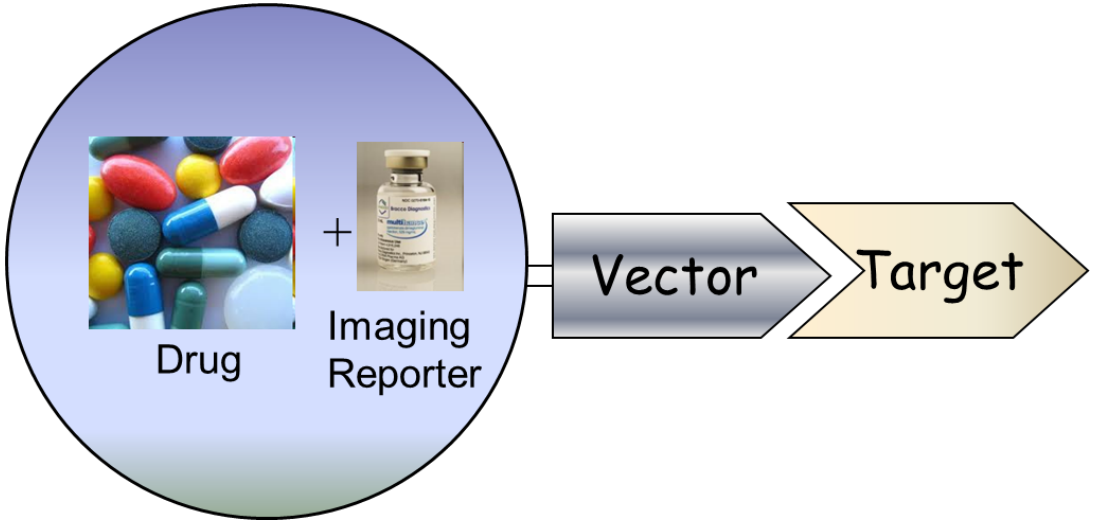
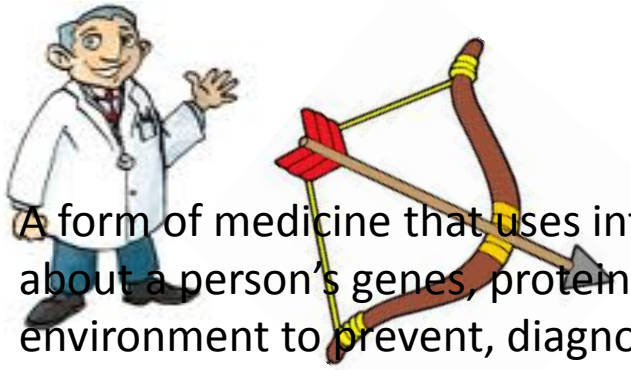
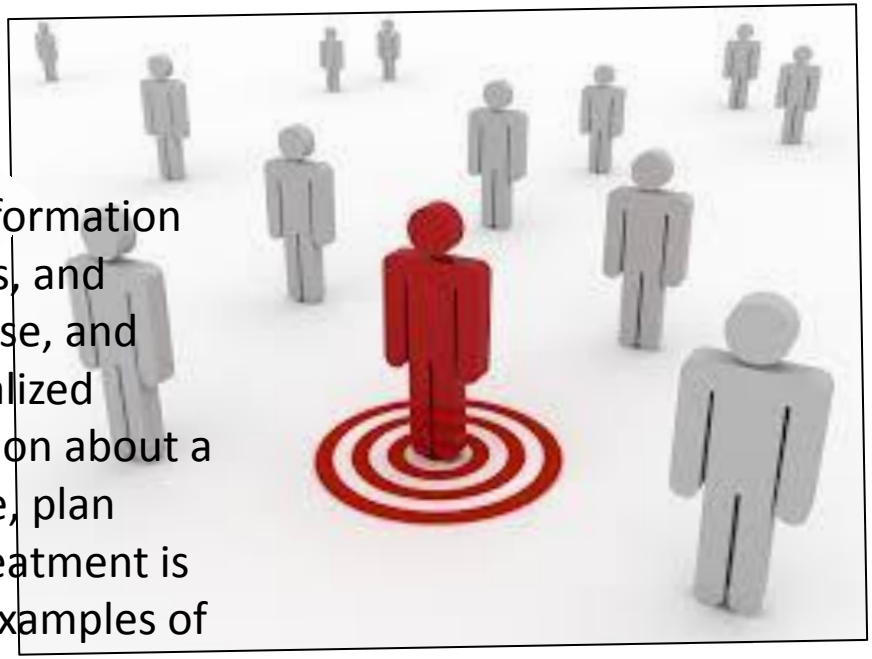
In vivo preclinical Imaging Guided Therapy



Simonetta Geninatti Crich
University of Torino, (Italy)

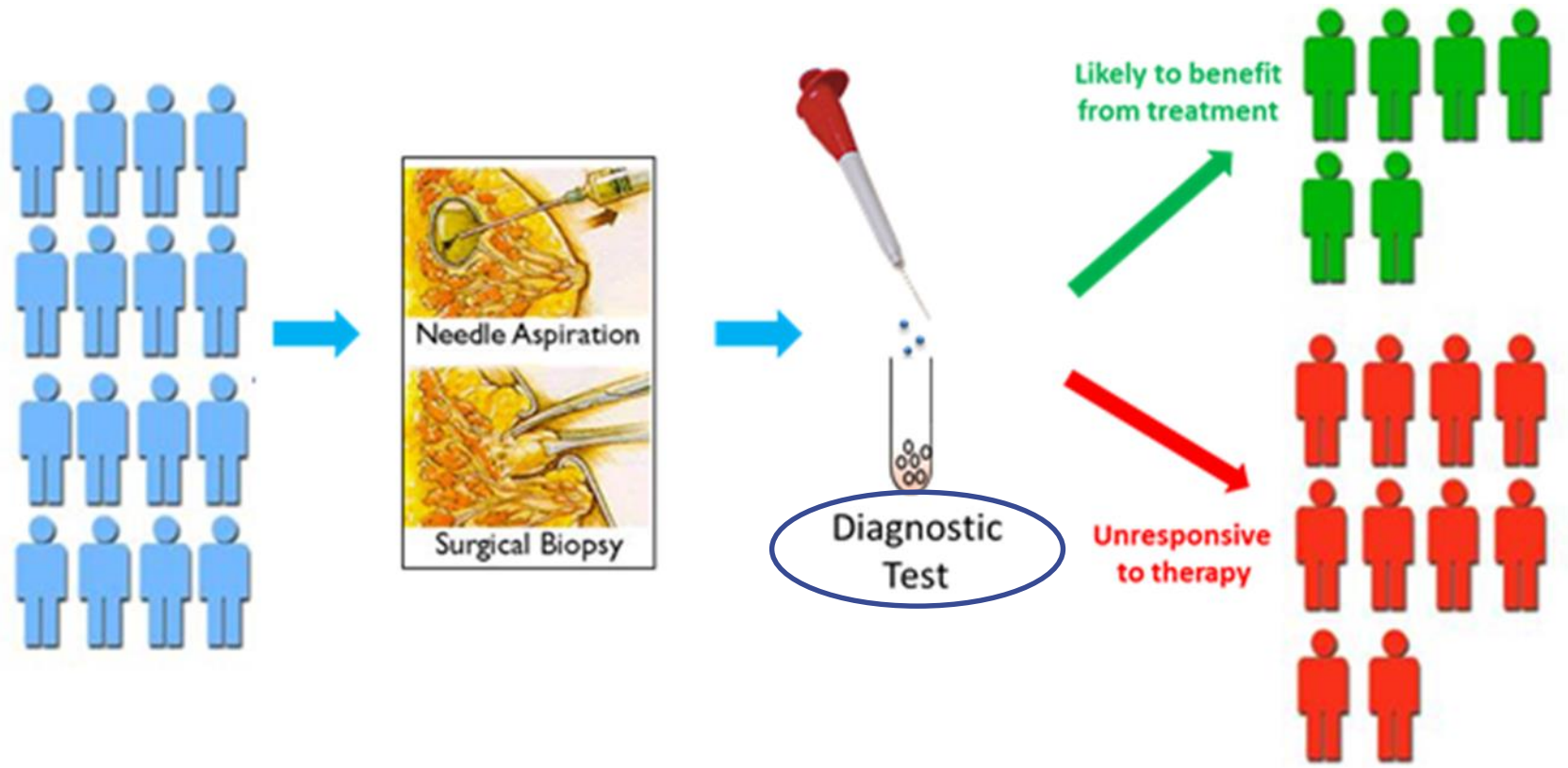
PERSONALIZED MEDICINE



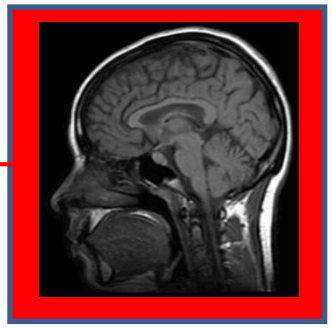


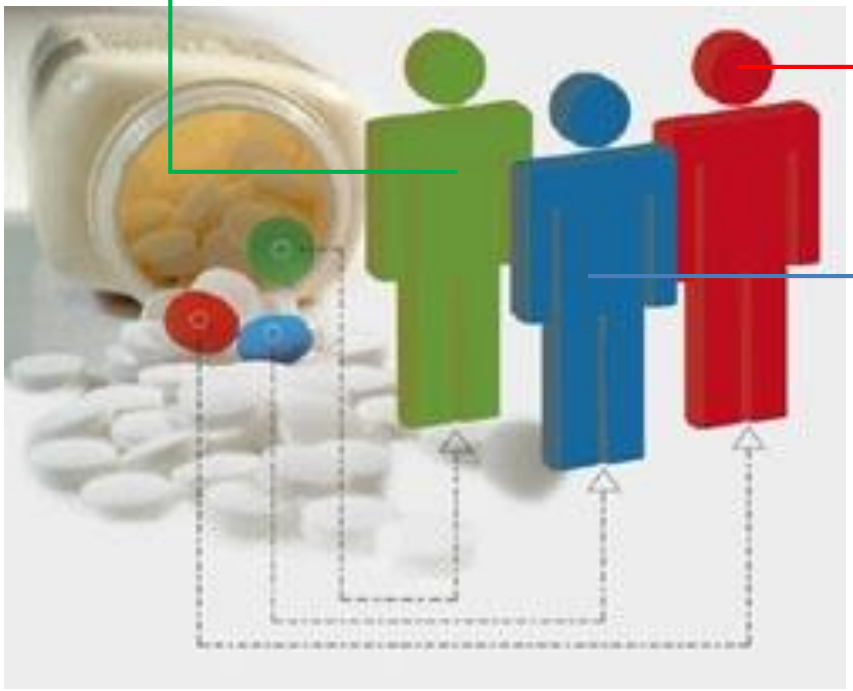
A form of medicine that uses information about a person's genes, proteins, and environment to prevent, diagnose, and treat disease. In cancer, personalized medicine uses specific information about a person's tumor to help diagnose, plan treatment, find out how well treatment is working, or make a prognosis. Examples of personalized medicine include using targeted therapies to treat specific types of cancer cells, such as HER2-positive breast cancer cells, or using tumor marker testing to help diagnose cancer. Also called precision medicine.

