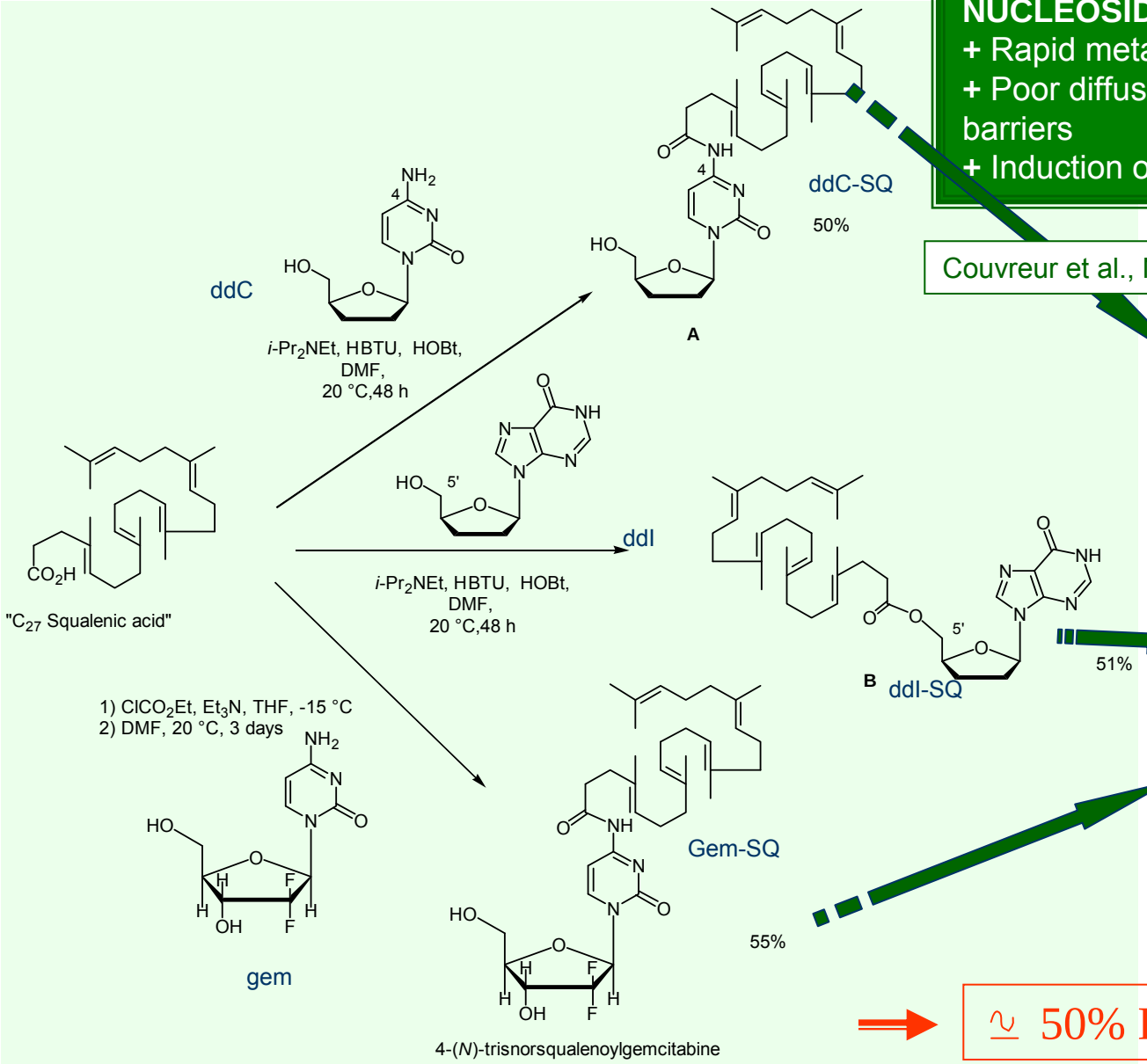
↓

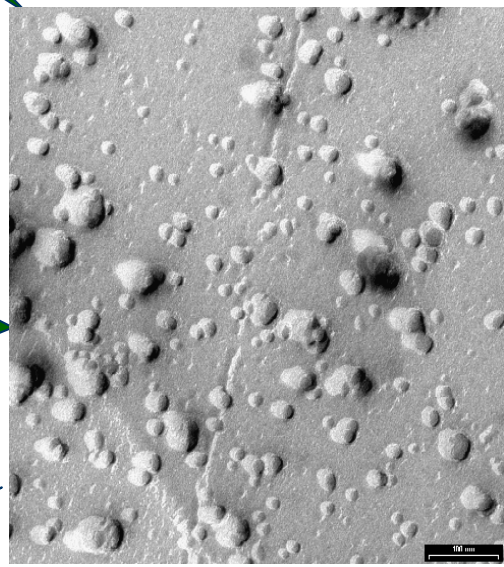
INEFFICACITE OU TOXICITE

THE CONCEPT OF "*SQUALENIZATION*"

