Combining nanoparticles with radiation rationally – are we there yet?

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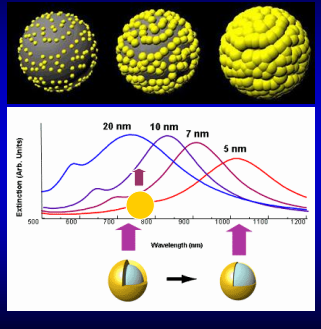
Disclosure Information
Sunil Krishnan

I have the following financial relationships to disclose:

Grant or research support from:
Genentech, Merck, Hitachi, Shell, MPOB, FUSF
Honoraria from:
Carestream Molecular Imaging

I **WILL** include discussion of investigational or off-label use of a product in my presentation.

Gold nanoshells



- Dielectric silica core
- Thin gold coating
- Light absorbed by the free electrons on the gold is converted to heat
- Core-shell ratio determines the optical characteristics

Electromagnetic spectrum

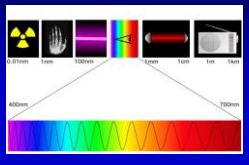
Light – non-ionizing, safe, affordable, non-invasive

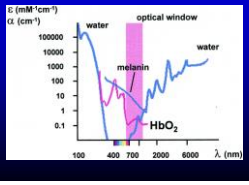
Penetration depth in tissues depends on the wavelength and tissue type

Near infrared region

Clinical optical window

Tissue penetration up to 2-3 cm





Why gold nanoshells?

Robust structure
less susceptible to chemical/thermal denaturation

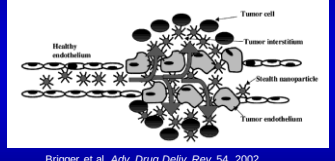
Biocompatiblity (silica, noble metal surface)
acceptable toxicity at high concentrations (up to 3% of body weight) of gold in the body

Very high absorption cross section
~ 3.8 x 10^{-14} m^2 vs. 1.66 x 10^{-20} m^2 for ICG
L.R.Hirsch et al. PNAS, 100 (23), 13549-13554.

Ease of surface modification for bioconjugation and PEGylation
less uptake in liver
longer biological half-life in blood due to slower clearance from the body

Accumulation in tumors

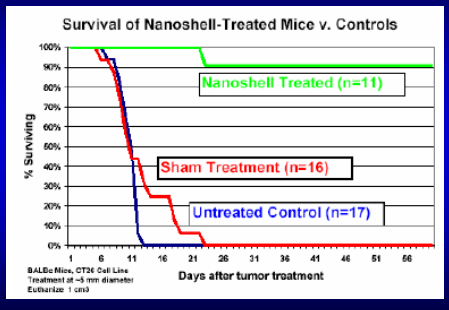
Enhanced Permeability and Retention (EPR) effect through leaky vasculature and inefficient lymphatic drainage of tumors (size : 60 to 400 nm size)



Brigger et al, *Adv. Drug Deliv. Rev.* 54, 2002

Wide interendothelial junctions, incomplete or absent basement membrane, a dysfunctional lymphatic system and large number of transendothelial channels.

Gold nanoshells



O'Neal P et al. Cancer Lett 209(2):171-6, 2004

Is thermoradiotherapy underutilized ?

Thermoradiotherapy is underutilized for the treatment of cancer

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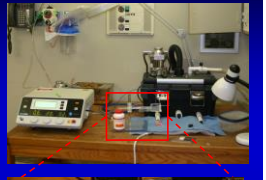
Invasive techniques
No real-time temperature monitoring or dosimetry
No uniform description of dose, time attributes

Gold nanoshell mediated hyperthermia

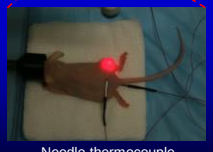
Laser	Diomed – 15 plus
λ_{em}	808 nm
P_{max}	15 W
Delivery	Fiber optic cable collimating lens
Beam Dia	1 cm
Exp time	15 to 20 minutes
Aiming beam	632 HeNe laser
Class 3b or 4	



Diagaradjane et al. Nano Lett. 2008 8(5):1492-500

Temperature measurements

Invasive method



Needle thermocouple

Non-invasive method

Magnetic Resonance Thermal imaging (MRTI)

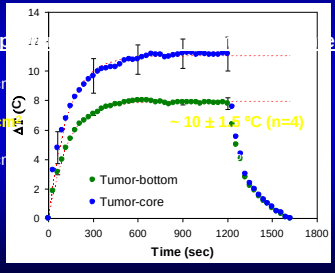
Thermocouple measurements

Laser p **er)**

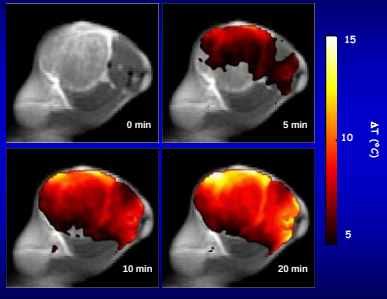
0.8 W/ cr

0.6 W/ cm

0.4 W/ cr

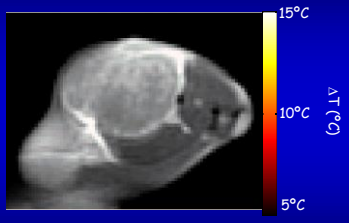


MRTI



- **MRTI & thermocouple measurements demonstrated a ΔT ~ 11 °C (from a baseline of ~ 30 °C)**
- Irradiation with laser alone (no nanoshells) demonstrated a **ΔT ~ 2 to 3 °C**

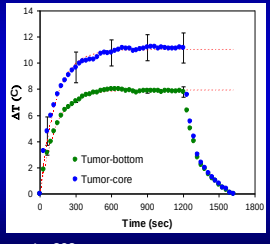
Real time MRTI



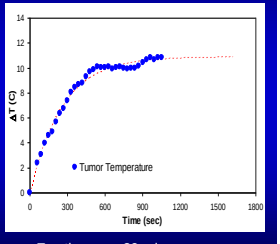
(0.6 W/cm^2 for 20 min at 808-nm)