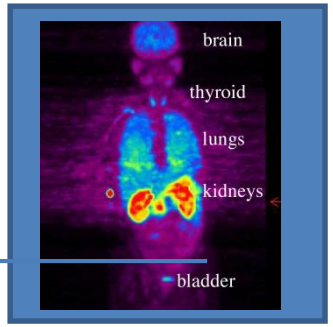
(1) Pre-treatment diagnostic test



(2)Imaging Guided Therapy







The current challenge for MRI contrast agents is in the field of Molecular Imaging

NANOTECHNOLOGY for IMAGING GUIDED DRUG DELIVERY

IMAGING

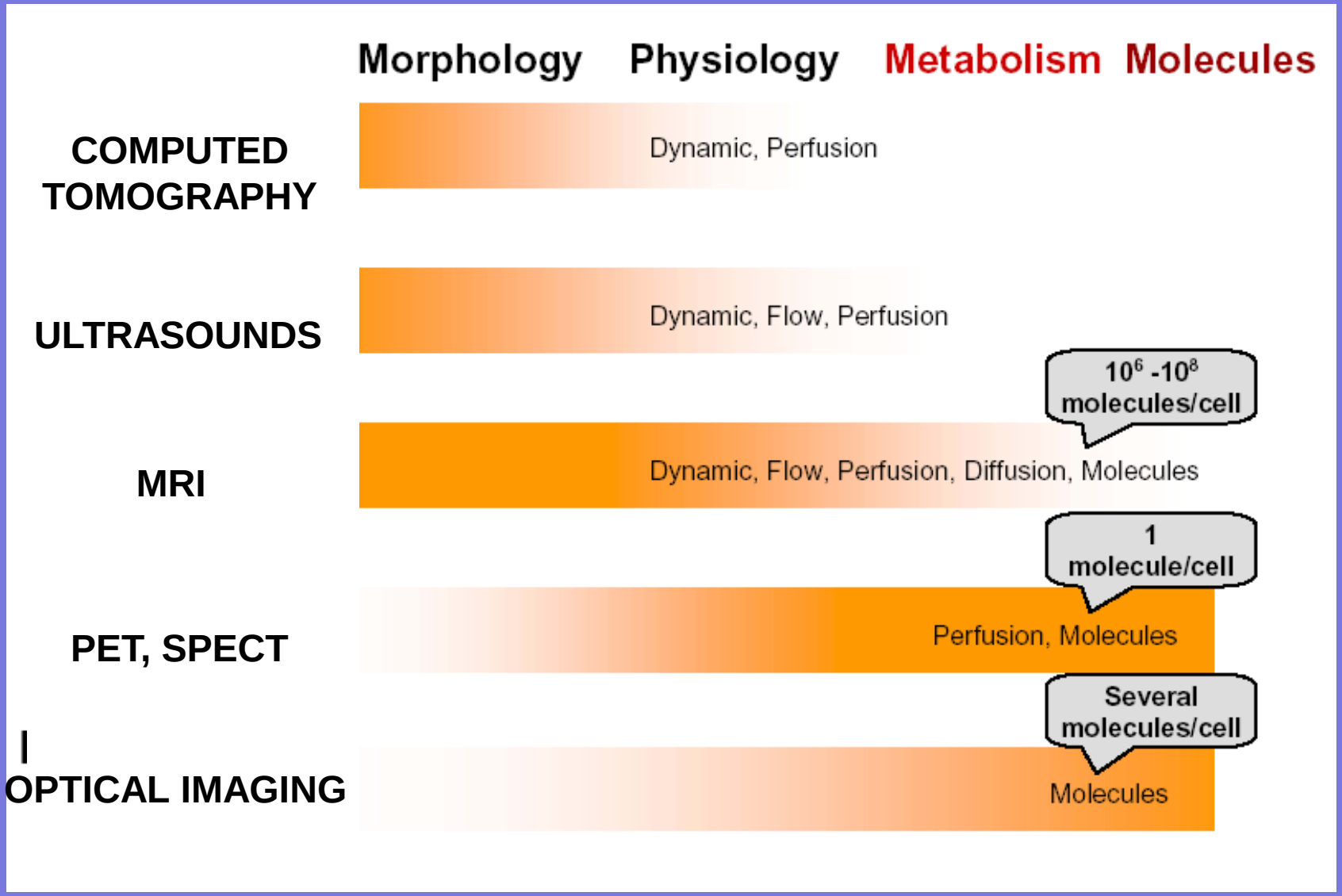
+

THERAPY

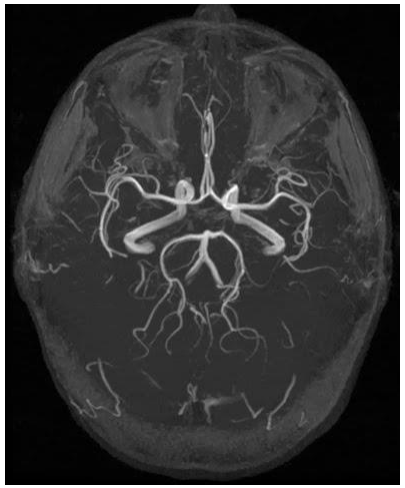
=

THERANOSTICS

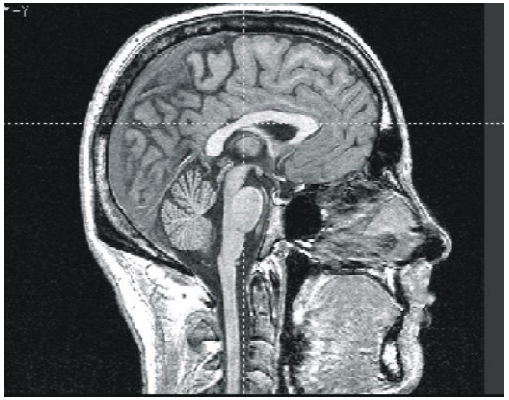
Imaging Modalities: range of detection



Courtesy of H. Siebold, Siemens Medical Solutions

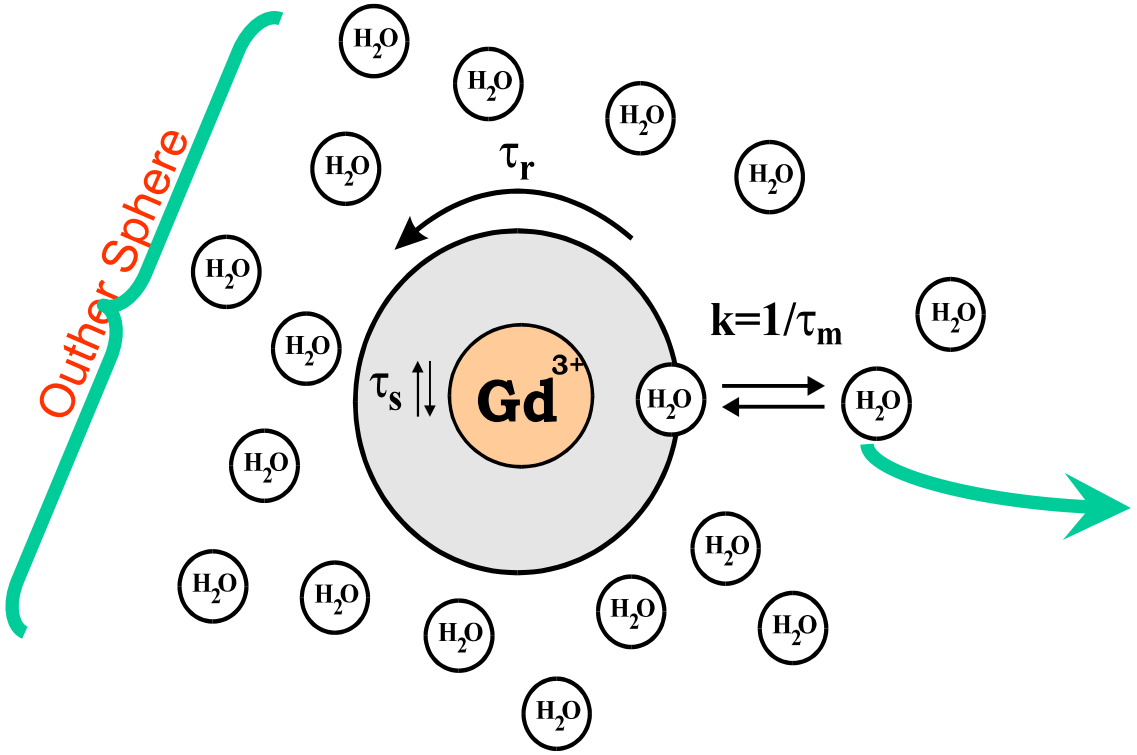


Magnetic Resonance Imaging



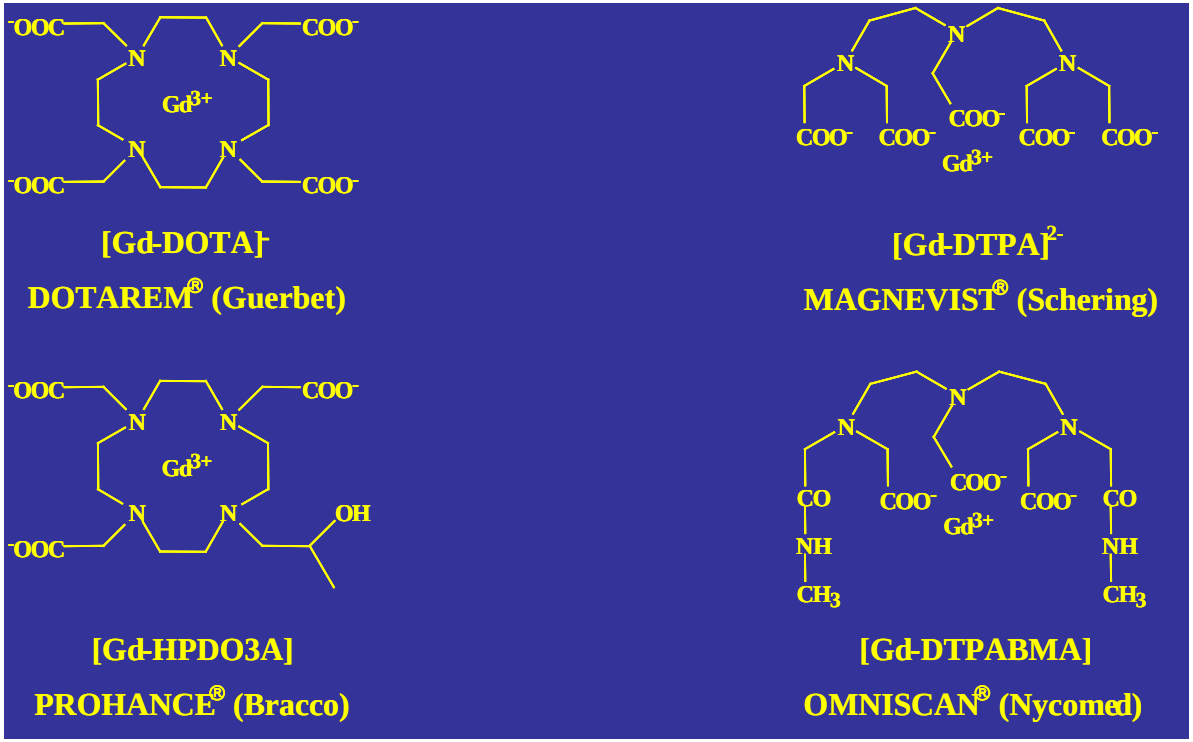
- Non invasive and repetitive imaging
- High resolution
- Absence of radiation
- Total tissue penetration
- Low sensitivity

MRI Contrast Agents

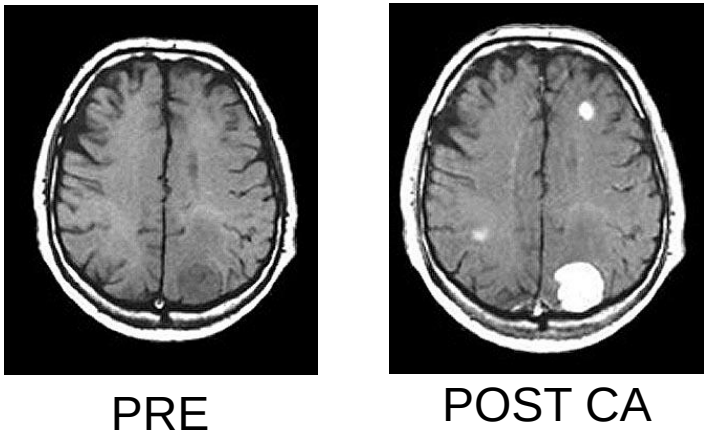


Clinical MRI Contrast Agents

Clinical dose 0.1 mmol/Kg



BRAIN MRI IMAGES

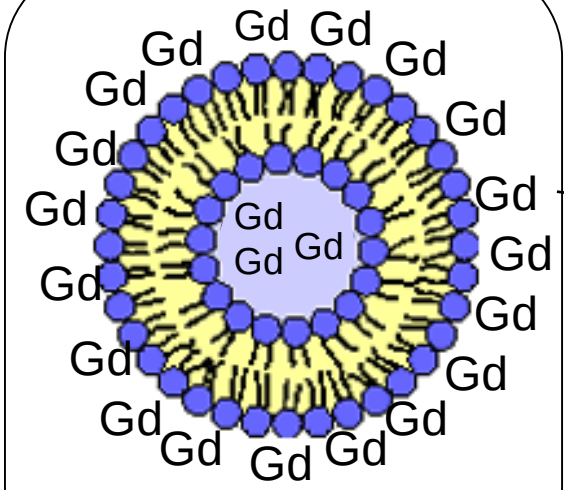


PRE

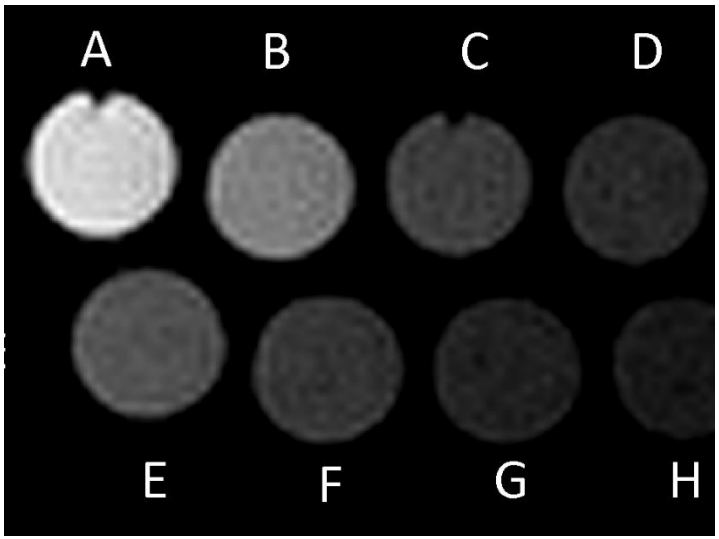
POST CA

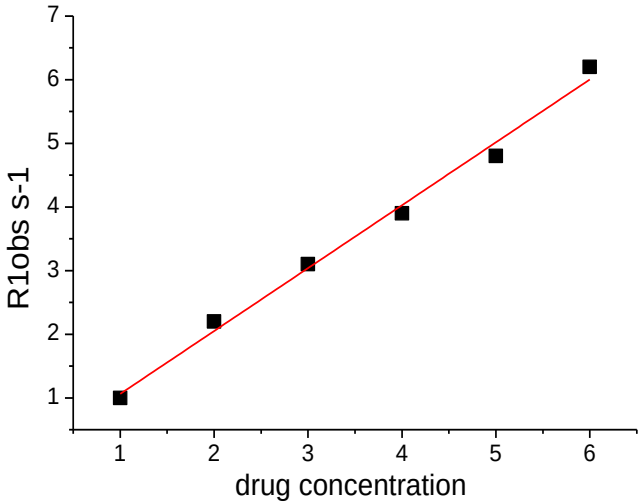
$SI \propto [CA]$

Signal Intensity (SI) ∝ [CA]