NUCLEOSIDES ANALOGUES
+ Rapid metabolism
+ Poor diffusion through biological barriers
+ Induction of resistances

Couvreur et al., Nano Letters, **6**, 2544-2548 (2006)

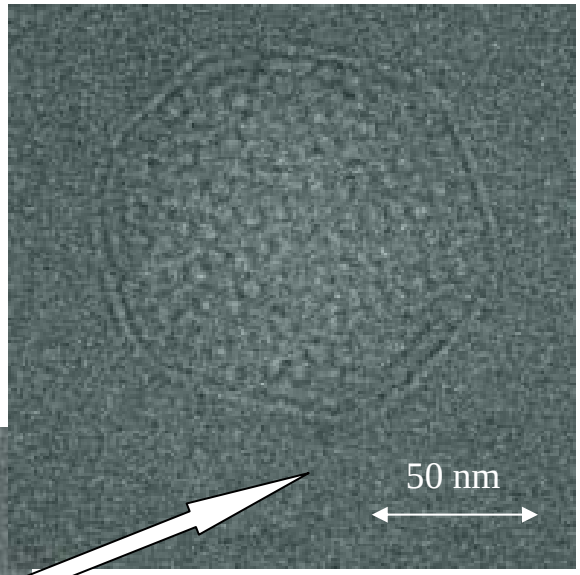


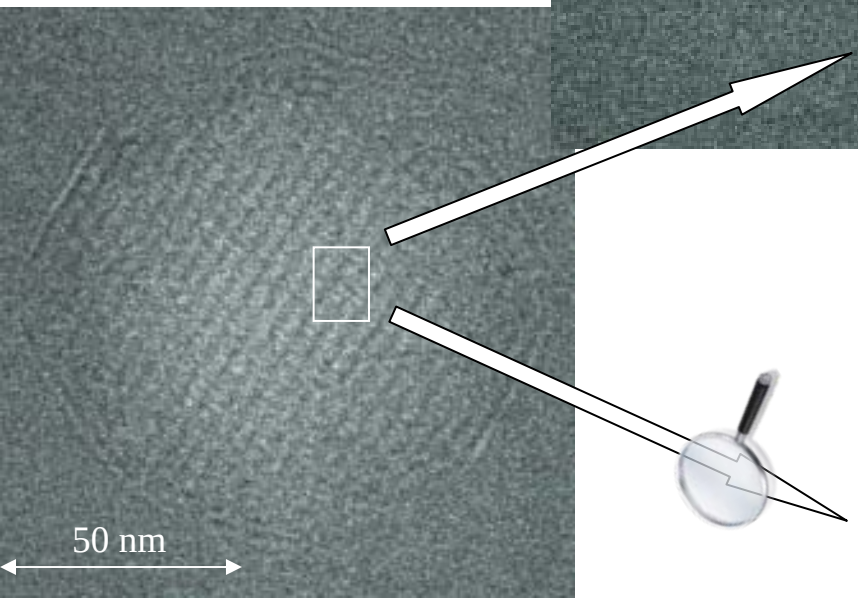


Nanoparticles
100-150 nm

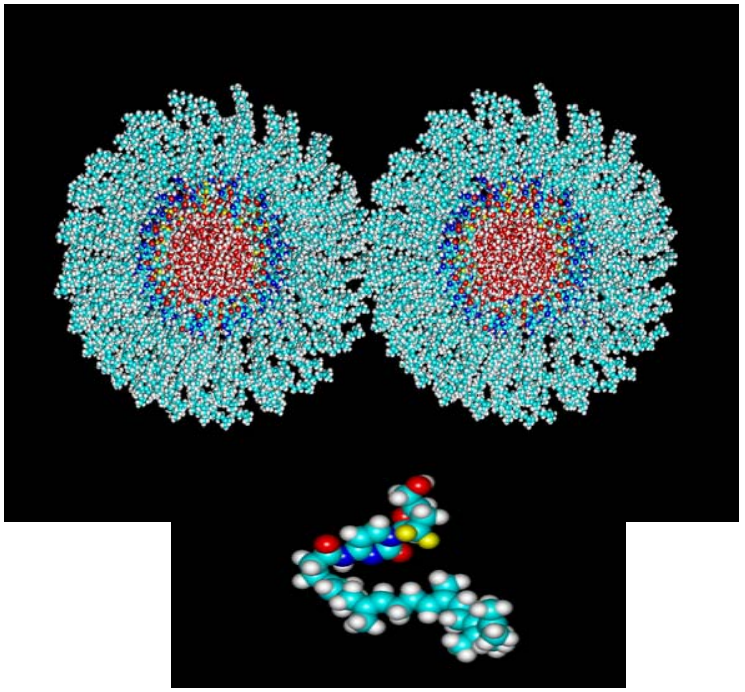
≃ 50% Loading !