Temperature profiles

Thermocouple



MRTI

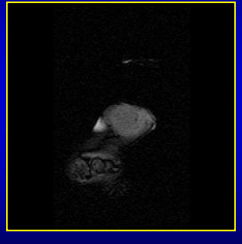


λ= 808 nm;
Power = 0.6 W (75 % duty cycle);
Power density = 350 mW/cm^2

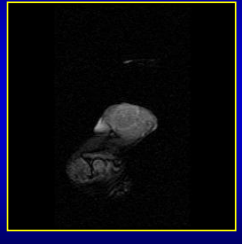
Exp time = 20 min
Beam Dia = 1 cm

Dynamic contrast enhanced MRI

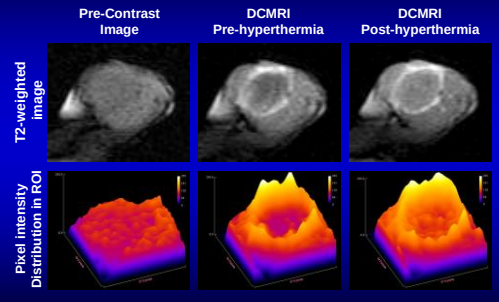
Pre-Hyperthermia



Post-Hyperthermia



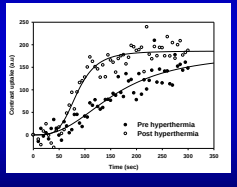
Dynamic contrast enhanced MRI



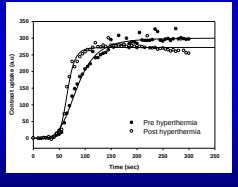
Increased perfusion with ~ 2-fold increase in the contrast enhancement was observed immediately (3 to 5 min) after gold nanoshell mediated hyperthermia.

Contrast uptake

Tumor Center



Whole Tumor



Experimental groups

Control	(n=7)
Hyperthermia	(n=7)
Radiation	(n=7)
Hyp + Rad	(n=7)

Radiation Dose

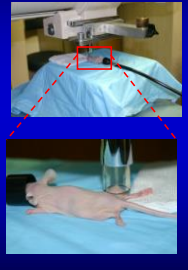
Phillips RT-250 Orthovoltage X-ray Unit

125 Kv; 20 mA ; 2 mm Al filter

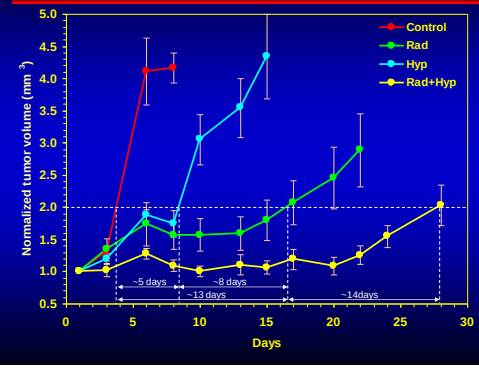
Skin cone – 1.5 cm diameter

Total delivered dose = 10 Gy

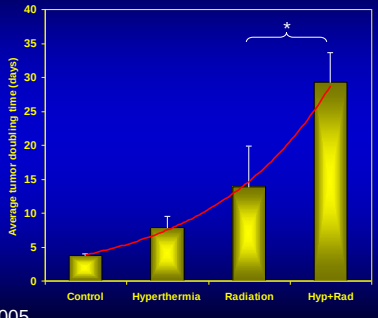
Radiation



Normalized tumor volume

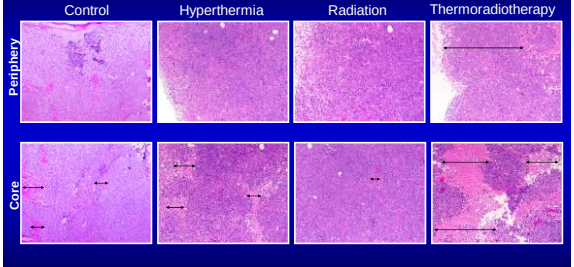


Tumor doubling time

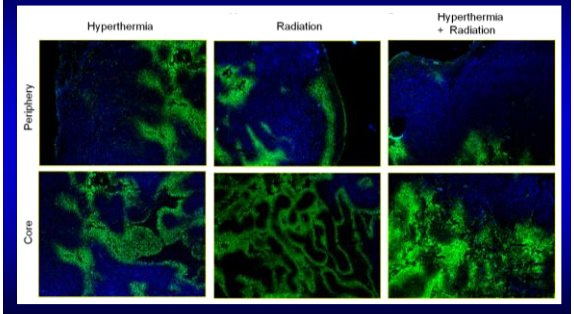


* P < 0.005

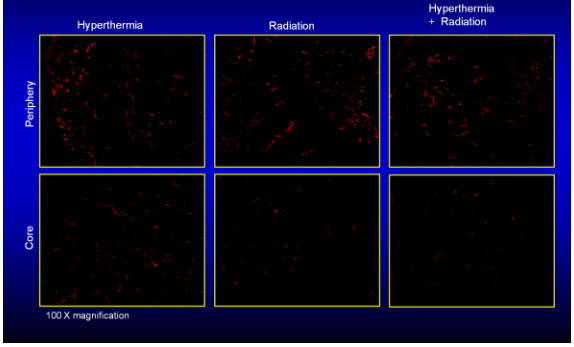
H&E



Hypoxia, cell proliferation, perfusion

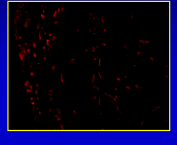


Microvessel staining – CD31

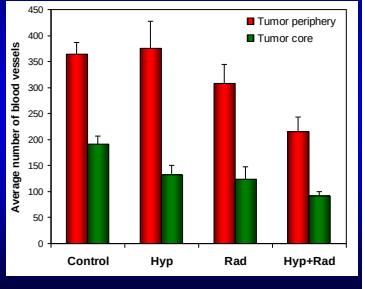


Microvessel Density

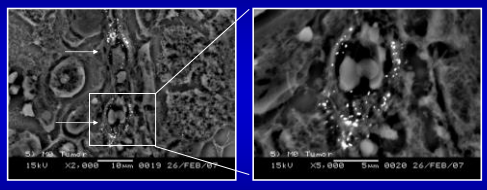
Raw Image



Processed Image



Scanning Electron Microscopy

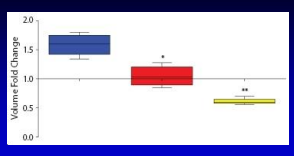


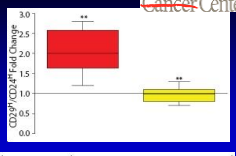
Conclusions

- Optically activated gold nanoshells serve as a novel means to non-invasively generate hyperthermia.
- Temperature profiles can be monitored regionally and globally within tumors using MRTI.
- Combining low-dose hyperthermia with radiation therapy leads to potent radiosensitization that is characterized by the **dual effect** of:
  - **(a) an initial increase in vascular perfusion of the hypoxic core of the tumor resulting in tumor cell radiosensitization, and**
  - **(a) a subsequent disruption of vasculature that results in a profound increase in the size of the necrotic core of the tumor.**

