Radiosensitizing effects

with ultra small gadolinium based nanoparticles

Theragnostic AGuIX

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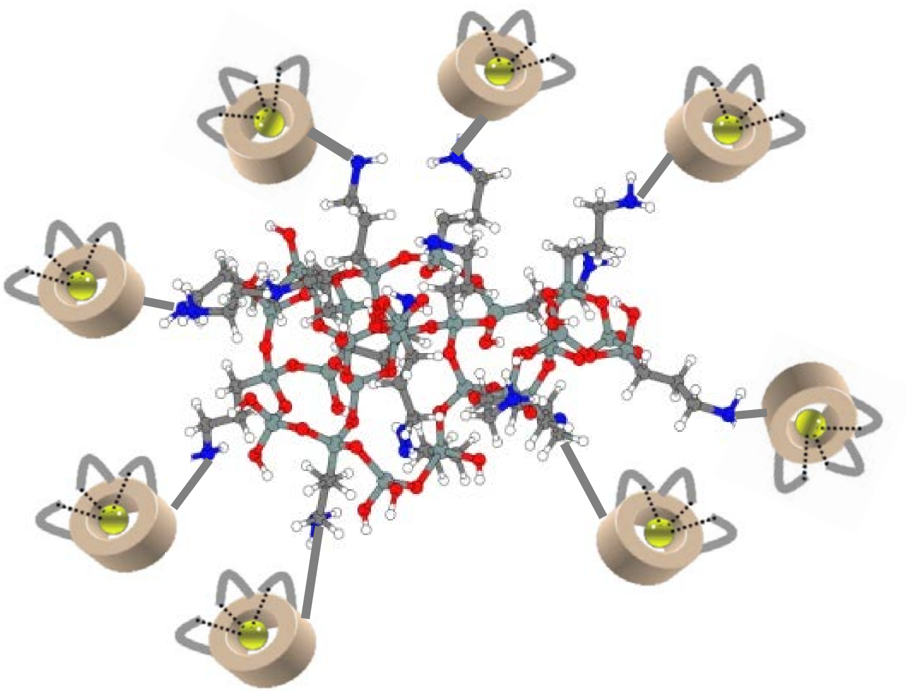
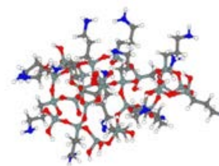
Chemical description of AGuIX® Nanodrug

AGuIX® Nanodrug

Ultra small sub-5 nm particles
Polysiloxane (silica) skeleton grafted with Gd-chelates

Polysiloxane Skeleton (with amino functions)
grafted with high chelating species (DOTAGA (Kind of "DOTAREM®))
including some gadolinium ions




Polysiloxane network (APTES/TEOS)

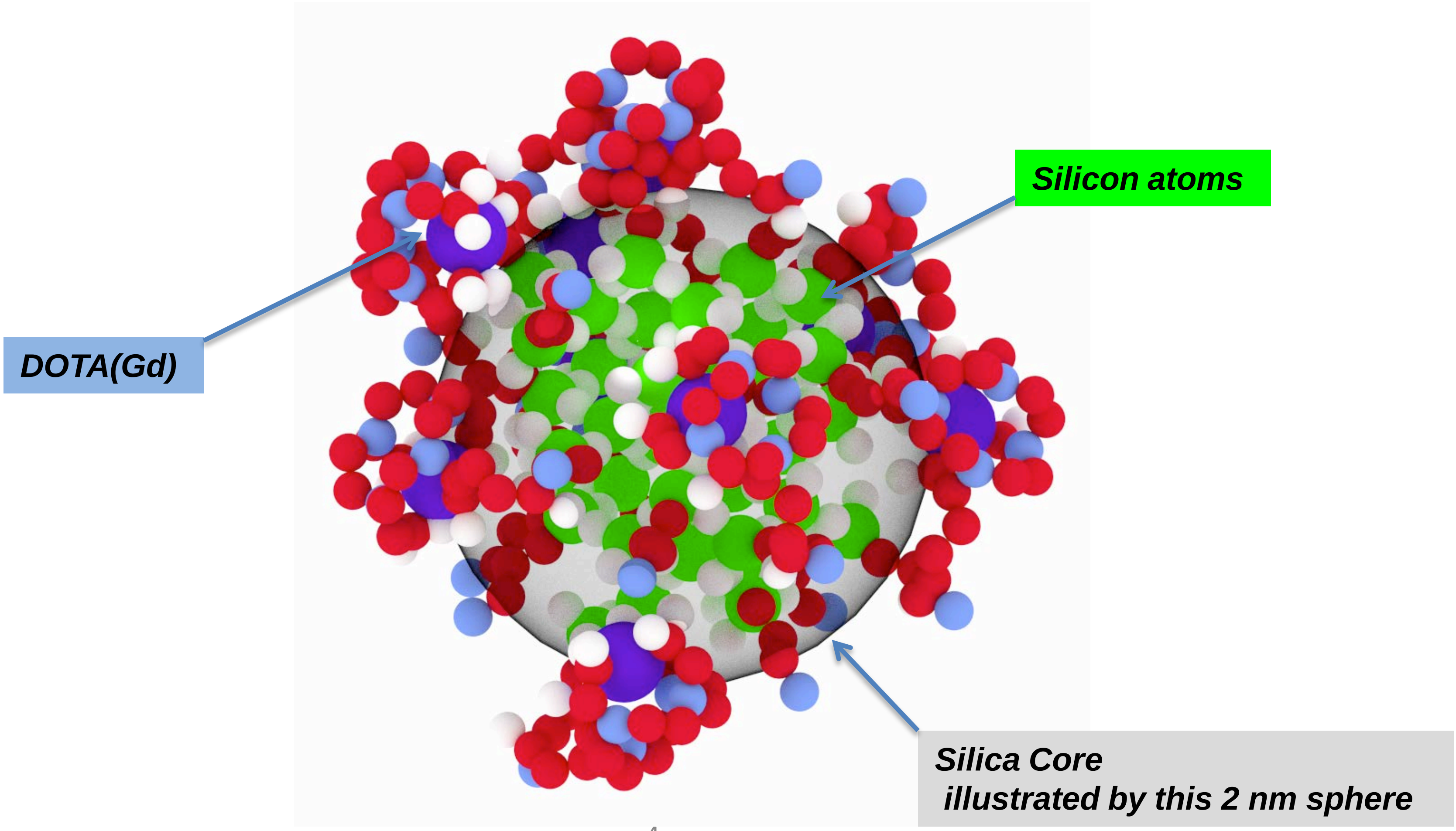

DOTA(Gd) (a kind of DOTAREM®)

Size **: 2-5 nm – 5/10 kDa**
High colloidal stability and freeze drying ability

AGuIX® Nanodrug

$$Gd_{10}Si_{30}C_{200}N_{50}O_{150}H_x$$

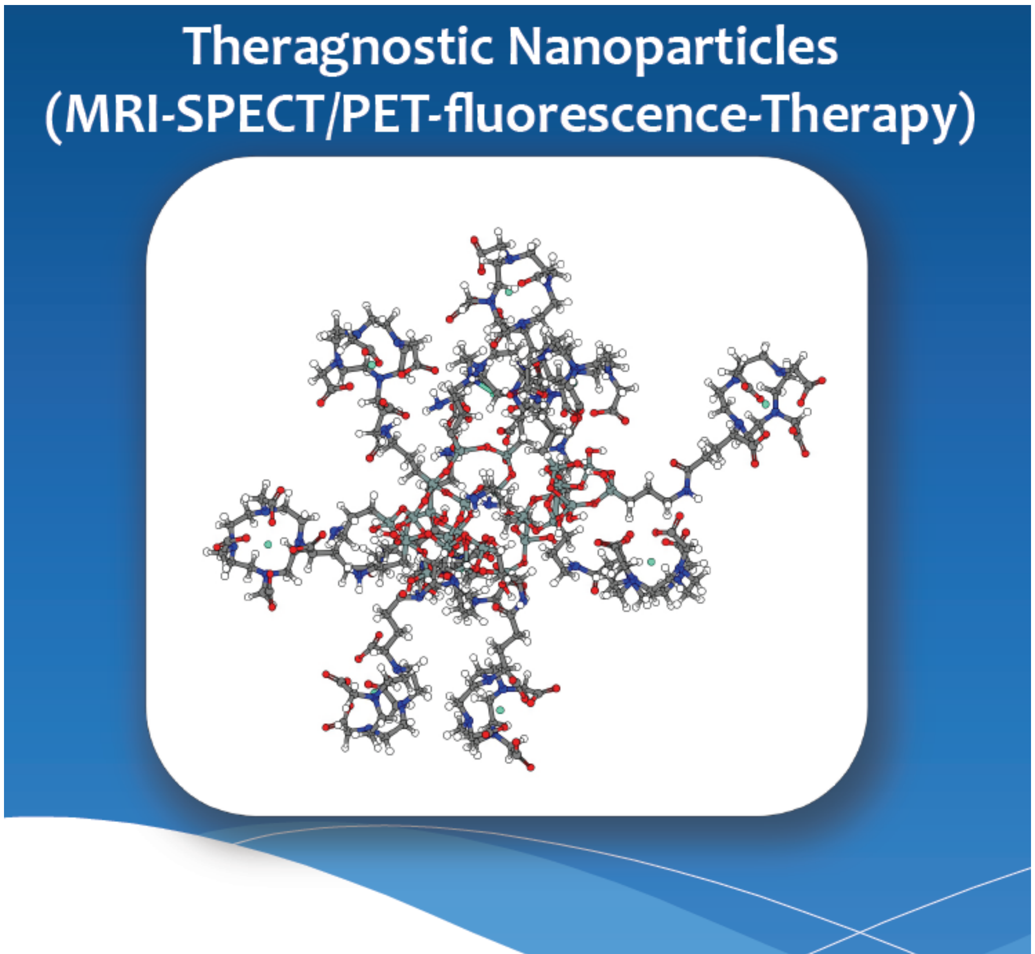
High gadolinium content ≈15% with a typical size ≈3 nm