Also AZT, ARA-C, Thymidine…



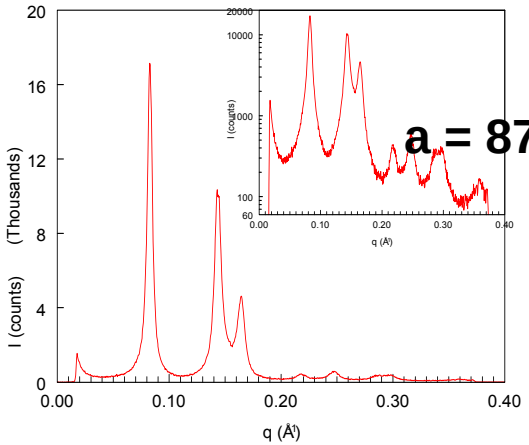


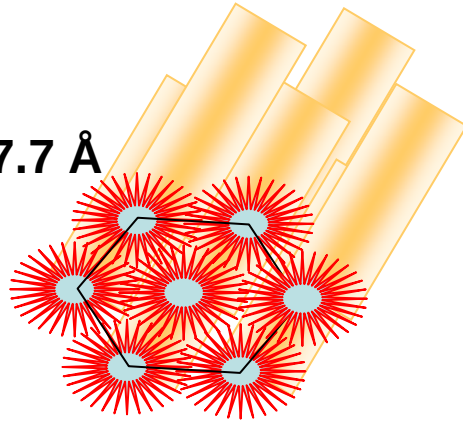
Cryo-TEM



Molecular Modelling

STRUCTURE OF SQUALENOYLATED GEMCITABINE





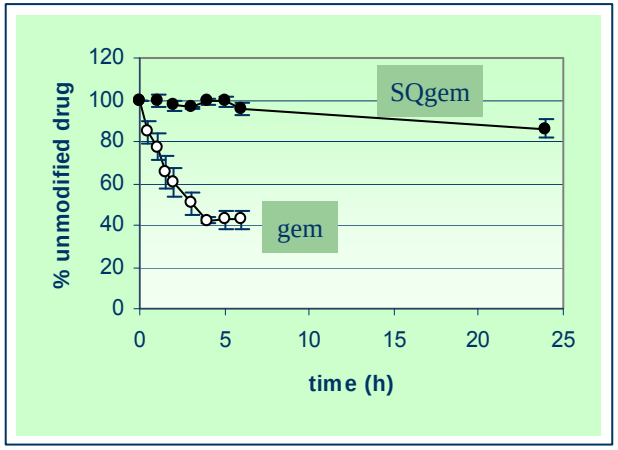
Structural Analysis by SAX

Couvreur et al., Small, **4**, 247-253 (2008)
Aoun et al., Adv Funct. Mater., in press (2008)

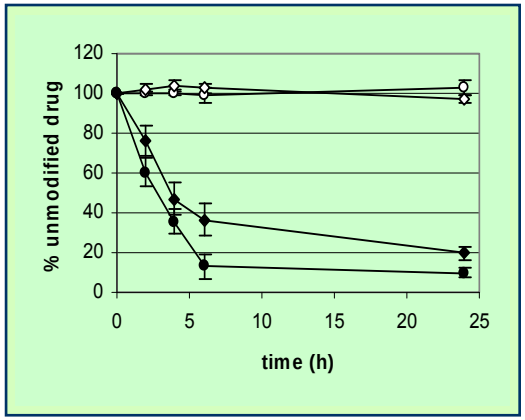
STABILITY IN PLASMA AND PHARMACOKINETICS OF GEMCITABINE-SQUALENE VERSUS GEMCITABINE FREE

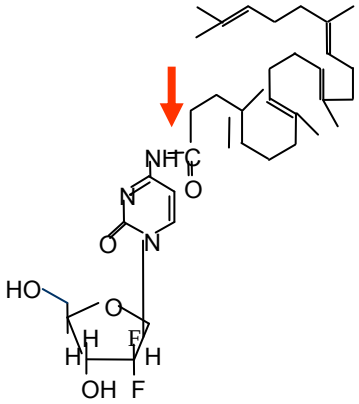
Harivardahan et al., Drug Metab and Disposit, **36**, 1570-1577 (2008)
H. Khoury et al., J Chromatography B,858, 71-78 (2007)

Stability in plasma (37°C)