Conclusions

Early effects	Late effects
Anti-hypoxic effect	**Vascular disrupting effect?**

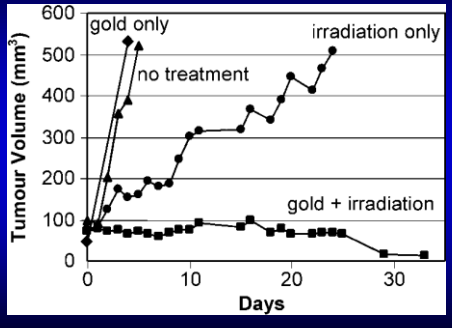




Treatment (Tumor T7)	10000	1000	100	10	Tumor Initiation Cell Frequency (TIC) 95% CI
Mock	6/6	6/6	1/6	0/6	1/323 (128-814)
6 GY	6/6	6/6	2/6	1/6	1/175 (61-498)
6 GY + 42°C	6/6	3/6	0/6	0/6	1/1626 (575-4602)*

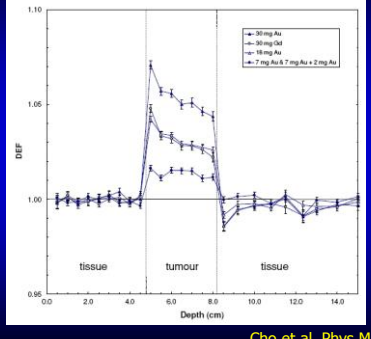
Atkinson RA, et al. Sci Translat Med, 2010; 2(55):55ra79

Physical dose enhancement



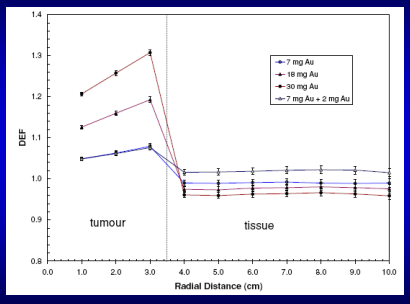
Hainfeld et al. Phys Med Biol 2004; 49: N309-15

Physical dose enhancement



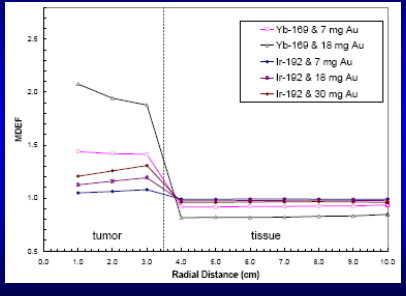
Cho et al. Phys Med Biol 2009

Physical dose enhancement



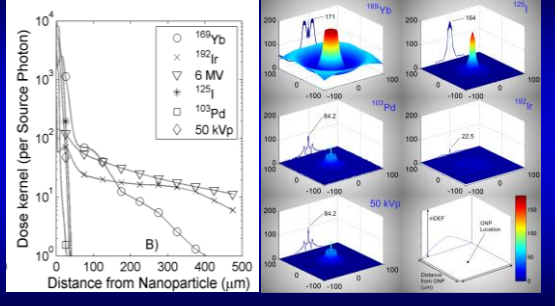
Cho et al. Phys Med Biol 2009

Physical dose enhancement



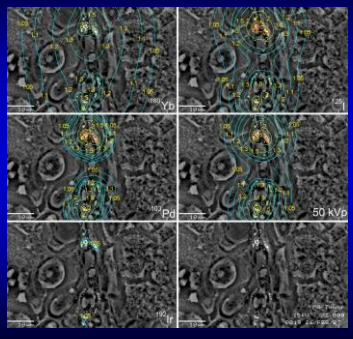
Cho et al. Phys Med Biol 2009, 54(16):4889-905.

Physical dose enhancement



Cho, Krishnan Med Phys 2010

Physical dose enhancement



Enhancing physical dose enhancement

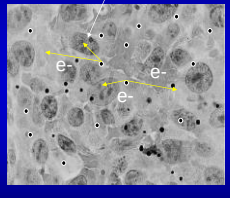
nanoparticles

Passive targeting

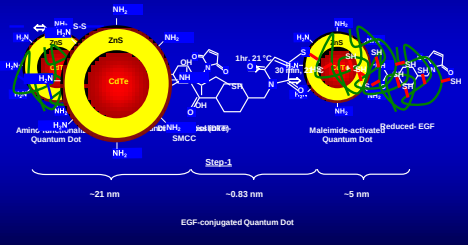
nanoparticles
+ peptides

Active targeting

on the order of 10 μm

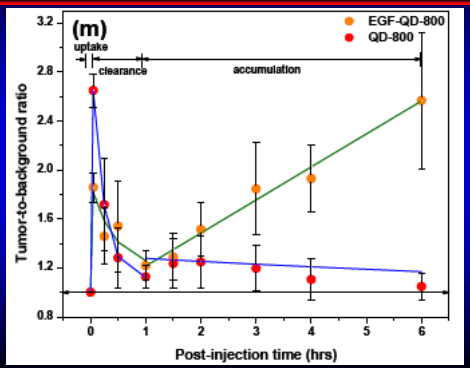


Peptide-nanoparticle bioconjugates

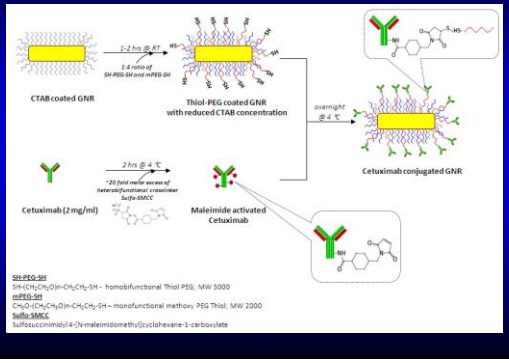


Diagaradjane et al. Clin Cancer Res. 2008, 14(3):731-41.