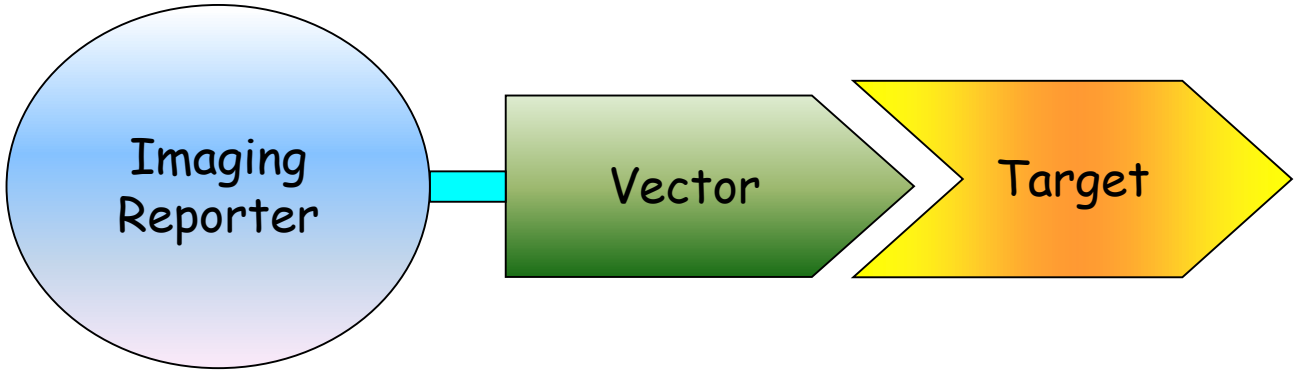
T1-weighted SE image



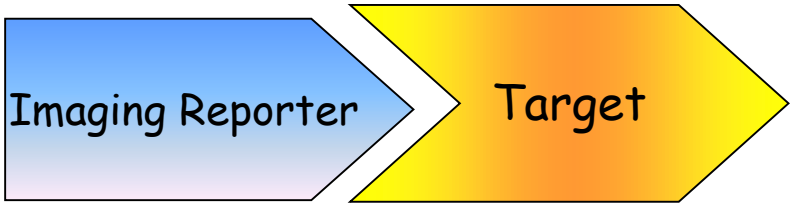


Nanoparticles for imaging guided drug delivery

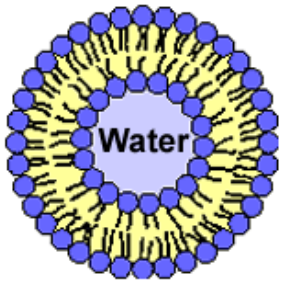
-Liposomes
-micells
- PLGA polymers
-perfluoro nanoparticles



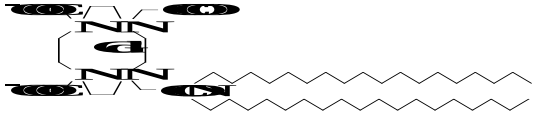
Natural Carriers (Proteins)



NANOPARTICLES → LIPOSOMES



IMAGING AGENT → Gd-DOTAMA(C_{18})$_2$



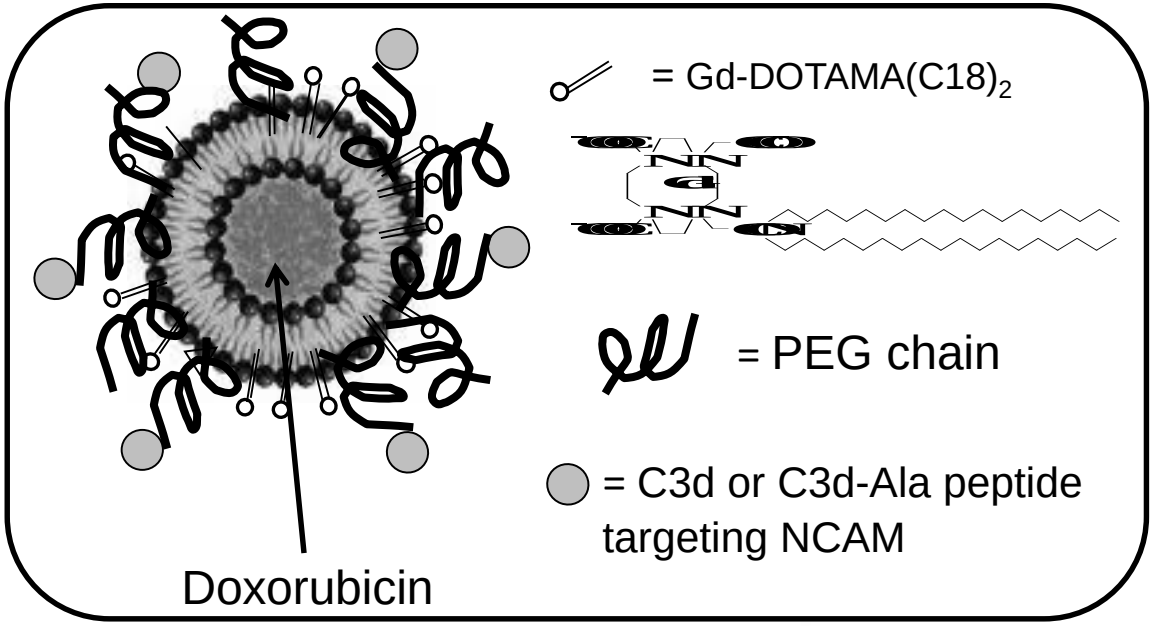
DRUG → DOXORUBICIN

TARGETED EPITOPE → Neural Adesion Molecules (Kaposi's Sarcoma)

CARRIER → C3d peptide

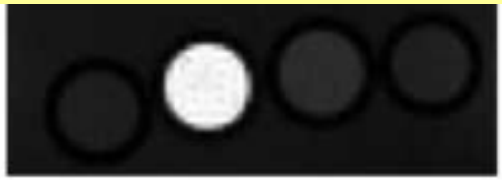
Grange, Geninatti-Crich, Esposito, Alberti, Tei, Bussolati, Aime, Camussi, Cancer Res, 2010

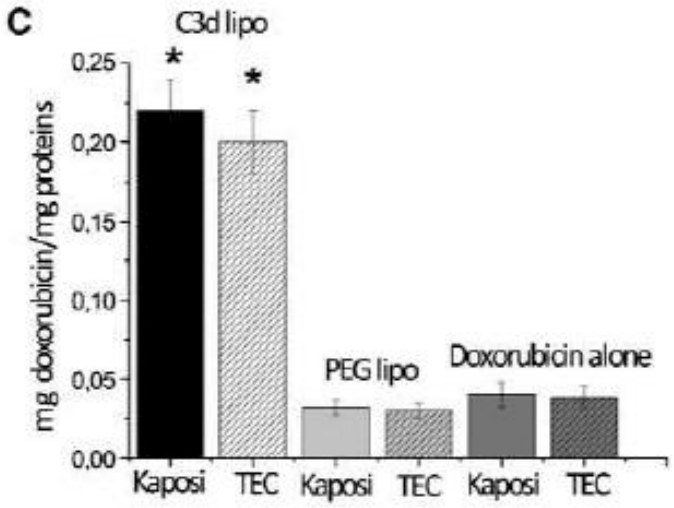
Combined Delivery of MRI contrast agents and doxorubicin through in Experimentally Induced Kaposi's Sarcoma



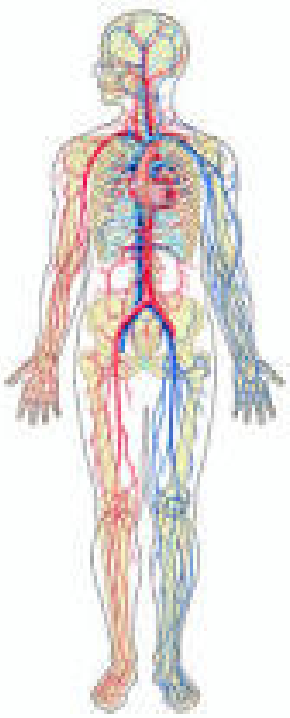
T1 weighted MRI IMAGE of CELLS Incubated with different liposomes

CTRL C3d C3d-ala peg



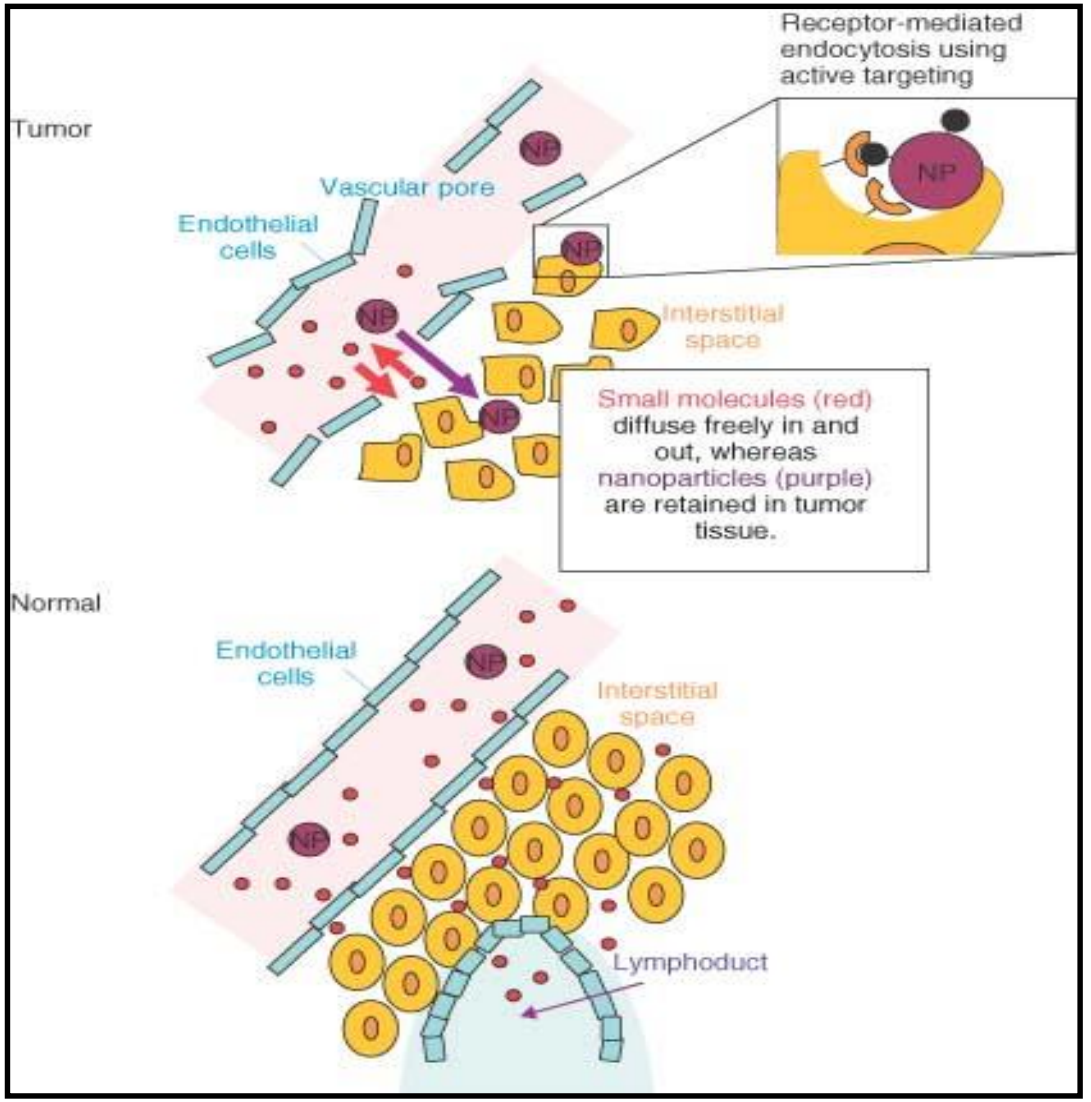


If the target receptor is expressed by cells in solid tumors the extravasation of the theranostic agent is needed



-In solid tumors the vessels formed by the process of angiogenesis show an Increase permeability due to large fenestrae (up to 400 nm)

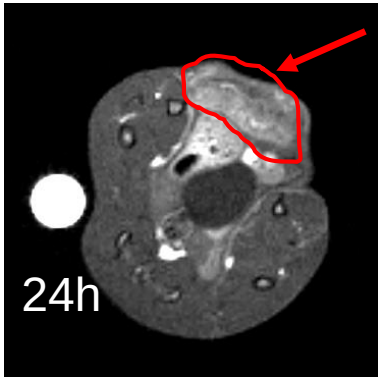
-**N**ormal vasculature endothelium consists of a continuous lining of endothelial cells tightly connected with each other.