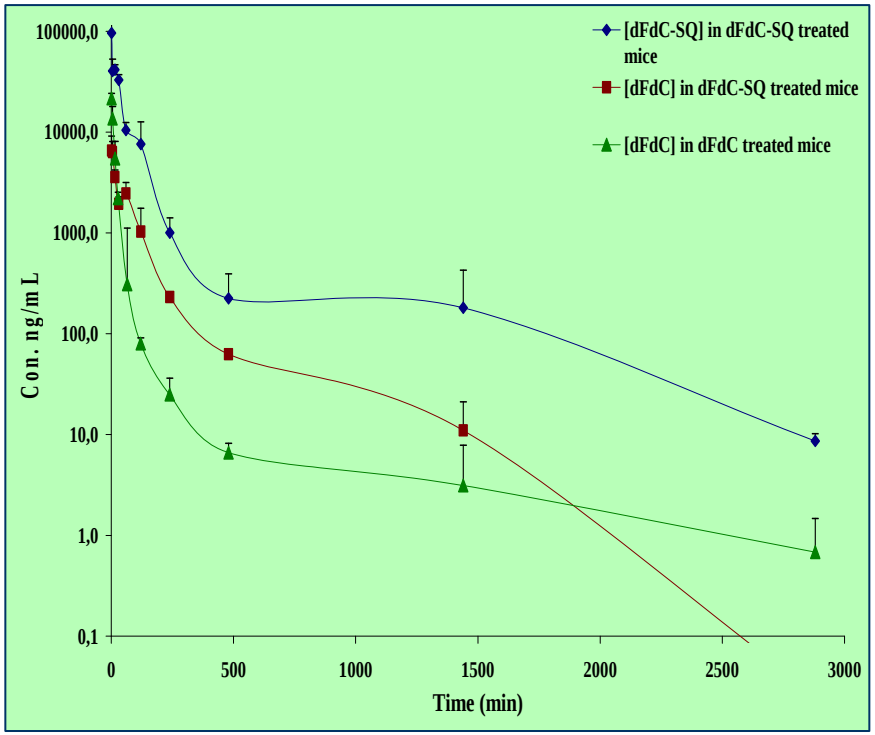
Release in the presence of cathepsins B et D (37°C)





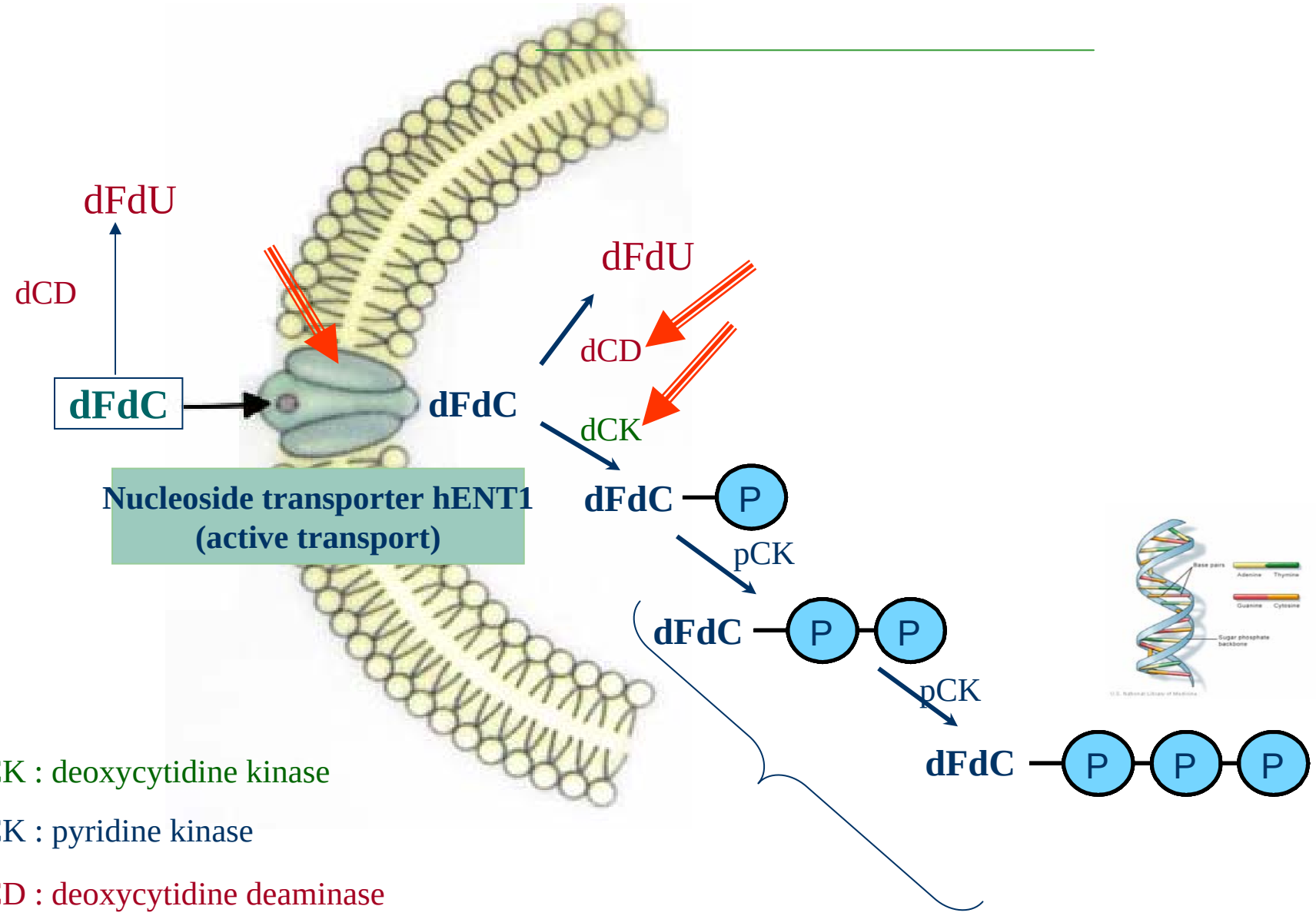
Squalenoylgemcitabine

Pharmacokinetics
(IV administration 15 mg/Kg)



