ICTR-PHE 2016

New Technologies session

Nanoparticle Enhanced MRI-Guided Radiation therapy for brain melanoma metastases.

Proof of Concept before Phase I Trial.

Shady KOTB
3rd year Ph.D. candidate

Supervisors : Dr. Lucie Sancey
Pr. Olivier Tillement

Lyon, France.

16th February 2016

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1.1 Concept of radiosensitizers in radiotherapy.

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2. Radiosensitization

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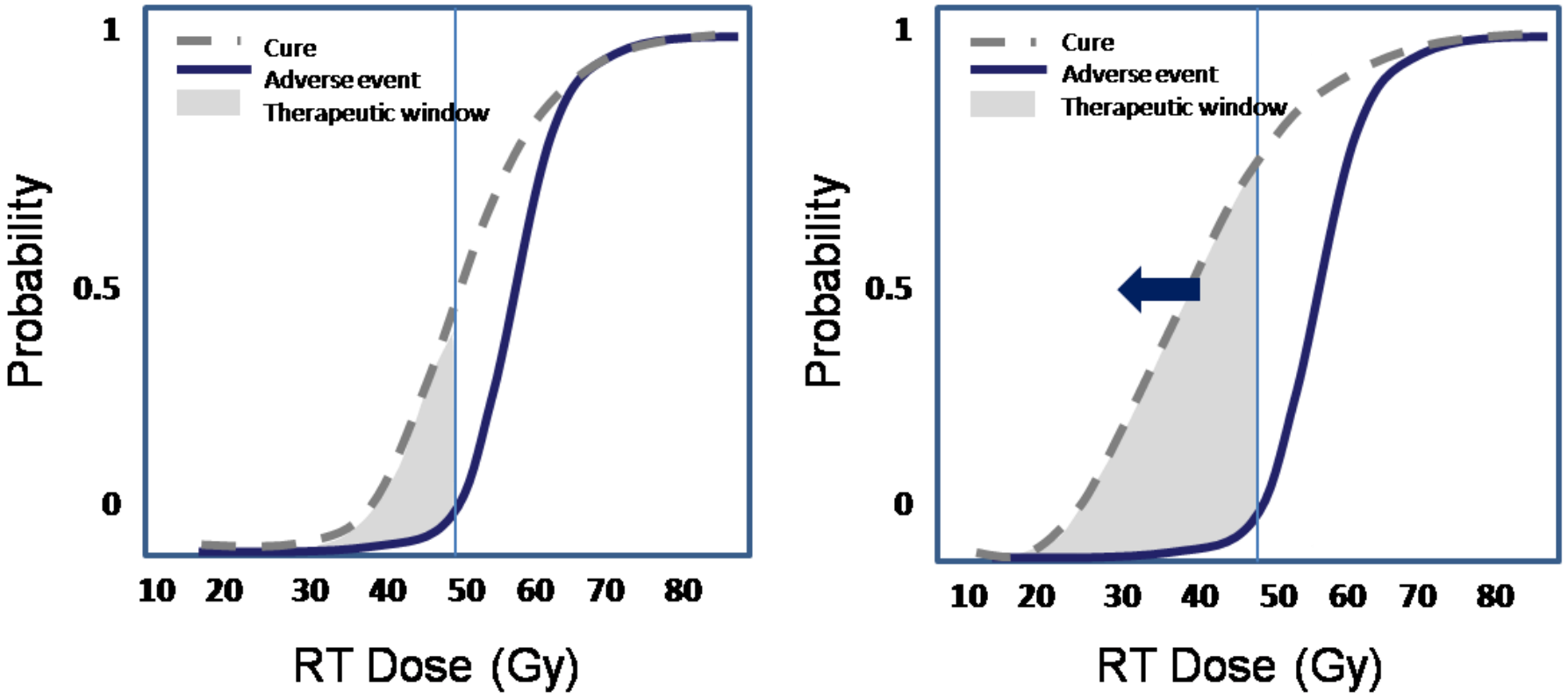
2.2 *In-vivo* intracerebral studies of B16-F10.

3. Conclusion

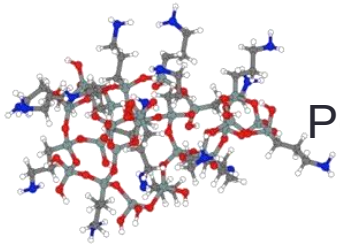
1- Radiosensitizers concept

WHY? ➡ RADIOTHERAPY OPTIMIZATION



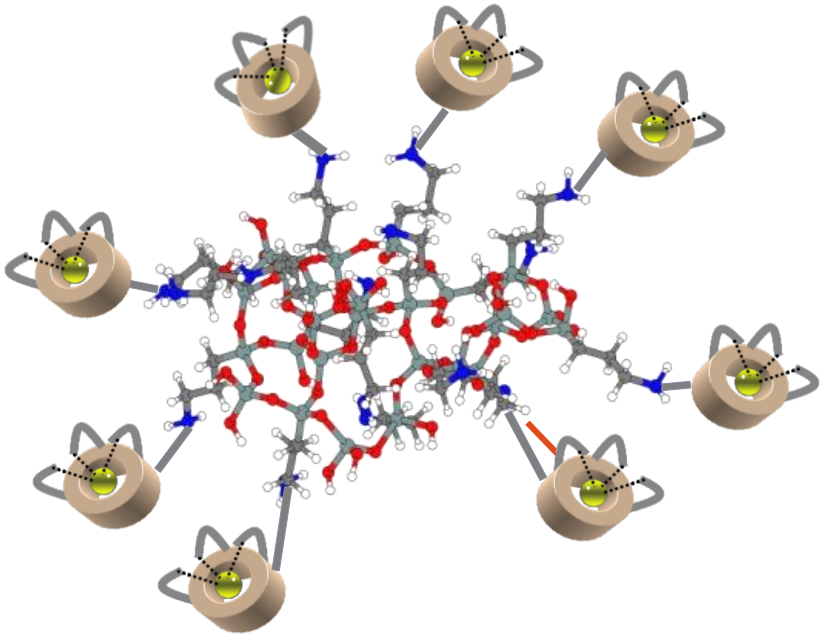
Schematic representation of opening the radiotherapy therapeutic window provided by radiosensitizers (left: radiotherapy alone; right: radiotherapy in the presence of radiosensitizers).

AGuIX®: Theranostic Nanoparticles



Polysiloxane network

DOTA(Gd)



Ultra-small Size : 3-5 nm

High colloidal stability

Small enough for renal elimination

Patent : *1053389*

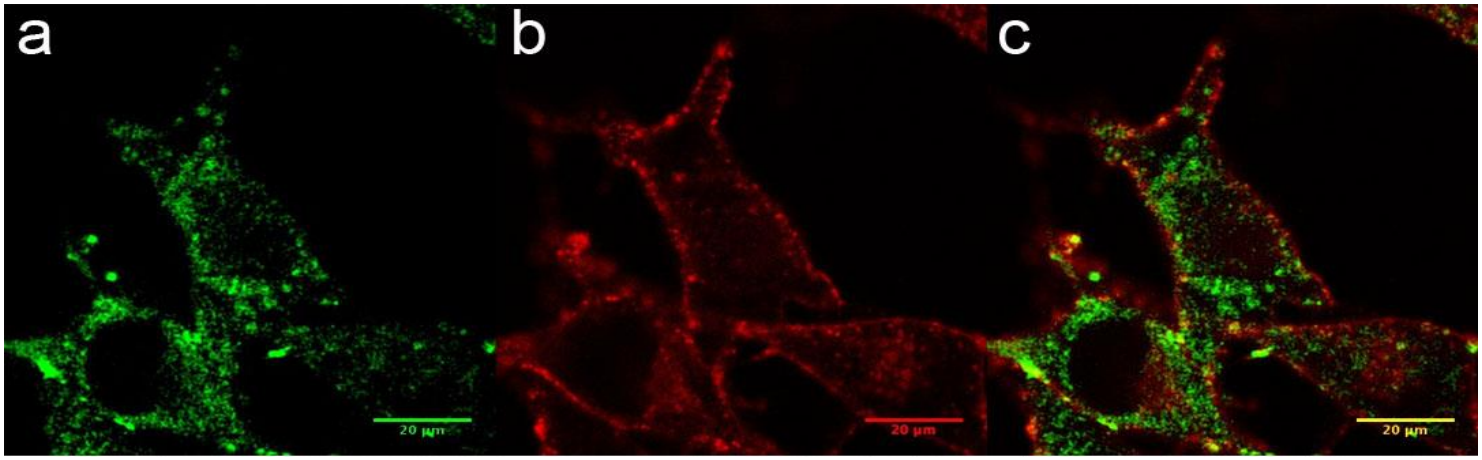
2- Radiosensitization of melanoma B16-F10 ***In vitro***

Mouse model for human melanoma

In vitro investigations – Cells uptake

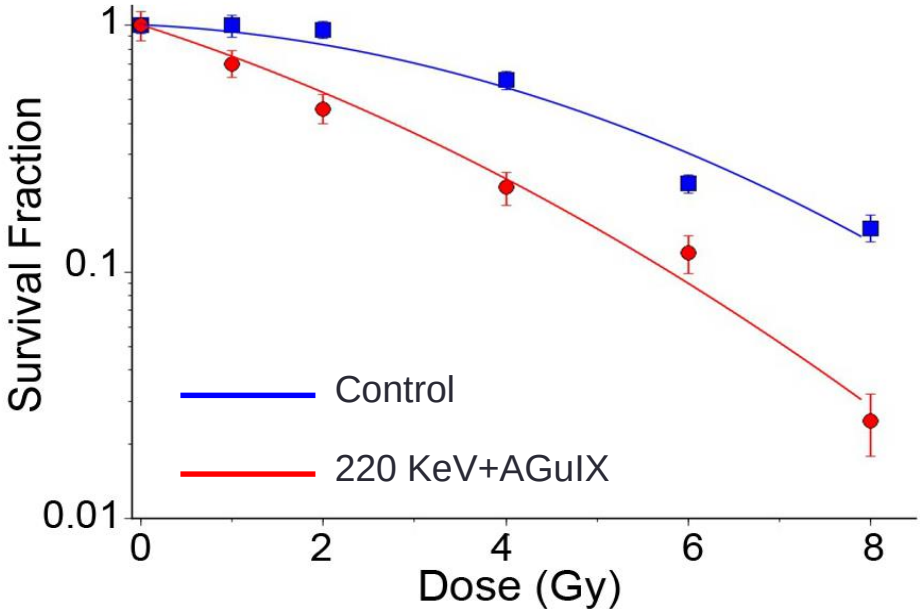
Confocal Microscopy on live cells

Nanoparticles uptake after incubation with 0.6 mg/L AGuIX for 1 hour (Corresponds to 0.4 pg Gd/cell internalized)



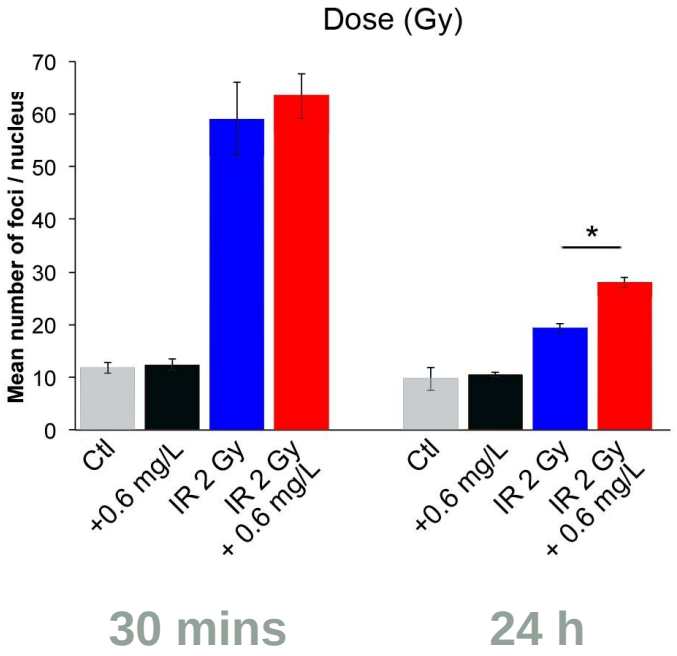
Fluorescence images of B16F10 1 hour after incubation with AGuIX coupled to FITC **(a)**, Plasma membrane labeling in red **(b)** and the merged image **(C)**.