AGuIX®

Preclinical Multimodal Nanoparticles

Laboratory batches of ≈50 g



Ultrasmall size
4±1 nm - renal excretion
MW 8.5±2 kDa

Polysiloxane composition
Easy further functionalization

DOTA (Gd) (MRI - Radiotherapy)
FDA approved
About 10 DOTAs/nanoparticle

Radiometals (M*) chelation
PET, SPECT, Therapy

WO 1053389 & F. Lux et al., Angew. Chem. Int. Ed., 2011 & A. Mignot et al., Chemistry Eur. J., 2013

Biodistribution
&
MRI contrast properties

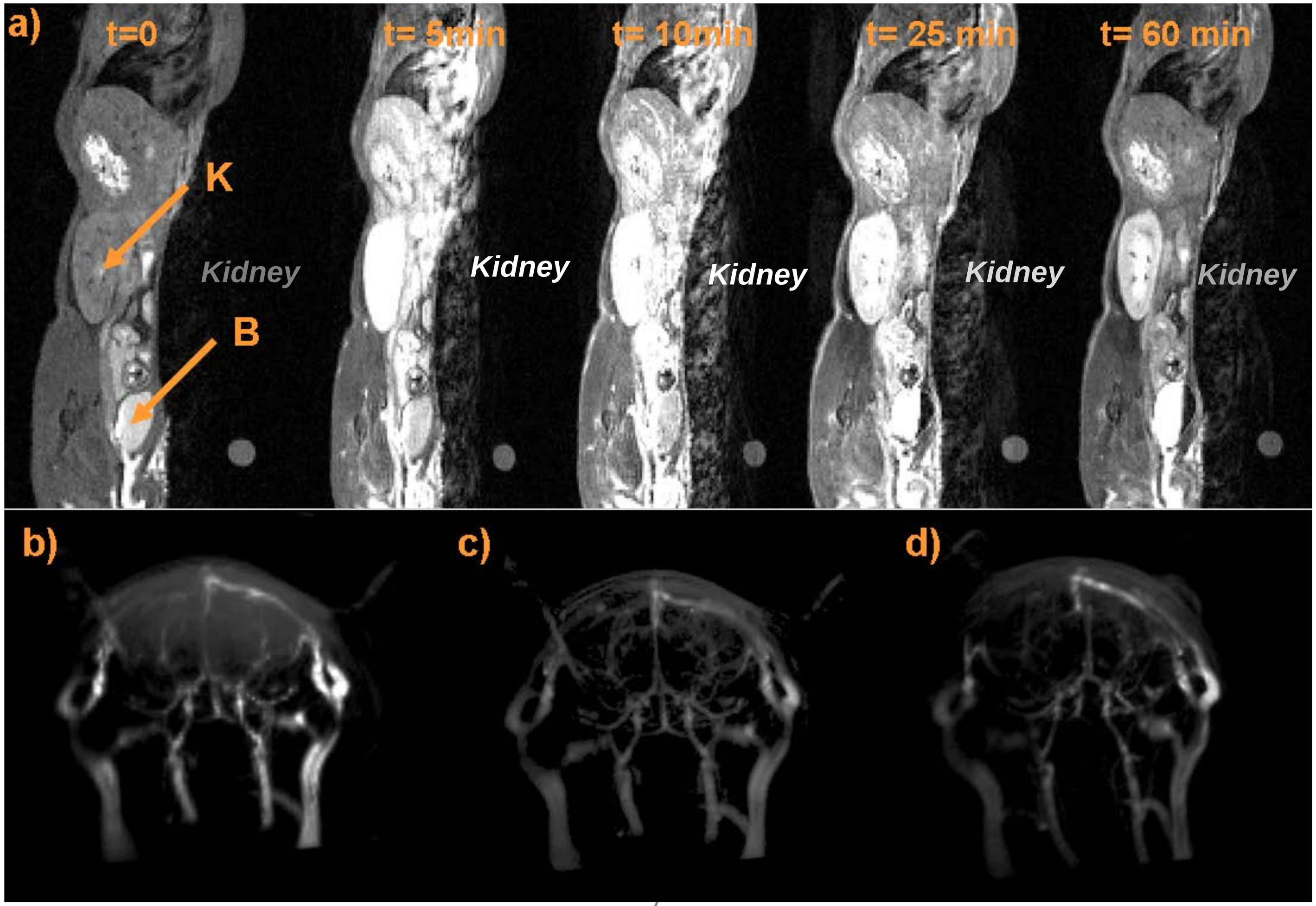
Two points

Gadolinium compounds are efficient T_1 MRI Contrast agents

AGuIX® presents very small size for particles

MRI images after intravenous injection in mice

Gadolinium based contrast Agent : MRI T1 effect
"Interesting" biodistribution associated to the 1-5 nm size