AUC dFdC/dFdC-SQ = 0.1288

INTRACELLULAR ACTIVATION OF GEMCITABINE AND RESISTANCE



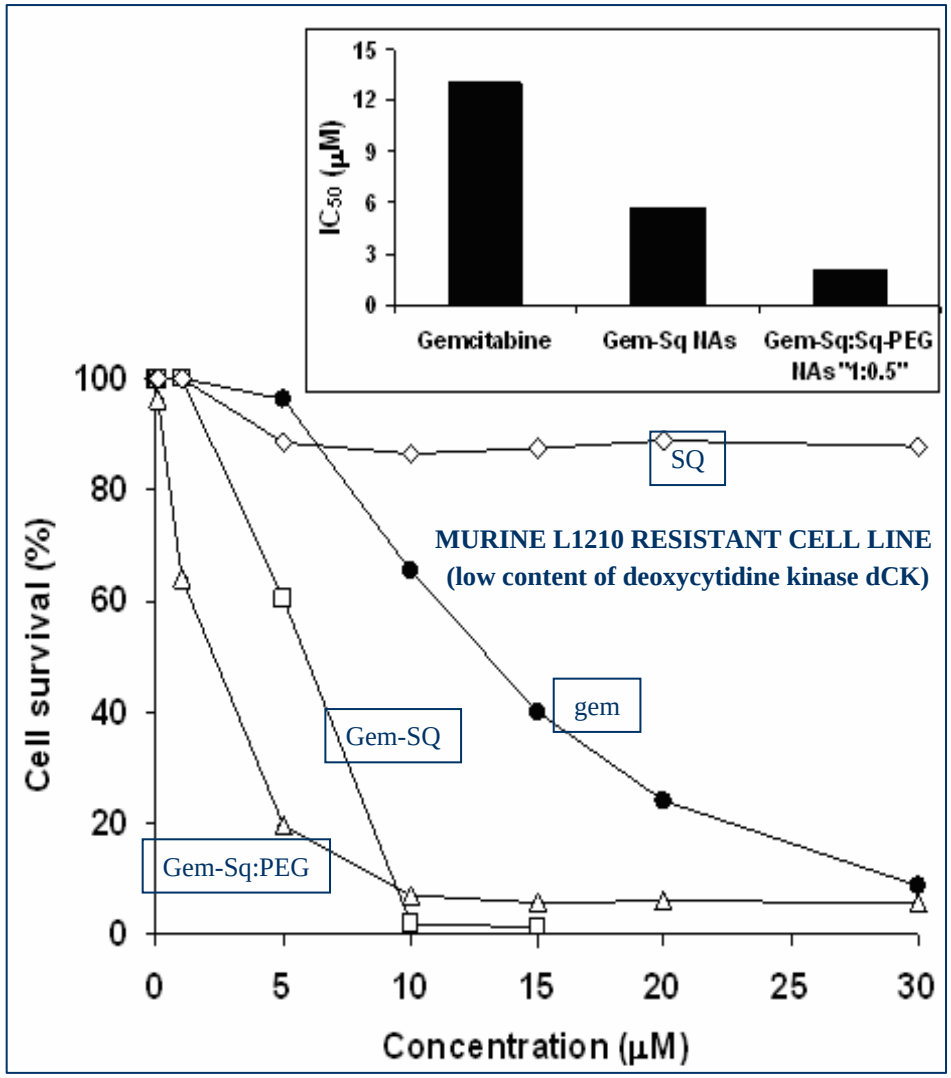
dCK : deoxycytidine kinase

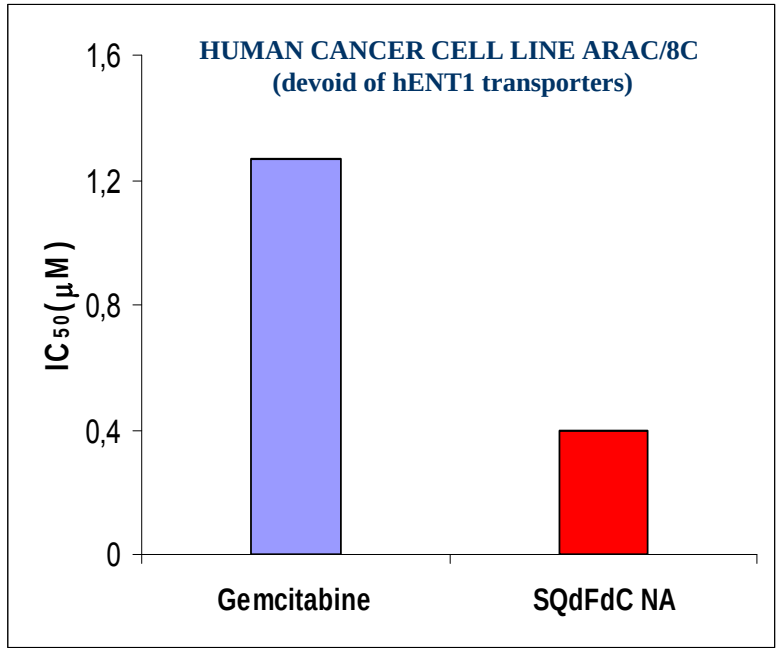
pCK : pyridine kinase

dCD : deoxycytidine deaminase

CYTOTOXICITY ON RESISTANT CANCER CELL LINES (L1210 AND ARAC/8C)

