



# AGuIX

## *Nanoparticules hybrides théranostiques, premier essai clinique phase 1b*

Olivier TILLEMENT

Université Lyon 1

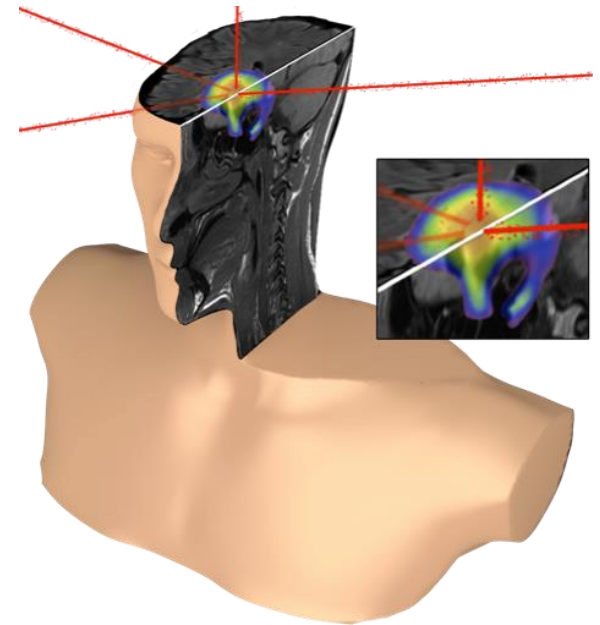
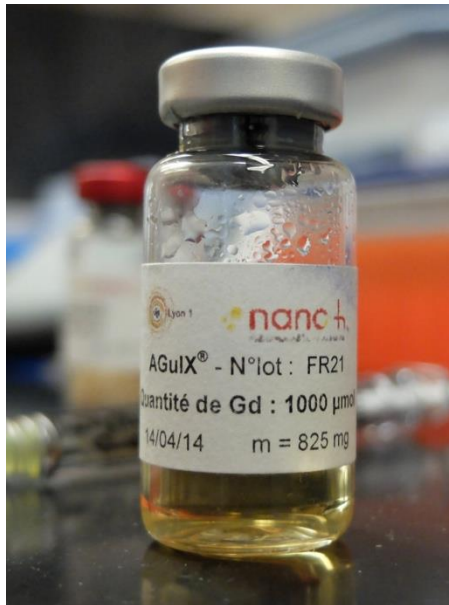


NH TherAguix SAS





# Nanomedicine



## ***AGuIX nanoparticles*** ***Activation and Guiding of Irradiation by X-Rays***



# AGuIX

## *Nano Hybrid Gd-particles*

### Composition

polysiloxane and DOTA(Gd)

high doping in gadolinium:  $\sim 15$  w%

### Size

$\sim 3$  nm

Good monodispersity

### Weight

$\sim 10$  kDa

### Conservation

Freeze-Drying

### Reconstitution

Colloidal solution

(+) 100 g/l

### Complexation constant

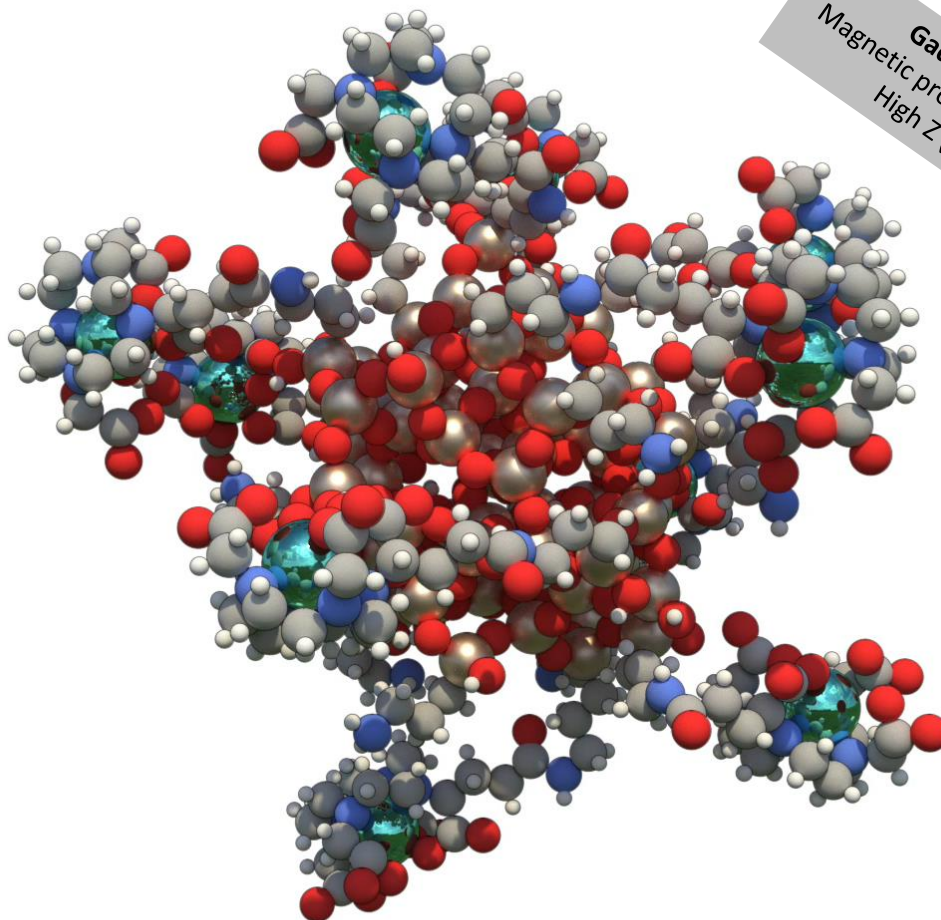
of DOTAGA on the Np

$\ln \beta \sim 24.78$

### Relaxivity

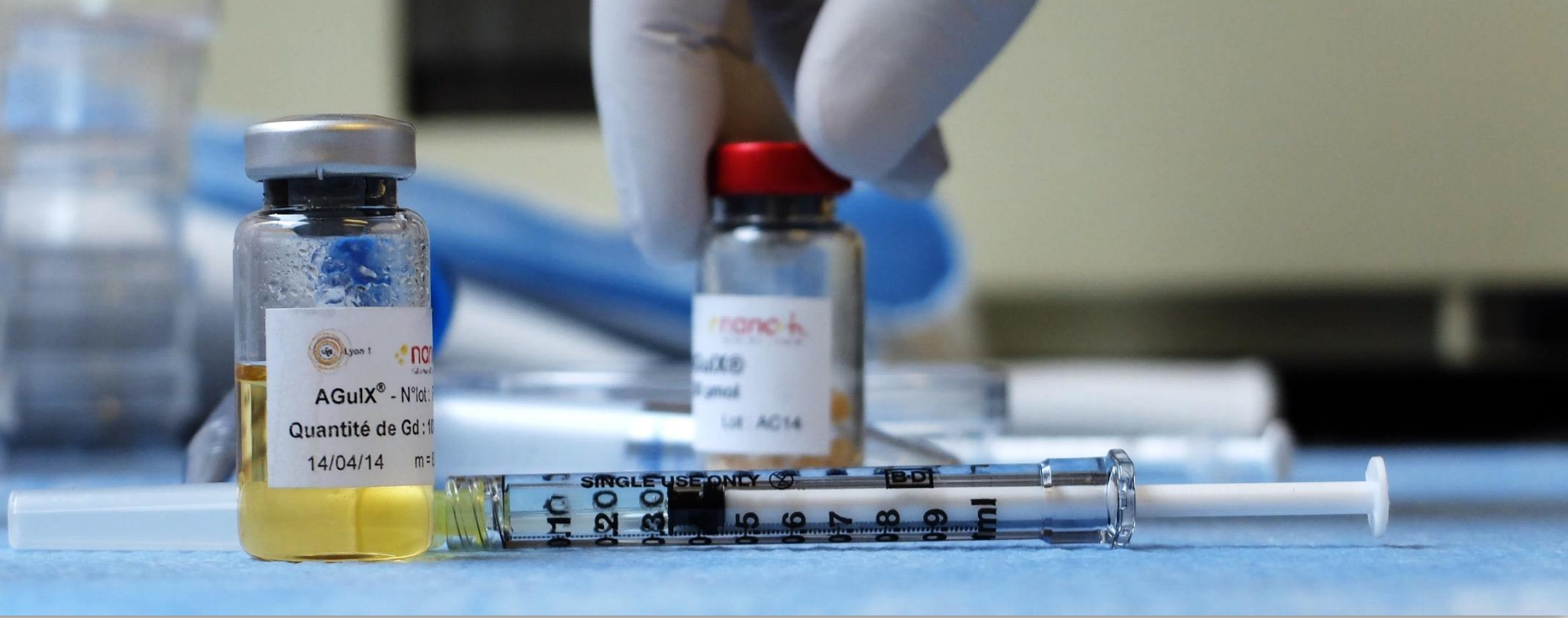
MRI-T1

$r_{1(\text{Gd})} \sim 10 \text{ s}^{-1} \cdot \text{mmol}^{-1}$  (60 MHz)