In vitro investigations - Clonogenic Assay 220-keV X-ray (2 Gy/min)



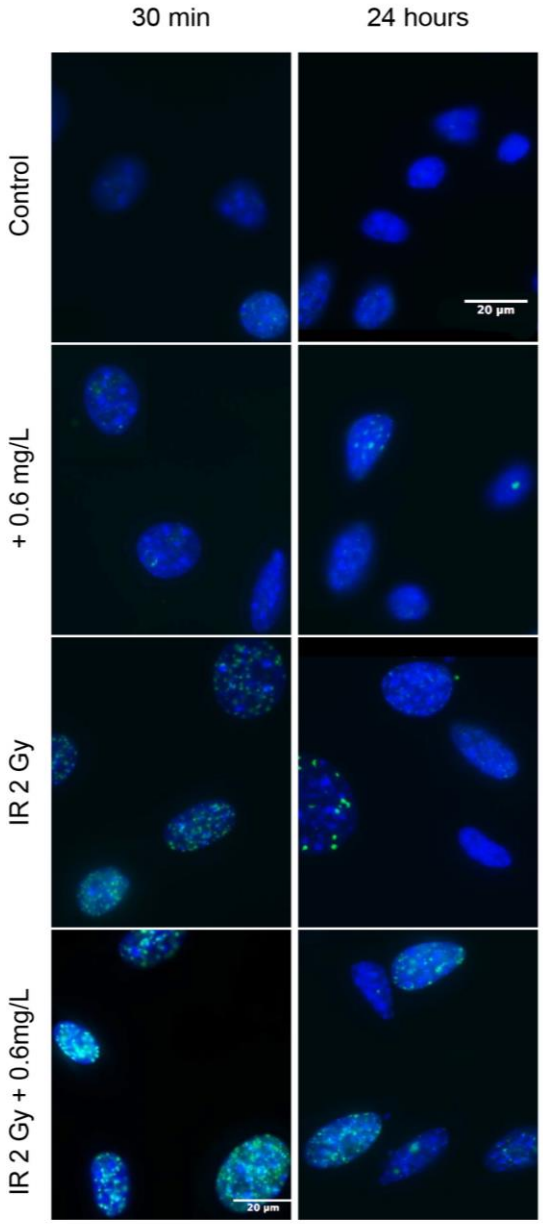
	α (Gy $^{-1}$)	D50% Gy	SF 2 Gy	% EF 2 Gy	SER 2 Gy	DEF
Control	0.04	4.5	0.96	-	-	-
0.6 mg/L AGuIX	0.26	1.8	0.56	**52 %**	2.08	1.3

In vitro investigations γ-H2AX immunofluorescence assay



30 mins **24 h**

	No. of DSBs.	% Enhancement
2 Gy	19 ±0.8	-
2 Gy+0.6 mg/L AGuIX	28 ± 1	**45**

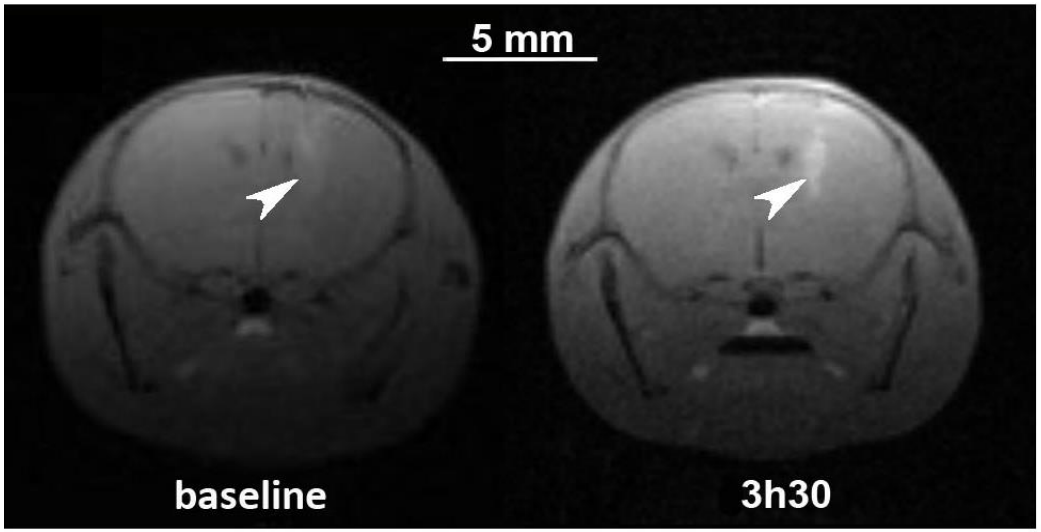


2- Radiosensitization of melanoma B16-F10

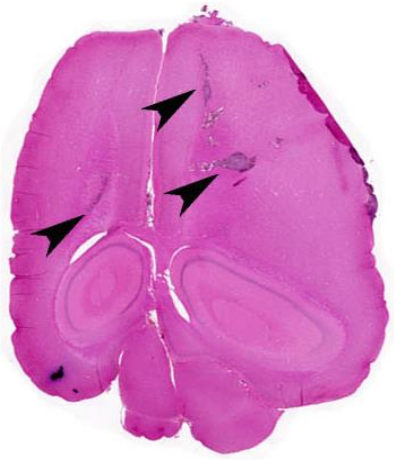
In-vivo, model for multiple brain metastases

In vivo investigations - Radiation protocol adjustment

4.7 Tesla scanner MRI



IV injection of 50 mg/L in 0.2 mL



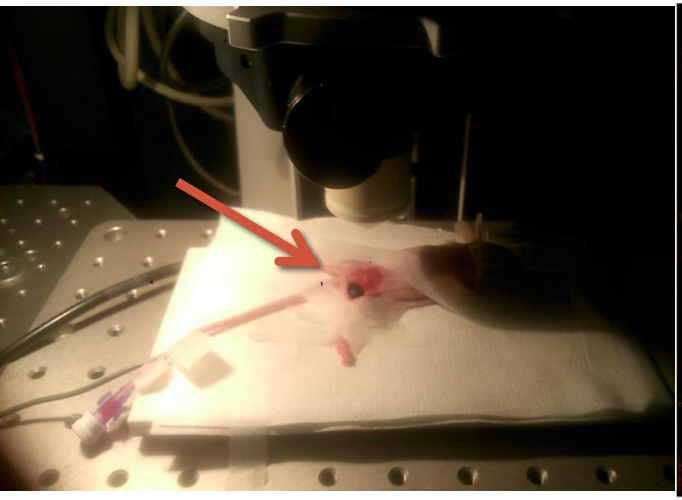
H&S staining

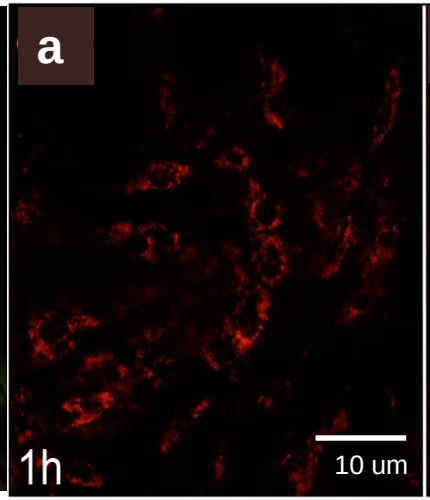
T_1 MR imaging of mice bearing B16-F10 day 5 post tumor implantation.

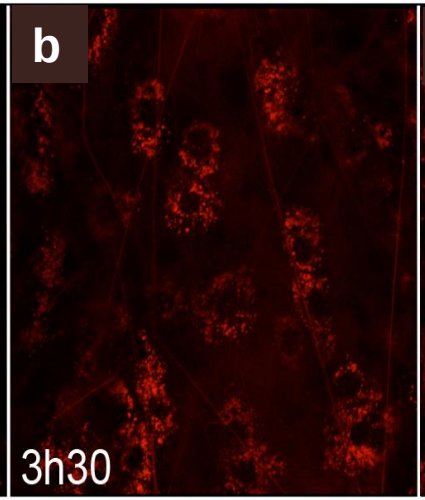
CNR for tumor *vs.* Contralateral hemisphere **21**
CNR tumor *vs.* muscle **59**

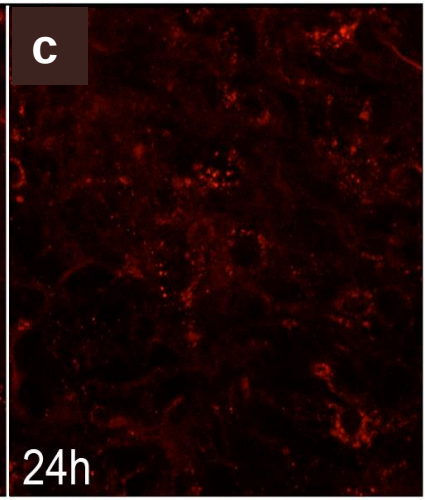
In vivo investigations - Radiation protocol adjustment

Intravital two-photon microscopy

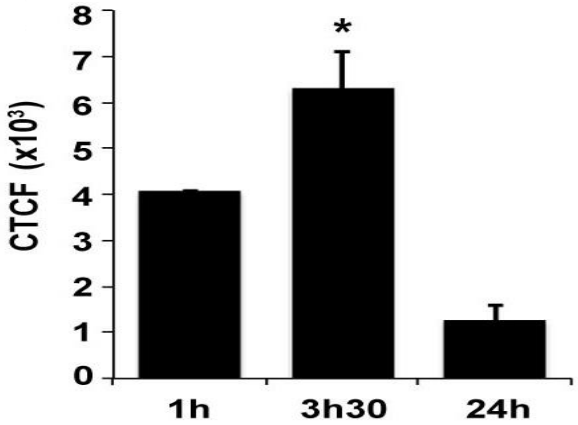






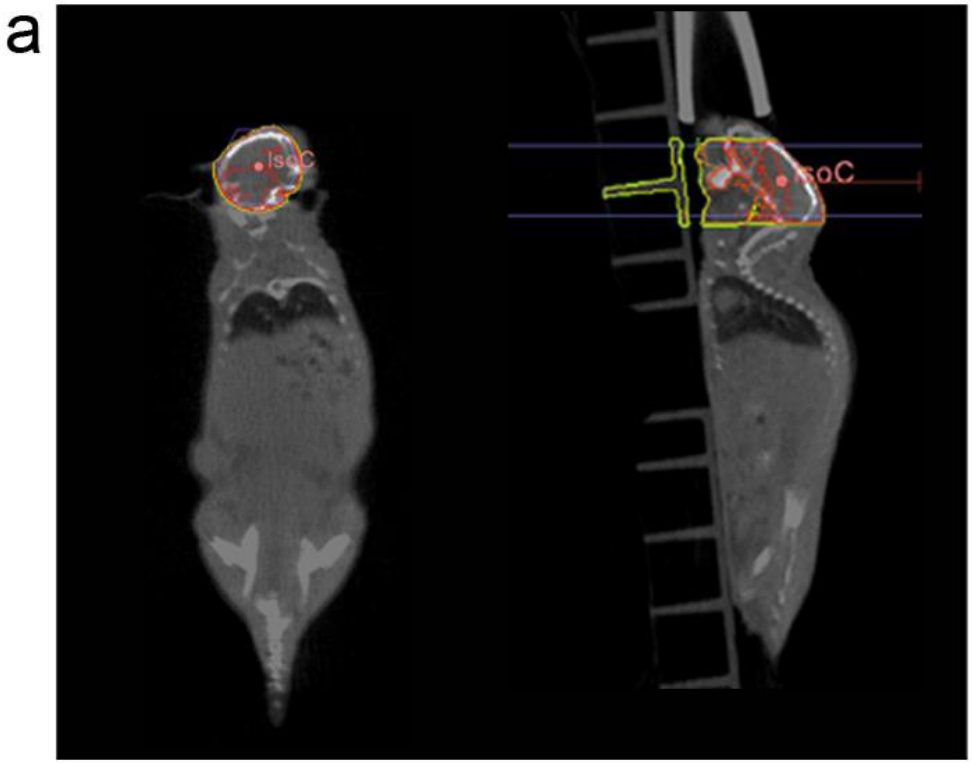


Intravital two-photon microscopy of labeled particles (Rhodamine-B) in subcutaneous B16F10 tumor.