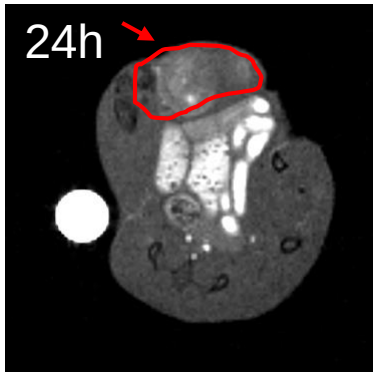


Osamu I. et al International Journal of Pharmaceutics 190 (1999) 49–56
Pavan P. Nanomedicine and Nanotechnology, 2010

THERANOSTIC AGENTS BIODISTRIBUTION DETECTED BY MRI

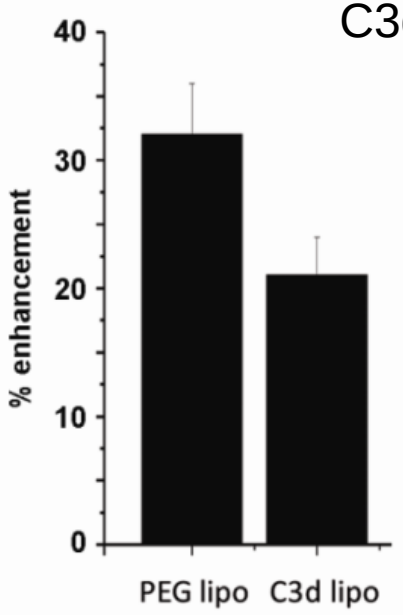
TUMOR

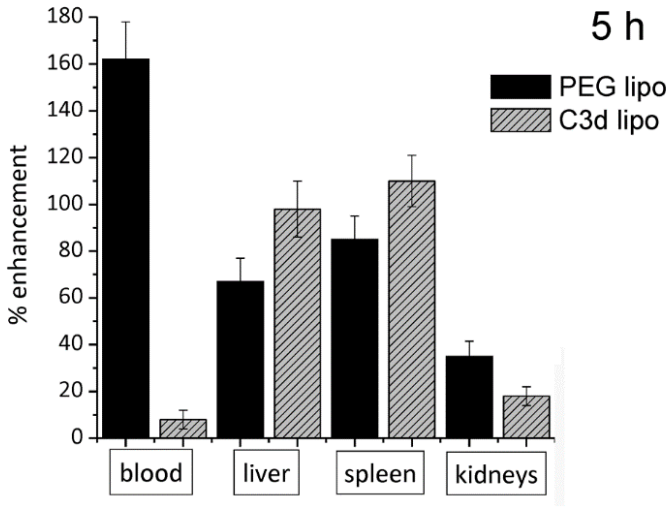


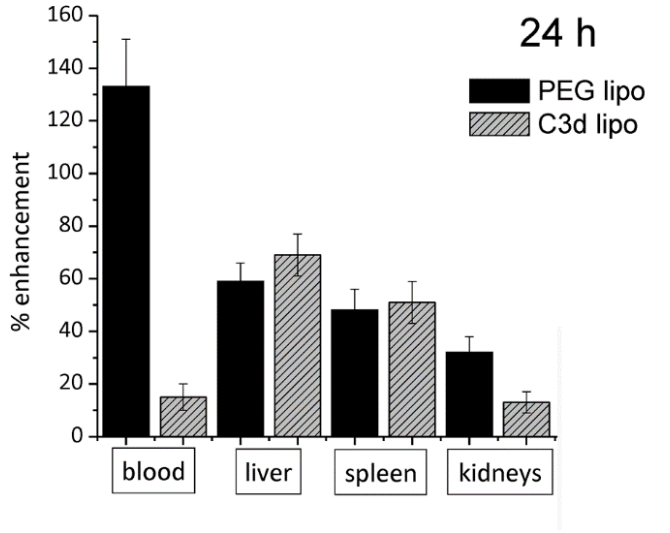


NOT TARGETED
PEG LIPO

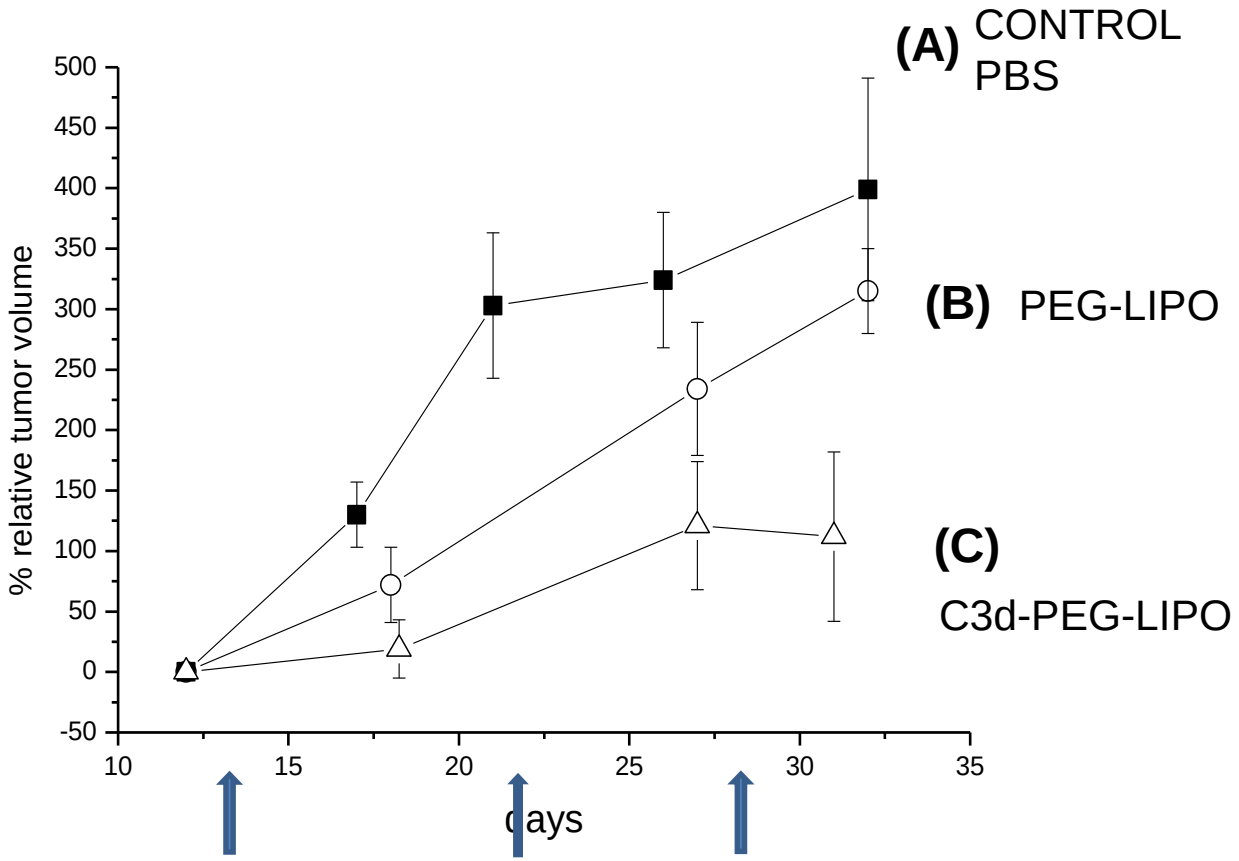
TARGETED
C3d LIPO





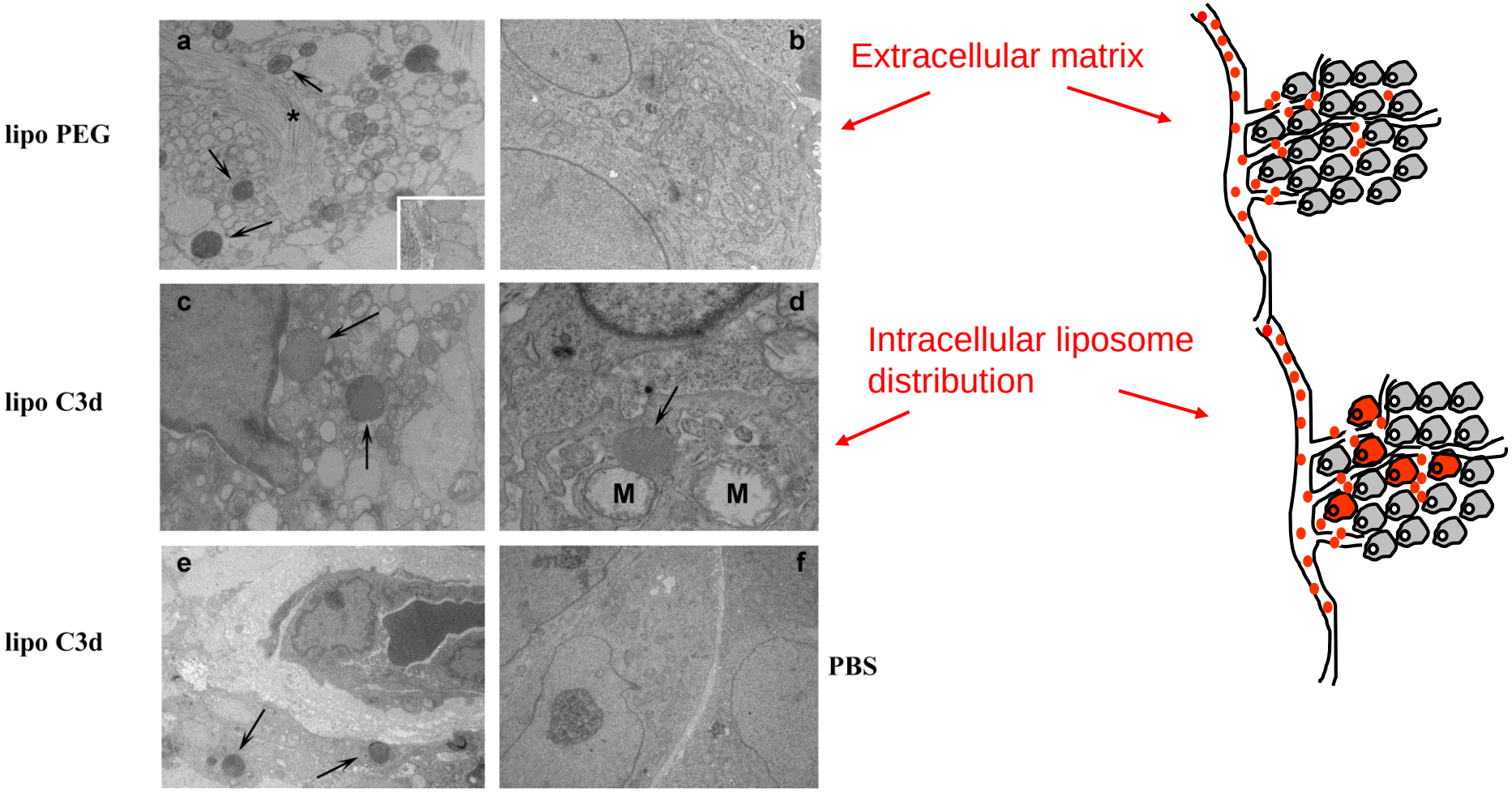


Therapeutic responses of SCID mice inoculated with Kaposi cells



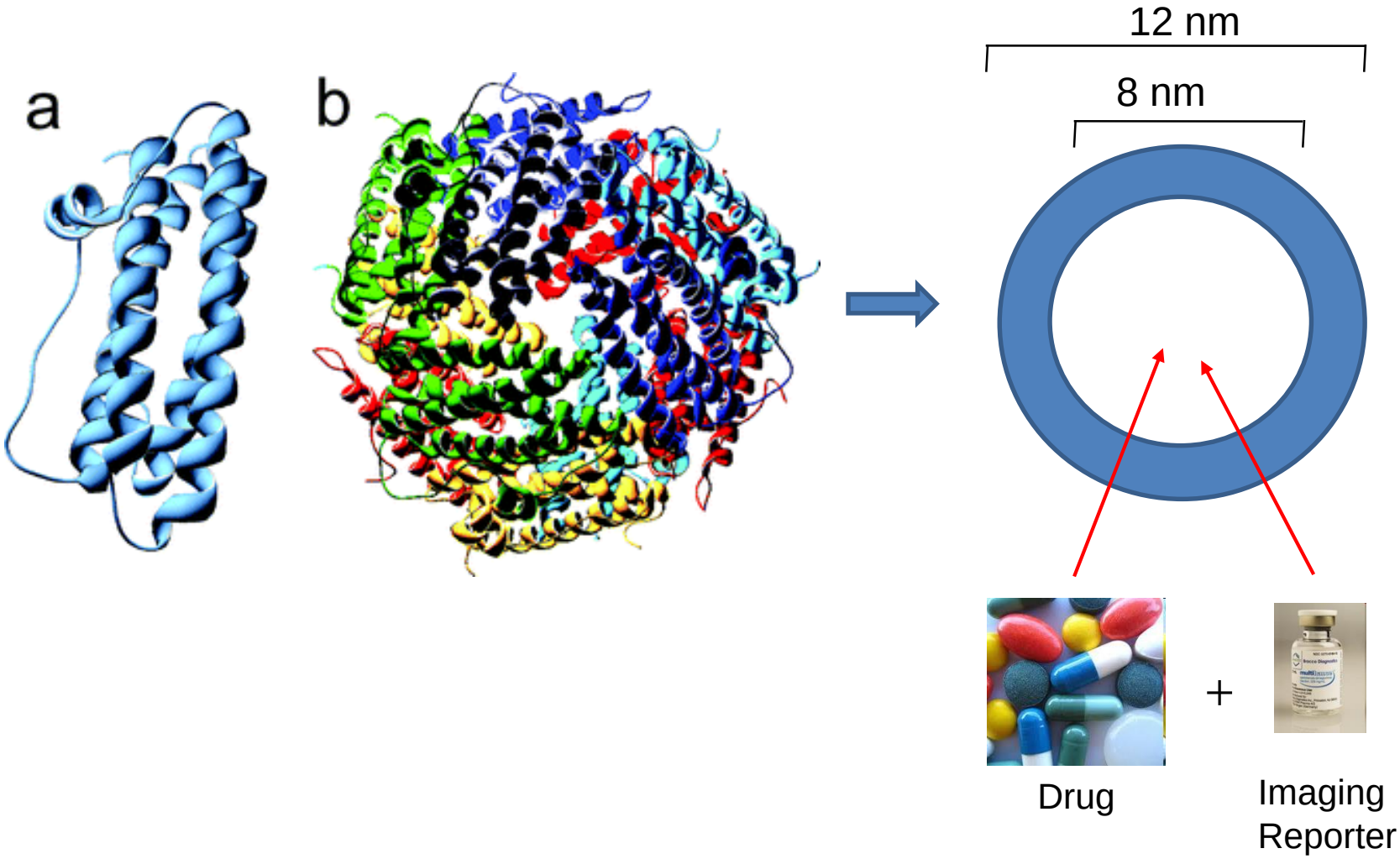
Treatments (5 mg/kg doxorubicin) were on days 12, 19, 26 (indicated by the arrows).

Electronmicroscopy analysis of tumors

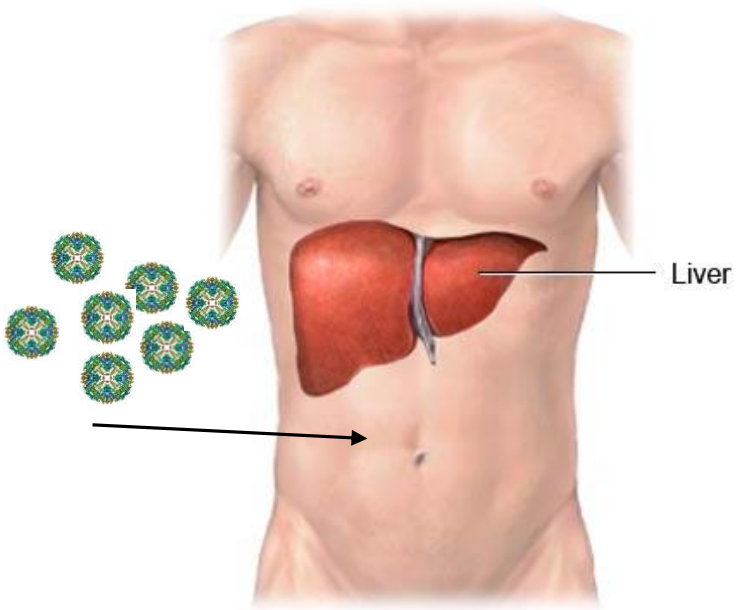


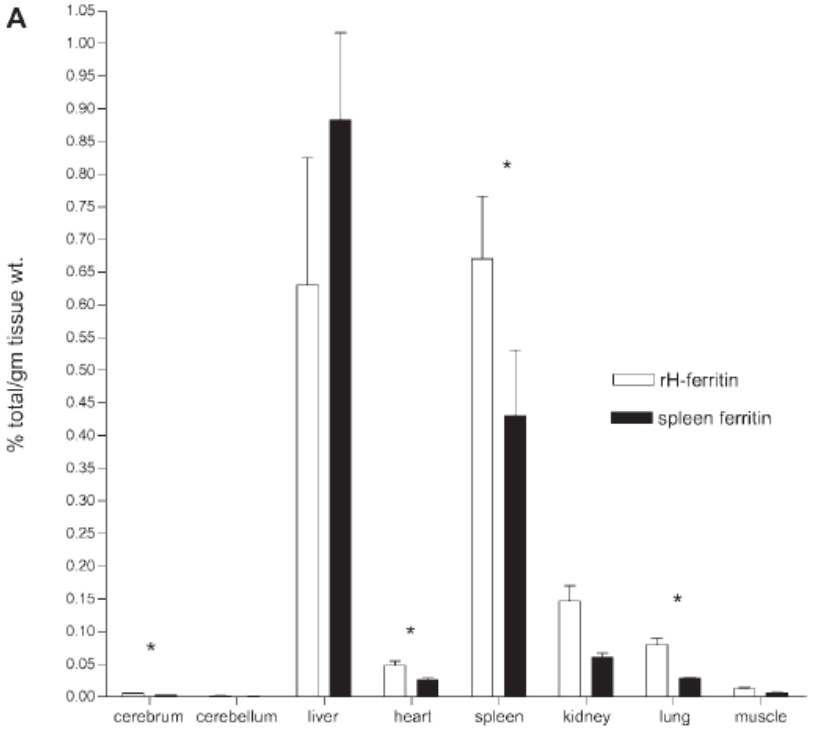