**Two safe compounds**  
Silica (polysiloxane)  
Stable Chelates (Gd)

**Gadolinium**  
Magnetic properties (MRI)  
High Z (64)



# Theranostic nanoparticles

## *Targeting – Imaging - Treatment*



## 1 - Theranostic

# Tumors targeting and Imaging

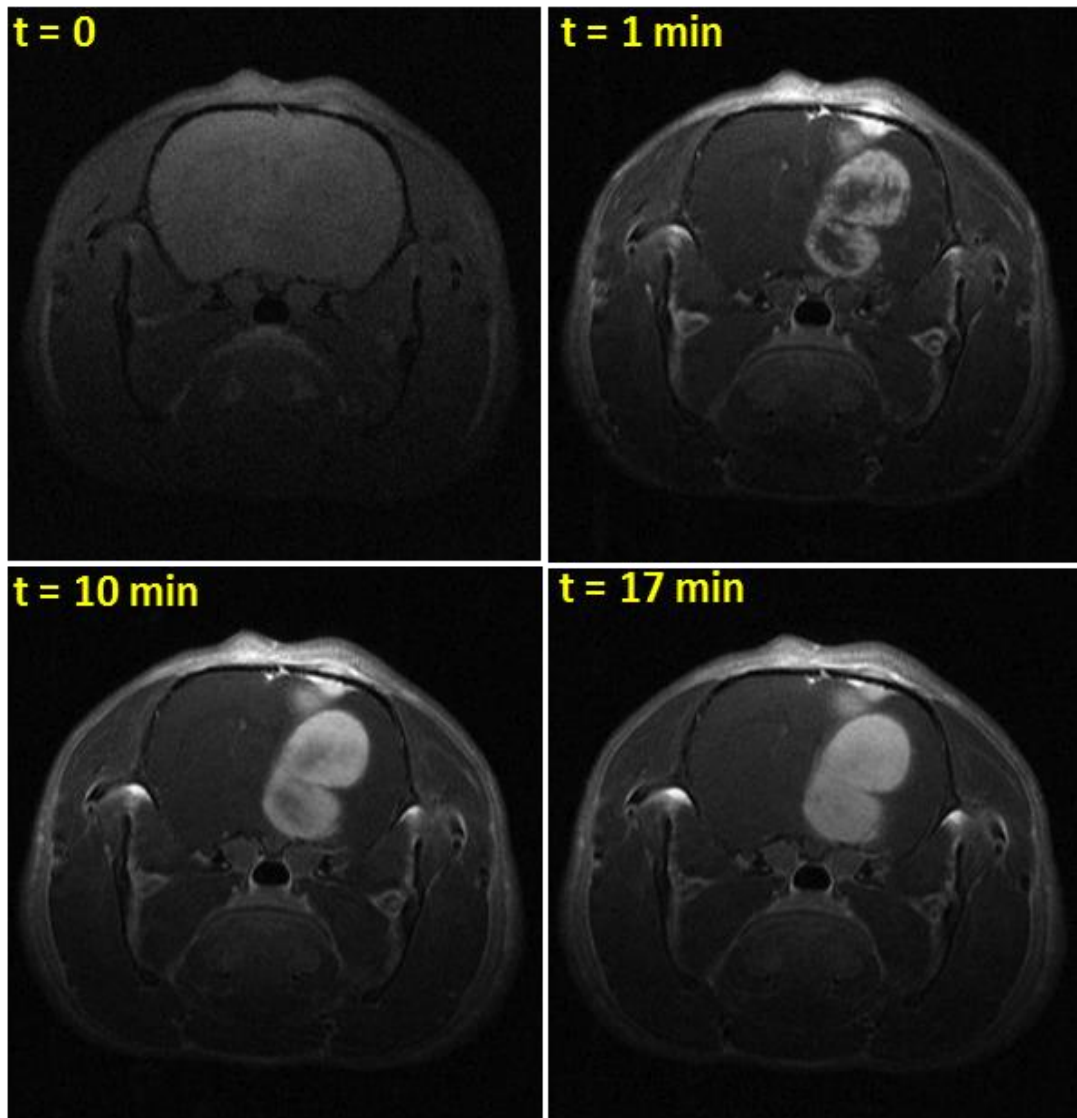
*Intravenous injection of AGulX to target (and see) all solid tumors*

# Preclinical results of Passive targeting after IV injection

6

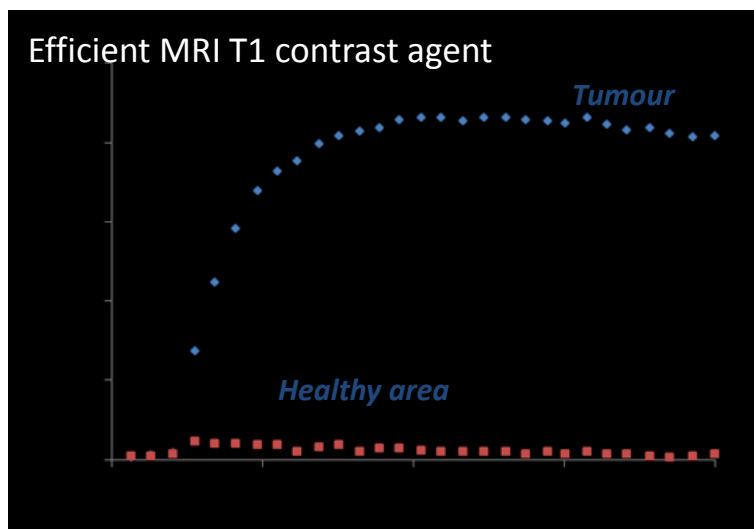
*Orthotopic Gliosarcoma 9L Fisher Rat*

G. Le Duc team – ESRF – Grenoble



*EPR effect*

Leaky tumor - Passive diffusion



TUMOUR IMAGING  
MRI T1



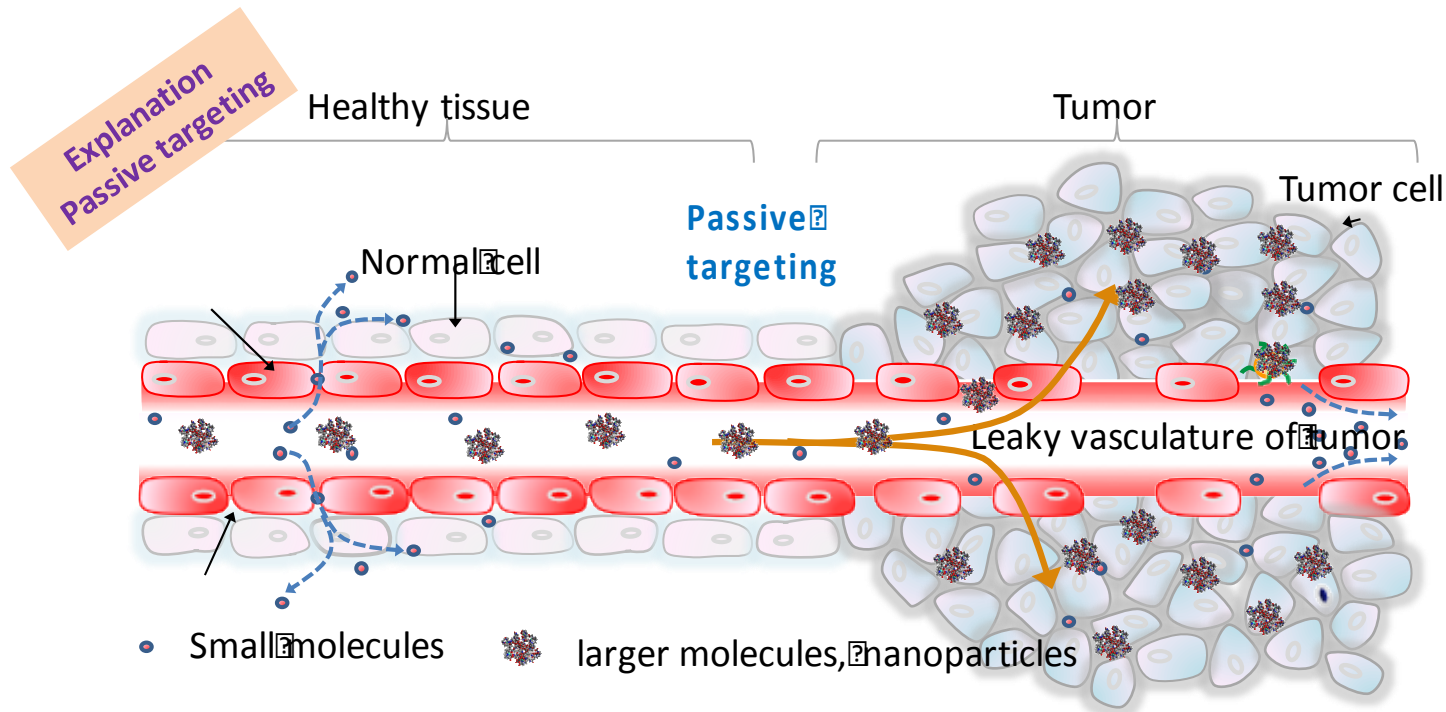
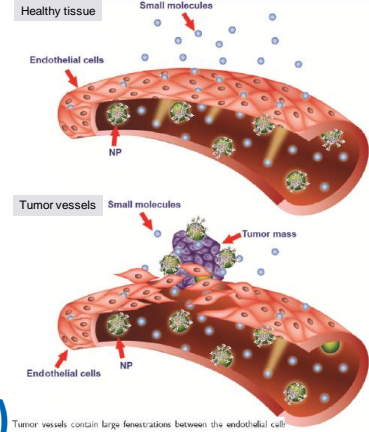
*EPR*  
*PASSIVE DIFFUSION*

# Target the tumours ?

## Nanomedicine & EPR Targeting

Tumour vessels contain large fenestrations between the endothelial cells

Well known passive targeting of the tumor by Enhanced Permeability Retention effect – 5<sup>th</sup> m<sup>3</sup> (EPR effect)