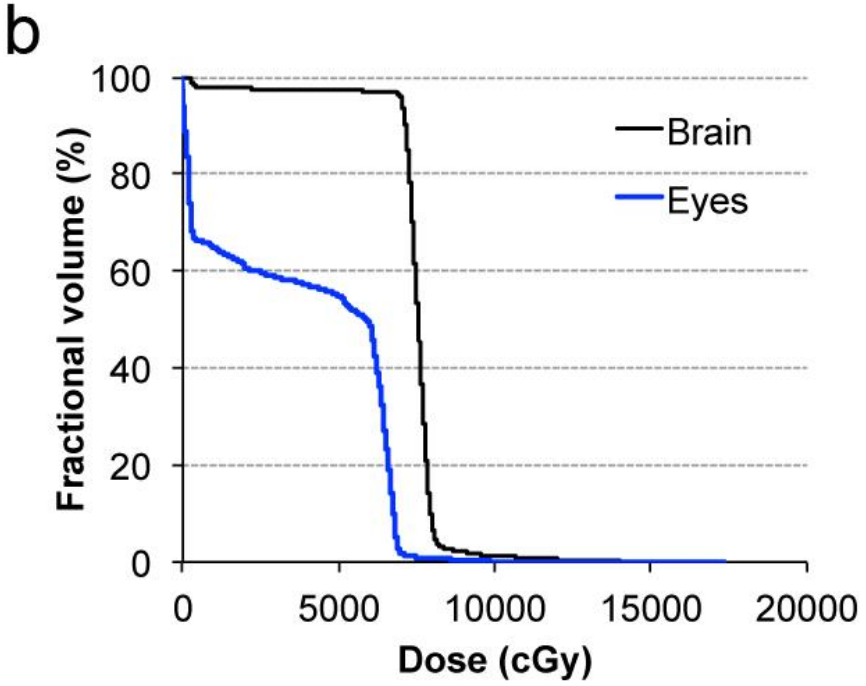


In vivo investigations - Radiation protocol adjustment

Image-guided cone beam CT after single 7-Gy exposure



Axial view Coronal view



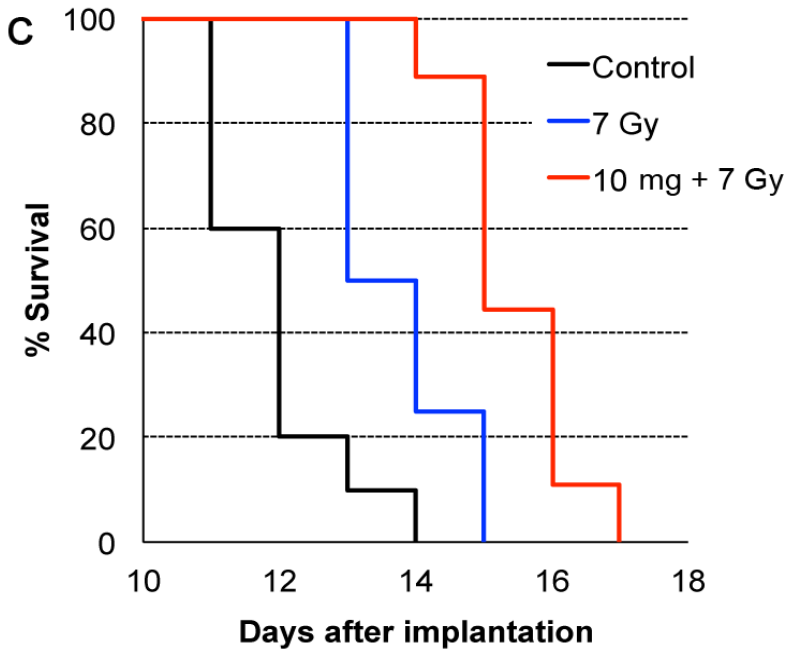
Dose volume histogram

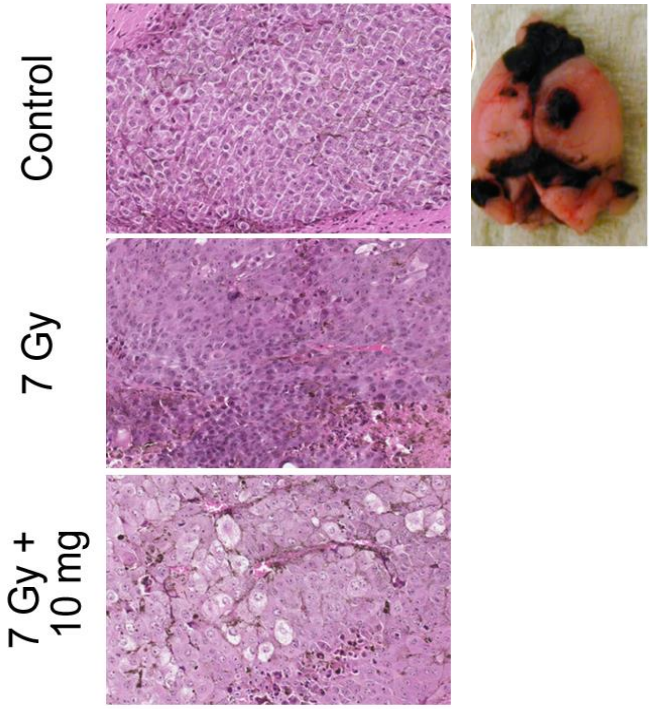
95 % of prescribed dose inside the brain.

51.5 % of prescribed dose in the eyes.

In vivo investigations - Single 7-Gy exposure.

Irradiation with 220 keV X-rays at dose rate 2 Gy/min.





	n	MeST (days)	ILS vs. Control	ILS vs. IR
Control	10	12	-	-
7 Gy	8	13	8.3 %	-
AGuIX + 7 Gy	9	15	25 %	**15.4 %**

S.Kotb et al., thno.2016Jan 20;6(3):418–27.

Conclusion: Translation to clinical applications

French agencies 02/2016

Phase I trial, Dr. Camy Verry (Grenoble's University Hospital):

- Enhancement of the radiation efficacy .

- **Multiple brain metastases,** from primary tumors; Skin melanoma, lung, and breast cancer (n>3, or +3 cm)
  Life expectancy < 6 months
  Excluded from stereotactic approaches and surgery

- Current treatment: 10 Fractions of 3 Gy , 5 days a week for 2 weeks.

- Dose escalation:15, 30, 50, 75, 100 mg/kg (3 patients / dose) .

- 1 AGuIX IV injection at day 1 + MRI + standard treatment
  - **Safety and Pharmacokinetics profile**
  - MRI contrast properties
  - Survival without brain progression

Acknowledgments

Olivier Tillement
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Françoise Peyrin
Denis Dauvergene

Ross Berbeco

Chantal Rémy
Emmanuel Barbier

Thanks for your attention

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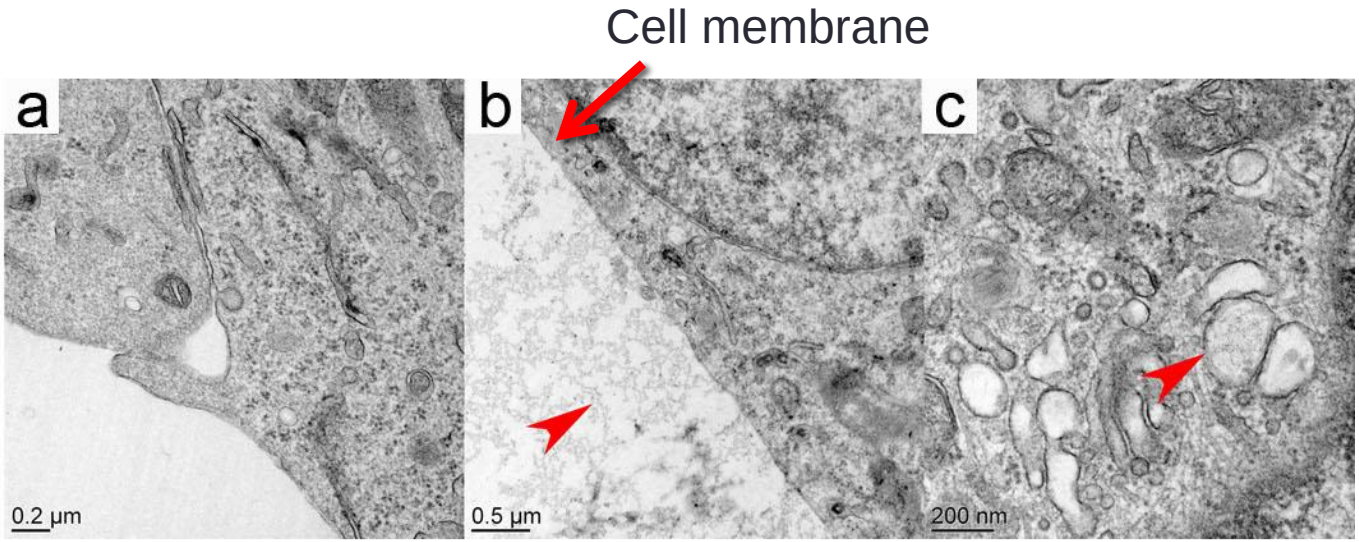
- **1 AGuIX IV injection at day 1 + MRI + standard treatment**
  - Safety and Pharmacokinetics profile
  - MRI contrast properties
  - Survival without brain progression

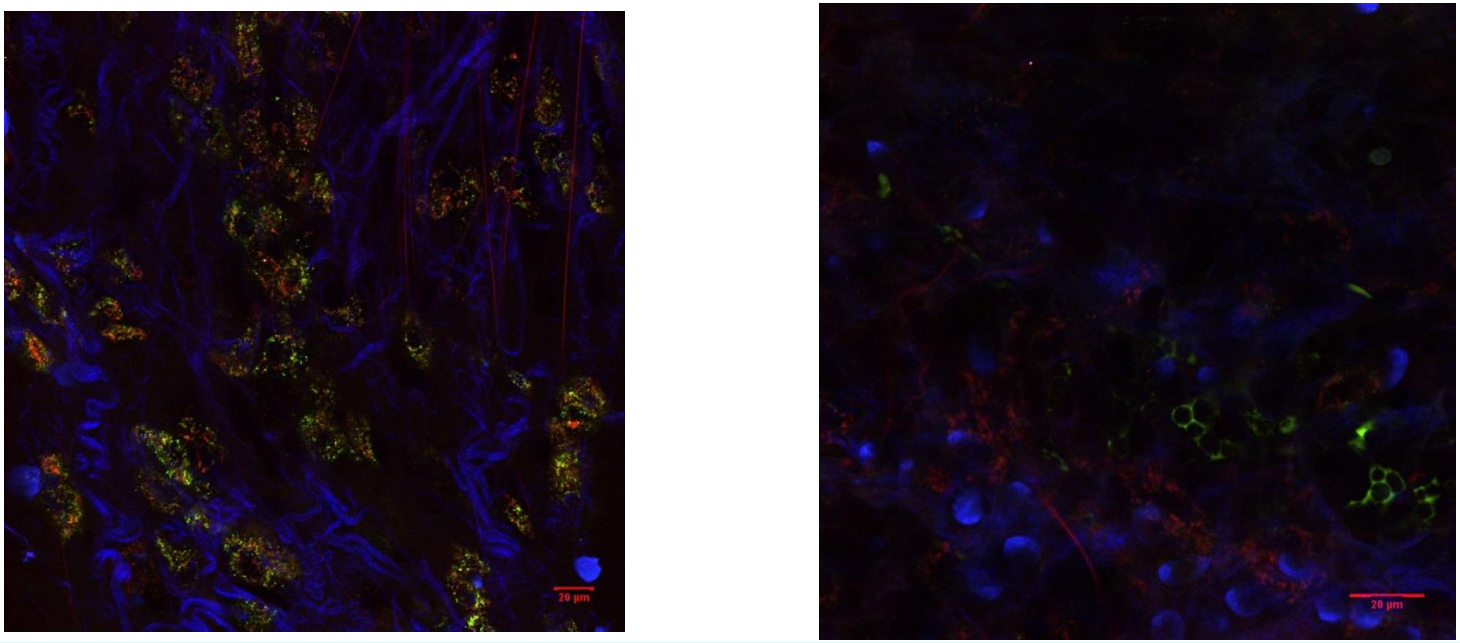
In vitro investigations – Cells uptake

Nanoparticles uptake after incubation with 0.6 mg/L for 1 hour (Corresponds to 0.4 pg Gd/cell internalized)