- TARGETED LIPOSOME → INTRACELLULAR
- NOT TARGETED LIPOSOME → EXTRACELLULAR

Apoferritin as carrier for imaging and therapeutic agents



Ferritin receptors (SCARA-5) are highly expressed on hepatocytes



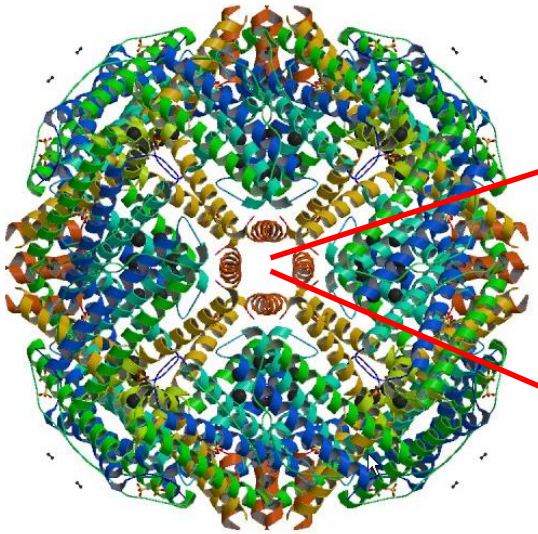


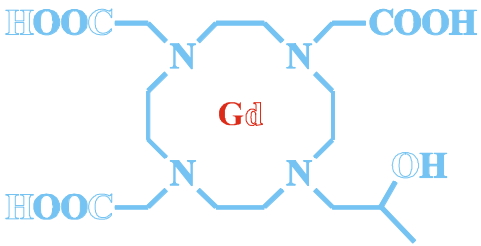
Fisher J et al Am. J. Physiol. Cell. Phisiol, 293, 2007.

Jian Huang et al *The Journal of Clinical Investigation, 120 ,2010*

Jau Yi Li et al, Developmental Cell 16, 35–46, January 20, 2009

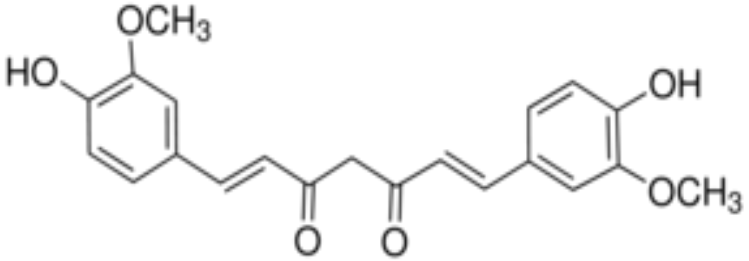
Apoferritin





Gd-HPDO3A

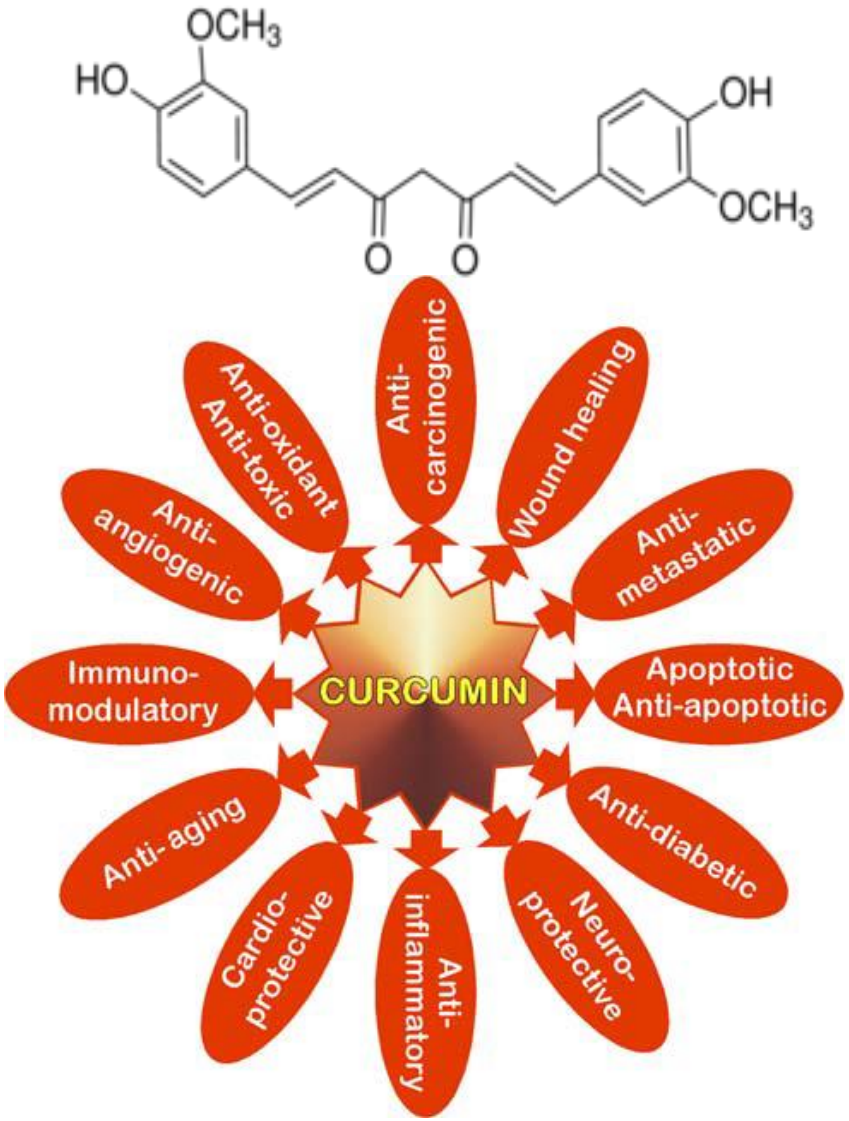
curcumin



Curcumin polyphenolic molecule extracted from the rhizomes of the plant Curcuma Longa.

Cutrin JC, Geninatti Crich S, Burghelea D, Dastrù W, **Aime S,** Mol Pharm. 2013;10(5):2079.