Diffusion in tumor No extravasation in healthy area

Selective distribution

<1% ID but very specific and stable

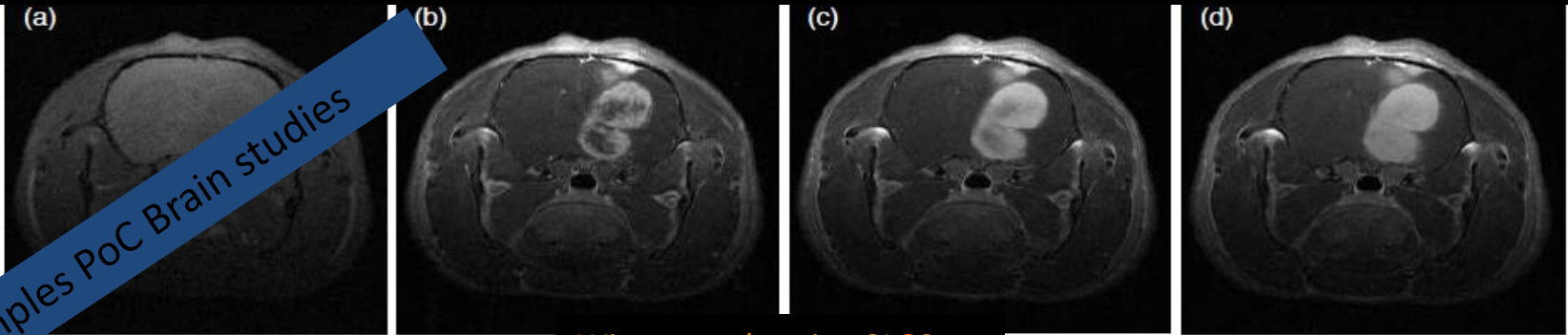
Maeda et al, Microvasc, 2016

Maeda et al, Controlled Release, 2000

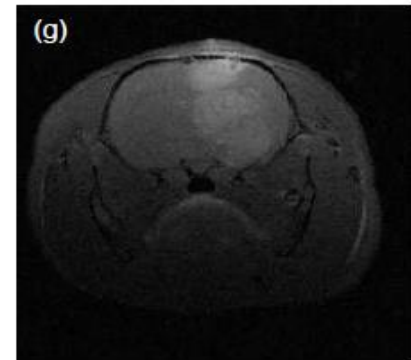
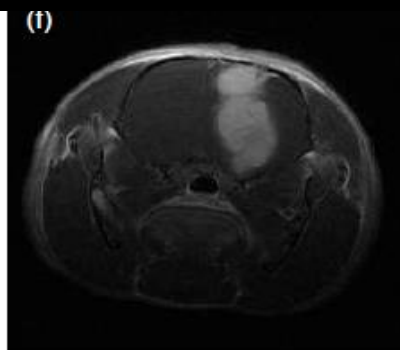
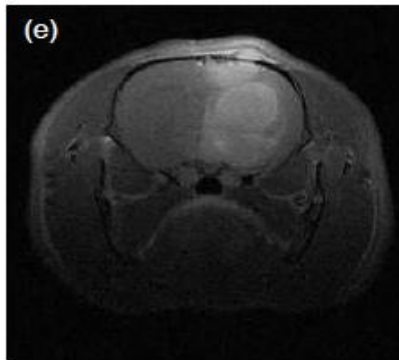
# T1-weighted images of Glioma after Intra Venous AGuIX Injection

8

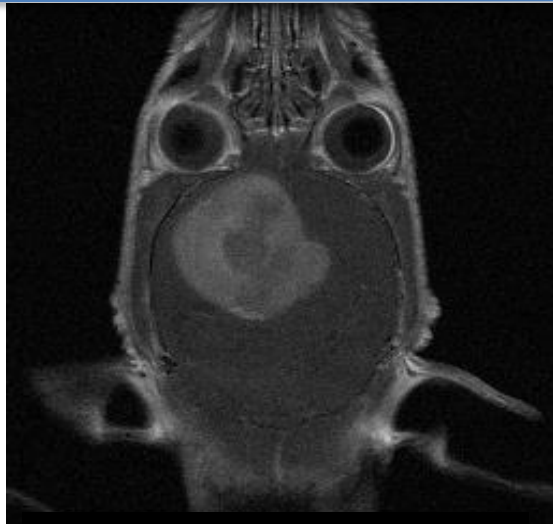
Examples PoC Brain studies



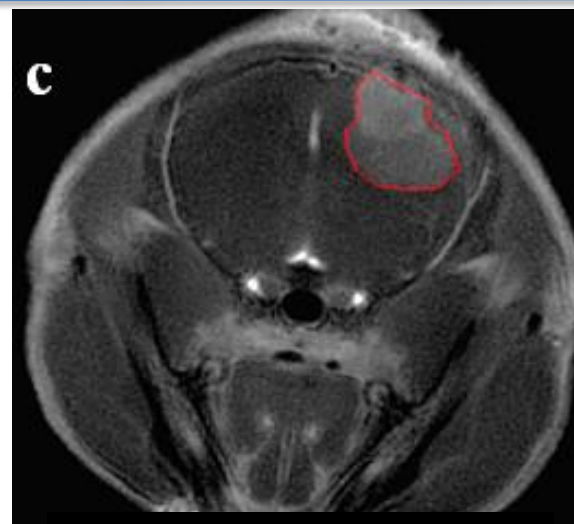
Wistar rats bearing 9LGS



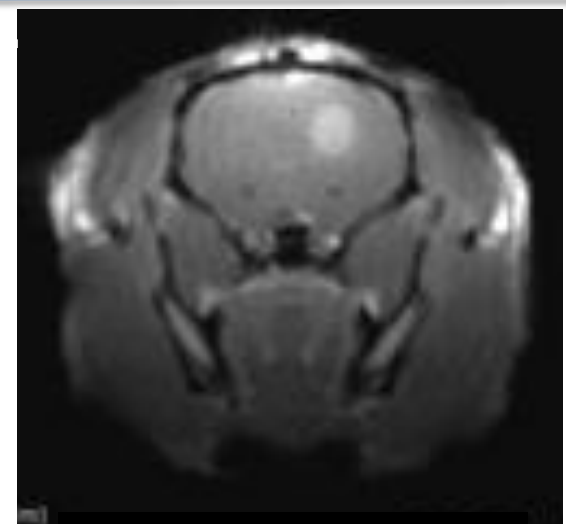
1



Nude Mice-bearing U87MG



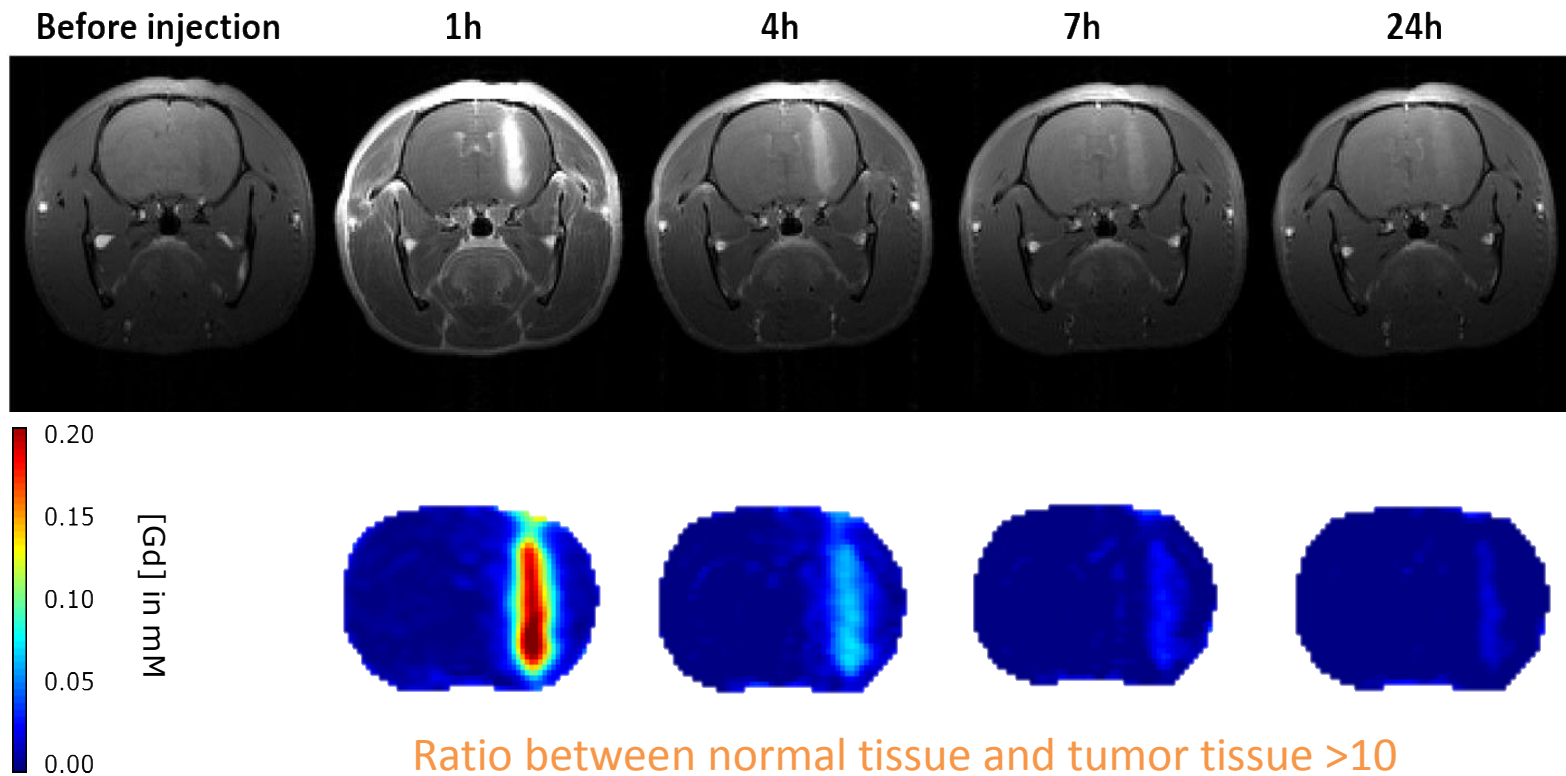
Nude Mice-bearing U87MG



Nude rat bearing U87MG

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# Tumour Specificity & Persistence in tumours tissues 24h after IV