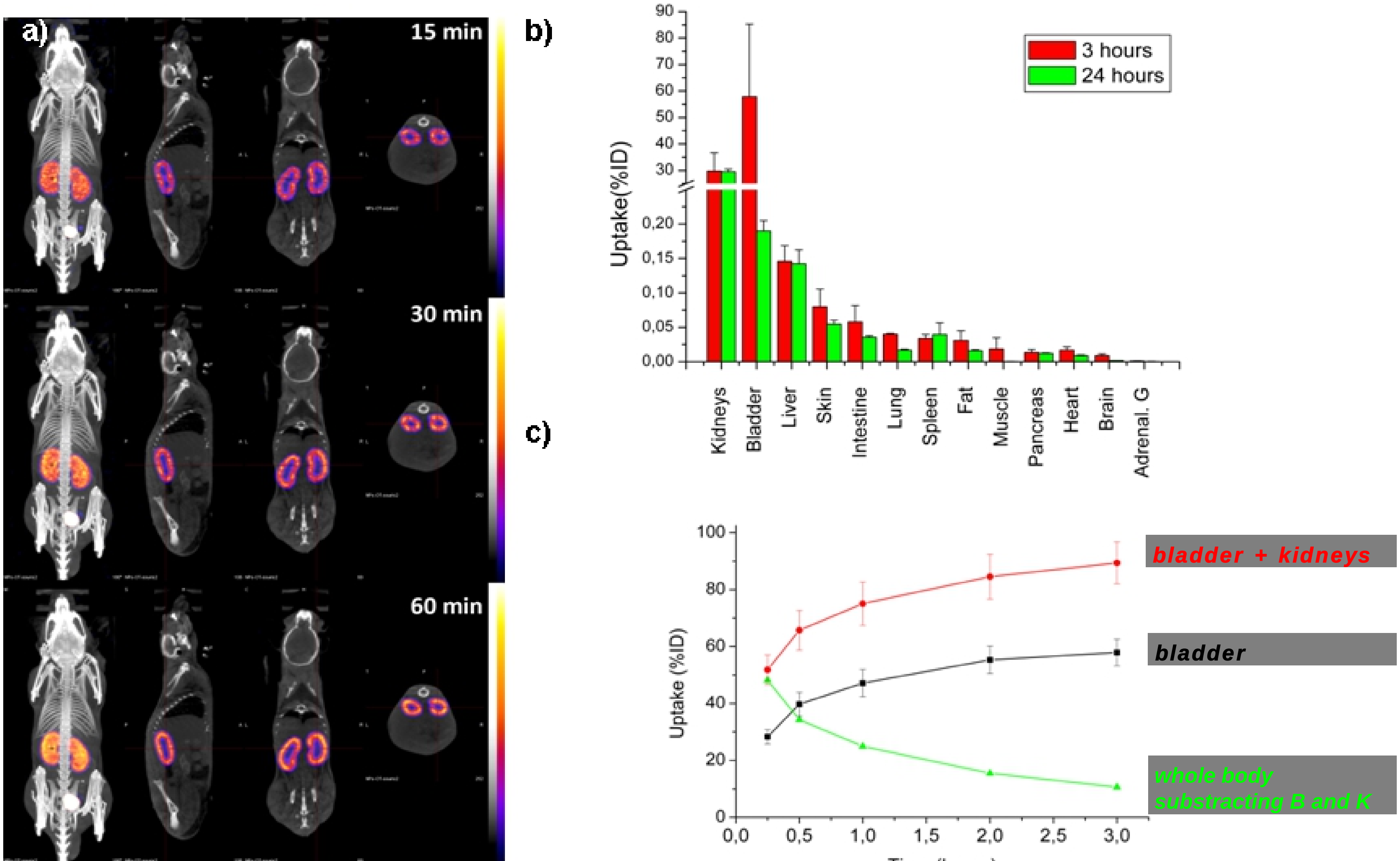


No contrast agent **AGuIX®** **DOTAREM®**

Injection IV: 80 µL at 40 mM in Gd - Male c57Bl/6J mouse T_1-weighted images- 7T

Biodistribution

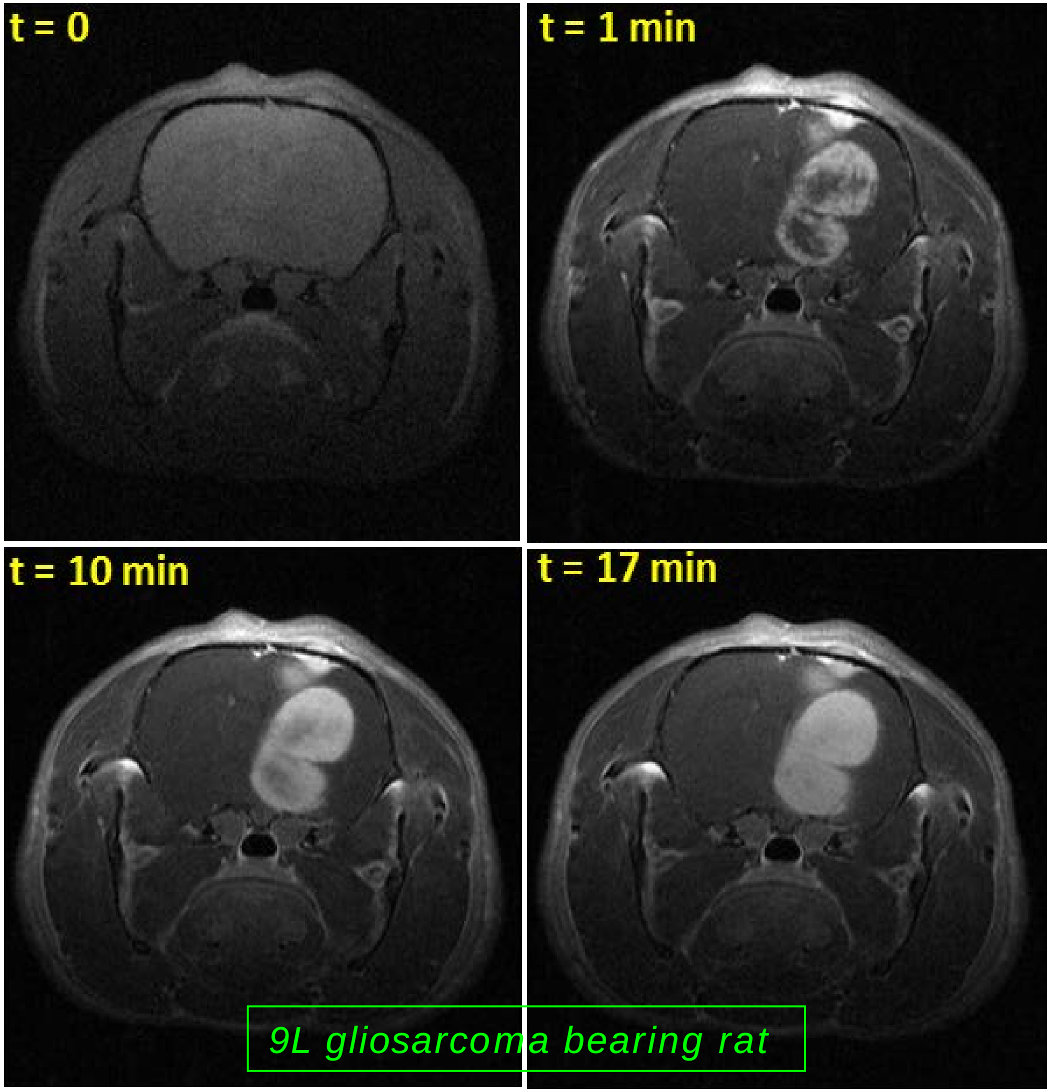
Renal elimination - No liver uptake - No extravasation
Blood residential time ≈ 2 times of classical molecular contrast agent

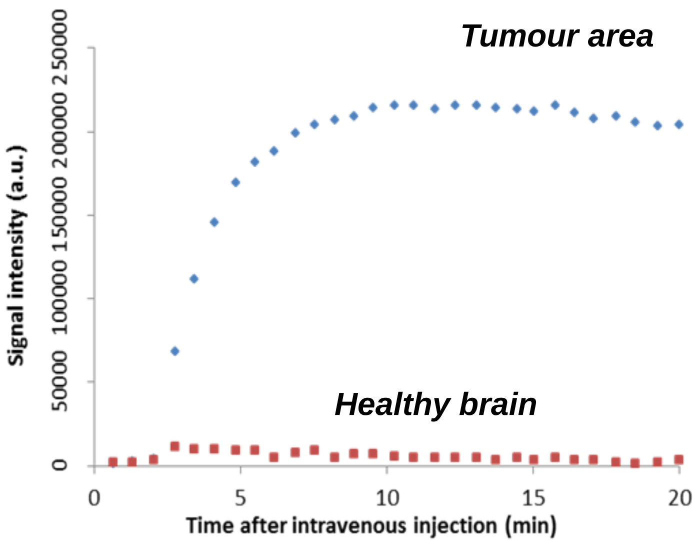


SPECT biodistribution ([111]In labeling) Male c57Bl/6J mouse

Tumor passive targeting

MRI T_1 – weighted images of the brain of a 9LGS-bearing rat after intravenous injection of AGuIX®





High efficient contrast agent

High R_1 value
Long tumour residential time
Low extravasation

High EPR effect !

Toxicological studies – Dose tolerance limits

IV injection – Clinical Dose CD ≈ 6 µmole Gd

Dose

Injected IV – Volume 150µl - Concentrations 200 to 500 mM – 6 mice/group for 10 Days

MTD - Maximum tolerated dose

MTD defined as the highest single dose that met all the following criteria:

zero death per group

maximal weight loss 10% in non-tumor bearing animals

CSS value as low as possible.

AGuIX®/ µmol (Gd)	Diarrhea	Lethargy	Closed eyes	Difficulty to wake up after anesthesia	CSS Clinical state score	Death	% weight variation
30	0	0	0	0	**0**	**0**	+3.2 %
40	0	0	0	1	**1**	**0**	+5.4 %
50	0	0	0	2	**2**	**0**	+0.8 %
75	0	0	0	3	**3**	**1**	+0.5 %

>10 CD

injection IV 500 g particles/l !

Lucie Sancey, Lot: FR16

Partial conclusion at this step

AGuIX®: Interesting small nano-compounds

Efficient Gd-MRI contrast agent

Multimodal access (*SPECT/PET*)

Tumour targeting (*high EPR effect*)

Well controlled synthesis

Only simple “classical” compounds (*Silica-Dota(Gd)*)

Access to IV injection

Renal elimination

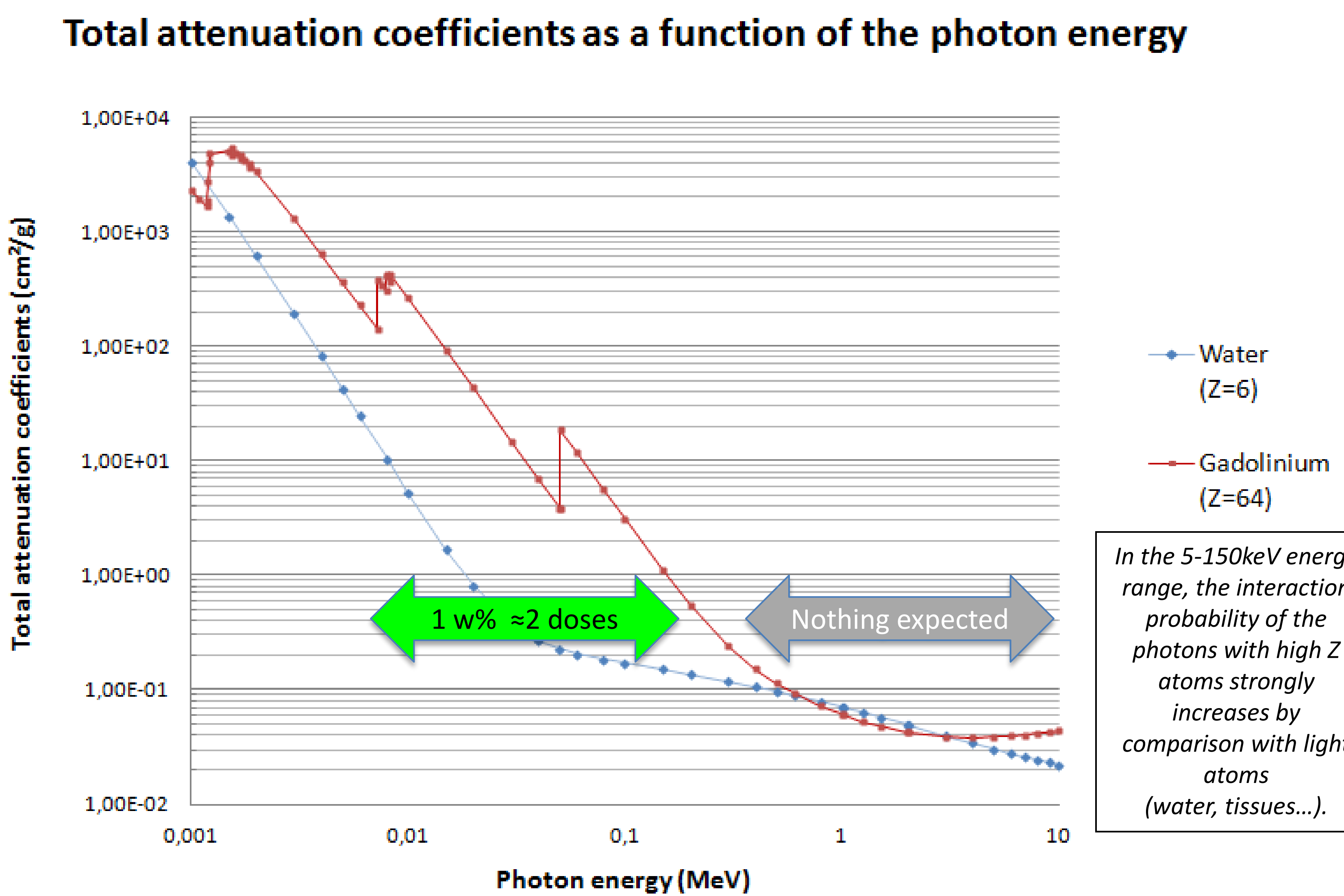
No toxicity evidence (*up to 10 times classical Gd-contrast dose*)

Therapeutical activation
&
Radiosensitization

Gadolinium is an element with a high atomic number
Z = 64

Dose enhancement can be expected with the presence of Gd (Z=64) atoms due to their greater X-ray absorption (attenuation coefficient)

1% by mass combined with keV X-rays have been suggested to increase the dose deposited by a factor of two (1 w% i.e. 10 g/l or 1000 ppm)



In the 5-150keV energy range, the interaction probability of the photons with high Z atoms strongly increases by comparison with light atoms (water, tissues…).