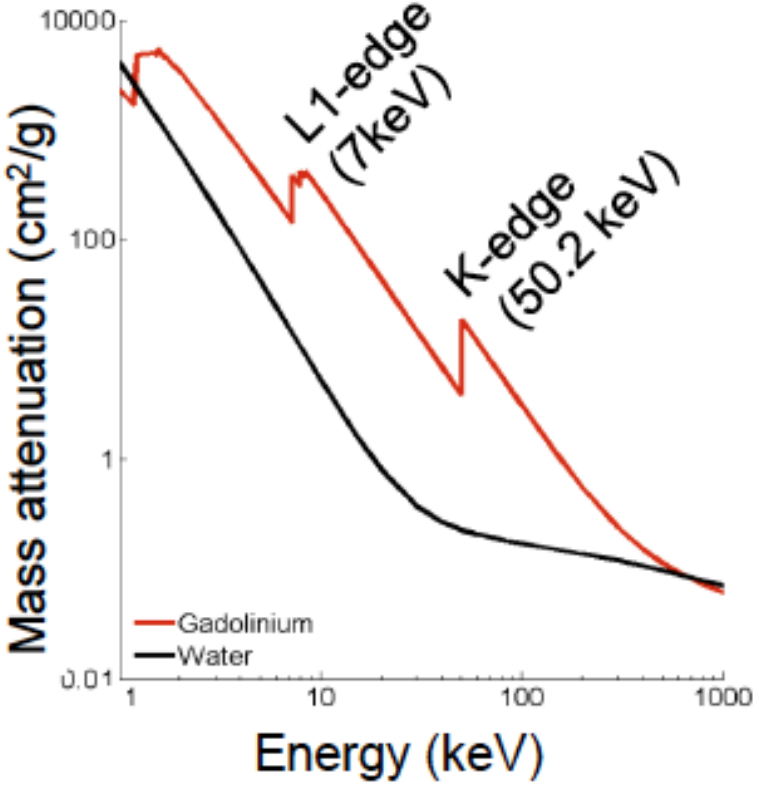
TEM images of B16-F10 cells in the control condition **(a)**, or 1 hour post incubation with 0.6 mM Gd **(b-c)**. Some aggregates are visible in close vicinity to the cell membrane **(b)** and internalized in vesicles **(C)**

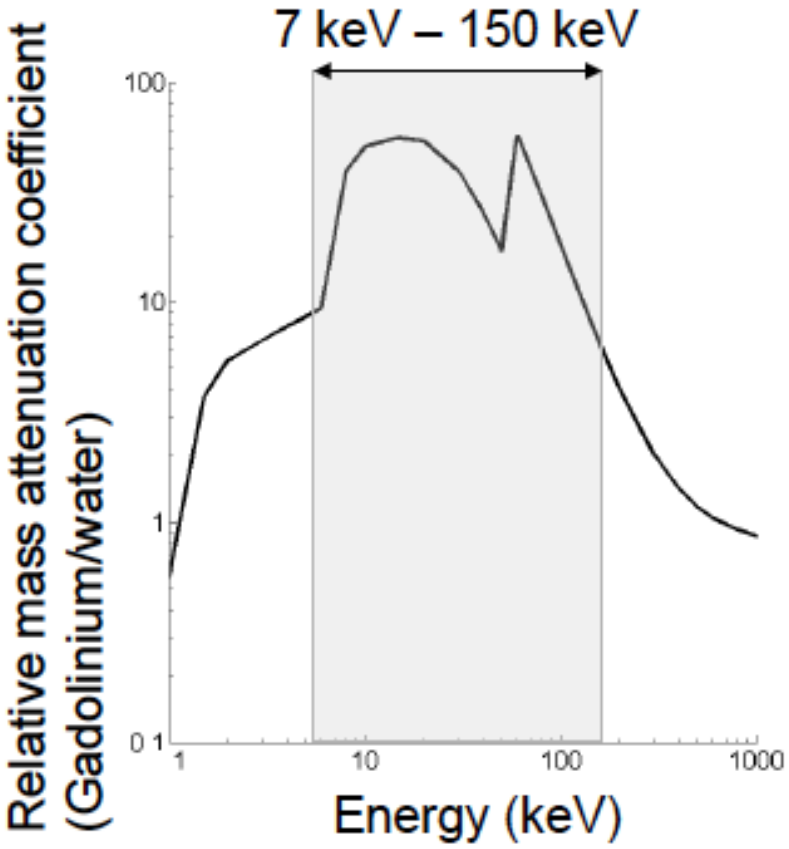
3h30 **24h**



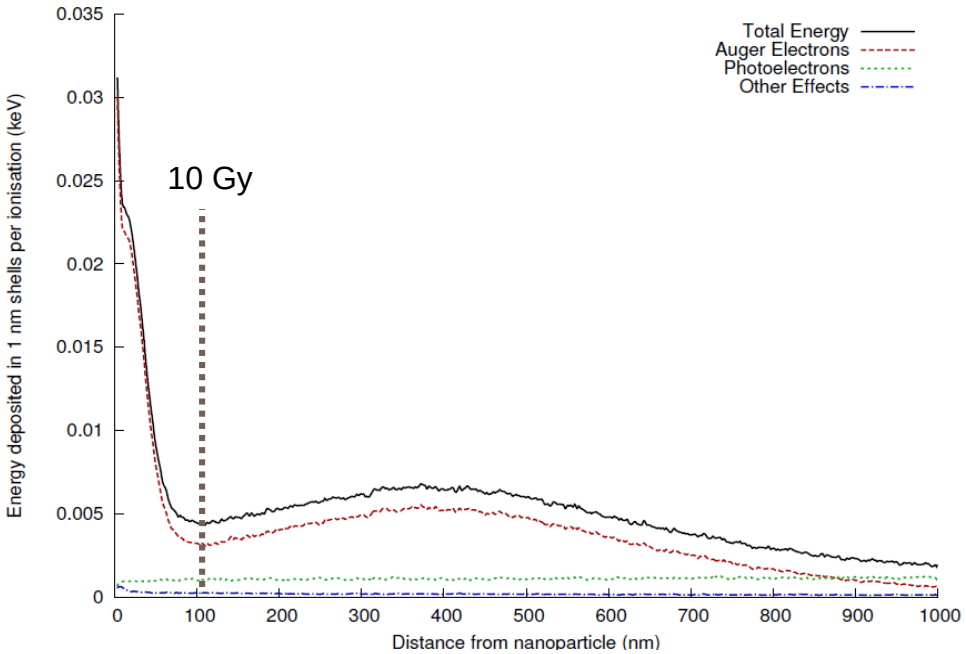
***Blue:** DAPI (DNA), **Green:** FITC-dextran (vessels), **Red:** RhoB-AGuIX®.*

AGuIX are either stick on the cell membrane, or diffused in the cytoplasm ; rather than being only on the extracellular matrix of the tumor.


Mass attenuation (cm²/g)
10000
100
1
0.01
1
10
100
1000
Energy (keV)
L1-edge
(7keV)
K-edge
(50.2 keV)
Gadolinium
Water


7 keV – 150 keV
Relative mass attenuation coefficient
(Gadolinium/water)
100
10
1
0.1
1
10
100
1000
Energy (keV)

Physical effect

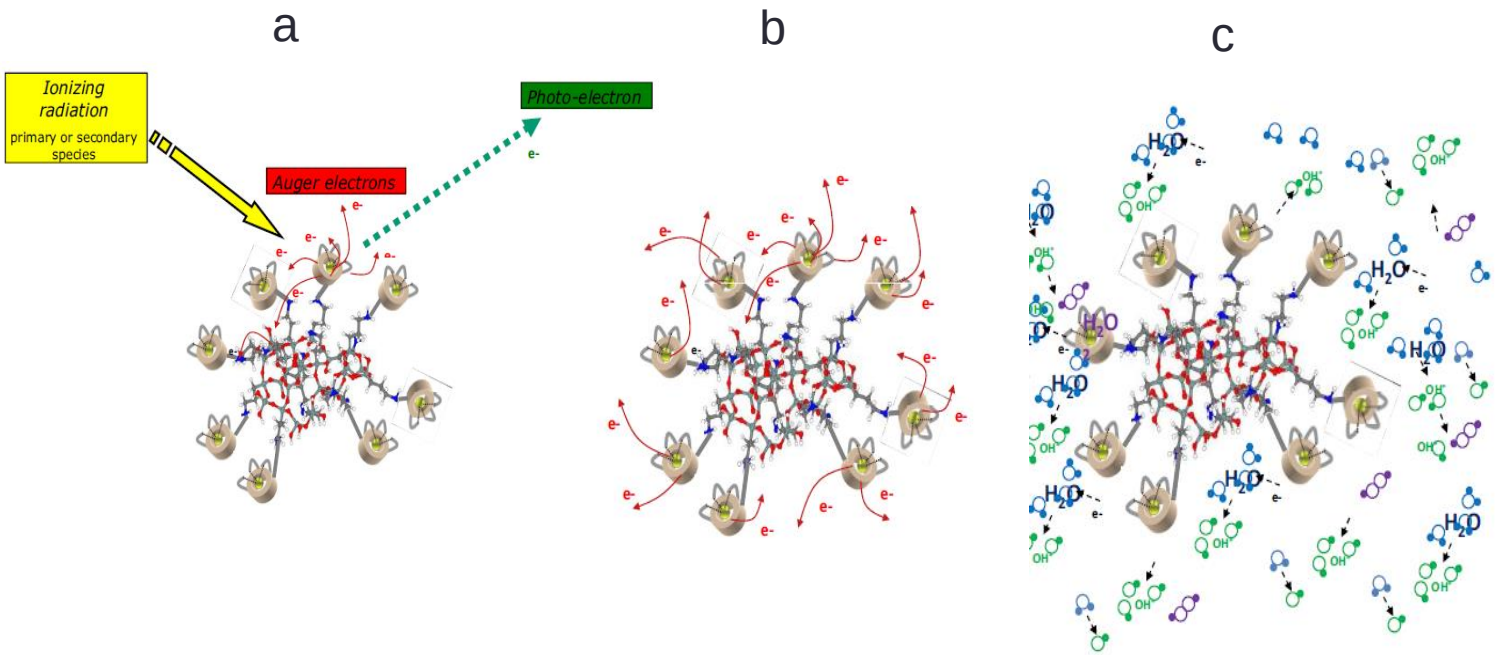


Nanoscale dose enhancement

Demonstration of nanoscale effects around irradiated AGuIX gadolinium nanoparticles. Using Geant4, the average energy deposited around an AGuIX nanoparticle following single ionising event has been calculated as a function of distance from the nanoparticle.