Biological activities of curcumin



Advantages:

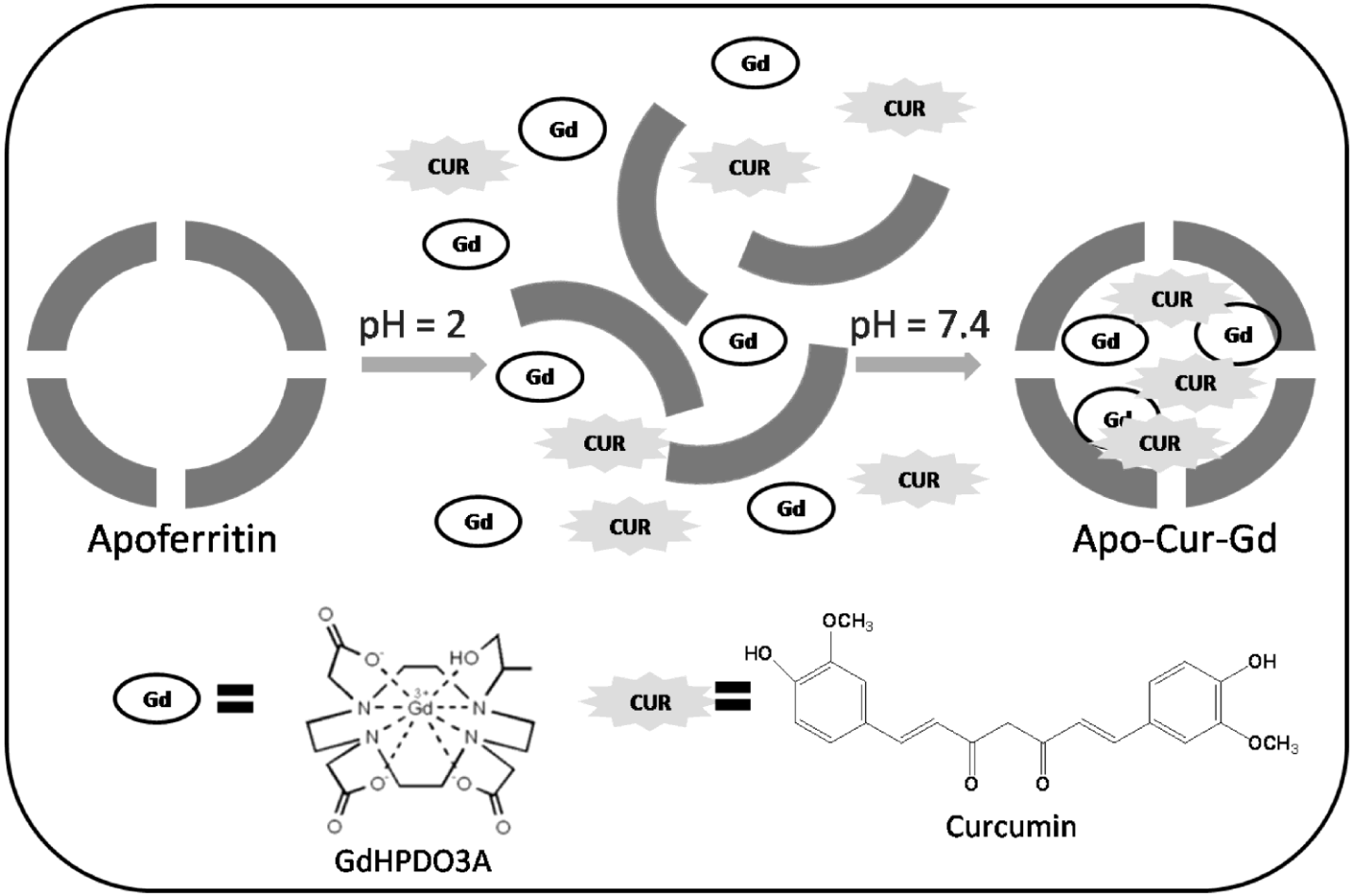
- Safety even at high doses (12 g/day)
- Good tolerability
- Multi-target compound with multiple therapeutic effect

Disadvantages:

- Low bioavailability
- Poor water solubility
- Low stability in water (in particular at neutral and basic pH)

Tanya Das et al Mol Cell Biochem (2010) 336:85–95; Marie-Hélène Teiten et al Toxins, 2010.

How to include Gd-HPDO3A and Curcumin in Apoferritin?



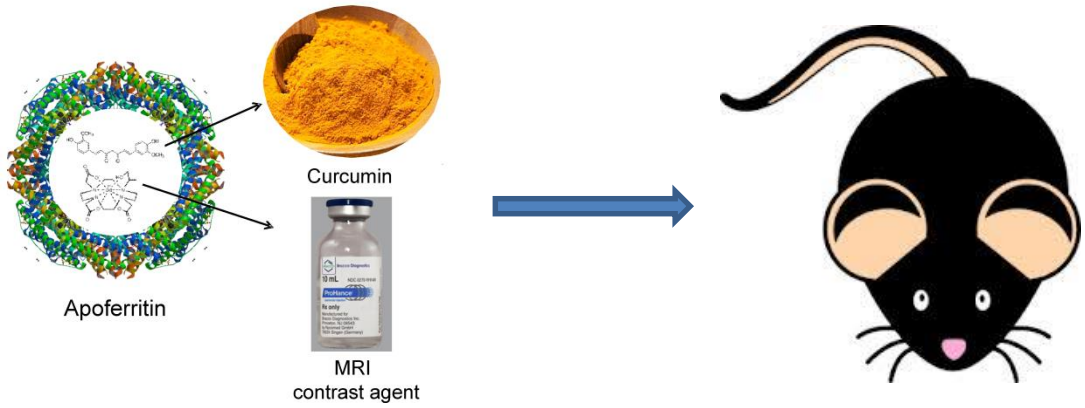
The number of molecules that remained entrapped in the apoferritin after dissociation/reassociation procedure is 9.5±2 and 0.4±0.1 for subunit (24 subunits/protein in the native form) for curcumin and Gd-HPDO3A, respectively.

Attenuation of thioacetamide-induced hepatitis by curcumin

- Thioacetamide (TA) has been employed for several years in the development of a model of acute liver injury in rodents.

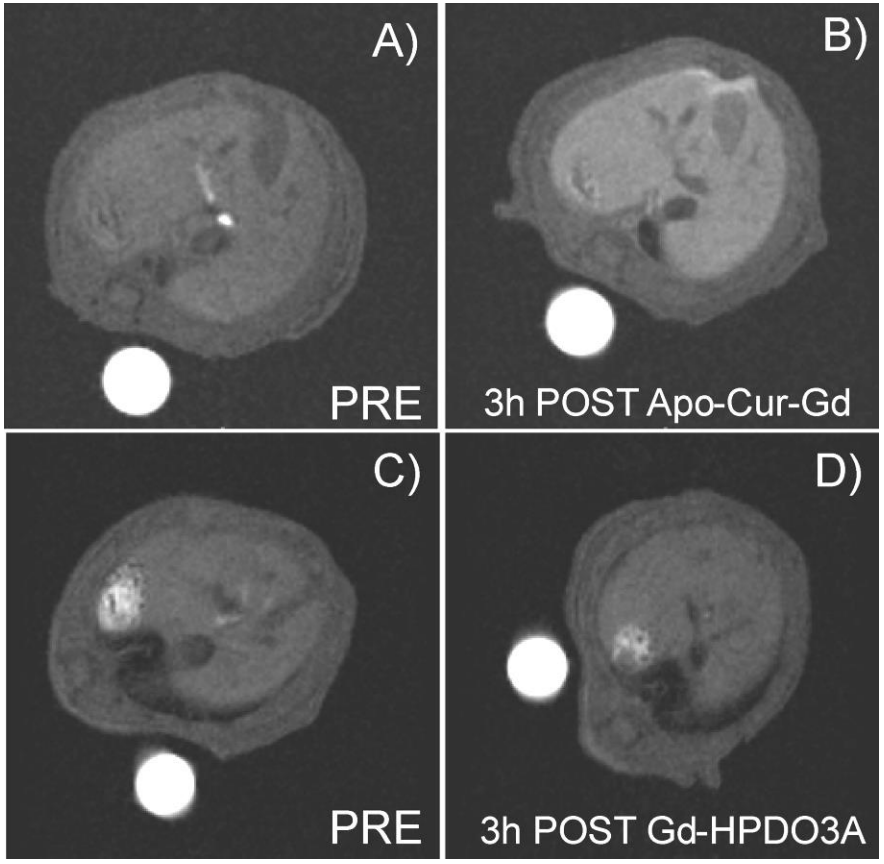
-The i.p. administration of high doses (60-100mg/kg) of TA causes fulminant hepatic failure as a consequence of enhanced ROS and lipid peroxides formation, and stimulation of NF-kb and resultant production of pro-inflammatory molecules. *(Rivera-Espinoza et al, Liver international 2009.)*

-In this study mice were divided into three groups.
Group A received TA (60 mg/kg) intraperitoneal (ip)
Group B was pretreated 24 h before TA ip administration (60 mg/kg) with APO-CUR-Gd ip (63 mg/kg)
Group C (control) received an equal volume of sterile 0.9% NaCl solution instead of TA

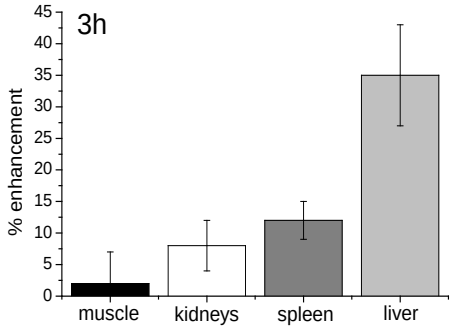


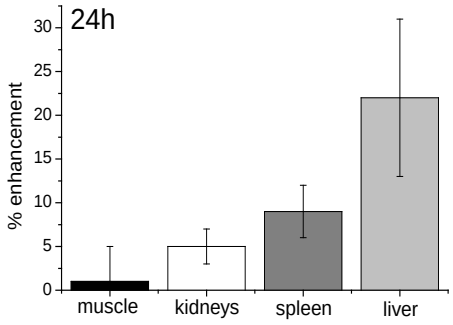
MRI evaluation of Apo-CUR-Gd biodistribution

$$\frac{SI_{PRE}}{SI_{POST}} = \frac{\{[1 - exp(-TR - TE)R1_{PRE}]\}\exp(-TExR2)}{\{[1 - exp(-TR - TE)R1_{POST}]\}\exp(-TExR2)}$$



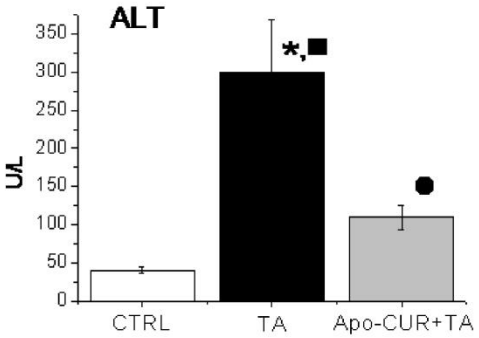
$[Gd]mM=(R1_{(POST)}-R_{1(PRE)})/r_{1p}$

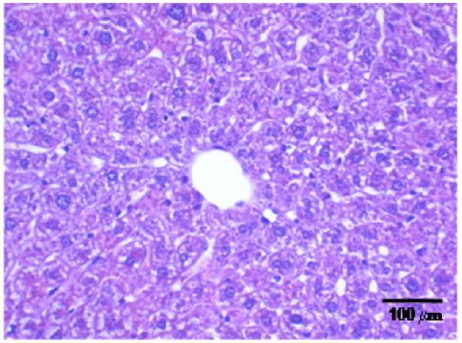




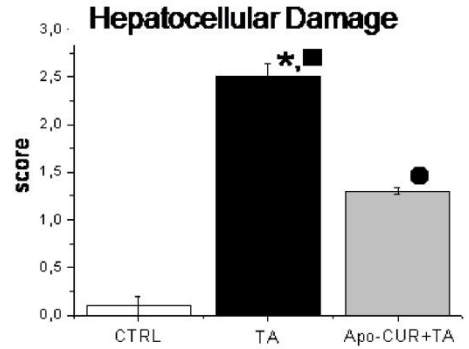
Liver [curcumin] = 250 μg/g (8 times higher than the amount found after the i.p. administration of curcumin alone (*A. Goel, Biochemical pharmacology 2008*.)