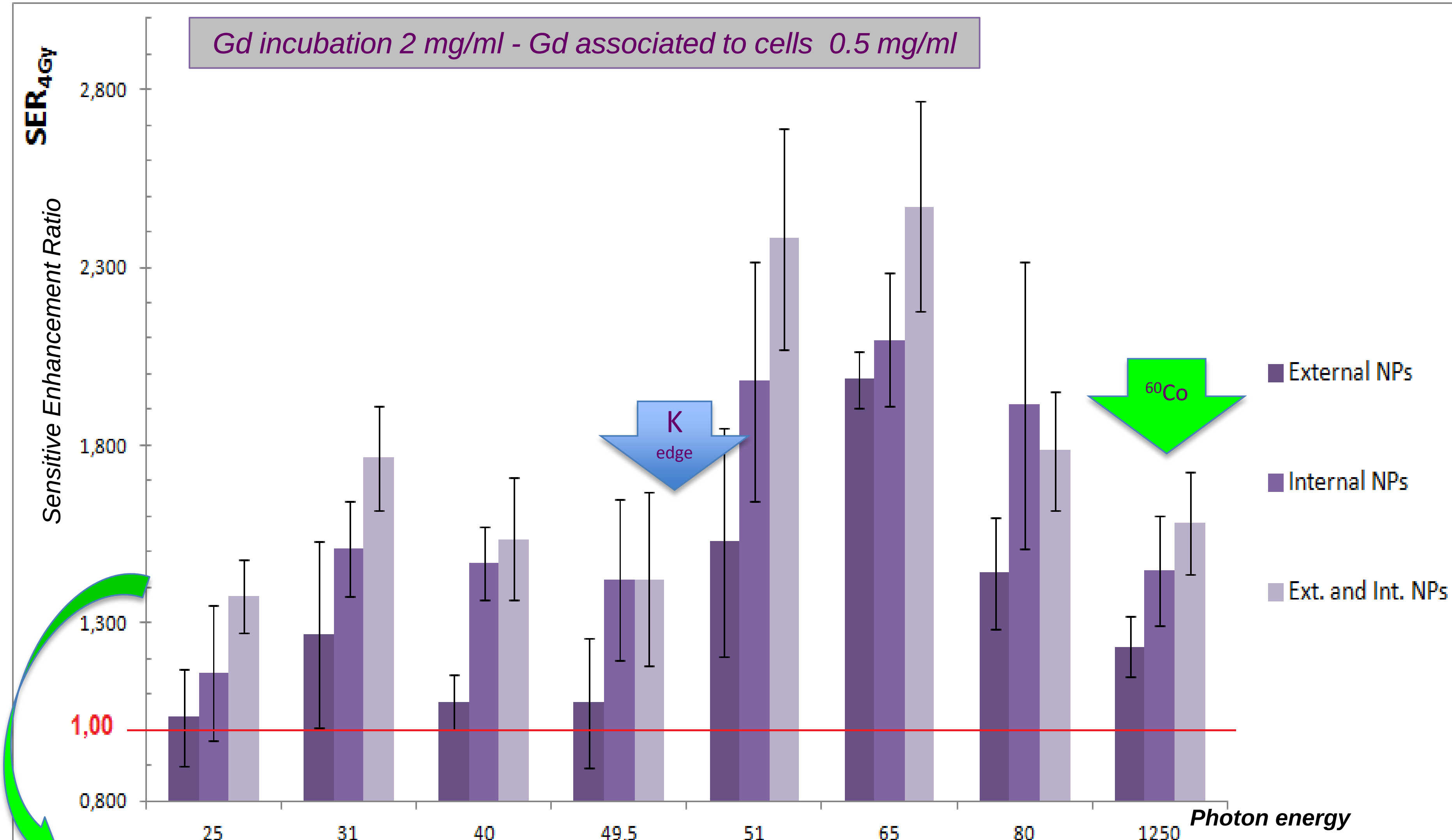
In Vitro Radiosensitization Experiments

Typical methods

Clonogenic assay to assess the *in vitro* viability of cells incubated either with or without AGuIX® nanoparticles and later irradiated.

Irradiation at ESRF with various energies around K_{edge} Gd

F 98 Rats Glioma cells



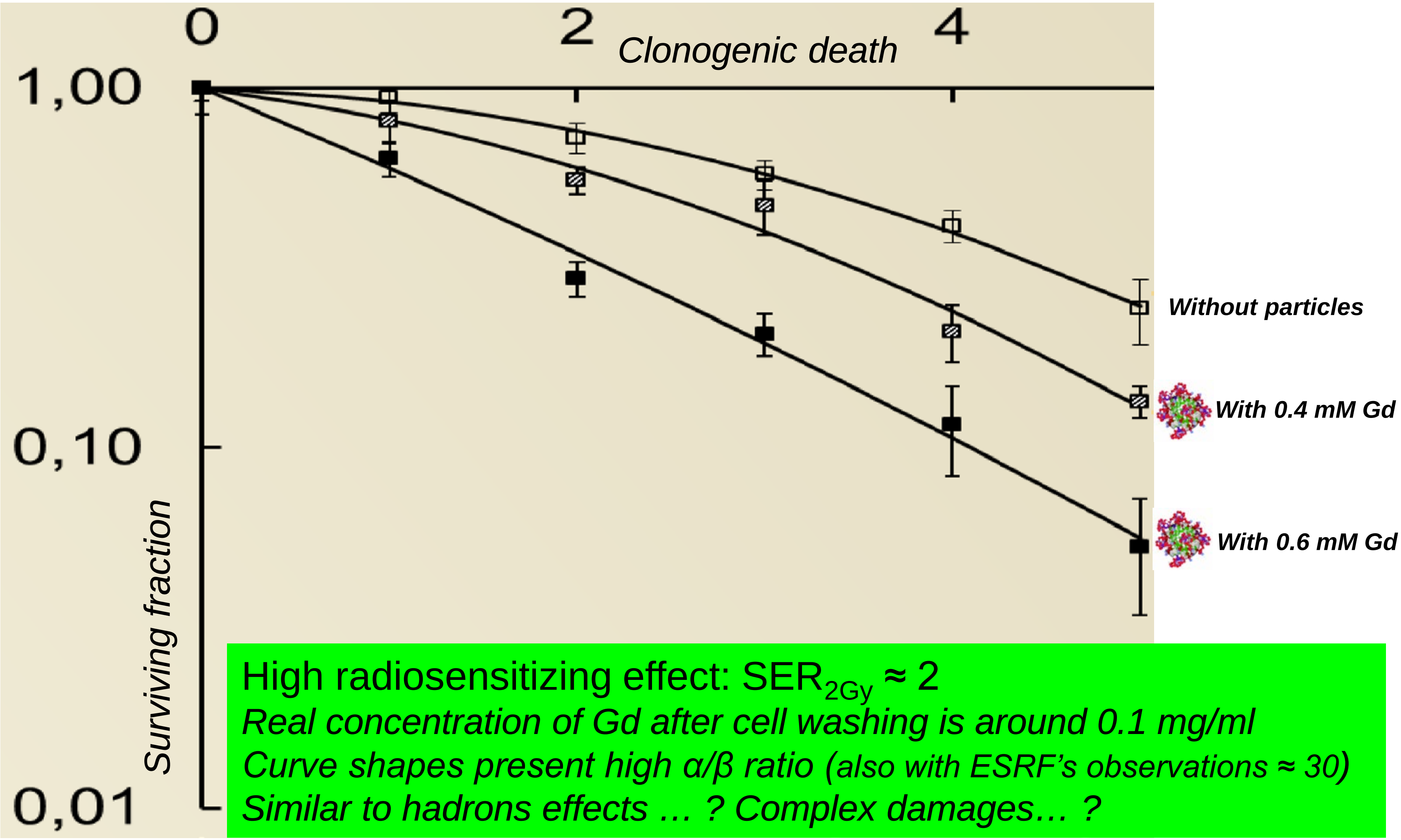
High radiosensitizing effect – expected evolution around the K-edge

Anyway: ten times more efficient than predictions in the keV range … and more if ***MeV****… and* ***outside*** *cells…*

F. Taupin *et al. Under review*

Irradiation at 250 kV (small animal irradiator facility Lyon)

SQ20B radioresistant Head and Neck Carcinoma



High radiosensitizing effect: $SER_{2Gy} \approx 2$

Real concentration of Gd after cell washing is around 0.1 mg/ml

Curve shapes present high α/β ratio (also with ESRF's observations ≈ 30)

Similar to hadrons effects … ? Complex damages… ?