## 2 – Theranostic

In Vivo and in Vitro preclinical Proof  
of Concept

Enhanced Radiation Therapy

*Booster of radiotherapy*

*Efficient therapeutic effect*

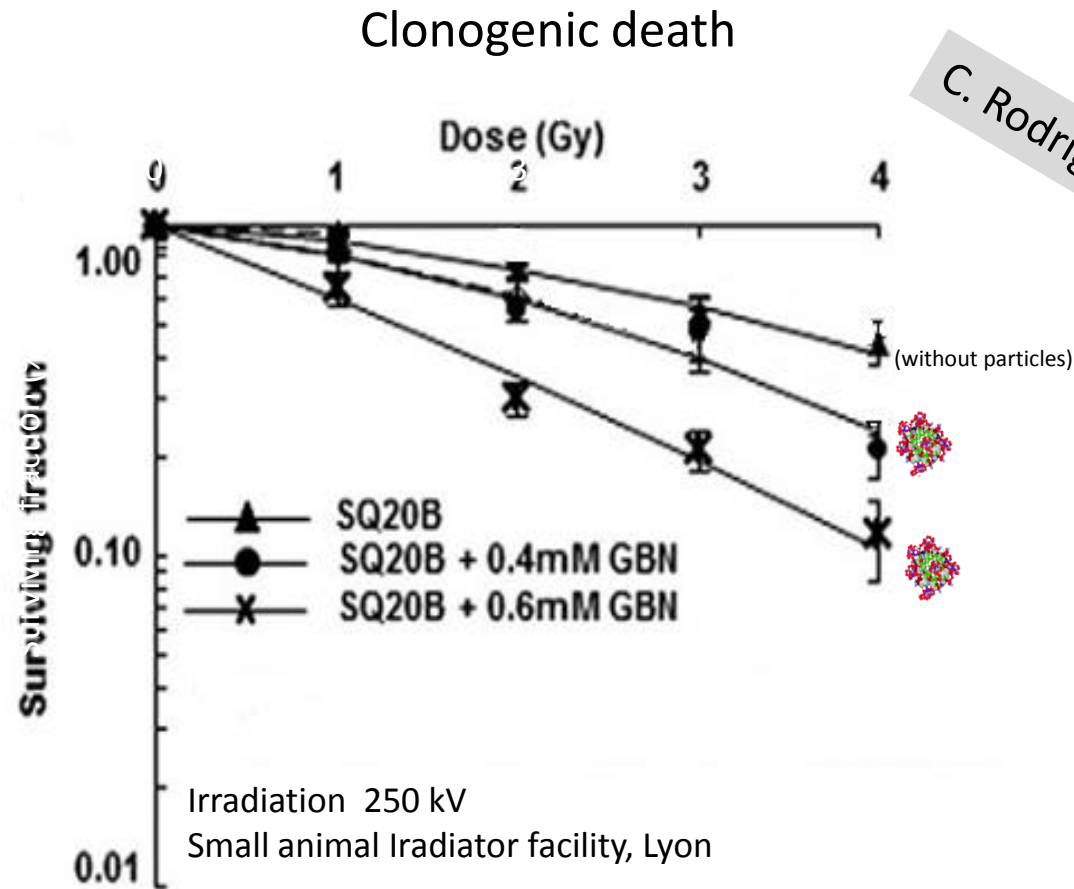


*In Vitro*  
Cell survival – Clonogenic assay

Nanoparticles Incubation  
&  
Irradiation

# *In Vitro*

## SQ20B Radioresistant Head & Neck Carcinoma



C. Rodriguez's HCL-team

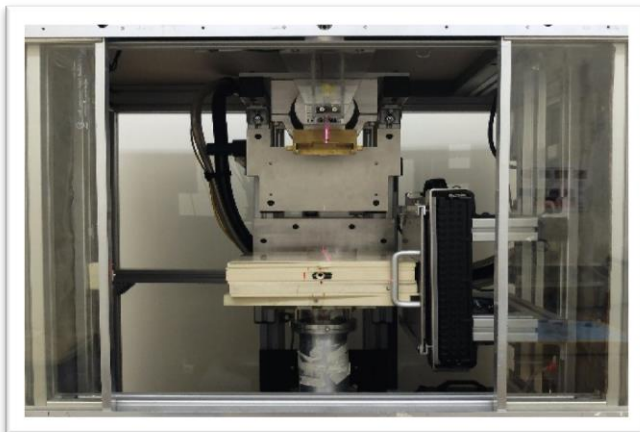
High radiosensitizing effect:  $SER_{2Gy} \approx 2$

# MV and kV Radiation Dose-enhancing effects of AGuIX<sup>®</sup>

*Panc1 tumor Cells*

*200 kVp SARRP & 6 MV linear accelerator (Boston)*

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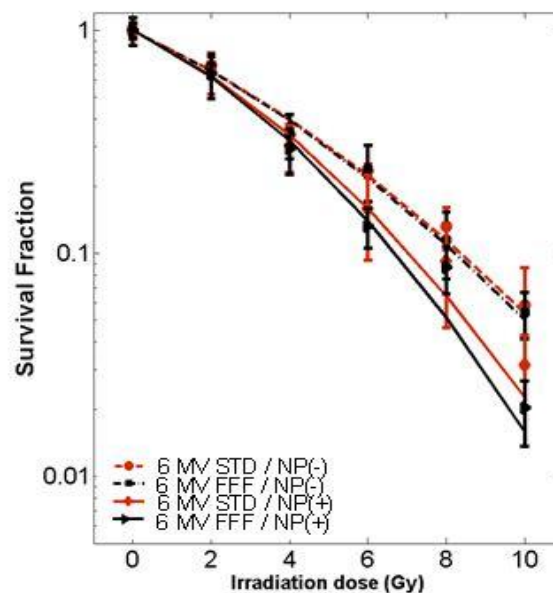
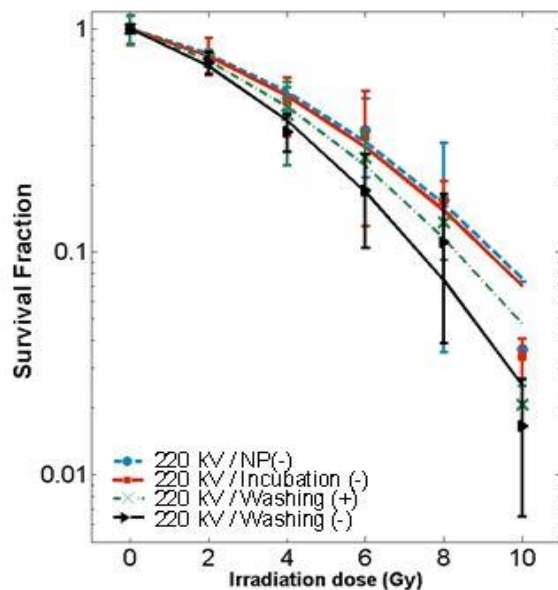