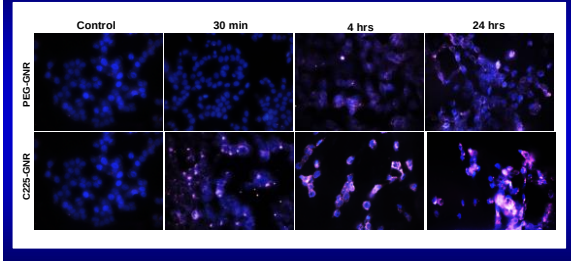
In vivo quantification



Conjugated gold nanorod

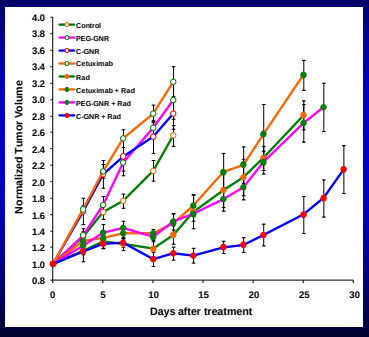


Gold nanorod

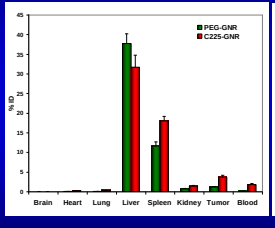


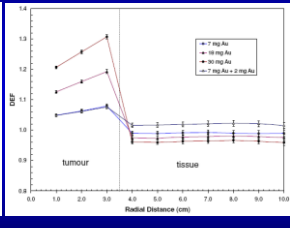
Krishnan lab, unpublished data

Tumor regrowth delay



Biodistribution

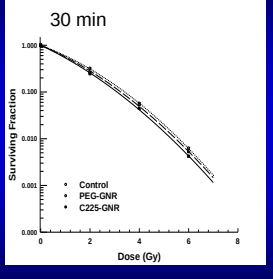




Cho SH. Phys Med Biol 2005; 50: N163-73

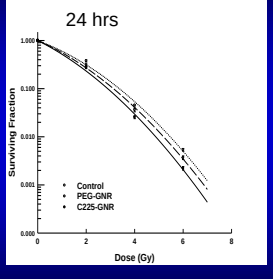
Clonogenic survival

30 min



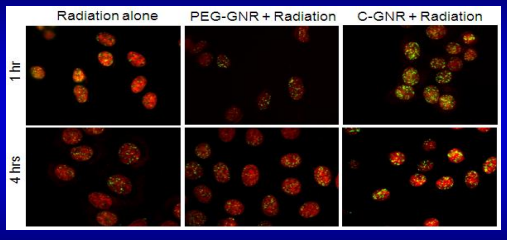
DEF 10%

24 hrs

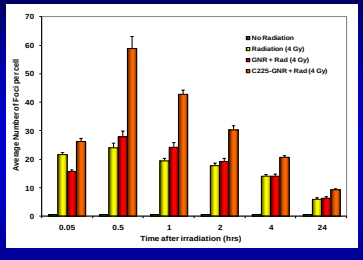


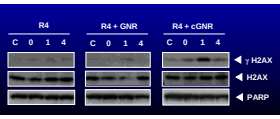
DEF 15%

DNA damage

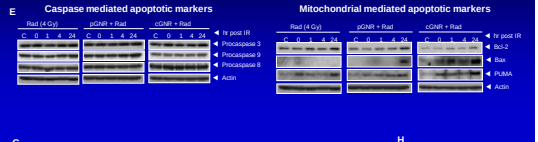


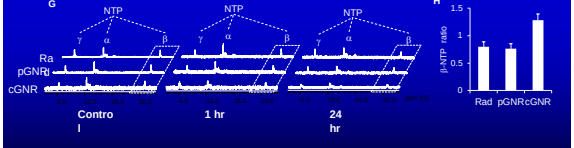
DNA damage



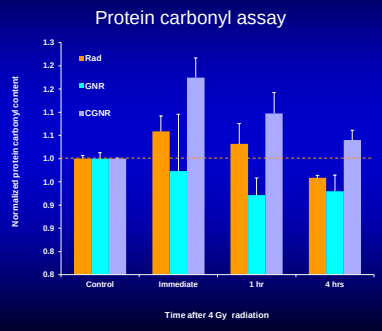


Apoptotic markers

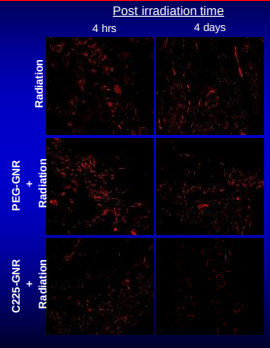




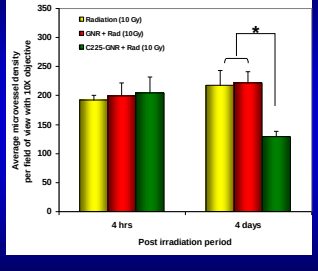
Total oxidative stress



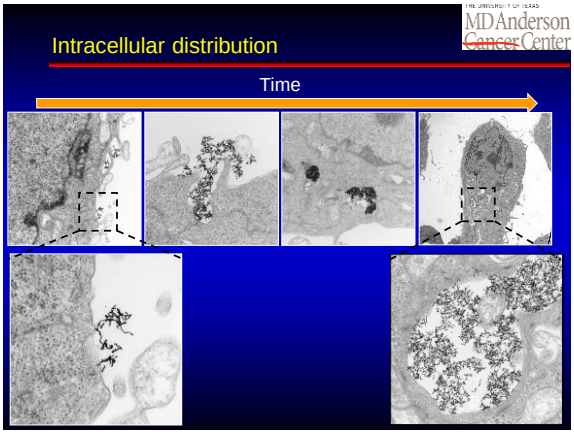
Tissue effects



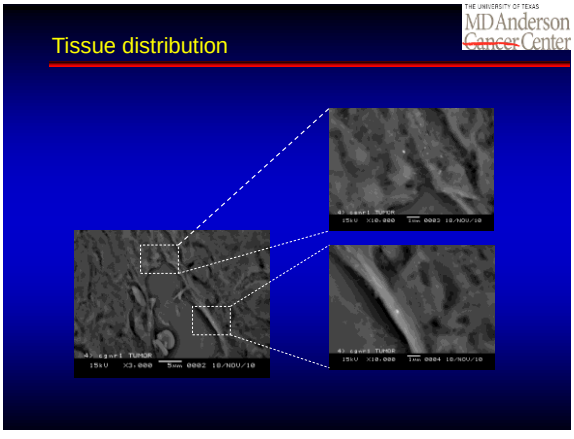
Tissue effects



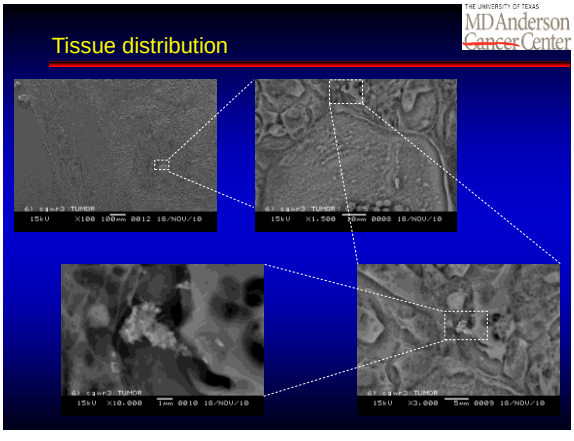
Intracellular distribution



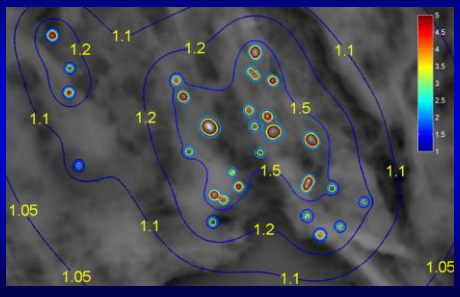
Tissue distribution



Tissue distribution



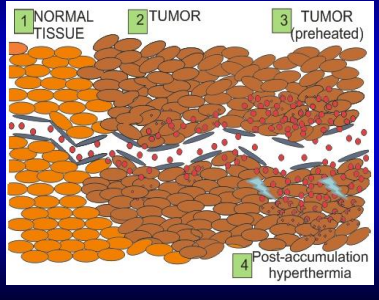
Modeling dose



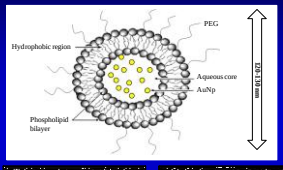
Summary

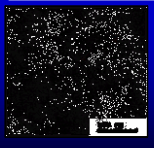
- Targeted payload delivery feasible with smaller nanoparticles bioconjugated to peptides/antibodies
- While the tumor accumulation does not increase dramatically, the distribution is altered at the cellular (internalized) and tissue (more perivascular) levels