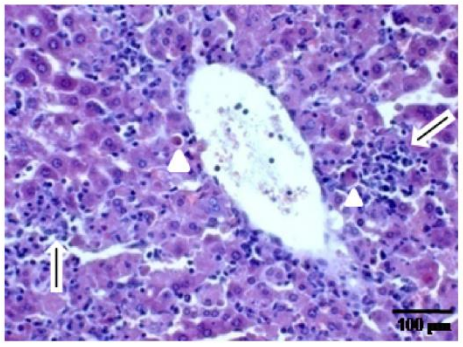
Hepatic Injury Evaluation 24h after TA administration



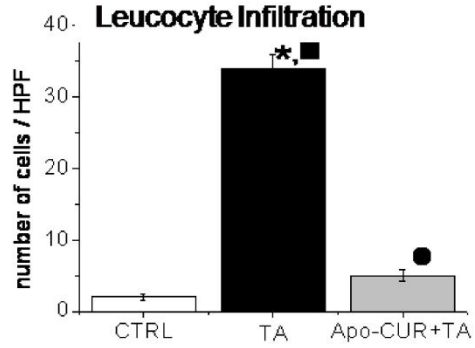


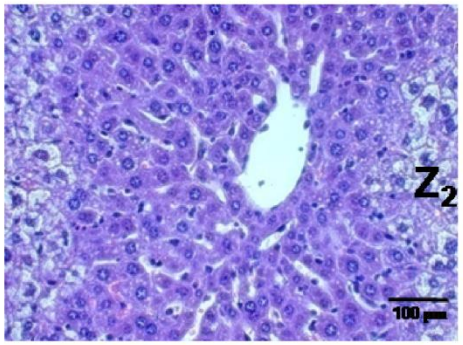
UNTREATED CTRL LIVER





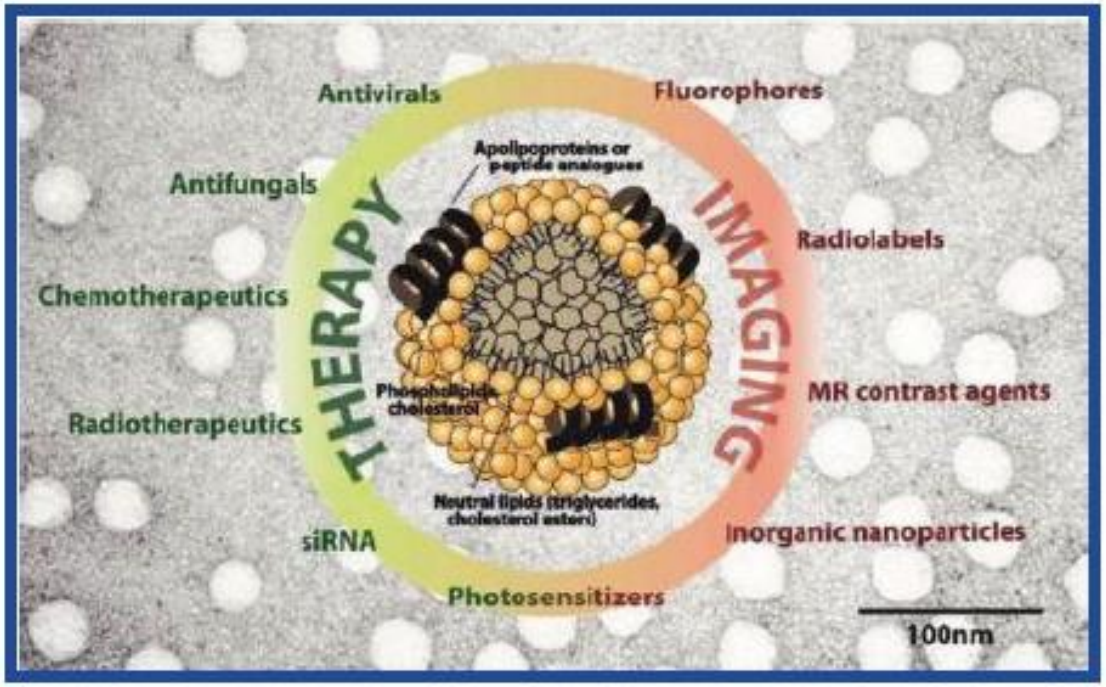
TA TREATED LIVER





TA + APO-CUR-Gd
TREATED LIVER

Low Density Lipoproteins as Theranostic Agents

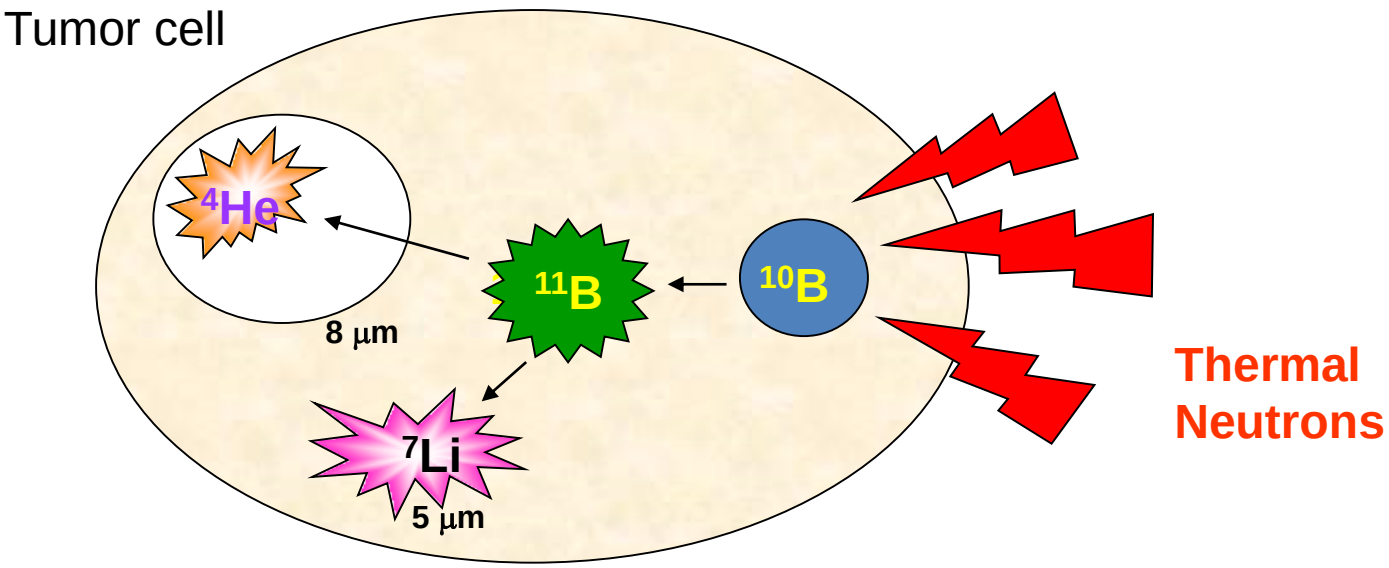


size = 20 nm

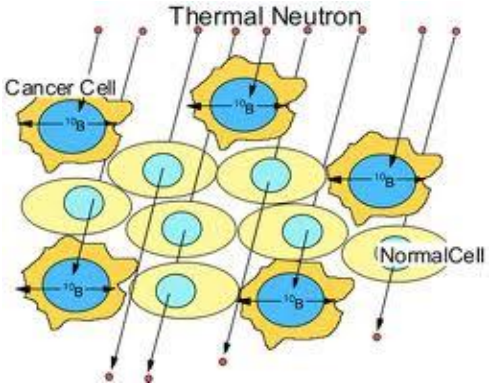
-Several examples of successful <u>delivery of drugs and imaging agents</u> through targeting of <u>LDL receptors</u> have already been reported.
-Altered LDLr levels are found in a variety of pathological conditions.
- Several rapidly dividing tumor cells over-express LDLr to supply the high cholesterol demand.

Kenneth et al Accounts of Chemical Research 2011

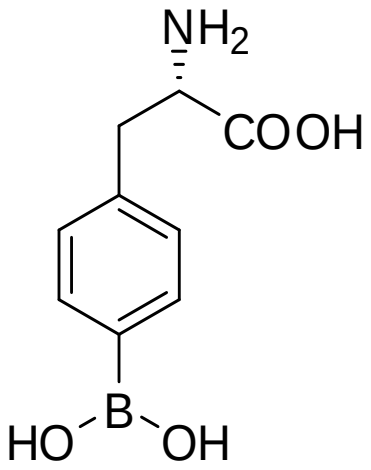
Boron neutron capture therapy (BNCT)



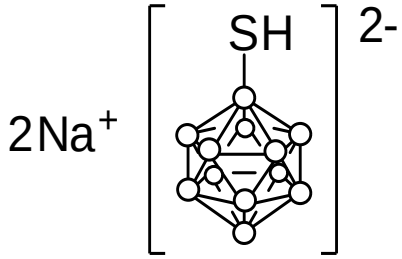
In order to be successful, a sufficient amount of ^{10}B must be selectively delivered to the tumor (ca. 20-30 ppm) whereas ^{10}B concentration in the surrounding normal tissues should be low (<5 ppm).