Pre-clinical irradiation set-up



Clinical irradiation set-up

DANA-FARBER/BRIGHAM AND WOMEN'S



# In Vitro radiosensitizing experiments with AGuIX

Teams <sub>10</sub> - Cells <sub>20</sub> - Radiations <sub>10</sub>

Experimental conditions <sub>50</sub>

SER<sub>1,2</sub> 2,5

photons

Investigator (team, town)	Radiation/energy	Cell line	NP/incubation time	Biological effect
K. Butterworth (personal communication) (Queen's University, Belfast, UK)	225 keV	Prostate—DU145	From 0.1 to 5.0 mM <sup>a</sup> /1 h	1.17 < SF < 2.50
		Glioblastoma—T98G		SF = 1.25
		Prostate—PC3		1.25 < SF < 1.33
R. Berbeco <sup>58</sup> (Harvard, Boston, MA)	220 kVp X-ray	Cervical carcinoma—HeLa	0.5 mM <sup>b</sup> /1 h	SER <sub>4Gy</sub> = 1.50 DEF = 1.5
C. Rodriguez-Lafrasse <sup>57</sup> (University Lyon, Lyon, France)	250 kV	Head and neck squamous cell carcinoma—SQ20B	0.4 mM <sup>a</sup> /1 h	SF <sub>2</sub> = 0.60 vs 0.72 (SER = 1.20)
			0.6 mM <sup>a</sup> /1 h	SF <sub>2</sub> = 0.35 vs 0.72 (SER = 2.00)
		SQ20B cancer stem cells	0.6 mM <sup>a</sup> /1 h	SF <sub>2</sub> = 0.6 vs 0.82 (SER = 1.40)
C. Rodriguez-Lafrasse (University Lyon, Lyon, France) <sup>54</sup>	250 kV	Head and neck carcinoma— SQ20B	0.4 mM <sup>a</sup> /1 h	SF <sub>2</sub> = 0.61 vs 0.75 (SER = 1.22)
			0.6 mM <sup>a</sup> /1 h	SF <sub>2</sub> = 0.37 vs 0.75 (SER = 2.14)
M. Dutreix (Institute Curie, Paris, France) <sup>56</sup>	660 keV	Glioblastoma—U-87MG	0.1 mM/1 h 0.5 mM/1 h	γ-H <sub>2</sub> AX + 80% vs irradiation only
R. Berbeco <sup>56</sup> (Harvard, Boston, MA)	6 MV	Cervical carcinoma—HeLa	0.5 mM <sup>b</sup> /1 h	SER <sub>4Gy</sub> = 1.30 DEF = 1.2
M. Barberi-Heyob <sup>56</sup> (CRAN, Nancy, France)	6 MV	Glioblastoma—U-87MG	From 0.01 to 0.50 mM <sup>b</sup> /24 h	SER from 1.10 to 1.50
G. Blondiaux (CERI, Orléans, France)	Neutron cyclotron (Orléans, France)	Mouse lymphoma—EL4	From 0.05 to 0.30 mM	Estimated SER <sub>3Gy</sub> > 2.00
S. Lacombe <sup>59</sup> (University of Paris-Sud, Orsay, France)	Ions He <sup>2+</sup> beam (Chiba, Japan)	Chinese hamster ovary carcinoma—CHO	1.0 mM/6 h	SER = 1.14
S. Lacombe <sup>59</sup> (University of Paris-Sud, Orsay, France)	C <sup>6+</sup> beam (200 MeV/uma) (Chiba, Japan)	Chinese hamster ovary carcinoma—CHO	1.0 mM/6 h	SER <sub>4Gy</sub> = 1.50
C. Rodriguez-Lafrasse <sup>57</sup> (University Lyon, Lyon, France)	C <sup>6+</sup> (33.6 keV μm <sup>-1</sup> ) (Caen, France)	Head and neck carcinoma— SQ20B	0.3 mM <sup>b</sup> /1 h	SER = 1.33
			0.6 mM <sup>a</sup> /1 h	SER = 1.59

DEF, dose enhancement fraction; NP, nanoparticle; SER, sensitizing enhancement ratio; SF, survival fraction.

L. Sancey *et al.*, *Br. J. Radiol.* 2014

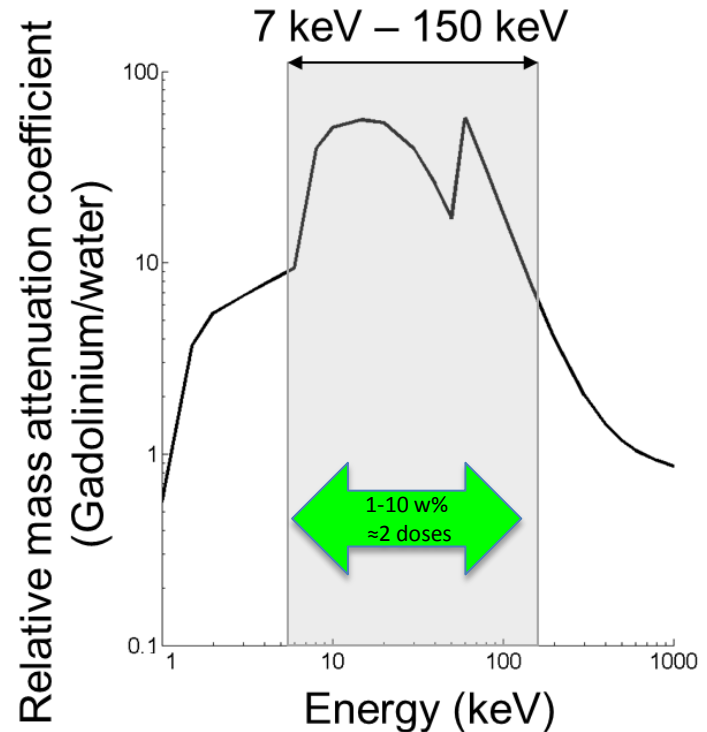
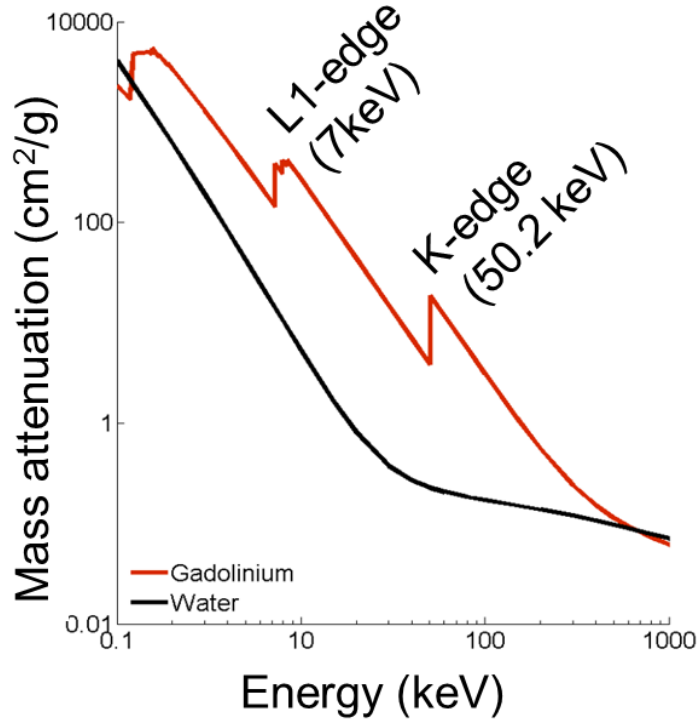
Clear evidences of radiosensitizing effects

Sensitizing Enhancement Ratio: +20%

# Why radiosensitizing effects ?

Simple dose increase ?

High Z element, Gadolinium:  $Z=64$



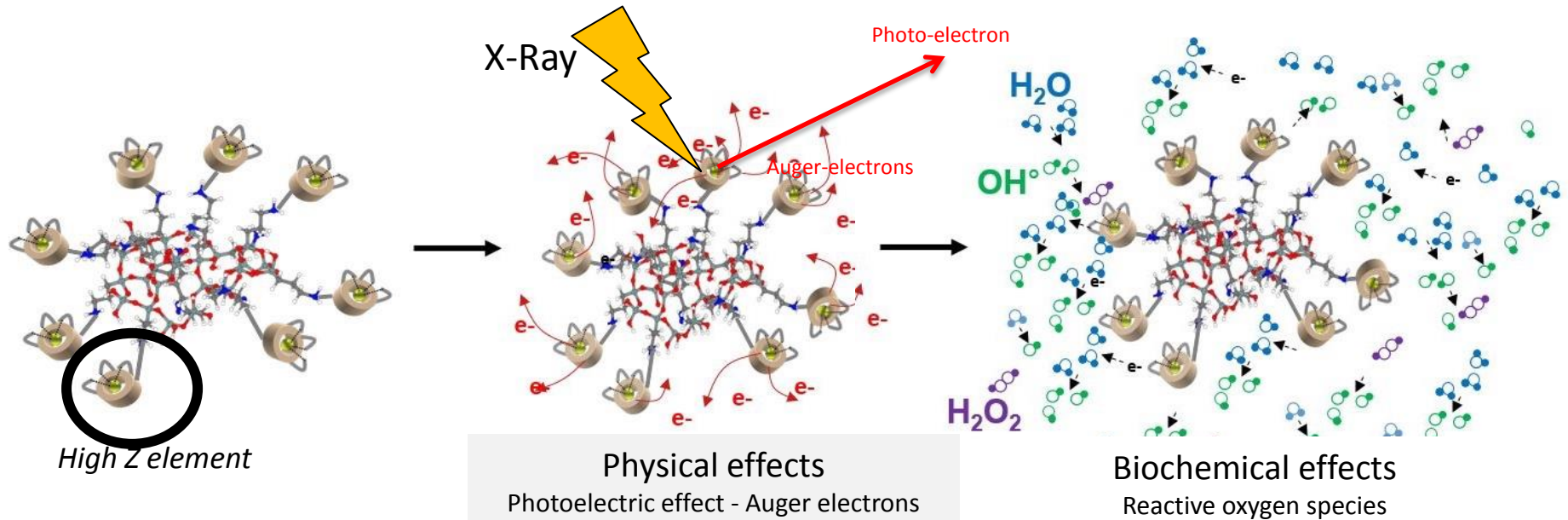
Possible dose effect ( $Z^{3-5}$ )...

Anyway only at high Gd-content (0.001 w% ?)... and nothing in the *MeV range*!