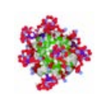
C. Rodriguez *et al. Under review*

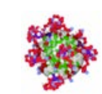
MV and kV Radiation Dose-enhancing effects of AGuIX®

Hela Human Cervix Carcinoma Cells
kVp SARRP & MV linear accelerator
Incubation 0.5 mM in Gd

Ross Berbeco – Boston

Dose	KV% Survival without NPs	KV% Survival with NPs	MV% Survival without NPs	MV% Survival with NPs
0 Gy				





High radiosensitizing effect

$SER_{4Gy} \approx 1.5$
for both the kV and MV irradiations

R. Berbecco *et al. To be published*

In Vitro experiments of dose-enhancing effects of AGuIX®

photons

Investigators	*Radiation / Energy*	*Cell line*	*Gd-AGuIX®*	*Biological effects*
H. Elleaume *et al.* *UJF/CEA - Grenoble*	31 to 80 keV Synchrotron ESRF	Rat malignant glioma F98	13.3 mM[c] – 5 h (washing or not)	**SER_{4Gy} 1.45 - 2.10**
K. Butterworth *et al.* *Queen's University - Belfast*	200/250 kV	Human Prostate Cancer DU145 & PC3	0.1-5 mM[c] - 1 h	**SF_{4Gy} 1.17 - 2.50** **SF_{4Gy} 1.25 - 1.33**
R. Berbeco *et al.* *Harvard MS - Boston*	200/250 kV Small animal Rad. Res. Plat.	Human Cervix carcinoma HeLa	0.5 mM[d] – 1 h	**SER_{4Gy} 1.6 (DEF 1.46)**
C. Rodriguez *et al.* *HCL - Lyon*	200/250 kV Small animal Rad. Res. Plat.	Human Head Neck carcinoma SQ20B & stem cells	0.4-0.6 mM[d] - 1 h 0.6 mM[c] – 1 h	**SER_{2Gy} 1.22-2.14** **SER_{2Gy} 1.4**
M. Dutreix *et al.* *Institut Curie - Paris*	660 keV Cesium (Institut Curie)	Human Glioblastoma U-87 MG	0.5 mM – 1 h	**γ-H_2AX + 80%**
H. Elleaume *et al.* *UJF/CEA - Grenoble*	1.25 MeV Cobalt - CEA	Rat malignant glioma F98	13.3 mM[c] – 5 h	**SER_{4Gy} 1.45 - 1.55**
R. Berbeco *et al.* *Harvard MS – Boston*	6 MV MV Linear Accelerator	Human Cervix carcinoma HeLa	0.5 mM[d] – 1 h	**SER_{4Gy} = 1.6 (DEF 1.44)**
M. Barberi *et al.* *CRAN – Nancy*	6 MV MV Linear Accelerator	Human Glioblastoma U-87 MG	From 0.01 to 0.5mM[d] – 24 h	**SER_{4Gy} 1.1 - 1.5**
G. Blondiaux *et al.* *CERI - Orléans*	Neutron Cyclotron Orléans, France	Mouse Lymphoma EL4	0.05-0.3mM - 1 h	**SER_{4Gy} > 2 (estimation)**
S. Lacombe *et al.* *Univ. Paris sud - Orsay*	Ions He^{2+} beam Chiba, Japan	Ch. Hamster ovary carcinoma CHO[a]	1 mM – 6 h	**SER_{4Gy} = 1.14**
Lacombe *et al.* *Univ. Paris sud - Orsay*	C^{6+} beam Chiba, Japan	Ch. Hamster ovary carcinoma CHO	1 mM – 1 h	**SER_{4Gy} = 1.5**
C. Rodriguez *et al.* *HCL - Lyon*	C^{6+} beam Germany	Human Head Neck carcinoma SQ20B	0.3[d]-0.6[c] mM – 1 h	**SER_{2Gy} 1.33 – 1.59**

c) AGuIX-DTPA; d) AGuIX-DOTA.
sensitizer enhancement ratio (**SER**) ; dose enhancement ratio (**DER**) ; dose enhancement fraction (**DEF**)

Partial conclusion at this In Vitro step

AGuIX® presents high radiosensitizing effects

Experimental evidences found by 8 different teams

Efficient with a large panel of radioresistant cells

Efficient with a large panel of Ionizing Radiations

Efficient at very low concentration (<<0.1 g/l in Gd)

<u>Last points</u>

Suspicion of activities even in the case of particles “outside” cells

&

During AGuIX® incubations, no evidence of any

cold toxicities neither *chemio-effects* neither *nano-stress* neither *nano-ROS*

neither *nano-toxicities* induced to cells…

without irradiation !

In Vivo
Preclinical Radiosensitization Experiments

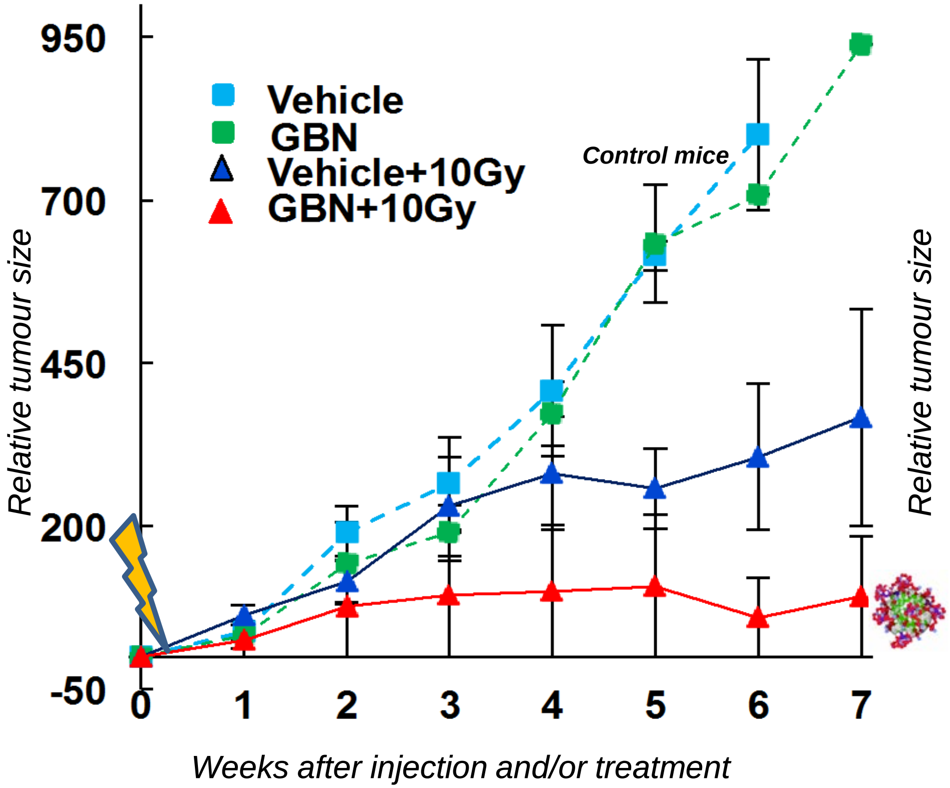
How can we reach efficient AGuIX® content in the tumour area ?

Injection IT, Intra Tumoural or Peritumoural (5-20% ID/g)
Nebulization for Lung – Administration via the Airways (1-5% ID/g)
Injection IV, Intravenous Injection (0.1-1% ID/g)

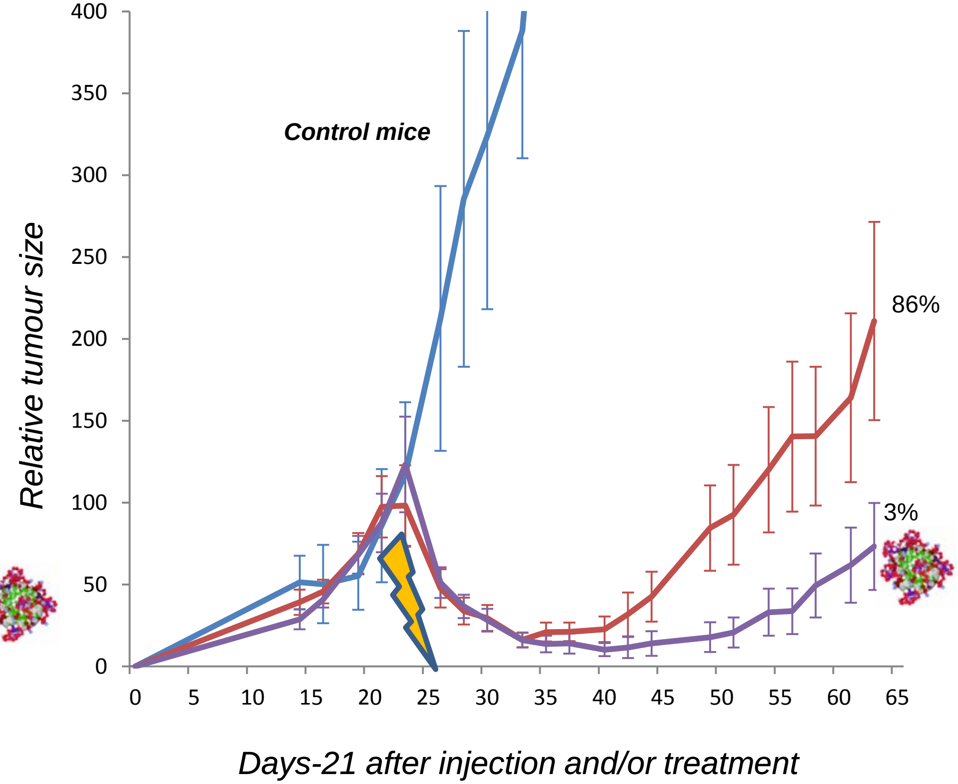
Irradiation after Intra Tumoural Injection of AGuIX®