BNCT drugs available for clinical investigation

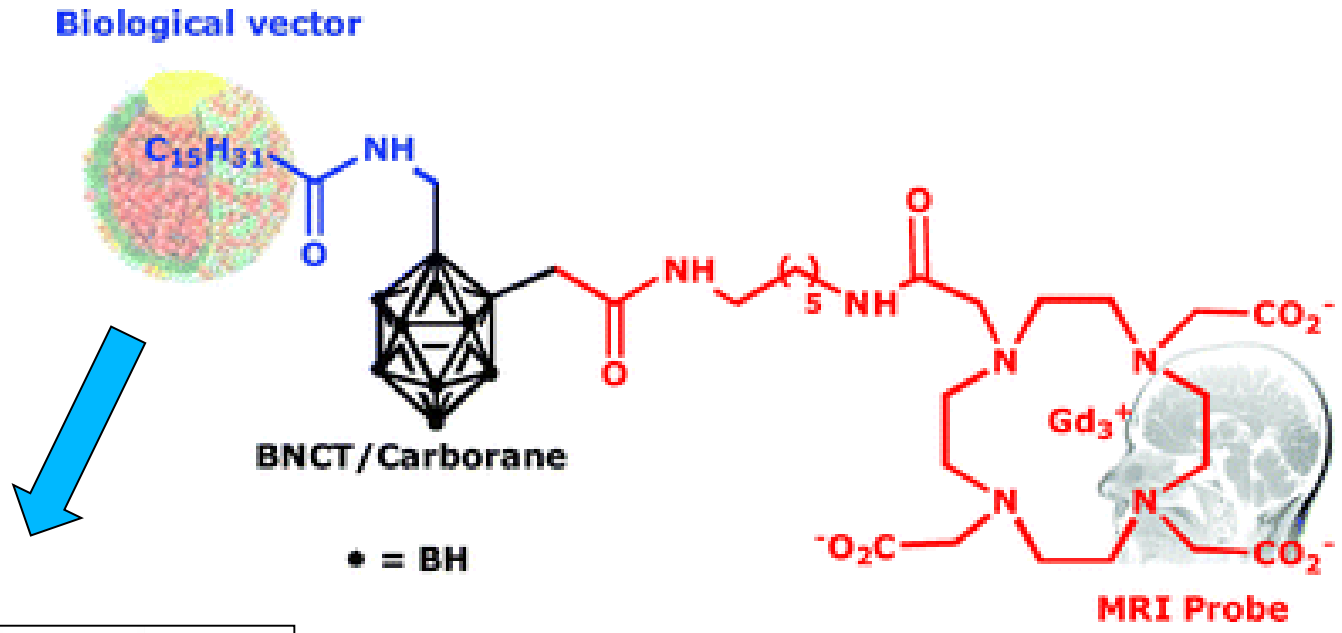


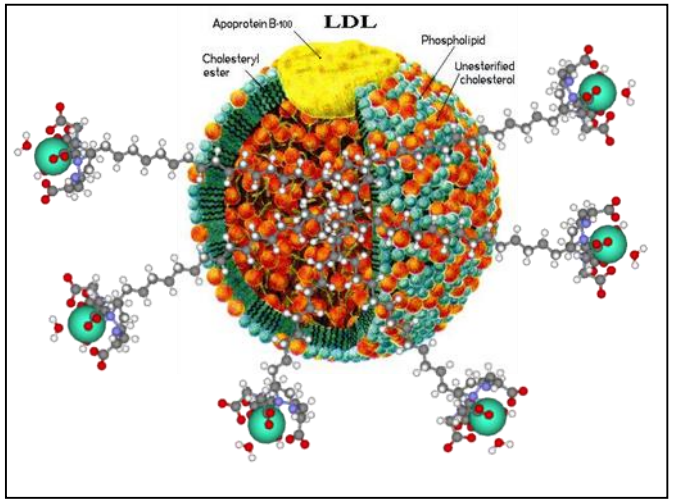
BPA



BSH

A Boron/Gd/LDL adduct for Imaging-guided Neutron Capture Therapy



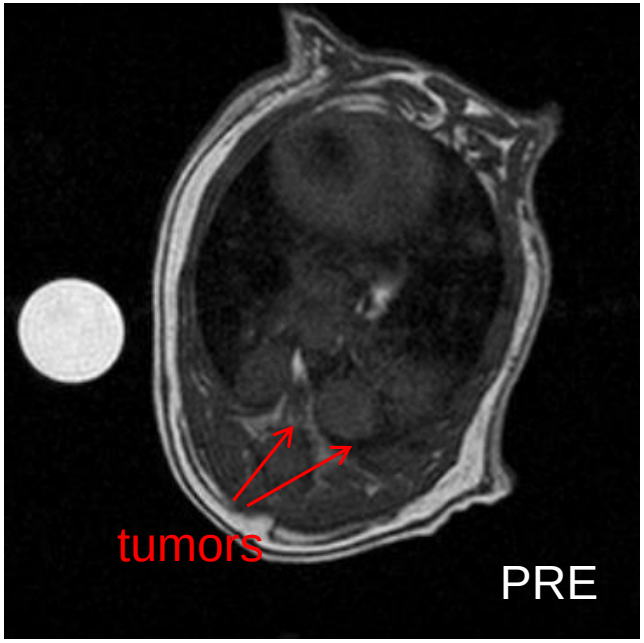


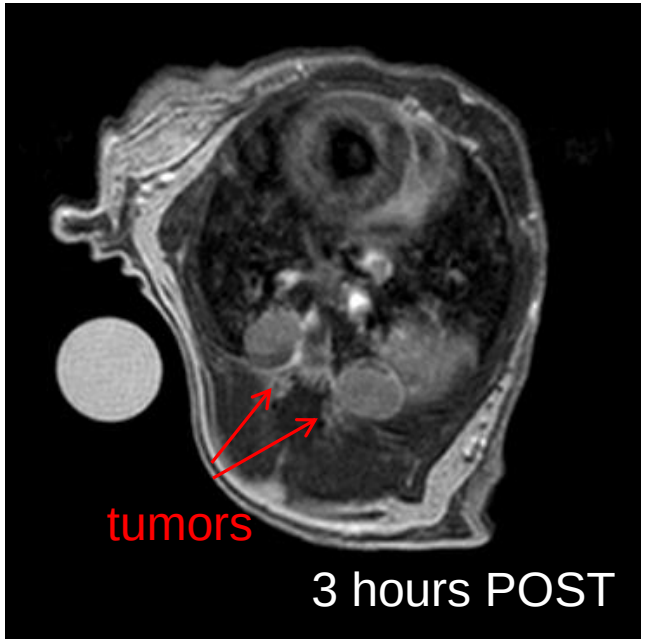
B : Gd = 10 : 1

S Aime, et al Org. Biomol. Chem., 2008, 6, 4460–4466
Geninatti-Crich et al. Chemistry. 2011 Jul 18;17(30):8479-86.

MRI analysis (Bruker 7T) on Pulmonary Metastasis obtained injecting i.v. 50000 TUBO cells (mammary carcinoma) three weeks before irradiation

T1 weighted AXIAL IMAGES





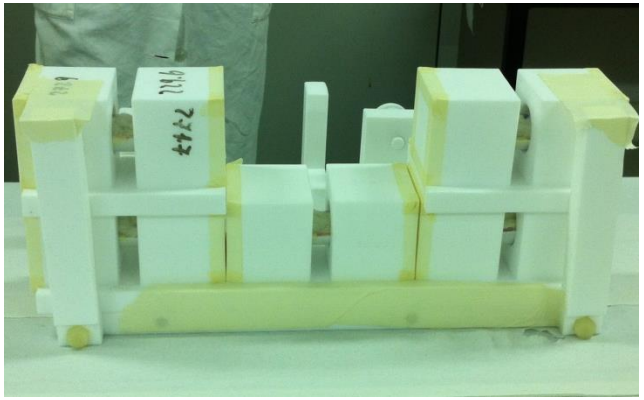
Boron concentration
Tumor: 43 ug/g
Muscle: 16 ug/g

BNCT at the TRIGA-Mark-II reactor, LENA, Pavia

Neutron irradiation 7 minutes; Reactor Power 250 kW

6h after Boron administration

95% ^{6}Li-enriched lithium carbonate



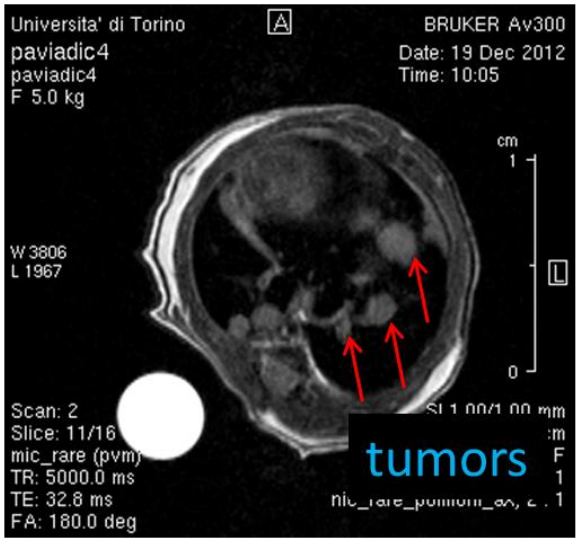
In collaboration with N. Protti, F. Ballarini, S. Bortolussi, S. Altieri, [1]University of Pavia, Department of Nuclear and Theoretical Physics

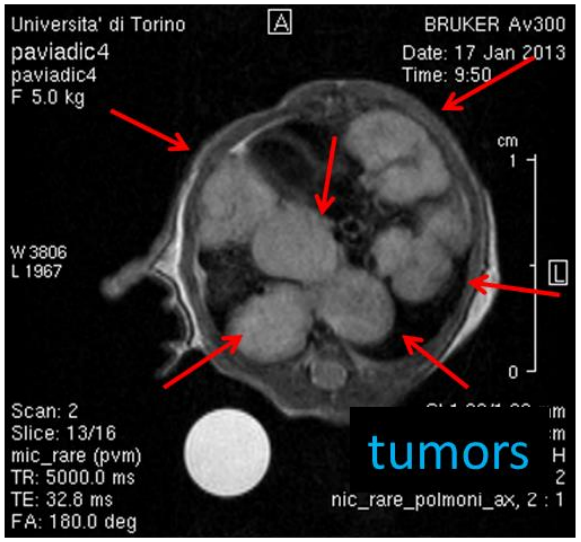
T2-weighted lung metastasis RARE images

Day 0

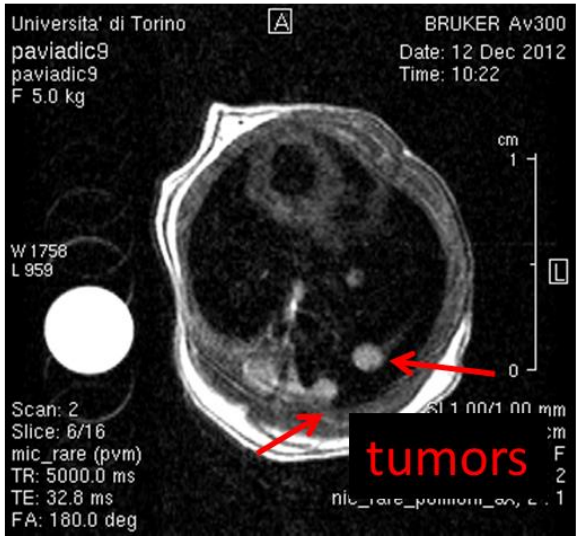
30 Days after BNCT

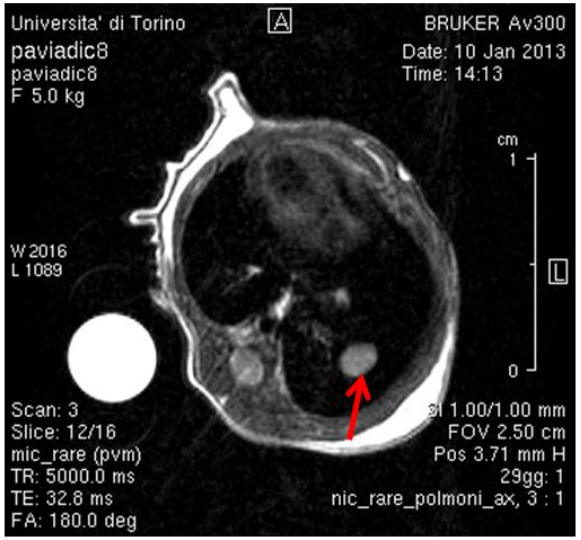
Irradiated Ctrl mice wo boron



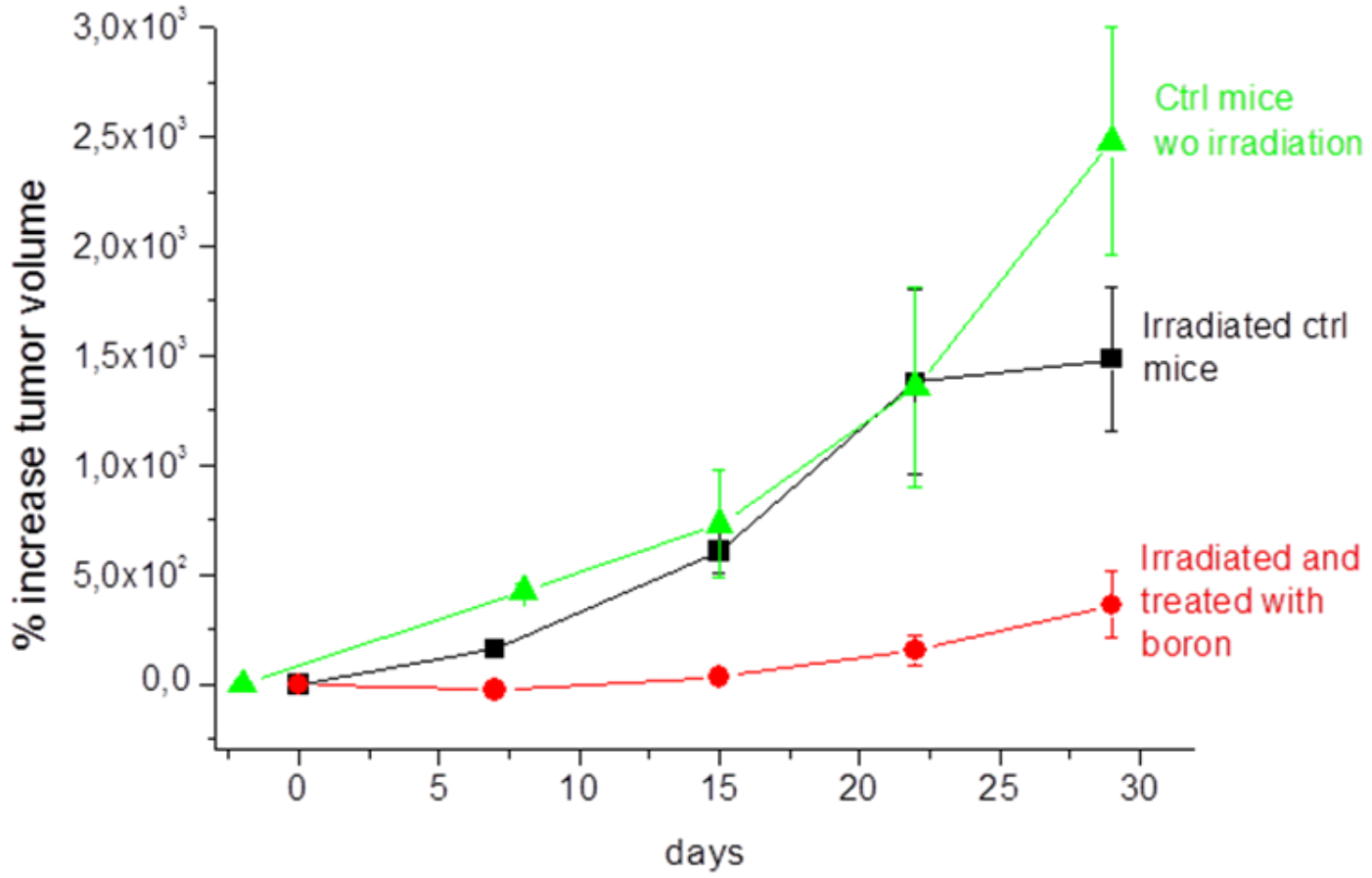


Irradiiated and boron treated mice





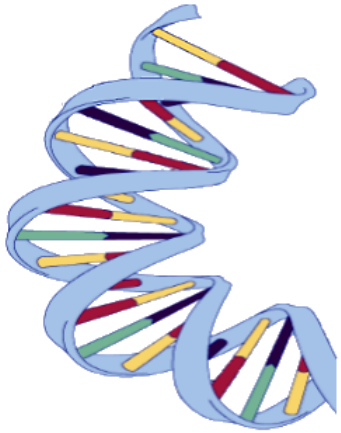
Relative tumor volume measured by MRI after irradiation (15 minutes, TRIGA-Mark-II reactor, LENA, Pavia)
Reactor power : 250 kW



Acknowledgements

Silvio Aime
Diego Alberti
Marta Cadenazzi
D. Burghelea
W. Dastrù

University of Torino
Department of Molecular Biotech
MOLECULAR IMAGING LAB



J. Cutrin
S. Lanzardo
L. Conti
F. Cavallo
Cristina Grange
Marta Tapparo
Giovanni Camussi

University of Torino
Department of Molecular Biotech

S. Altieri
N. Protti
S. Bortolussi

University of Pavia
Department of Nuclear and Theoretical Physics

cost
EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

Action TD1004

Regione Piemonte (PIIMDMT and nano-IGT projects), MIUR (PRIN 2009235JB7)