# Local Nano-Dose effects

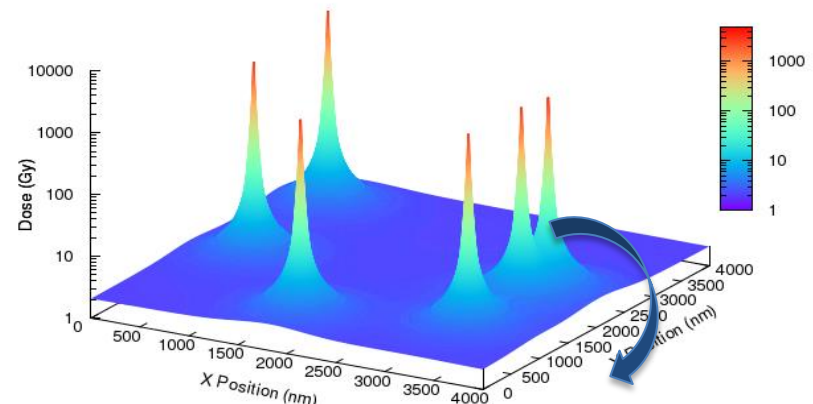
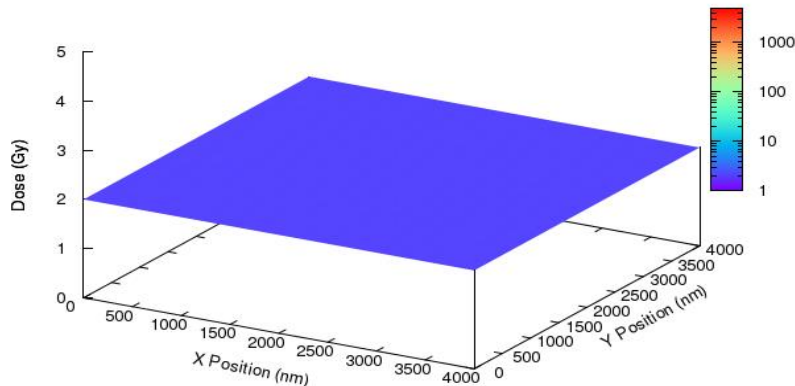
*Propagation of Auger shower around the nanoparticles*

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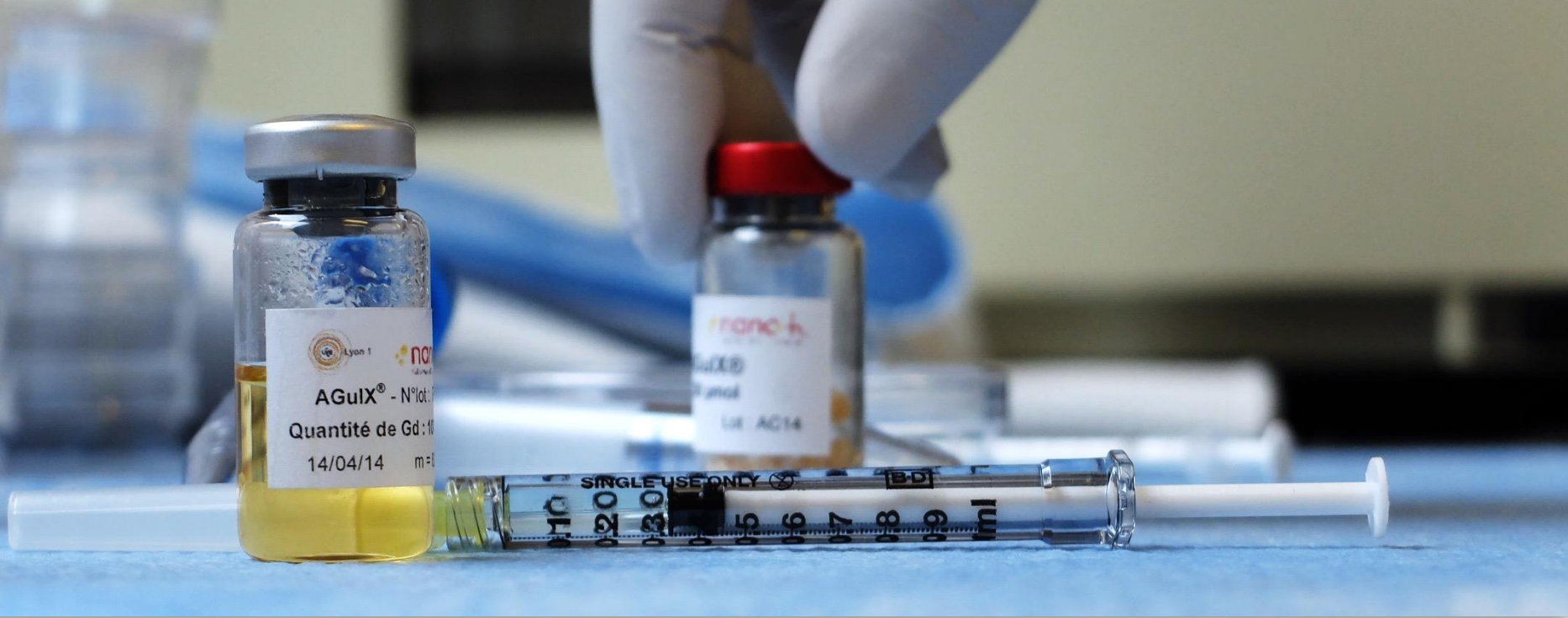


Without Nanoparticles

With Nanoparticles



*More biological effects of the dose !*



*In Vivo*

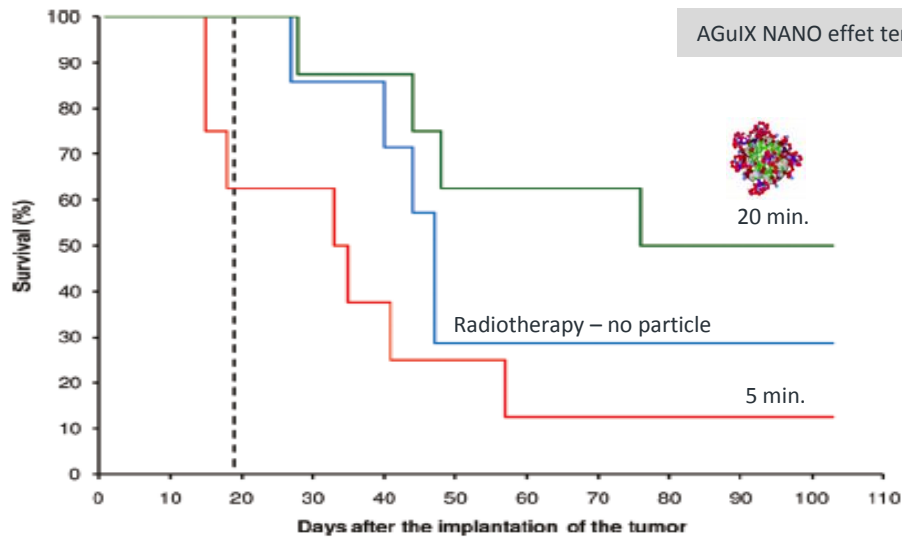
Preclinical animal radiosensitizing studies

Intravenous Injection (0.1-1 % ID/g)

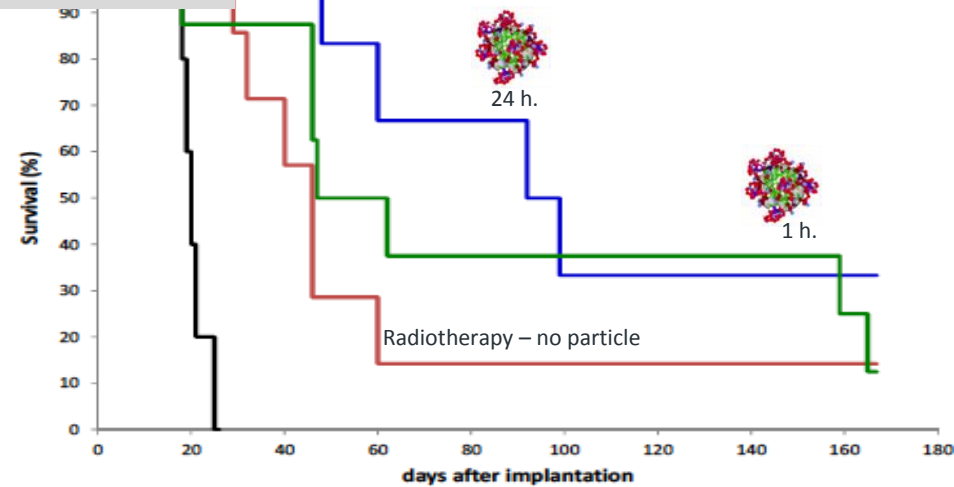
&

Irradiation

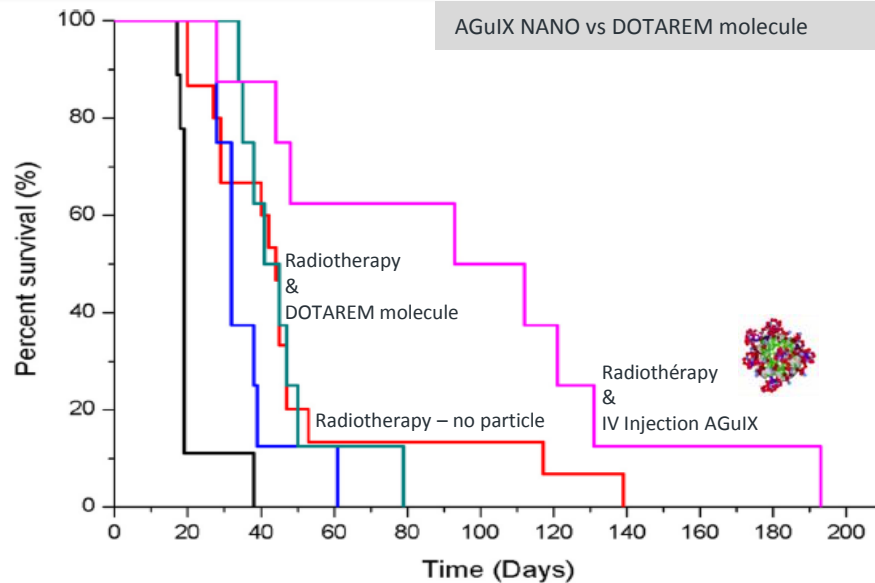
# Survival curves of orthotopic gliosarcoma bearing rats (9LGS)