Irradiation 200 kV 10 Gy after AGuIX IT injection SQ20B & A375sc

Head And Neck Carcinoma SQ20B
150 µl 6.6 mM Gd – 10 Gy



Melanoma A375 sc
40 µl 100 mM Gd - 2*10 Gy



In vivo radiosensitizing effects

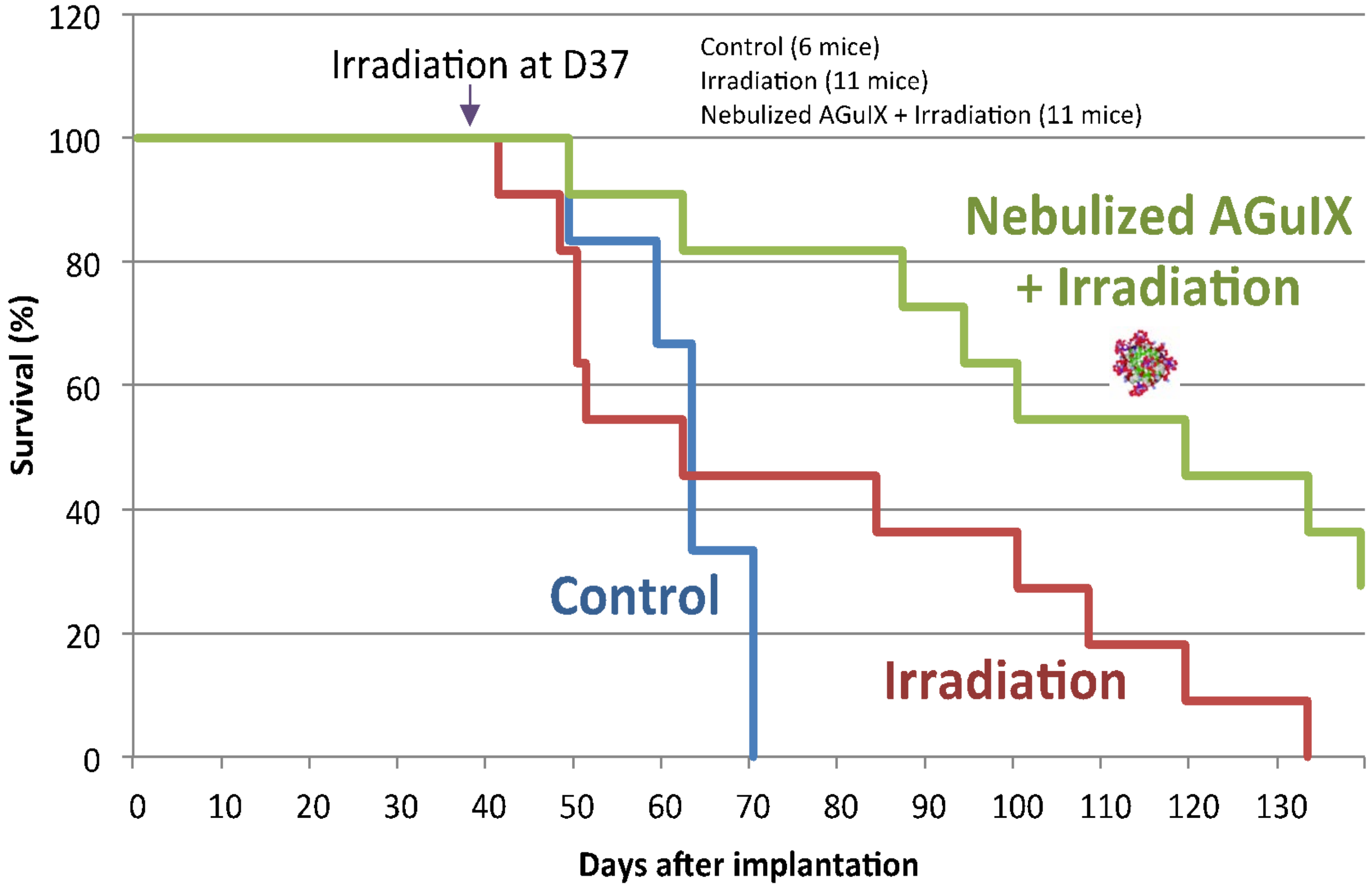
C. Rodriguez *et al. Under review*

S. Dufort *et al. Unpublished Results*

Irradiation after Inhalation: administration via the airways

Irradiation 200 kV 10 Gy 24 h after AGuIX® nebulization

Orthotopic Lung tumors, H358
50 µl 20 mM Gd – 10 Gy



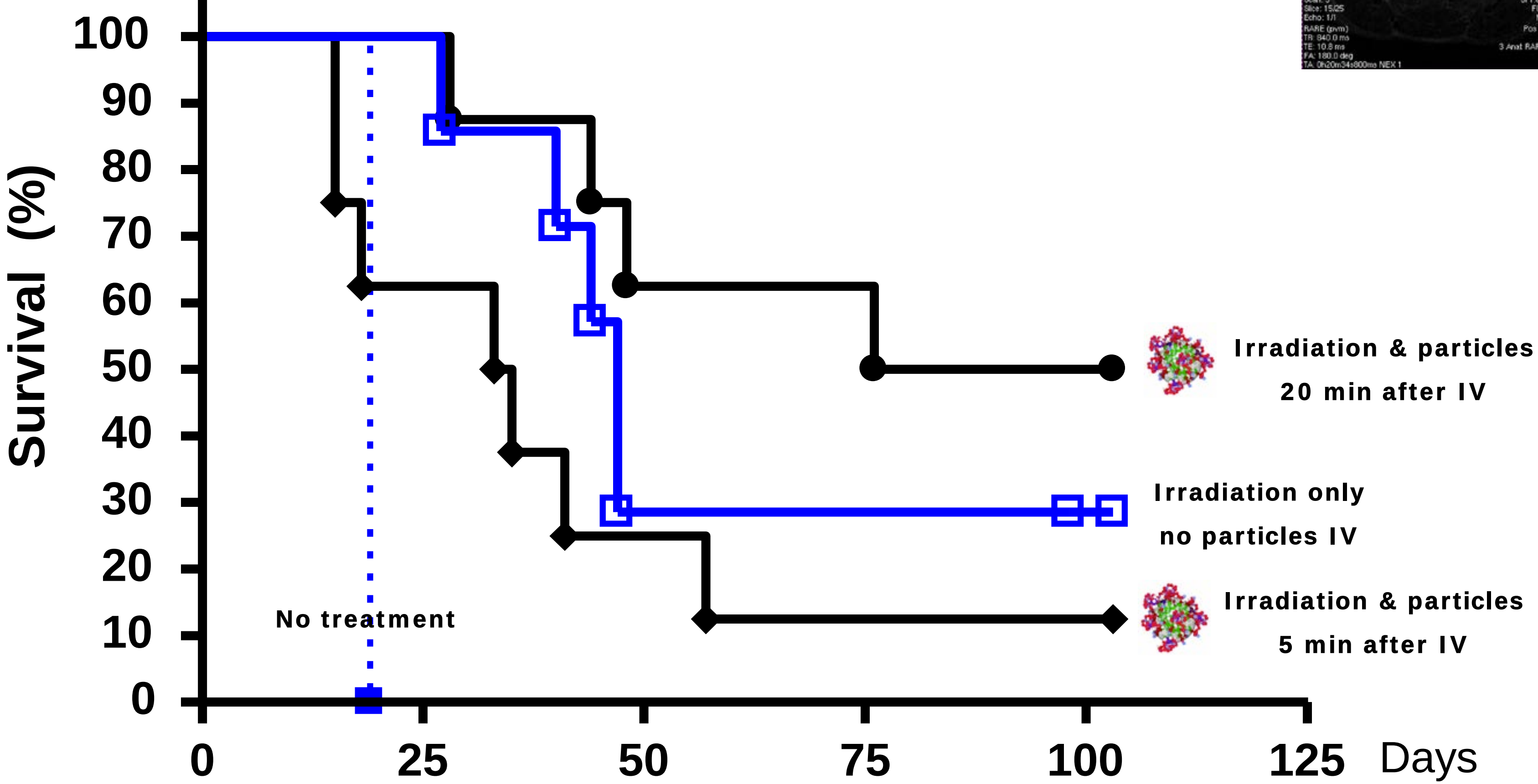
S. Dufort *et al. Unpublished Results*

Irradiation after Intravenous Injection of AGuIX®

Irradiation MRT after AGuIX IV Injection

Orthotopic Gliosarcoma 9L on Fisher Rats

1.4 ml 40 mM Gd, irradiation 5 or 20 min. after IV



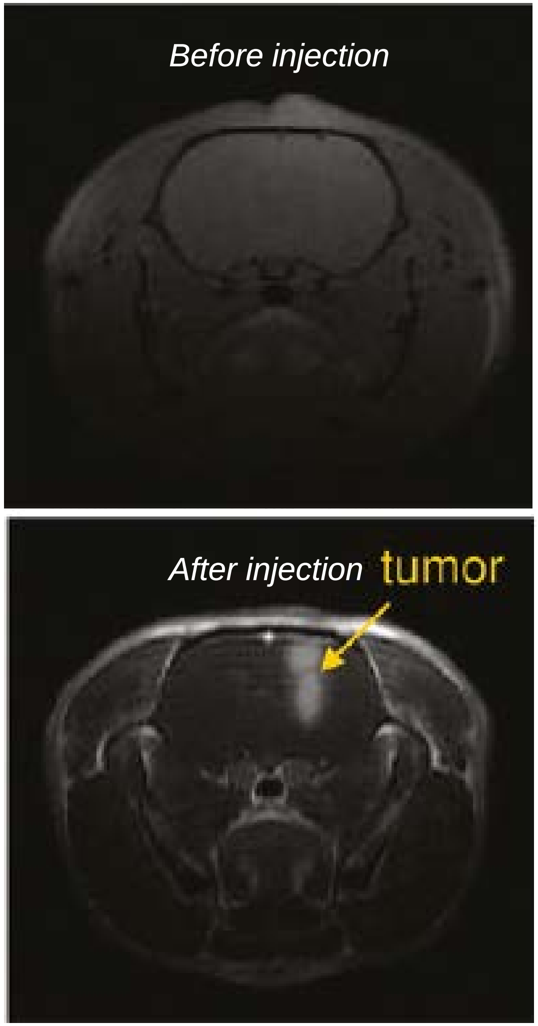
High radiosensitizing effect at 20 min.