Stephen McMahon
Centre of cancer research and cell biology
Queen's university Belfast

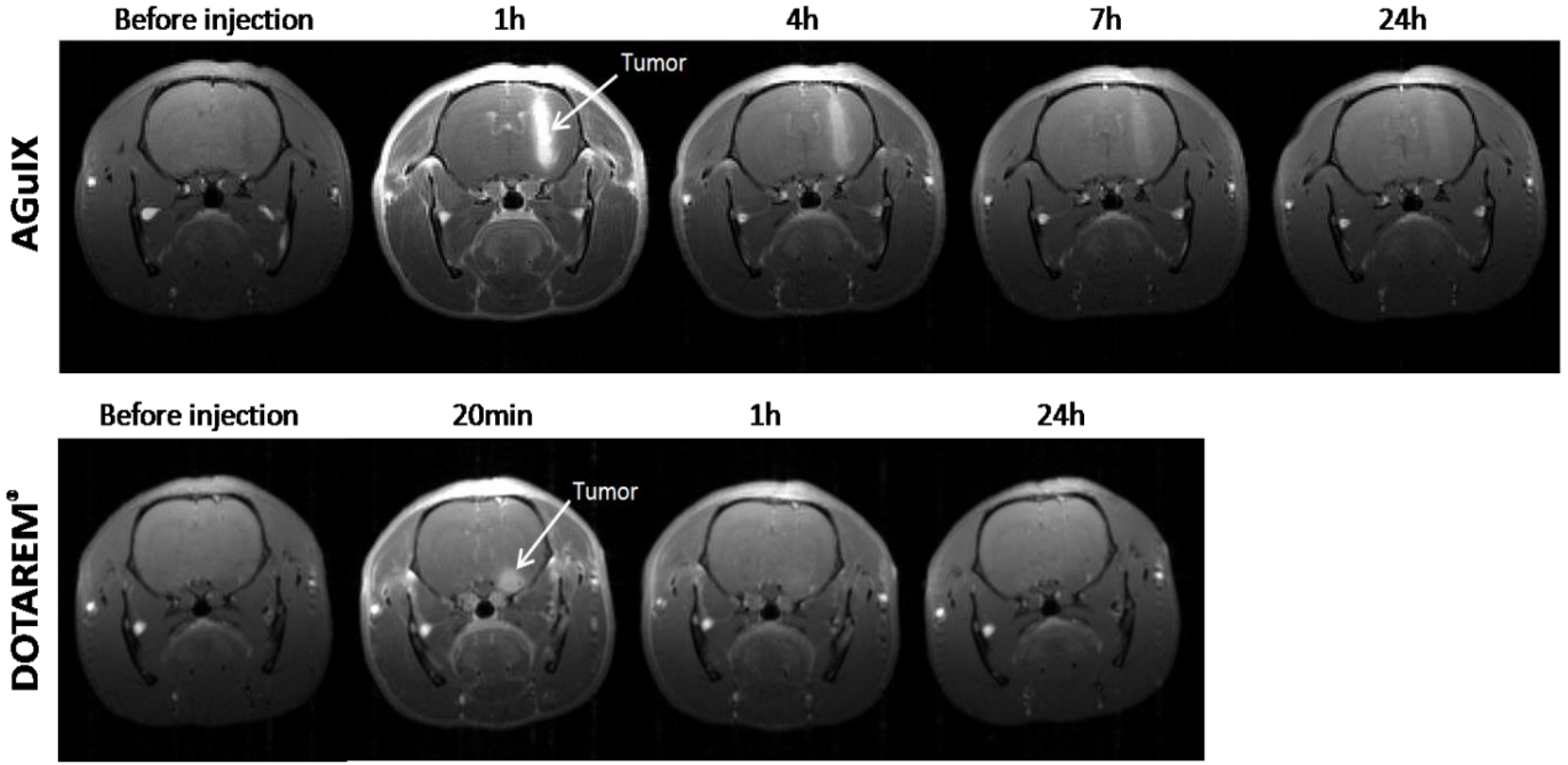
Physical effect



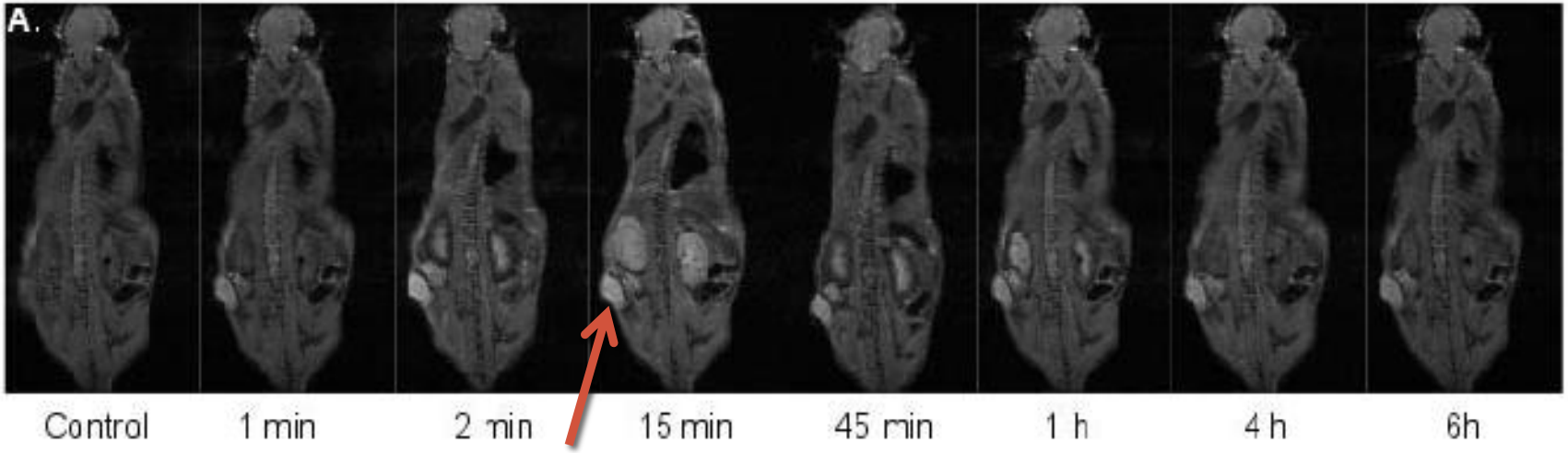
Complex Damages

High Biological effects

Intracranial 9LGS tumor detection by MRI (7T)

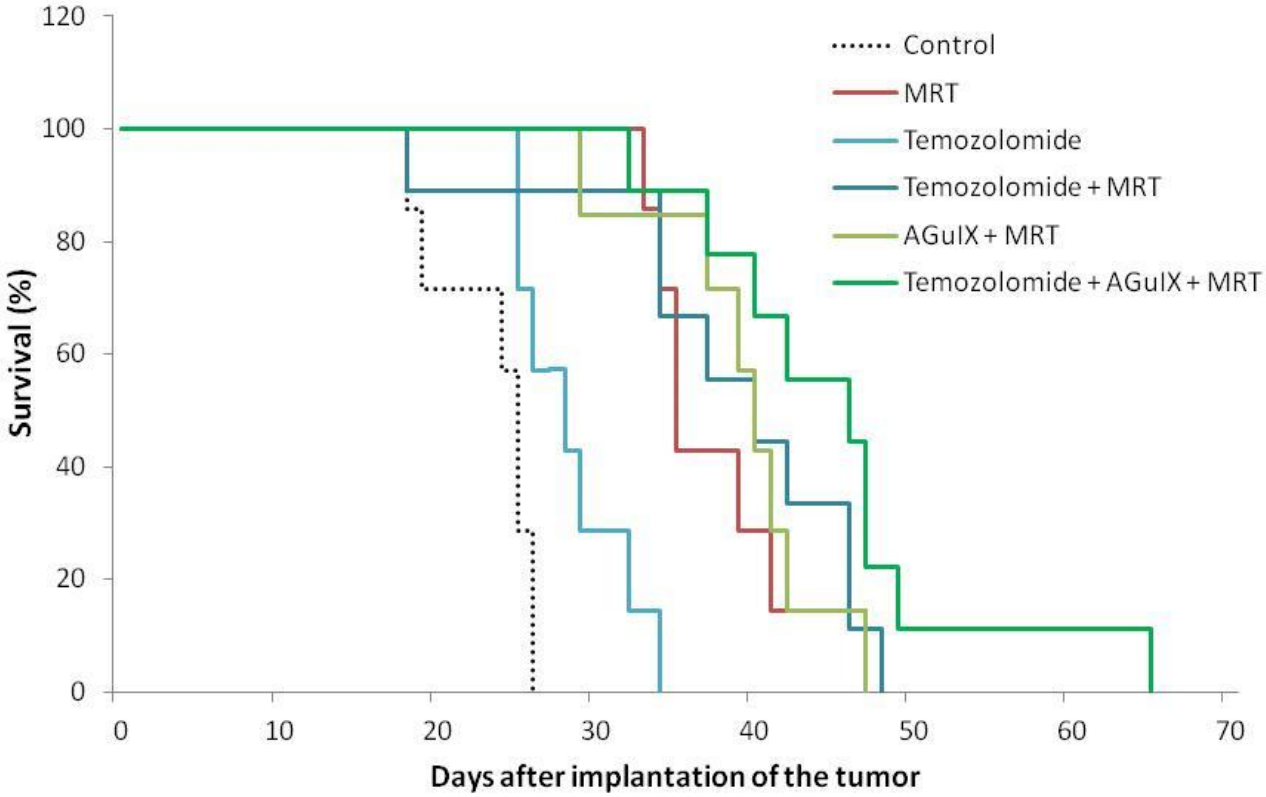


Le Duc et al., Cancer Nanotechnol, 2014



Whole body MRI T1- after IV injection of 40 mg/L of AGuIX (Pancreatic mouse model).