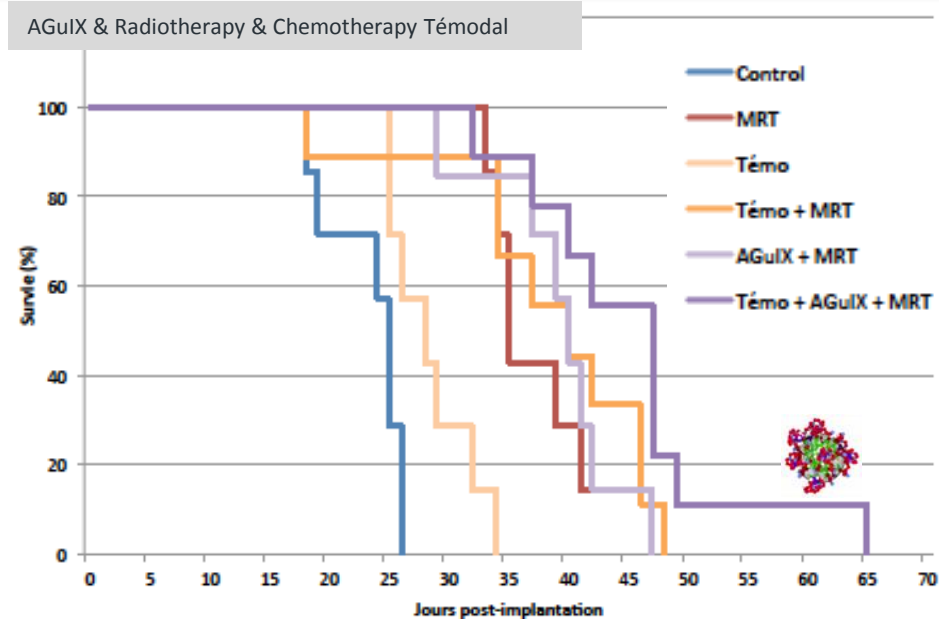
Without treatment (black, n=4), only treated by radiotherapy (blue n=7) and treated by Radiotherapy 5 min. (curve, n=8) and 20 min. (green, n=8) after AGuIX IV Injection



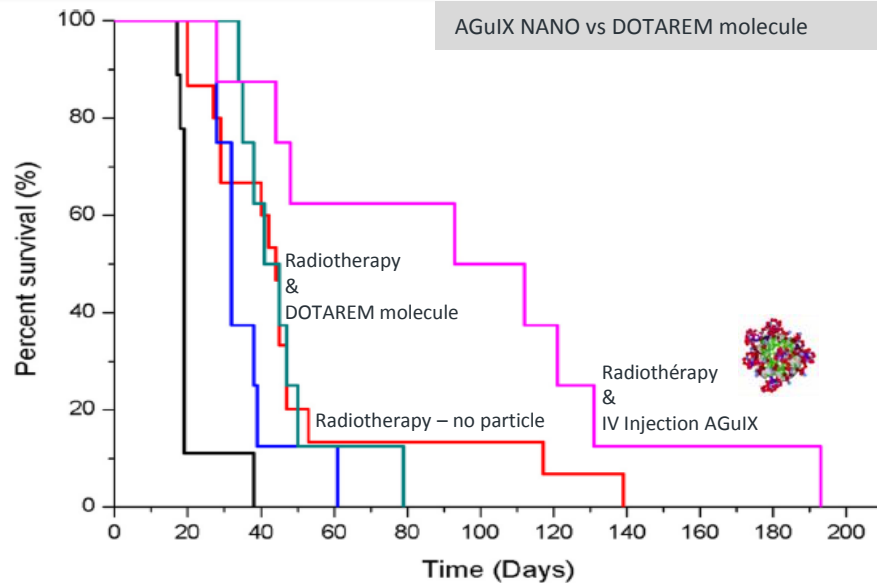
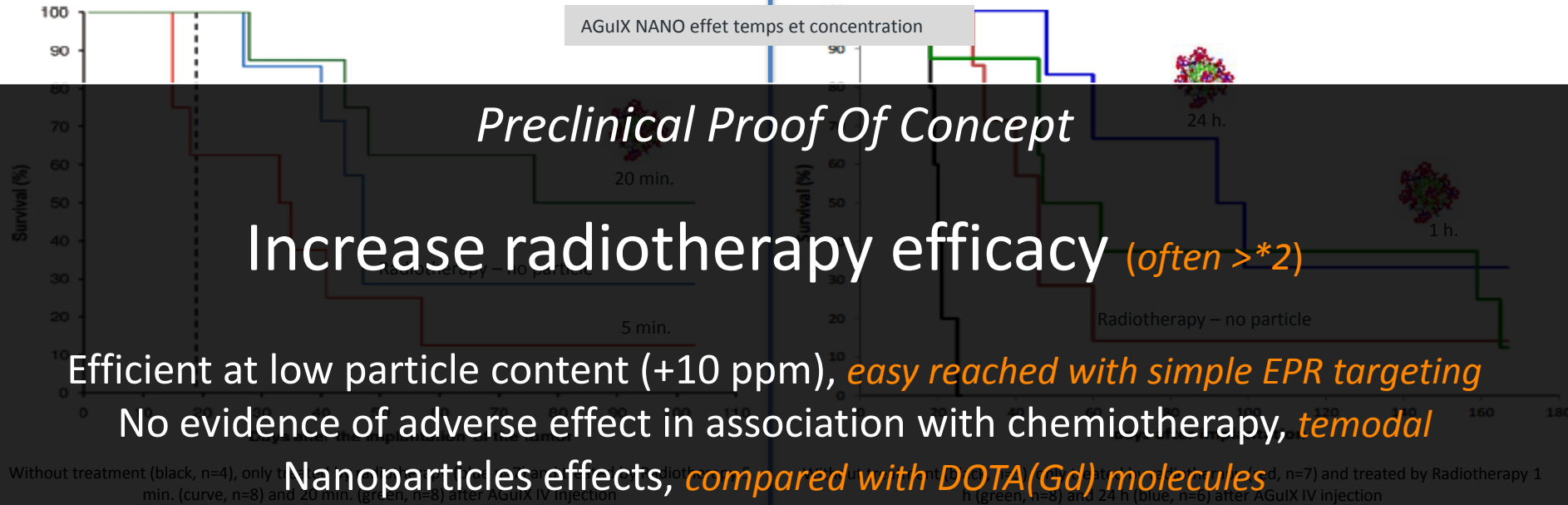
Without treatment (black, n=5), only treated by radiotherapy (red, n=7) and treated by Radiotherapy 1 h (green, n=8) and 24 h (blue, n=6) after AGuIX IV injection



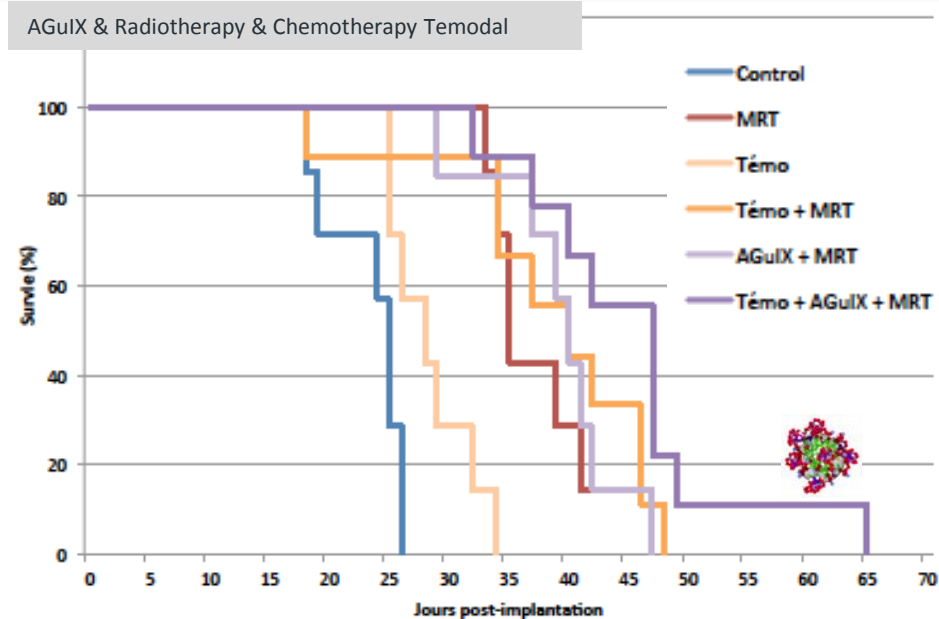
Without treatment (black, n=9), only treated by radiotherapy (red n=15) and treated by Radiotherapy 20 min. after injection of DOTAREM (blue, n=8 and green, n=8) and 20 min. (pink, n=8) after AGuIX IV injection.



# Survival curves of orthotopic gliosarcoma bearing rats (9LGS)



Without treatment (black, n=9), only treated by radiotherapy (red n=15) and treated by Radiotherapy 20 min. after injection of DOTAREM (blue, n=8 and green, n=8) and 20 min. (pink, n=8) after AGuIX IV injection.