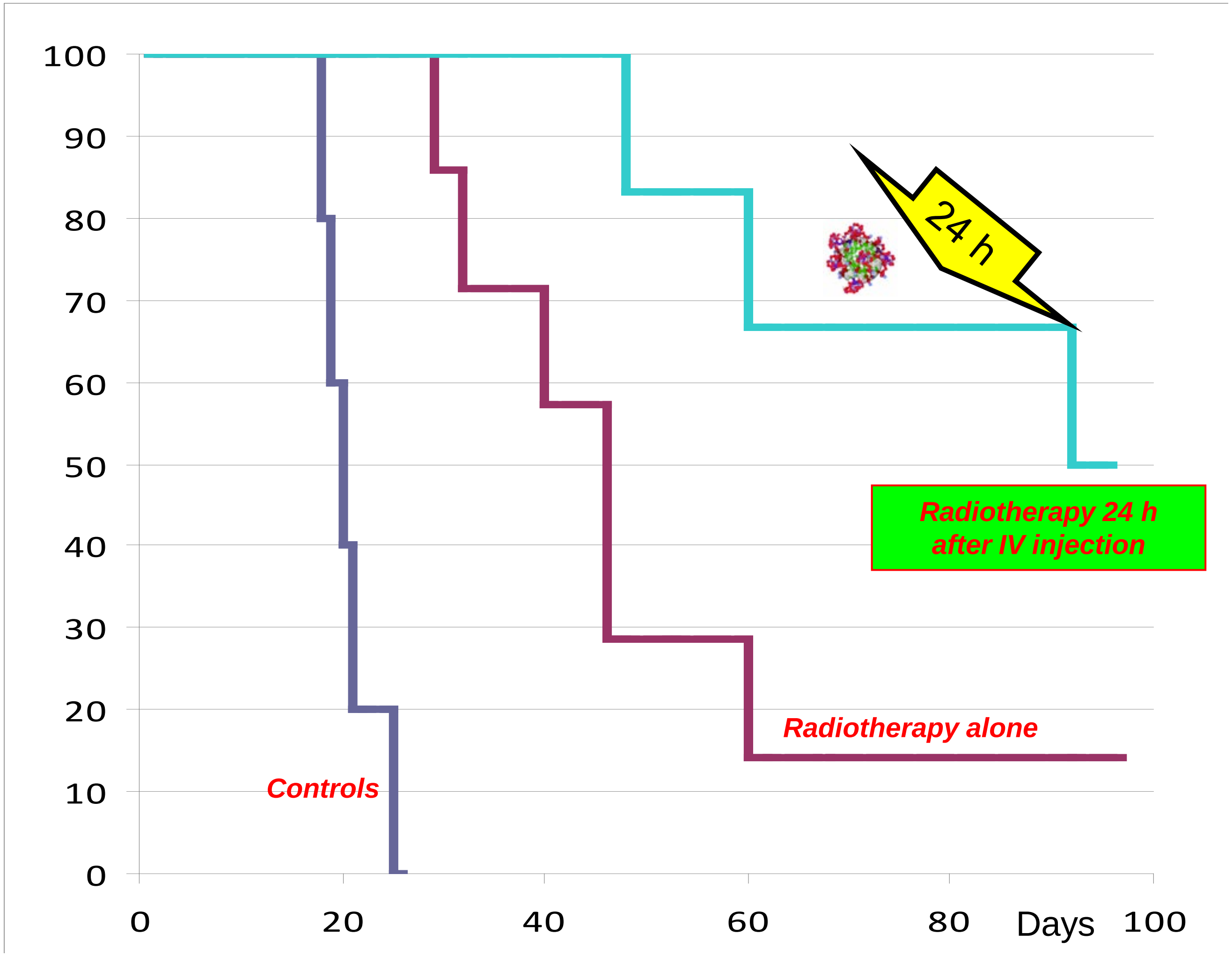
Result at 5 min. indicates an effect in the healthy area of the AGuIX® in blood stream… and outside cells…

G. Le Duc *et al.*, *ACS Nano*, **2011**, *5*, 9566-9574

Irradiation 24 hours after Intravenous Injection of AGuIX®

Orthotopic Gliosarcoma 9L Fisher Rat – 1.4 ml 40 mM Gd





Very high Radiosensitizing effect 24 h hours after IV injection of AGuIX®

Gadolinium concentration in tumours seems to be in the ***ppm*** *range µg/g…*

G. Le Duc *et al., Unpublished results*

Conclusion

AGuIX® radiosensitizer

High radiosensitizing effect

complex damages

No need of specific irradiations

conventional clinical apparatus

Efficient at low concentrations

ppm range - <0.01 w% - <1% of injected dose

No specific active targeting is needed and EPR alone can be enough

No need of specific cell internalisation

active outside the cells

No evidence of toxicity

renal elimination

MRI contrast agent: Theragnostic compounds

efficient MRI T_1 Contrast Agent

Mechanisms – Fundamentals studies
&
How can this work ?

Surprising very high radiosensitizing efficiency

Efficient with

Low concentrations,

large panel of Ionizing species,

large panel of tumour cells

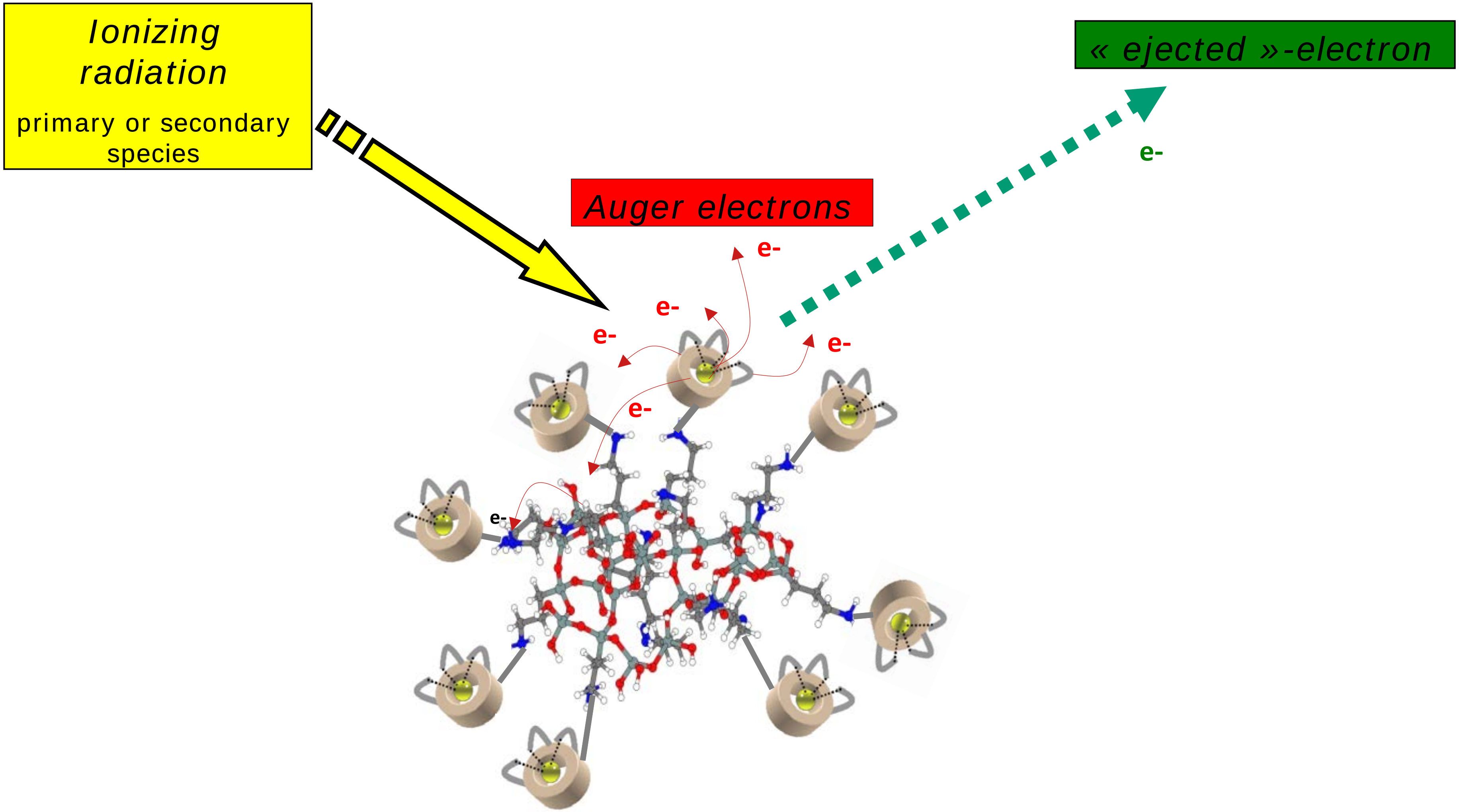
Outside cells

Complex damages

A possible mechanism story... draft schematic story...

Interaction with Ionizing radiation and a gadolinium

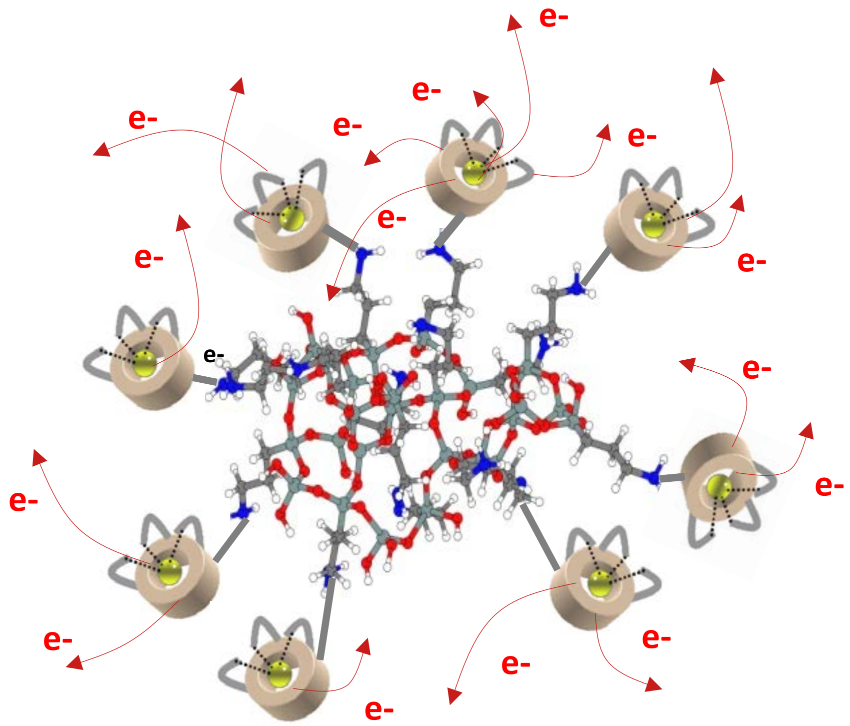
Initiation of a photon electron and some Auger electrons