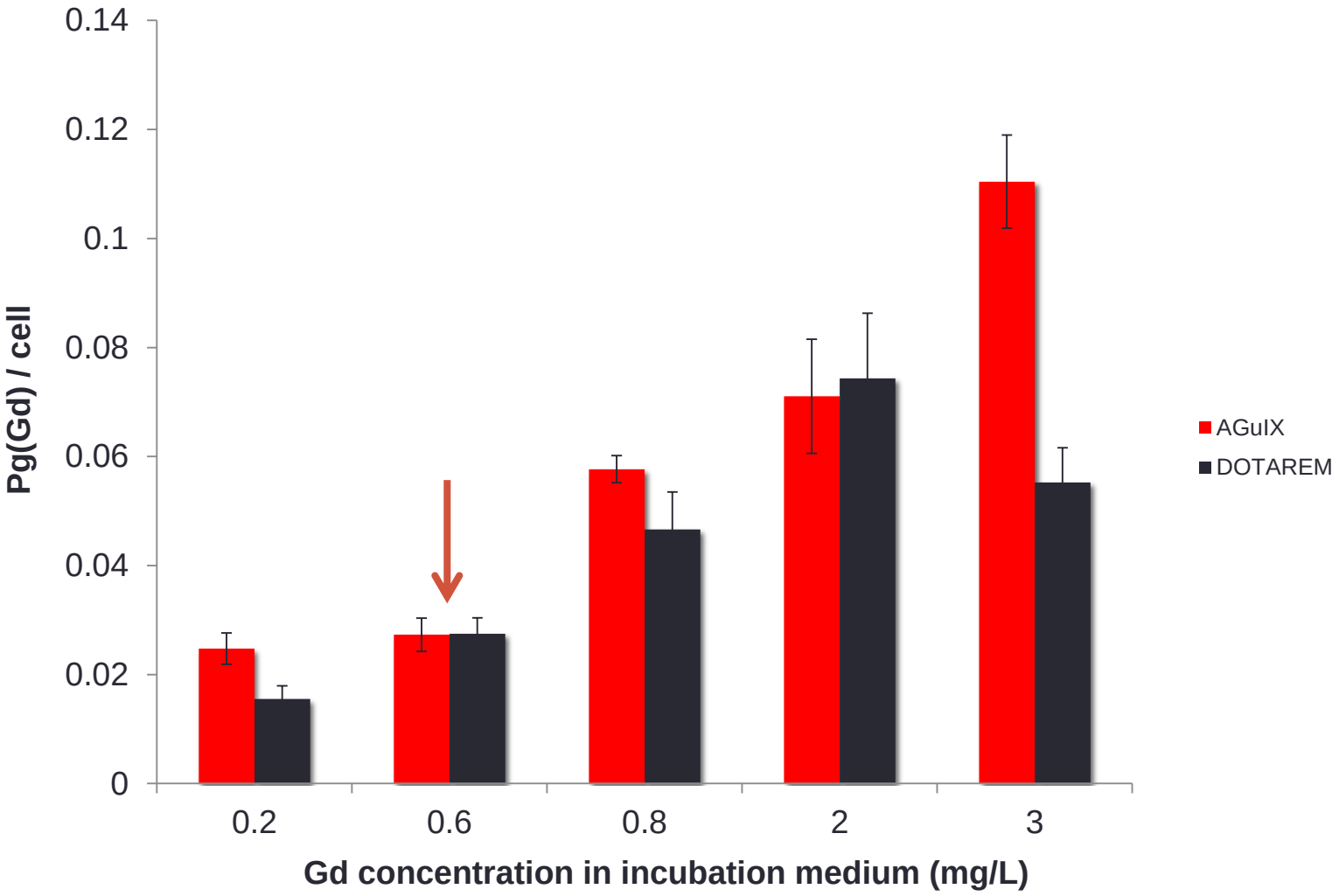
A.Detappe et al. submitted



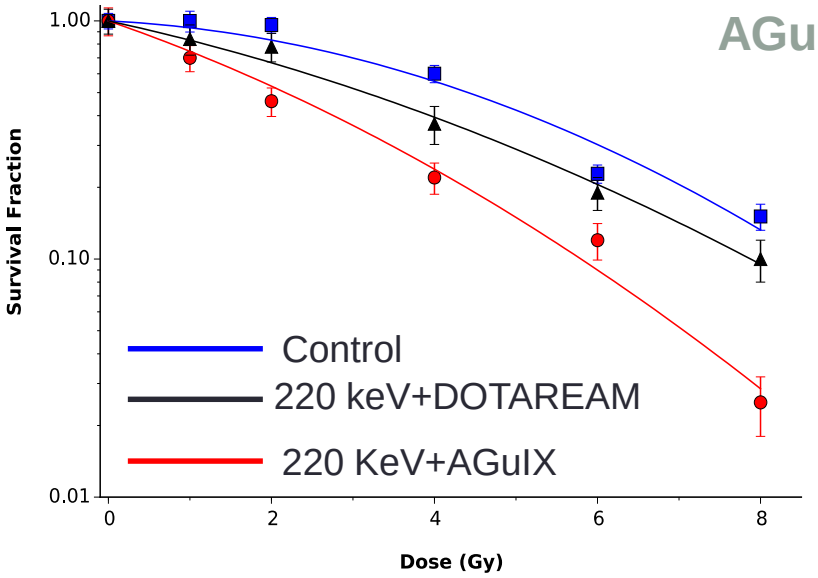
Survival of 9LGS bearing rats : Combination of MRT and temozolimide

Unpublished data.

Gadolinium NPs vs. Molecular chelates (DOTAREM®)



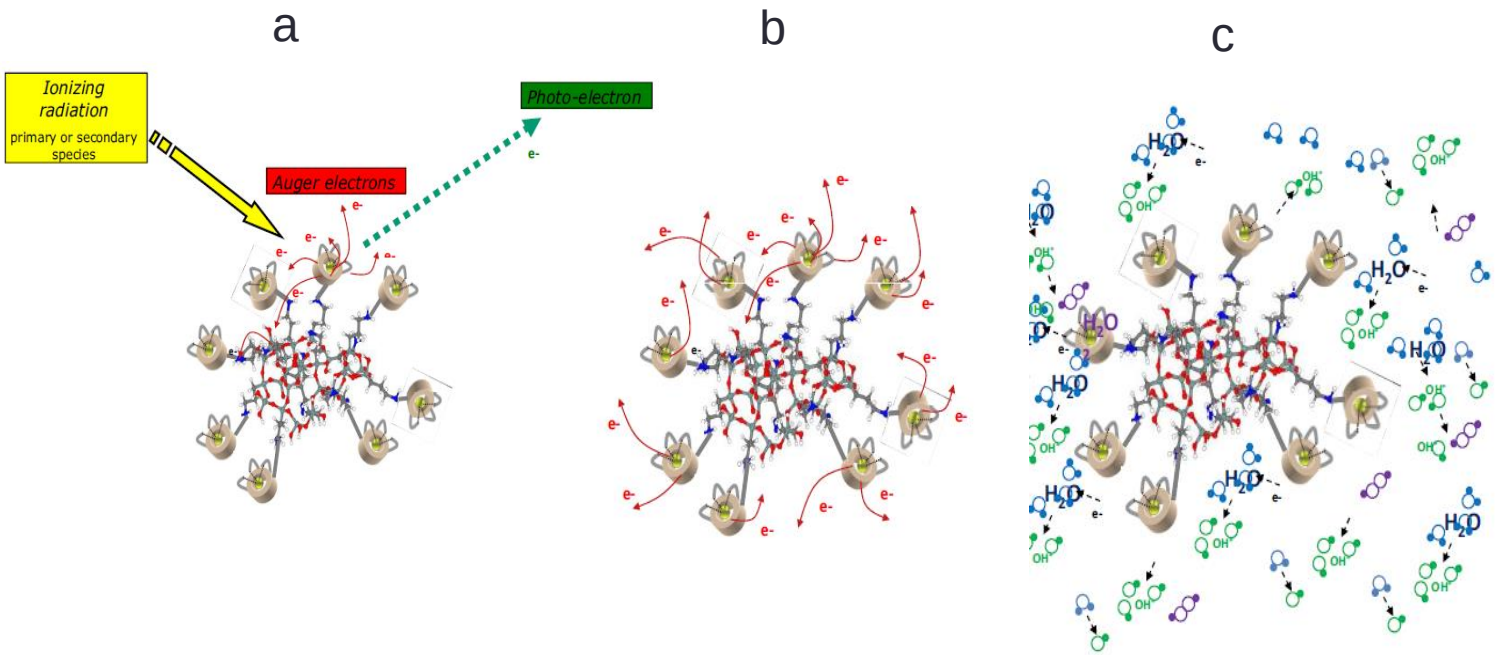
Gadolinium NPs vs. Molecular chelates 220-keV X-ray (2 Gy/min)



ROS enhancement production factor AGuIX *vs.* DOTAREM at 0.6 mg/L is 1.23

	α (Gy $^{-1}$)	D50% Gy	SF 2 Gy	% EF 2 Gy	SER 2 Gy
Control	0.04	4.5	0.96	-	-
0.6 mg/L AGuIX	0.26	1.8	0.56	52 %	2.08
0.6 mg/L DOTAREM	0.173	3	0.78	19.6 %	1.2