Harivardhan Reddy et al.,. J.Control. Rel., **124**,20-27 (2007)
Aoun et al., Adv Funct. Mater., **18**, 1-11 (2008)

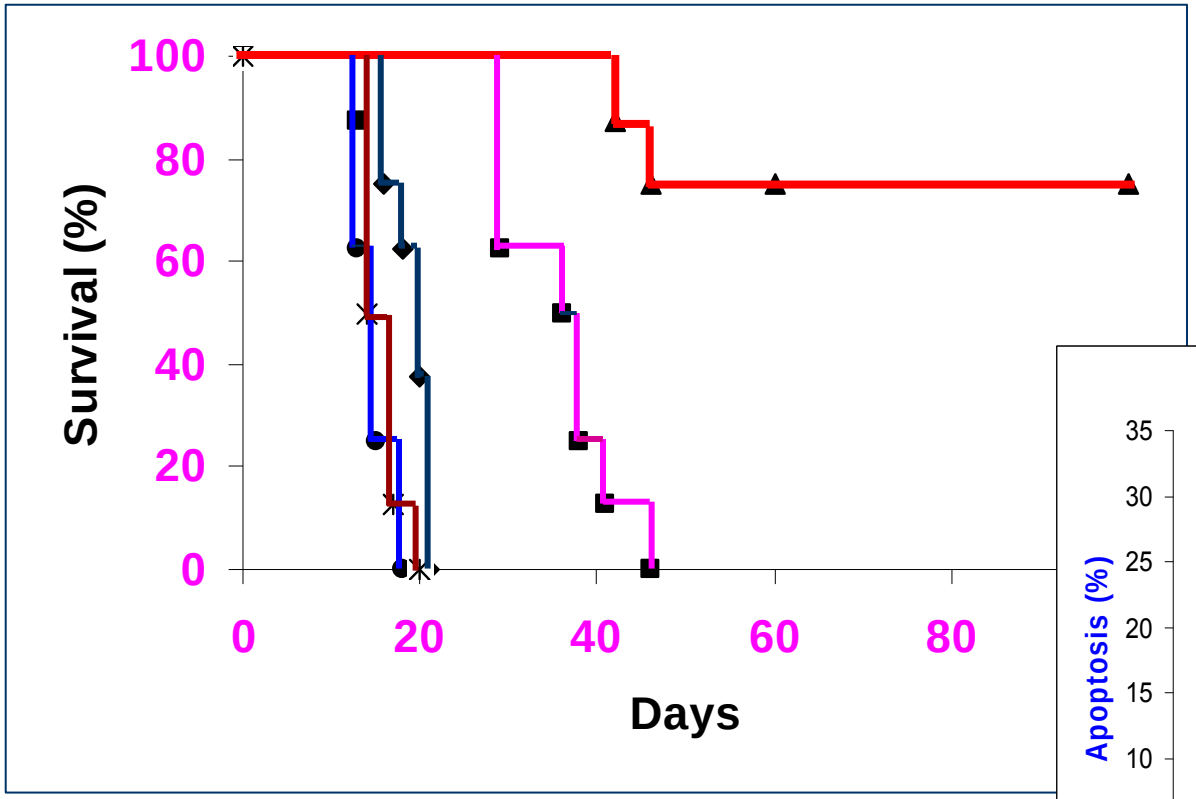


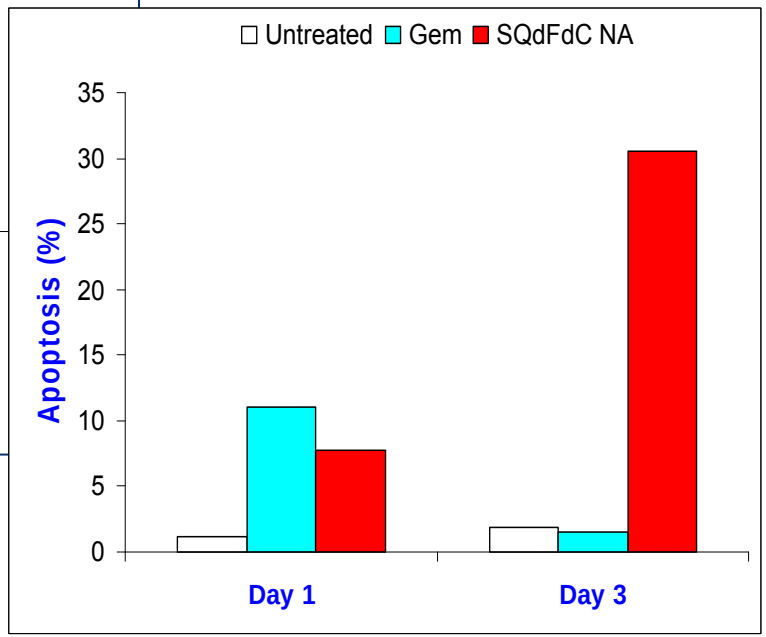


IN VIVO ANTICANCER ACTIVITY AT MTD

(L1210 leukemia iv/iv)