Propagation to neighbour High Z species

Nano particle effect

Auger shower propagation



Distance between two Gd neighbour ≈ 1 nm inn AGuIX®

(1 mM in a molecular complex form will give ≈10 nm)

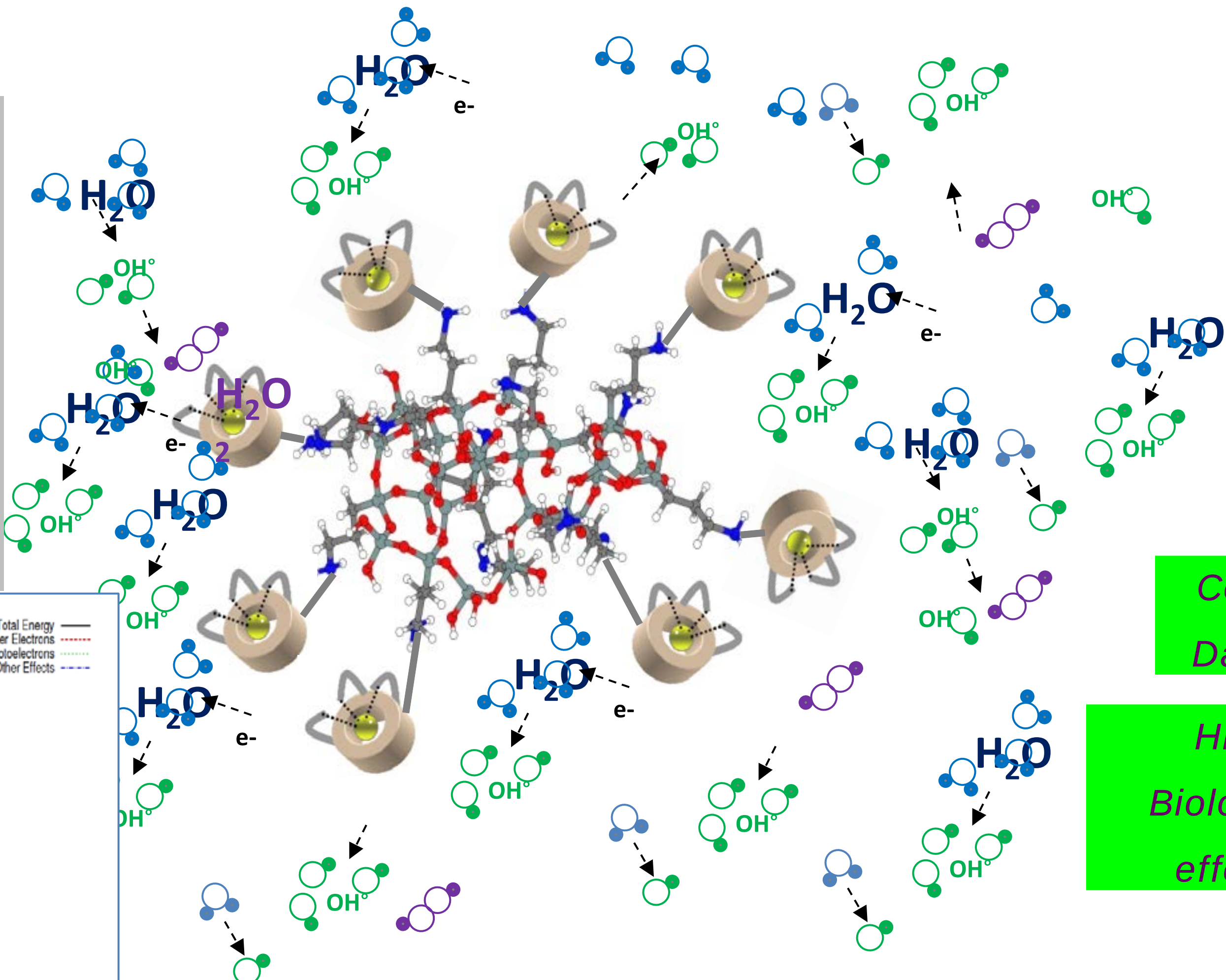
Delivery of high doses in the local zone around nanoparticles

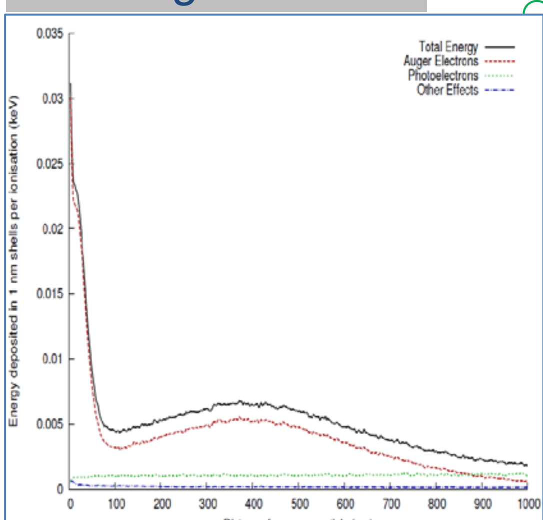
Formation of high concentration of active species

(radicals, peroxides,…)

Extremely large doses are expected in the vicinity of the Nanoparticles.

Hundreds of Gy <50 nm after 1 ionizing event…





Complex Damages

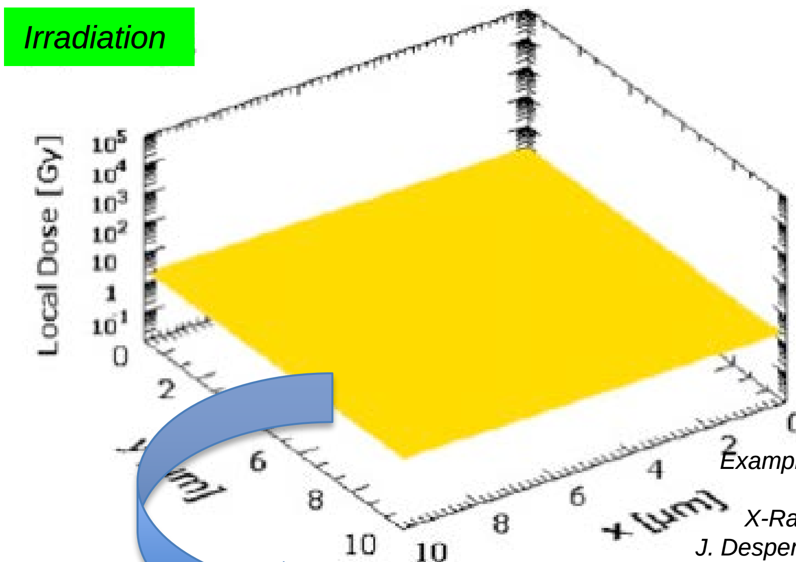
High Biological effects

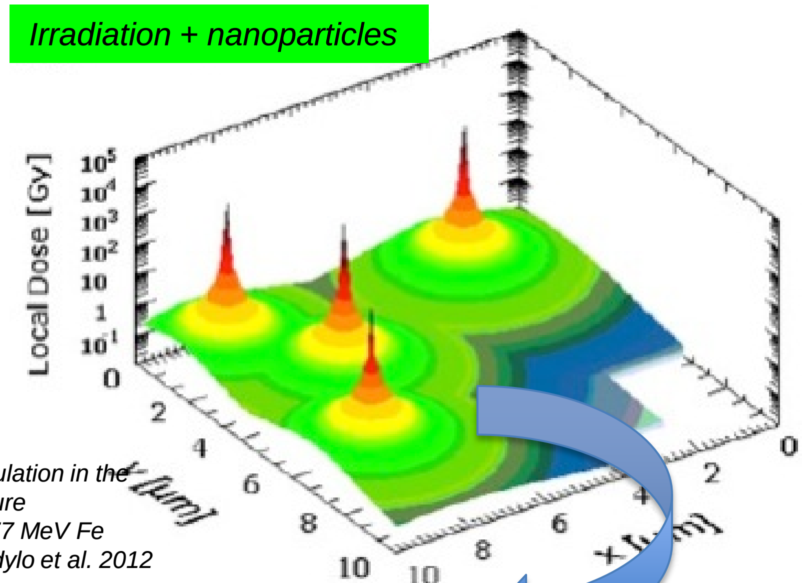
Calculations S. McMahon *et al.*, Scientific Reports 2011

Same global macroscopic dose

but some local modifications in the sub-micrometric / nanometer range

Same dose will create the same amount of ROS ° OH





Example of simulation in the literature
X-Ray and 177 MeV Fe
J. Desperas-Standylo et al. 2012

Formation of the same amount of ROS : °OH

$°OH + °OH \rightarrow H_2O_2$

H_2O_2 formation is related to the square of the °OH concentration…

local high ROS concentration can initiate secondary chemical species with high chemical stability (for example H_2O_2…HOCl…) and long range action…

Only hypotheses for a beginning of explanations !

I think there are tricky interesting points to understand, and we need helps…

Preclinical and fundamental studies

In 2013, we start 3 PhDs in collaboration with the teams of …

Ross Berbeco (Alex Detappe - Pancreas)

DANA-FARBER CANCER INSTITUTE

Eric Deutsch (Frédéric Law- Lung)

Institut de cancérologie GUSTAVE ROUSSY VILLEJUIF - www.igr.fr

Claire Rodriguez (Shady Kobt – Head & Neck)

Faculté de Médecine LYON SUD Charles Mérieux

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Et al. !