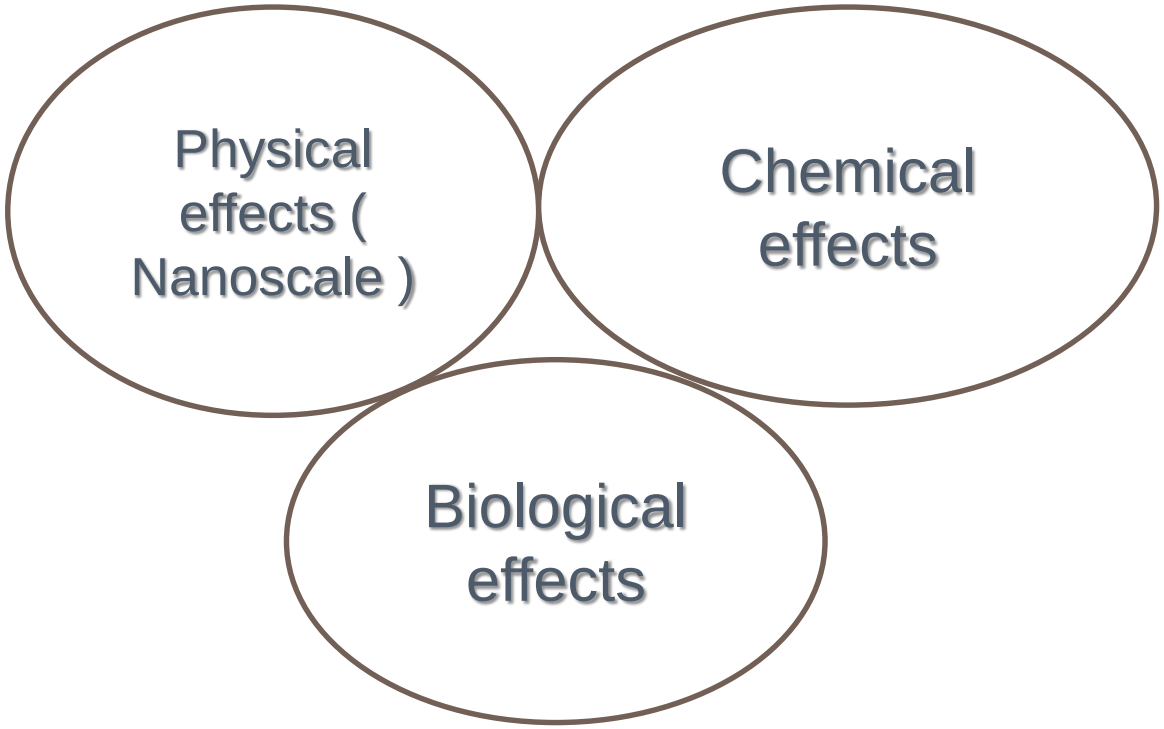
Physical effect



Complex Damages

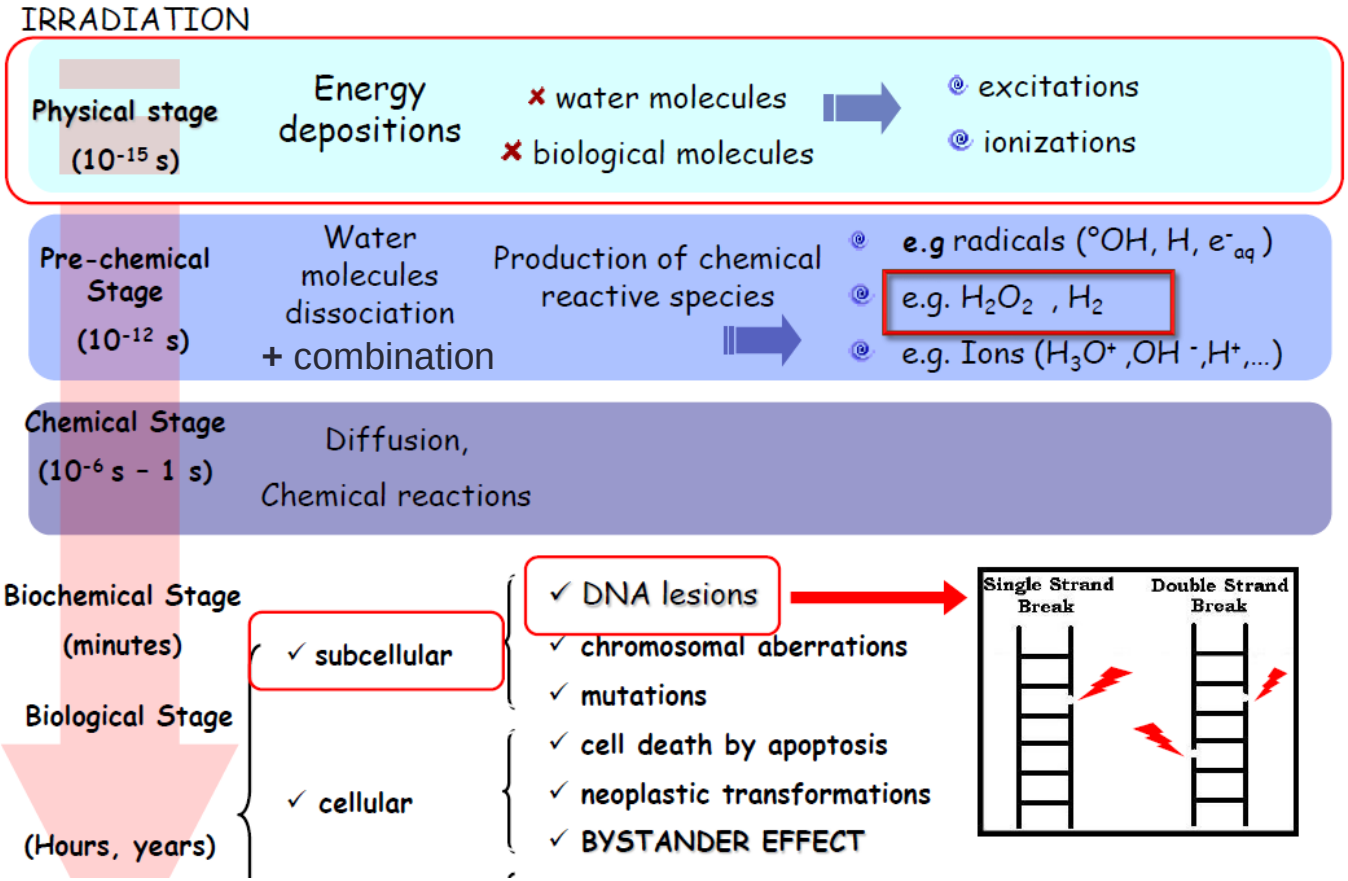
High Biological effects

Radiosensitization effect



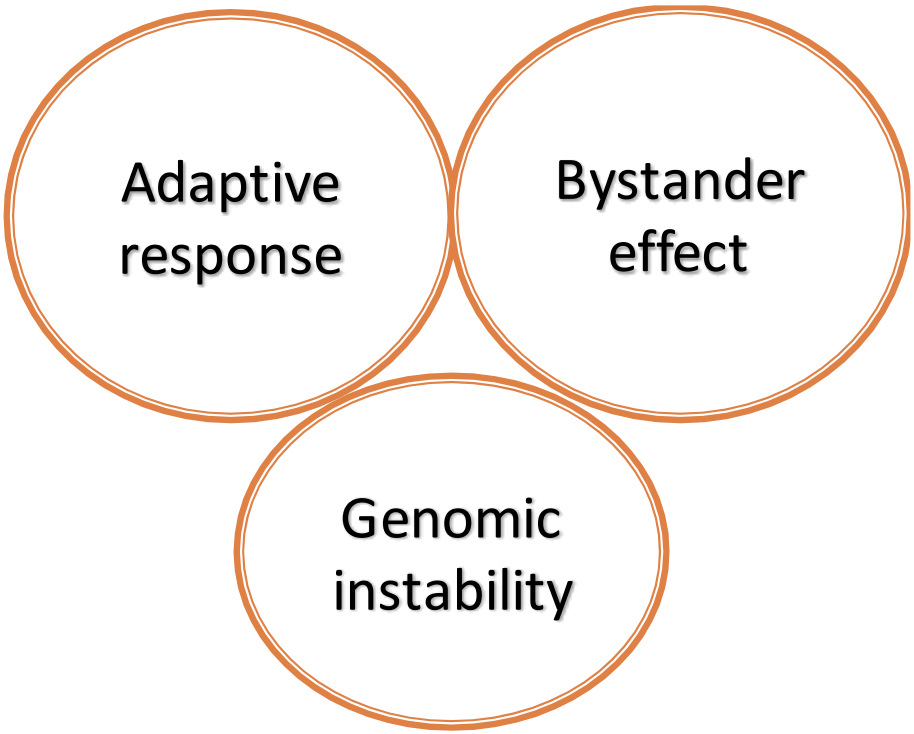
Chemical effect and biological effect

Time evolution of radiation-induced biological damage



Courtesy of D.Alloni, University of Pavia.

Radiation-induced non targeted effects



Bystander effect: Possible signals

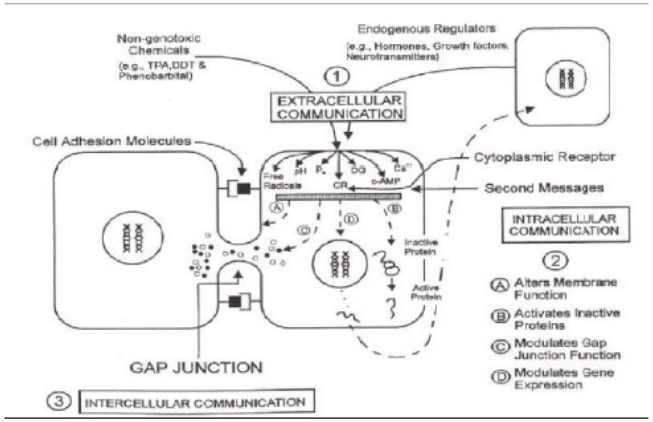
via Gap Junctions

- Ca^{++}
- c-AMP (cyclic-AMP)
- Antioxidants (thiols)
- (long-lived) organic radicals
- Nitric Oxide
- ……..

via Extracellular Environment

- Cytokines, e.g.:
  - IL-6, IL-8, IL-10 (Interleukin-6, 8, 10)
  - TNFα (Tumor Necrosis Factor- α)
  - TGFβ (Tumor Growth Factor- β)
- Lipid peroxidation products
- ……..

→ ROS (Reactive oxygen species: H_2O_2, O_2 , etc.)



Trosko & Ruch1998, Frontiers in Bioscience3

Microenvironment and cell communication have been studied by biologists since long time ago, but they have been extensively considered in radiobiology only after bystander-effect observations!

Acta Oncologica, 2010; 49: 1334–1343

informa
healthcare

ORIGINAL ARTICLE

A mathematical framework for separating the direct and bystander components of cellular radiation response

MARTIN A. EBERT[1,2], NATALKA SUCHOWERSKA[3,4], MICHAEL A. JACKSON[3] & DAVID R. MCKENZIE[4]

[1]*Department of Radiation Oncology, Sir Charles Gairdner Hospital, Western Australia, Australia,* [2]*School of Physics, University of Western Australia, Western Australia, Australia,* [3]*Department of Radiation Oncology, Royal Prince Alfred Hospital, New South Wales, Australia and* [4]*School of Physics, University of Sydney, New South Wales, Australia*

Abstract

A mathematical model for fractional tumor cell survival was developed incorporating components of cell killing due to direct radiation interactions and bystander signals resulting from non-local dose deposition. *Material and methods.* Three possible mechanisms for signal production were tested by fitting predictions to available experimental results for tumor cells (non-small cell lung cancer NCI-H460 and melanoma MM576) exposed to gradient x-ray fields. The parameter fitting allowed estimation of the contribution of bystander signaling to cell death (20–50% for all models). Separation of the two components of cell killing allowed determination of the α and β parameters of the linear-quadratic model both with and without the presence of bystander signaling. *Results and discussion.* For both cell lines, cell death from bystander signaling and direct radiation interactions were comparable. For NCI-H460 cells, the values for α and β were 0.18 Gy^{-1} and 0.10 Gy^{-2} respectively when direct and bystander effects were combined, and 0.053 Gy^{-1} and 0.061 Gy^{-2} respectively when the signaling component was removed. For MM576, the corresponding respective values were 0.09 Gy^{-1} and 0.011 Gy^{-2} for the combined response, and 0.014 Gy^{-1} and 0.002 Gy^{-2} for the isolated direct radiation response. The bystander component in cell death was found to be significant and should not be ignored. Further experimental evidence is required to determine how these results translate to the *in vivo* situation where tumor control probability (TCP) models that currently assume cellular independence may need to be revised.

	α(Gy^{-1})	%Cell death
Direct	0.014	16
Bystander	0.076	84

Design of repeated toxicity study in Monkeys

Table 1. ***Design of the repeated toxicity study of AGuIX in Cynomolgus Monkeys.***

Group	Dose level (mg/kg/adm)	Dose volume (mL/kg/adm)	Dose concentration (mg/mL)	Number of animals	
				Males	Females
1. Control	0	2.5	0	3	3
2. Low dose	**150**	2.5	**60**	3	3
3. Intermediate dose	**300**	2.5	**120**	3	3
4. High dose	**450**	2.5	**180**	3	3

Dose administrated once/week for two weeks. Monkeys were sacrificed two weeks post last injection. No any side effects observed at the above mentioned dose. The NOEL is 450 mg/kg.

Human equivalent dose

Formula for Dose Translation Based on BSA

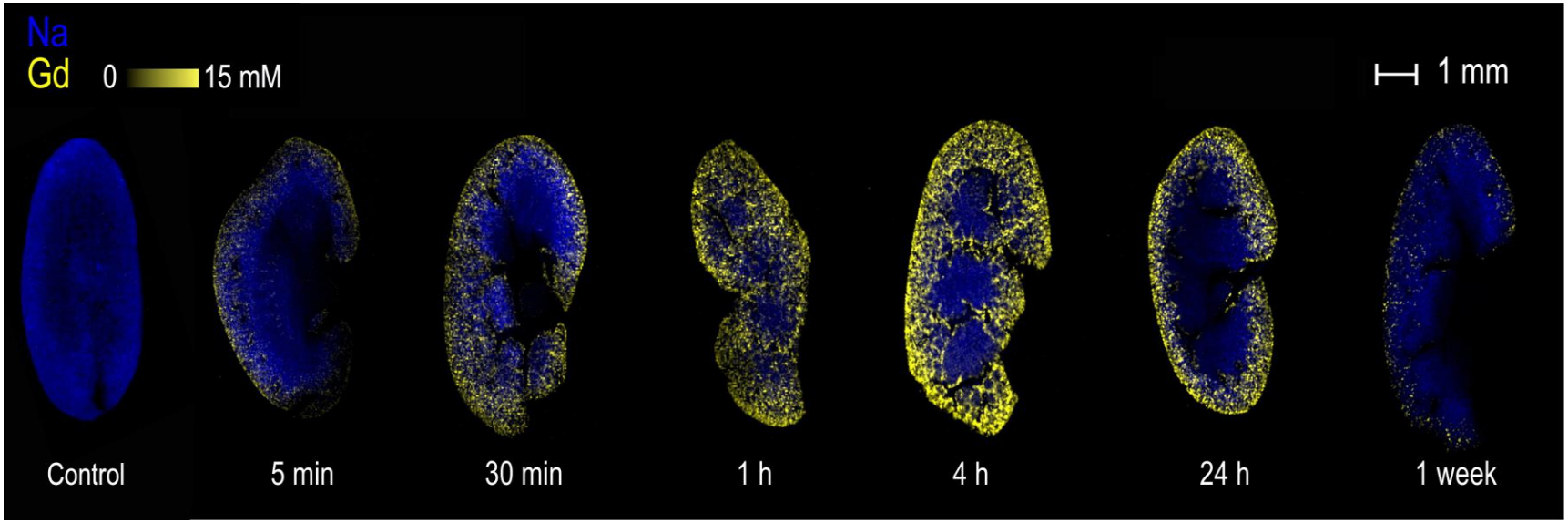
$$\text{HED (mg/kg)} = \text{Animal dose (mg/kg)} \textit{ multiplied by } \frac{\text{Animal } Km}{\text{Human } Km}$$

TABLE 1. *Conversion of animal doses to HED based on BSA*

Species	Weight (kg)	BSA (m^2)	K_m factor
Human			
Adult	60	1.6	37
Child	20	0.8	25
Baboon	12	0.6	20
Dog	10	0.5	20
Monkey	3	0.24	12
Rabbit	1.8	0.15	12
Guinea pig	0.4	0.05	8
Rat	0.15	0.025	6
Hamster	0.08	0.02	5
Mouse	0.02	0.007	3

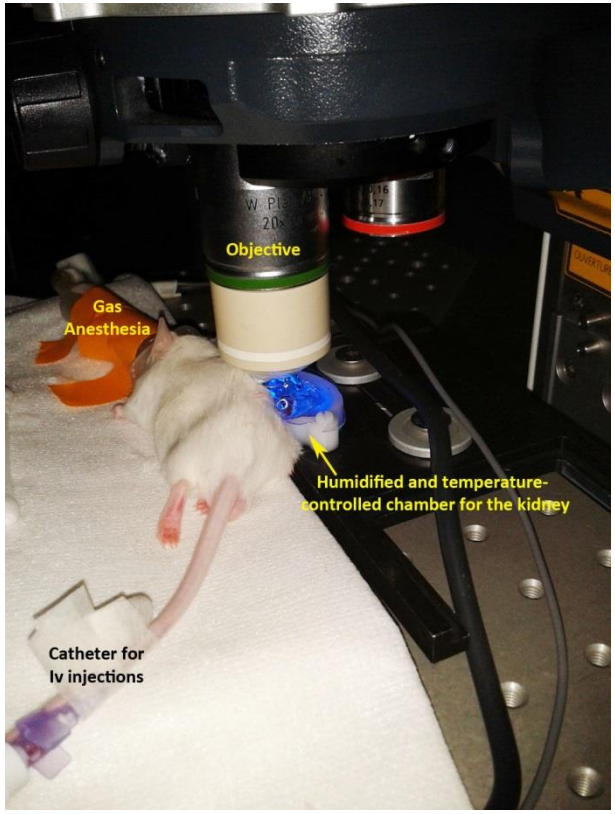
To convert dose in mg/kg to dose in mg/m^2 .