Harivardhan Reddy et al.,. J.Control. Rel., **124**,20-27 (2007)
Harivardahan Reddy et al., J. Pharmacol. Exp. Ther., **325**, 484-490 (2008)

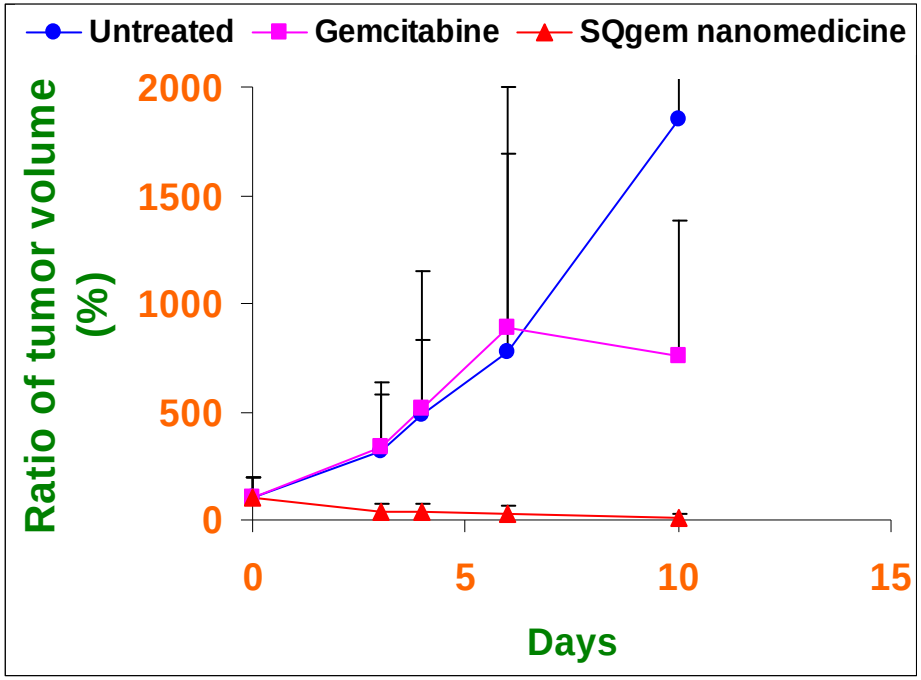




- Untreated
- Squalene 100mg/kg
- Cytarabine 100mg/kg
- Gemcitabine 100mg/kg
- SQgem nanoassemblies 20mg/kg

IN VIVO ANTICANCER ACTIVITY AT MTD
(L1210 leukemia sc/iv)

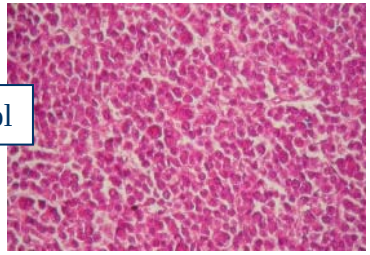
Harivardhan et al., Mol. Pharm., accepted 2009