- Both the intracellular localization and the perivascular sequestration result in greater radiosensitization at a biological level, mediated primarily by:
  - Increased DNA damage and downstream signaling
  - Increased oxidate stress
  - Increased vascular disruption

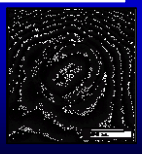
Another approach

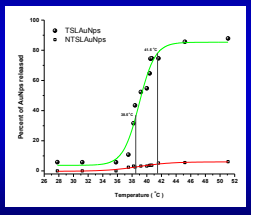


Thermosensitive liposome

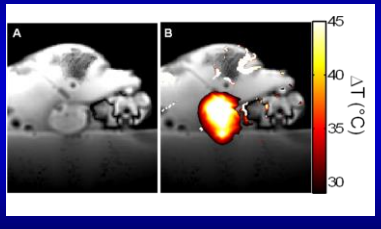




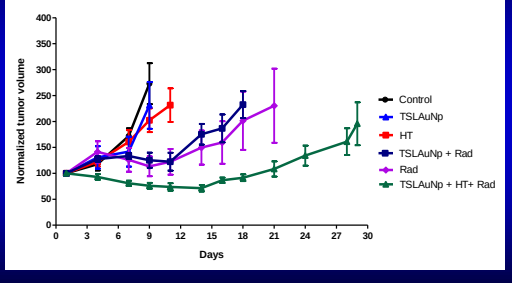




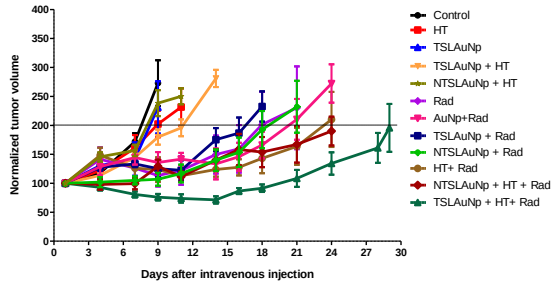
Focused ultrasound



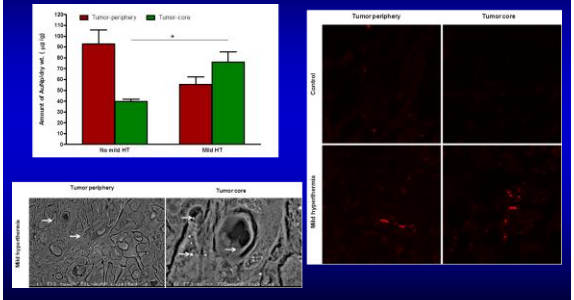
Radiosensitization



Radiosensitization



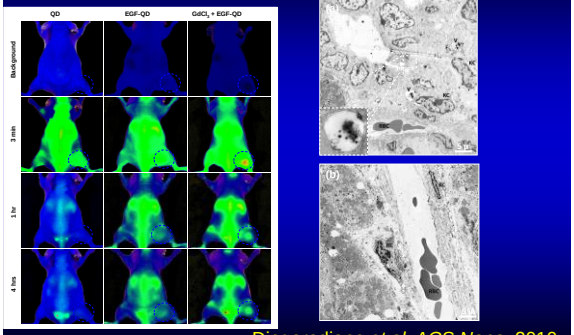
Deep penetration of tumors



Summary

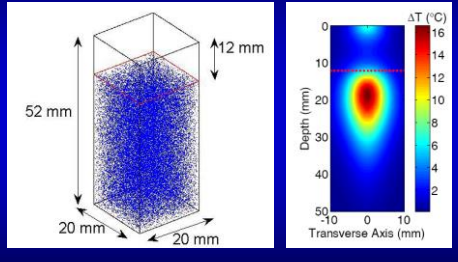
- Delivery of nanoparticles using thermosensitive liposomes enhances deep penetration of nanoparticles when triggered by hyperthermia
- Deep penetration of gold nanoparticles improves radiosensitization independent of the effect of hyperthermic radiosensitization
- In principle, this could be a class solution for a variety of tumors accessible by ultrasound

RES capture



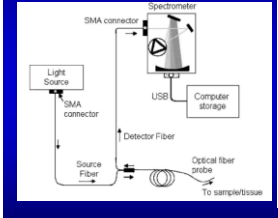
Diagaradjane *et al. ACS Nano*, 2010

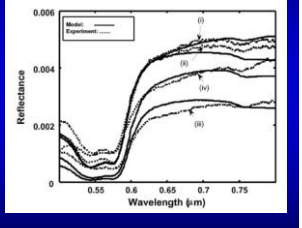
Thermal dosimetry



Cheong S-K et al. Med Phys 36(10):4664-71, 2009

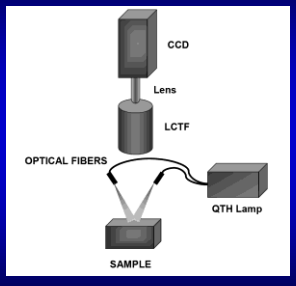
Quantifying gold nanoparticles in tumor





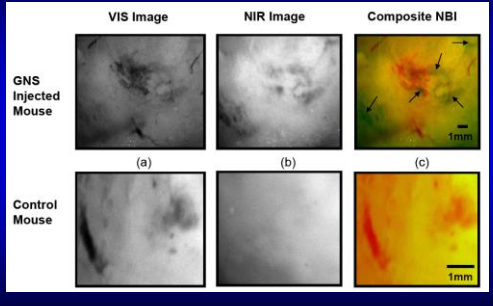
Zaman et al IEEE J Sel Top Quant Elec 13(6):1715-20, 2007.