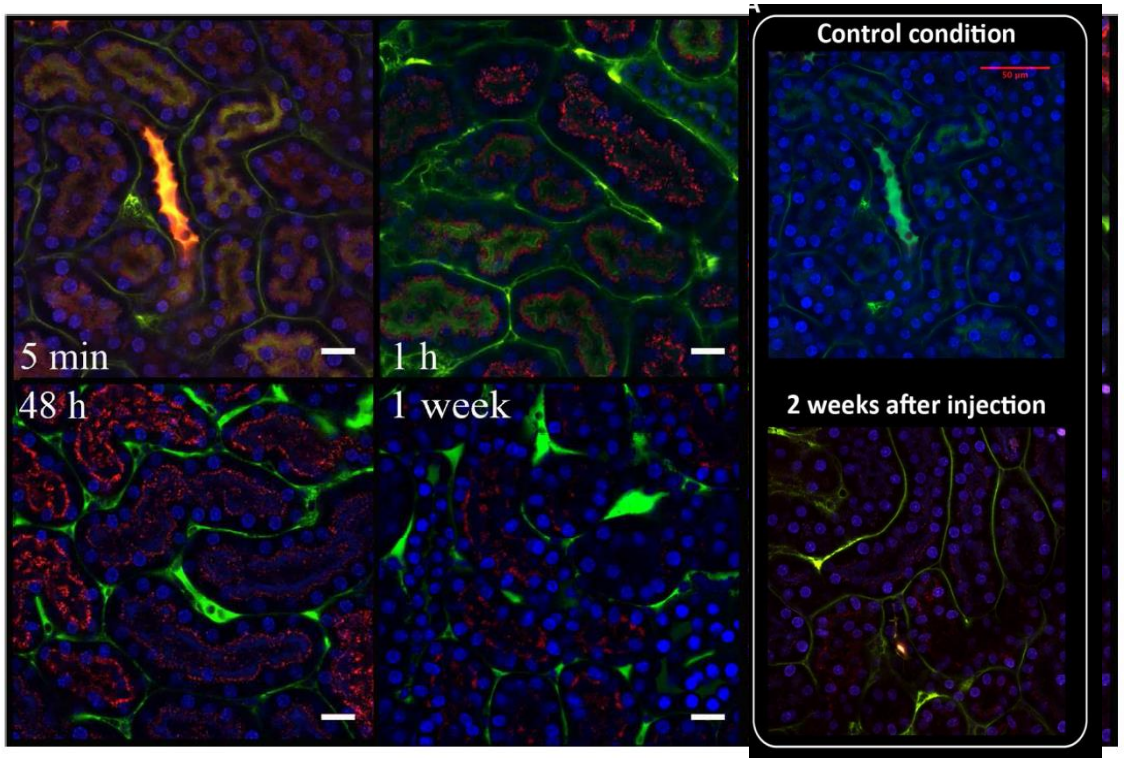
Laser-induced breakdown spectroscopy



Sancey & Kotb et al. ACS Nano. 2015 Mar 24;9(3):2477-88

Intravital two photon microscopy





***Blue**: DAPI (DNA),-**Green**: FITC-dextran (vessels),-**Red**: Rho B-AGuIX®. The AGuIX® reach the kidney within few minutes, with a maximum of intensity between 4-24hrs.*

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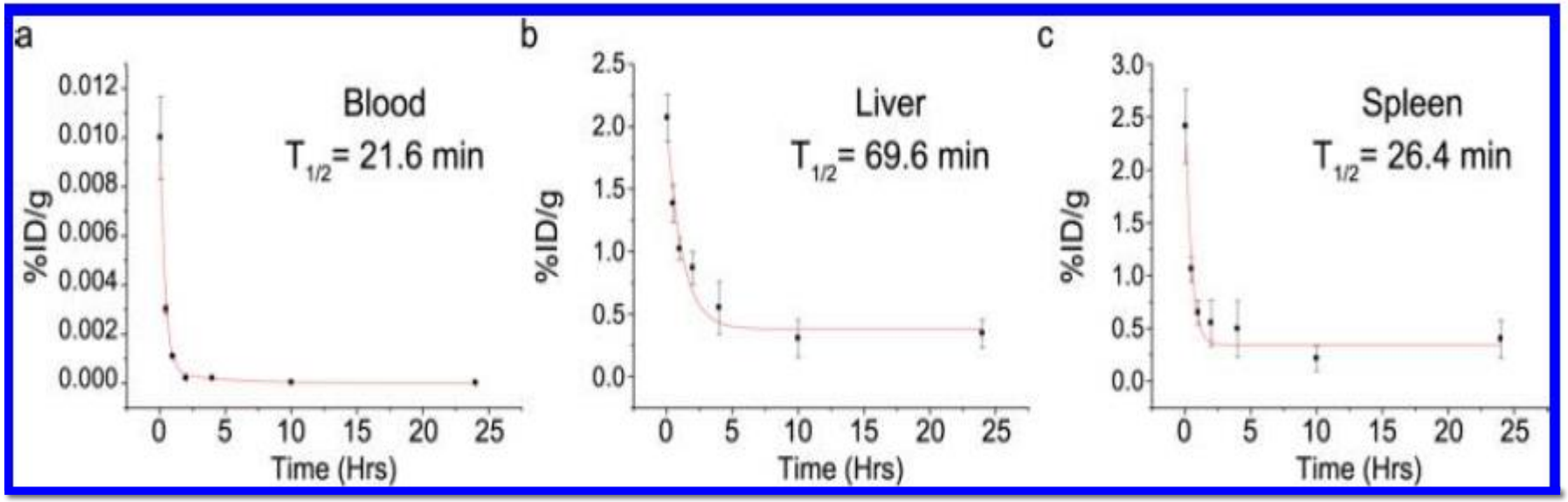
Retention in reticuloendothelial system (RES) and blood



The nanoparticle's half-life time was determined in blood, liver, and spleen as a function of time elapsed since administration of 8 µmoles by ICP-AES.

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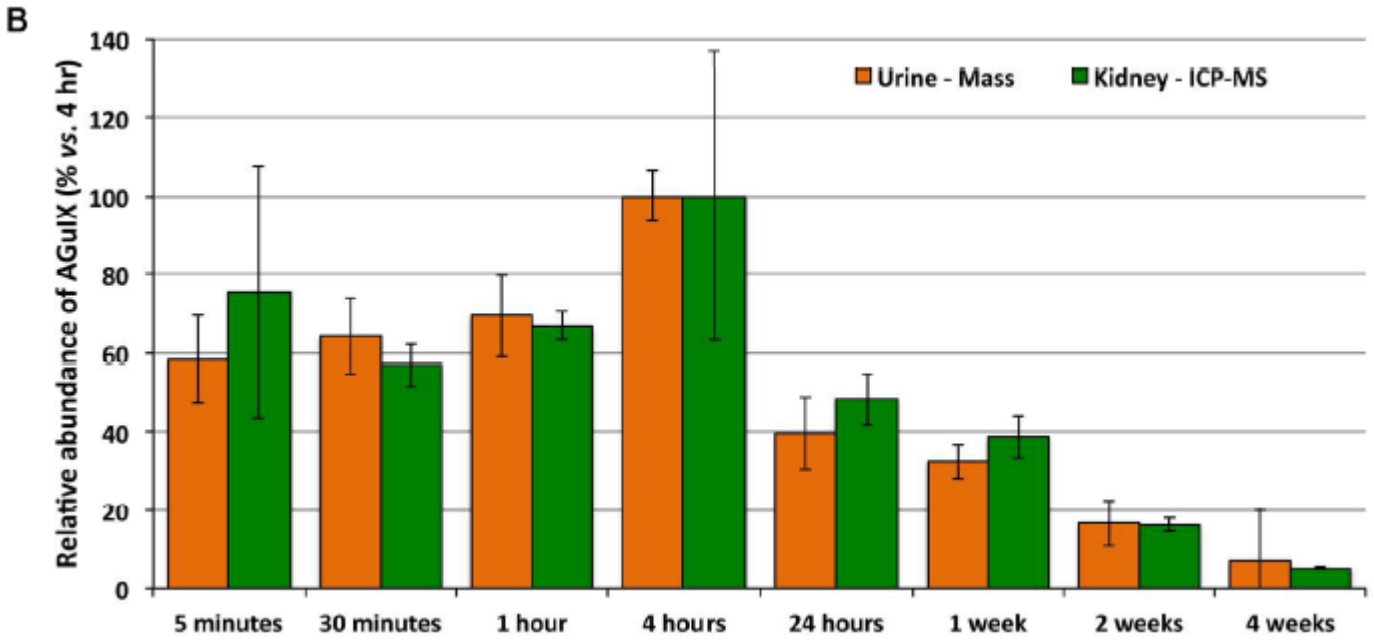
Relative quantity detected in urine and kidney



Comparison of the relative quantities of AGuIX measured in the urine and in the kidney by MS and ICP-MS respectively.

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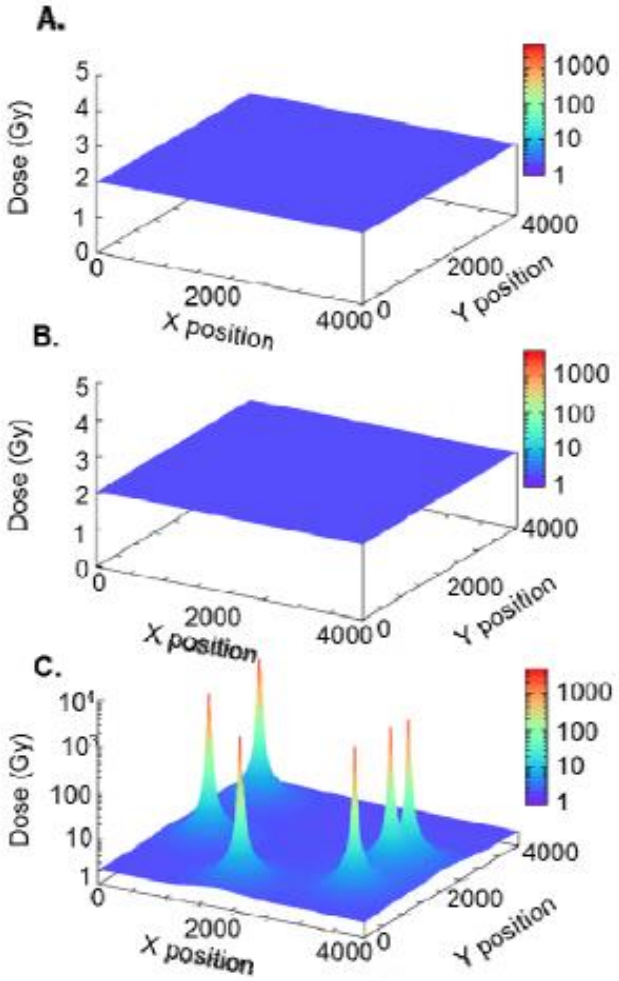
Nanoscale dosimetric effect in GdNP therapy

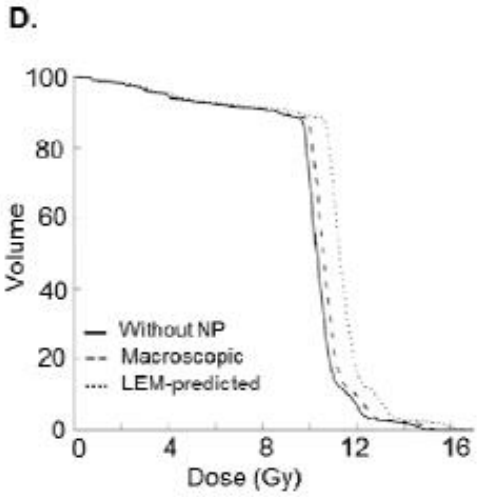
A.Detappe et al. submitted

Uniform exposure to Dose 2 Gy within a 5 μm cube

Dose enhancement 2.7% (Macroscopic dose model)

Dose enhancement delivered heterogeneously due to presence of GdNPs (total increase in the dose is the same,LEM model)





DVH according to Macroscopic model and LEM model

Due to the relatively low density of Gd in the tumor, physically driven enhancement is limited- **2.7 % physical dose enhancement** and **9.8 % predicted by LEM**, both too small to explain the observed biological effect.