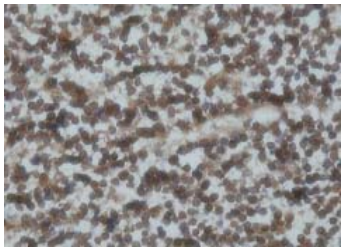


Hematoxylin-Eosin-Saffran (HES) staining

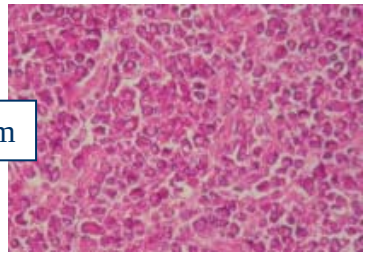
KI-67 nuclear antigen

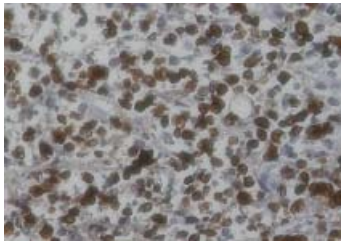
Control



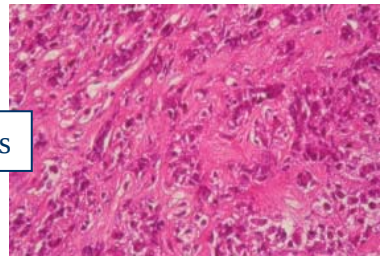


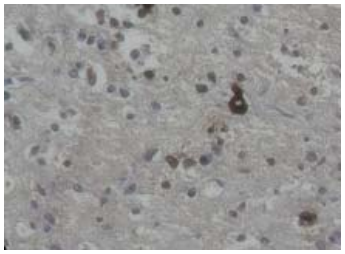
Gem





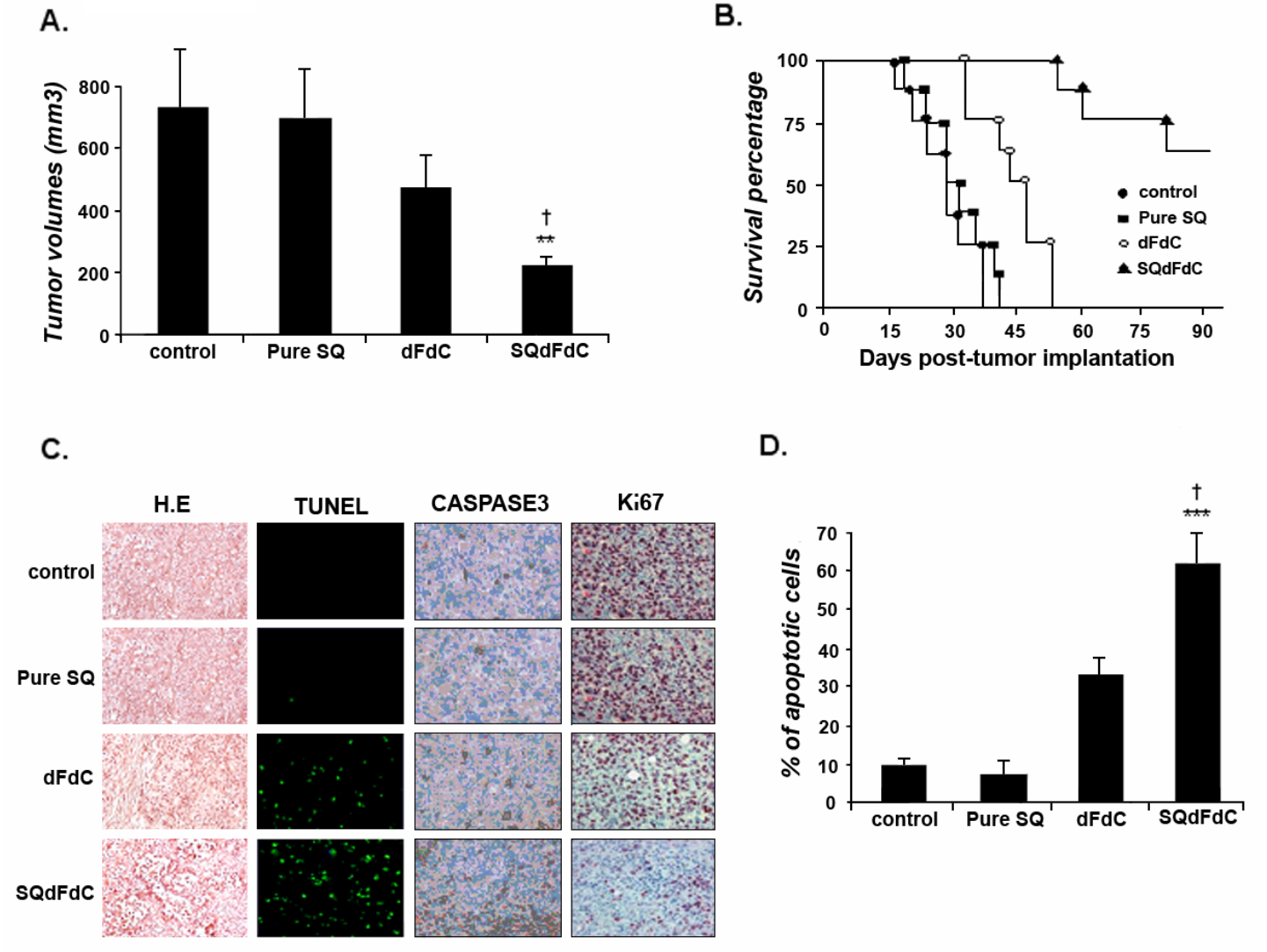
SQgem nanoassemblies





GEMCITABINE-SQUALENE NANOPARTICLES EXERTS ANTITUMOR ACTIVITY ON PANC-1 ORTHOPTIC TUMOR MODEL

Hajiri et al, Unpublished data (2009)



LES NANOMEDICAMENTS: UNE APPROCHE NOUVELLE POUR LE TRAITEMENT DU CANCER

- Permet de protéger le médicament de la métabolisation
- Permet le ciblage cellulaire/tissulaire
- Permet d'adresser aux tumeurs plusieurs molécules actives sur des cibles complémentaires (thérapeutique potentialisatrice)
- Permet de contourner les résistances
- Permet la conception de « nanothéranostics »