Imaging gold nanoparticles in tumors

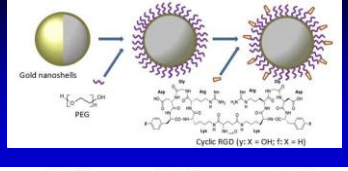


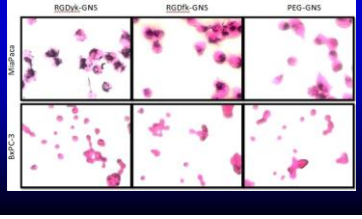
Puvanakrishnan P et al. J Biomed Optics 14(2):024044, 2009.

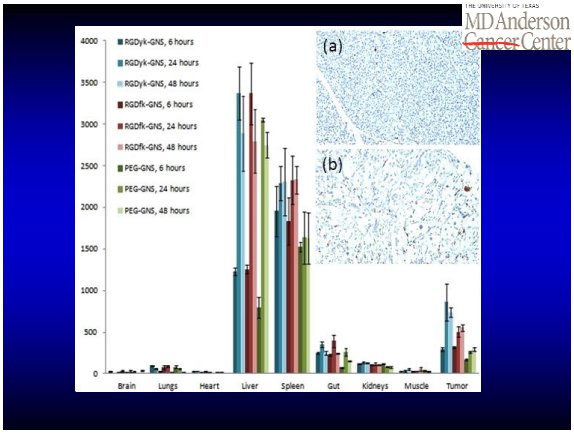
Imaging gold nanoparticles in tumors

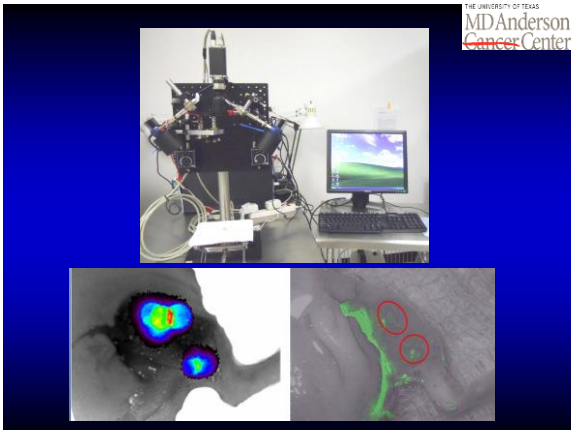


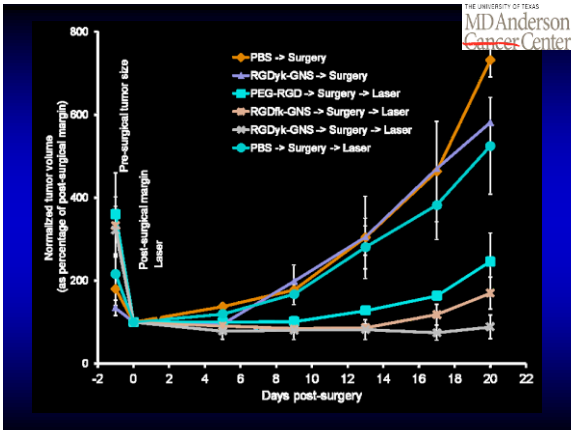
Puvanakrishnan P et al. J Biomed Optics 14(2):024044, 2009.



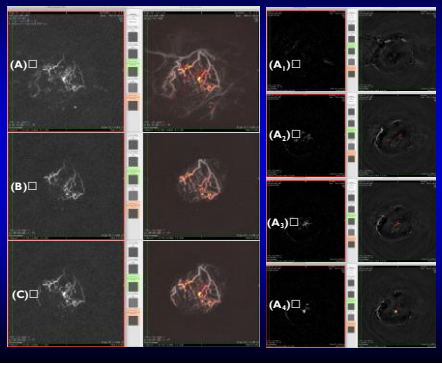



THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
RGDyk-GNS, 6 hours
RGDyk-GNS, 24 hours
RGDyk-GNS, 48 hours
RGDfk-GNS, 6 hours
RGDfk-GNS, 24 hours
RGDfk-GNS, 48 hours
PEG-GNS, 6 hours
PEG-GNS, 24 hours
PEG-GNS, 48 hours
(a)
(b)
4000
3500
3000
2500
2000
1500
1000
500
0
Brain
Lungs
Heart
Liver
Spleen
Gut
Kidneys
Muscle
Tumor


THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center


THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
PBS -> Surgery
RGDyk-GNS -> Surgery
PEG-RGD -> Surgery -> Laser
RGDfk-GNS -> Surgery -> Laser
RGDyk-GNS -> Surgery -> Laser
PBS -> Surgery -> Laser
Normalized tumor volume
(as percentage of post-surgical margin)
Pre-surgical tumor size
Post-surgical margin
Laser
800
600
400
200
0
-2
0
2
4
6
8
10
12
14
16
18
20
22
Days post-surgery

Photoacoustic imaging

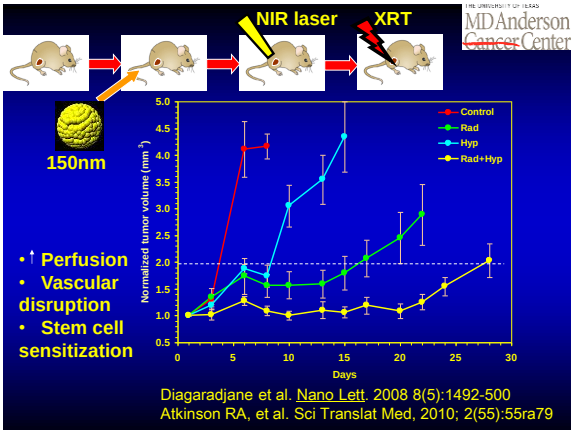


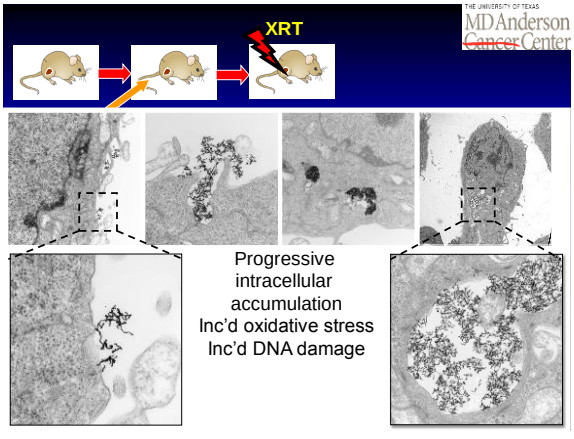
Summary

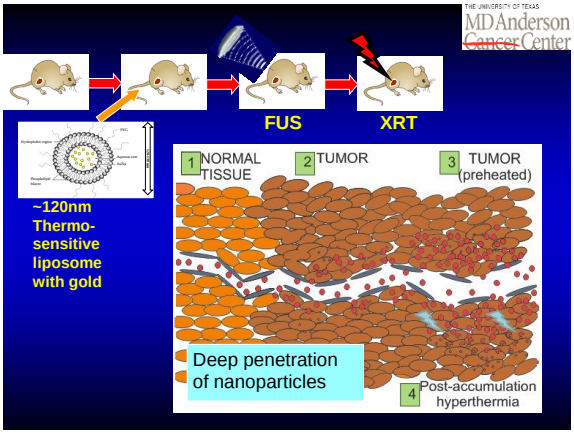
- Larger particles for vascular-targeted applications (thermo-ablation, hyperthermia, vascular imaging)
- Smaller particles for parenchymal applications (imaging, targeted payload delivery)
- Combinations of above
- Unresolved issues related to clinical translation

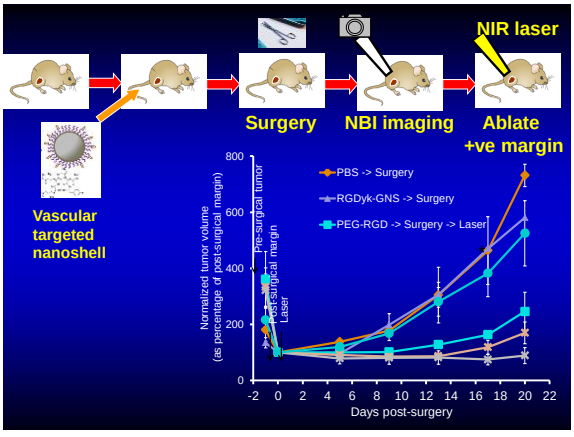
Summary

- Overcome radioresistance via
  - increased perfusion, reduced hypoxia
  - stem cell sensitization
  - vascular disruption
  - physical radiation dose enhancement
    - oxidative stress
    - DNA damage
  - triggering these effects deep within tumor core


NIR laser
XRT
MD Anderson Cancer Center
150nm
Control
Rad
Hyp
Rad+Hyp
Normalized tumor volume (mm³)
Days
• ↑ Perfusion
• Vascular disruption
• Stem cell sensitization
Diagaradjane et al. Nano Lett. 2008 8(5):1492-500
Atkinson RA, et al. Sci Translat Med, 2010; 2(55):55ra79


XRT
MD Anderson Cancer Center
Progressive intracellular accumulation
Inc'd oxidative stress
Inc'd DNA damage


FUS
XRT
MD Anderson Cancer Center
~120nm Thermo-sensitive liposome with gold
1 NORMAL TISSUE
2 TUMOR
3 TUMOR (preheated)
Deep penetration of nanoparticles
4 Post-accumulation hyperthermia



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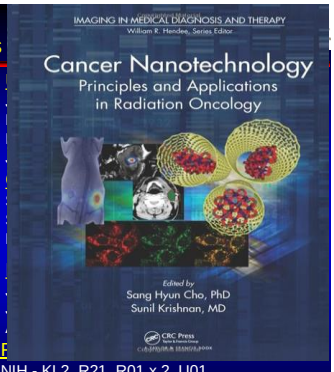
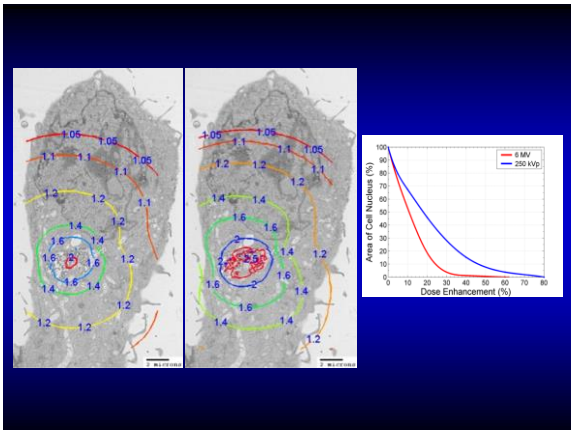
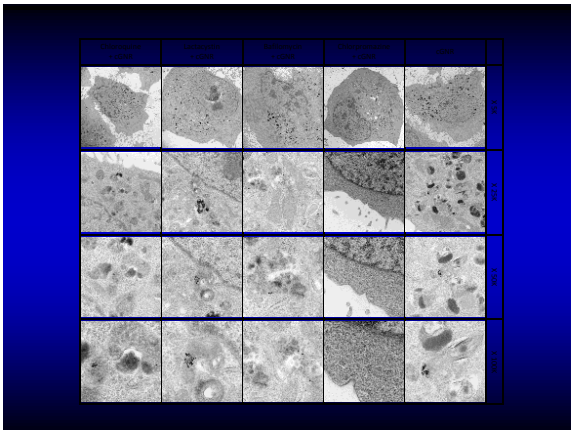
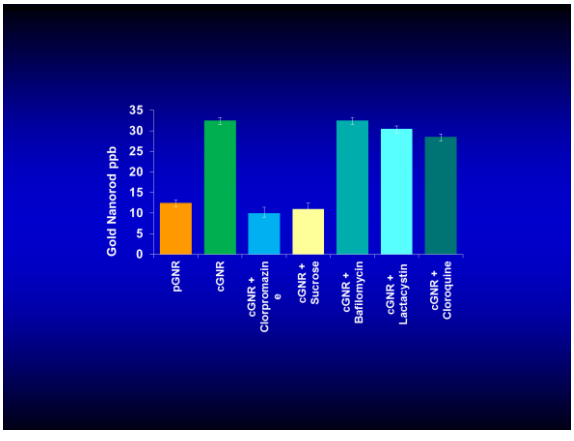
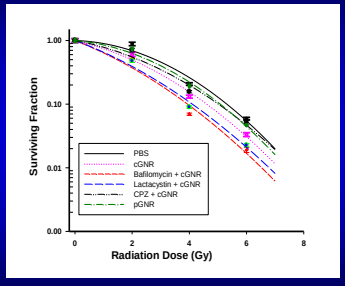


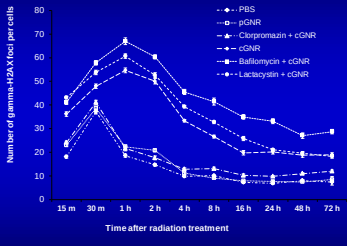
Thank you

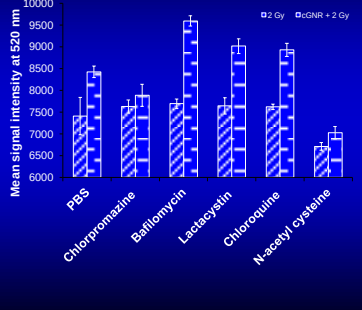

1.05
1.1
1.2
1.4
1.6
2
6 MV
250 kVp
Area of Cell Nucleus (%)
Dose Enhancement (%)

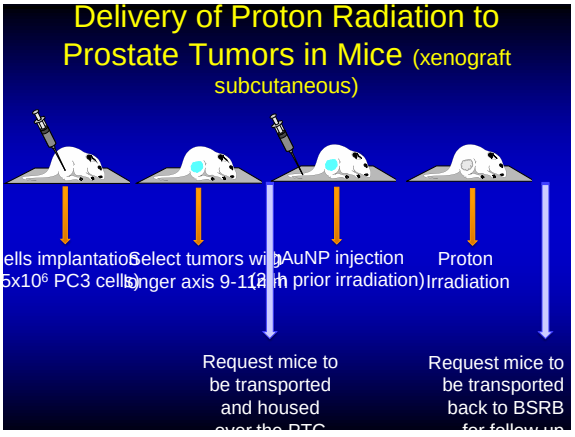


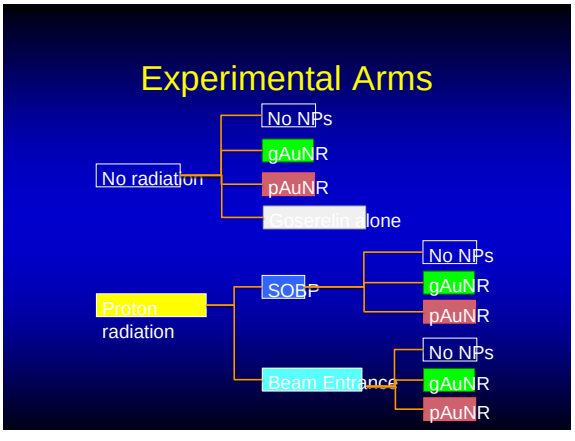

Gold Nanorod ppb
35
30
25
20
15
10
5
0
pGNR
cGNR
cGNR + Clorpromazine
cGNR + Sucrose
cGNR + Bafilomycin
cGNR + Lactacystin
cGNR + Cloroquine

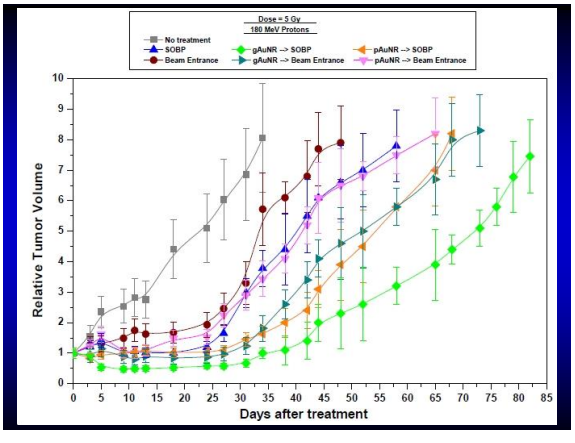

Surviving Fraction
1.00
0.10
0.01
0.00
0
2
4
6
8
Radiation Dose (Gy)
PBS
cGNR
Bafilomycin + cGNR
Lactacystin + cGNR
CPZ + cGNR
pGNR

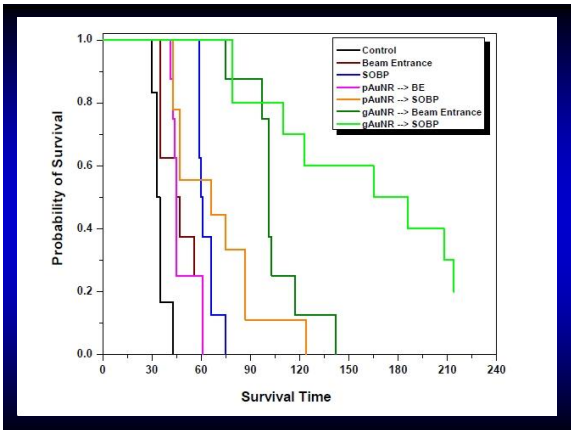

Number of gamma-H2A.X foci per cells
80
70
60
50
40
30
20
10
0
15 m
30 m
1 h
2 h
4 h
8 h
16 h
24 h
48 h
72 h
Time after radiation treatment
PBS
pGNR
Clorpromazin + cGNR
cGNR
Bafilomycin + cGNR
Lactacystin + cGNR


Mean signal intensity at 520 nm
10000
9500
9000
8500
8000
7500
7000
6500
6000
2 Gy
cGNR + 2 Gy
PBS
Chlorpromazine
Bafilomycin
Lactacystin
Chloroquine
N-acetyl cysteine


Delivery of Proton Radiation to Prostate Tumors in Mice (xenograft subcutaneous)
ells implantation
5x10⁶ PC3 cells)
Select tumors with
longer axis 9-11 mm
gAuNP injection
(2 h prior irradiation)
Proton
Irradiation
Request mice to
be transported
and housed
Request mice to
be transported
back to BSRB


Experimental Arms
No radiation
No NPs
gAuNR
pAuNR
Cetuximab alone
Proton
radiation
SOBP
No NPs
gAuNR
pAuNR
Beam Entrance
No NPs
gAuNR
pAuNR


Dose = 5 Gy
180 MeV Protons
No treatment
SOBP
Beam Entrance
gAuNR --> SOBP
gAuNR --> Beam Entrance
pAuNR --> SOBP
pAuNR --> Beam Entrance
Relative Tumor Volume
Days after treatment



Acknowledgment