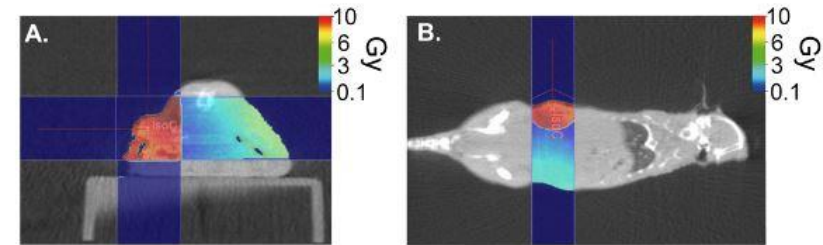
# Proof of Concept for MRI-Guided radiosensitization in Pancreas adenocarcinoma

## 1. MRI - Biodistribution

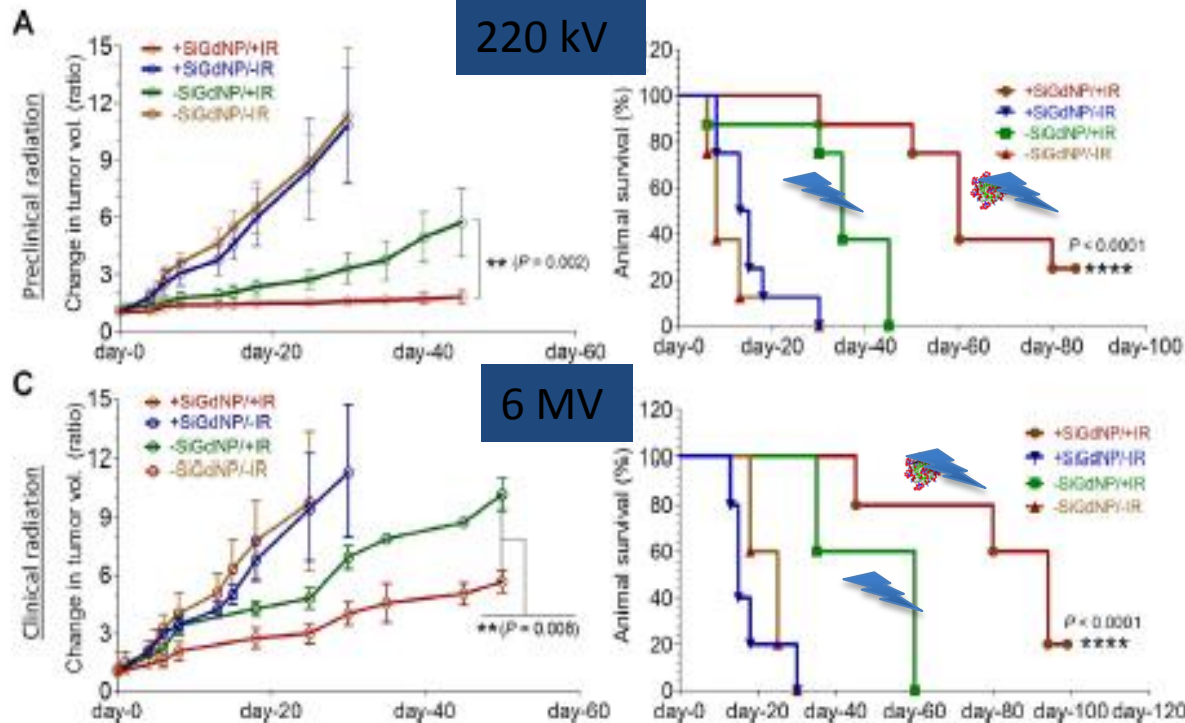
DANA-FARBER/BRIGHAM AND WOMEN'S



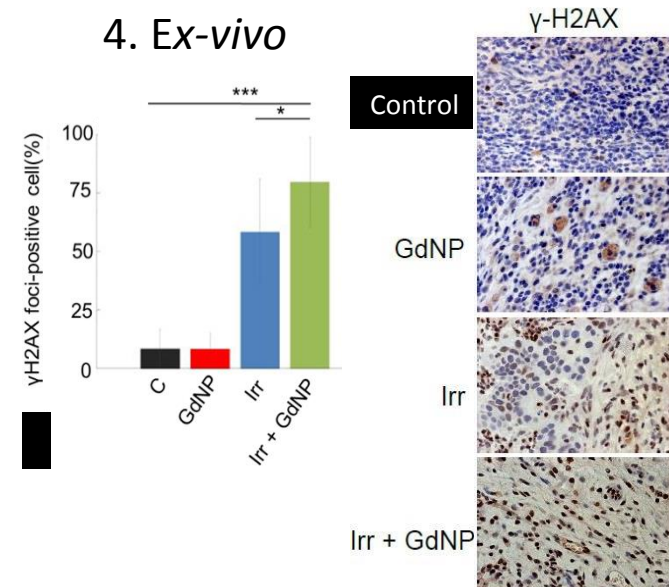
## 2. Dosimetry



## 3. Therapeutical effect (10 Gy)



## 4. Ex-vivo



# Preclinical PoCs

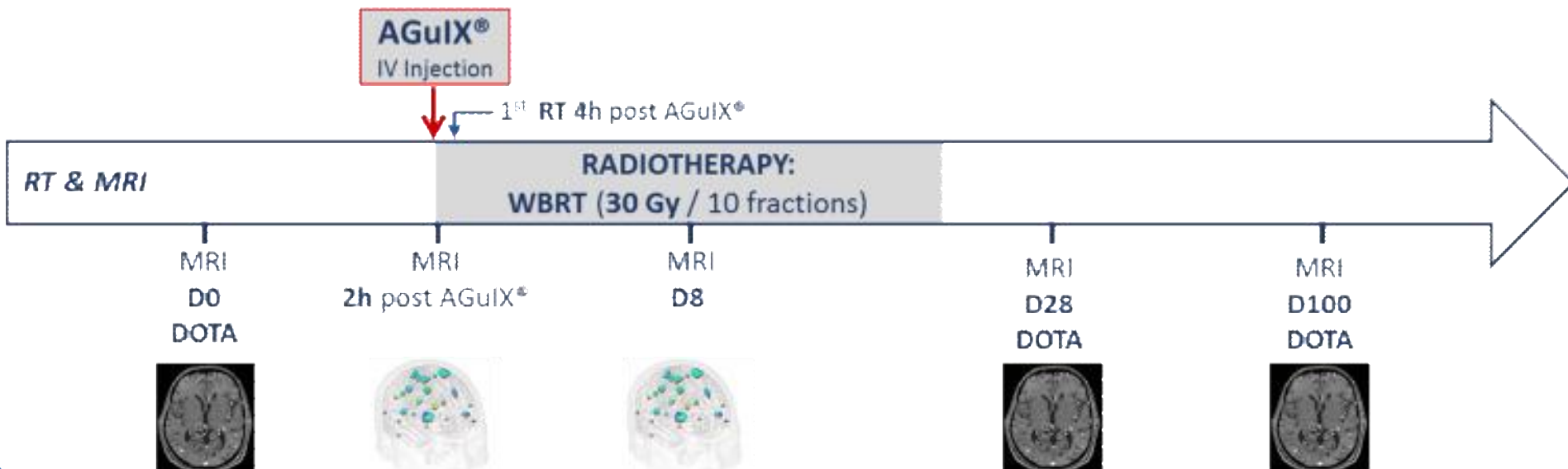
*Positive results in 12 different kinds of cancers*

Cancer	In vitro	Animal studies		Clinical trial
		Imaging	Therapy	
Glioblastoma	✓ F98, U87, T98G	✓ MRI- PET- SPECT	✓	BI preparation NANOGLIO (IGR, Guillaume Louvel, MD)
Brain metastases	✓ B16F10	✓ MRI	✓	✓ NANORAD- Phase I in progress (CHU Grenoble, Camille Verry, MD)
Cervical cancer	✓ HeLa	NE	NE	✓ NANOCOL- Authorization ANSM (IGR, Cyrus Chagari, MD)
Lung cancer	✓ A549	✓ MRI- Fluo	✓	NE Discussion and development device with Vectura
Prostate cancer	✓ DU145, PC3	✓ X (post IT)	NE	NE Discussions Queen's university Belfast (Suneil Jain, MD)
Head and neck cancer	✓ SQ20B, FaDu, CAL33	✓ Fluo (post IT)	✓	FUI Project Submitted Discussions CHU Lyon (Philippe Ceruse, MD)
Pancreatic cancer	✓ Capan-1	✓ MRI- SPECT	✓	NE
Chondrosarcoma	✓ SW1353	✓ SPECT	✓	NE
Liver HCC	✓ HepG2	✓ MRI- PET	✓	NE
Melanoma	✓ B16F10	✓ SPECT	✓	NE
Breast cancer	NE	✓ MRI- PET	NE	NE
Colorectal cancer	✓ HCT116	✓ MRI	NE	NE



## 3 – First In Man

# Phase 1b – NANORAD *Radiosensitization of multiple brain metastases using Gadolinium based Nanoparticles (AGuIX)*



Principal investigator: Dr C. Verry – (J. Balosso)    University hospital: CHU Grenoble

# The First Man » Injection

July 2016



Pharmaceutical form: Sterile lyophilized powder

Reconstitution with water for injection

1 hour before injection

Concentration 100 g/L

Intravenous injection

Syringe pump - injection rate: 1 mL/min

Duration of injection: 15 min / 1st dose → 90 min / 2nd dose





# SAFETY & PK results

# Good tolerance of the 4 first dose levels (12p/15p)



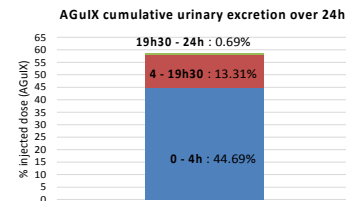
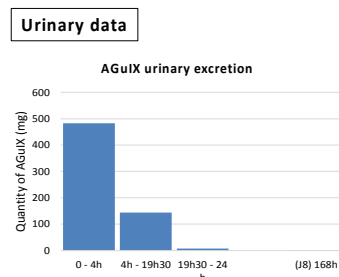
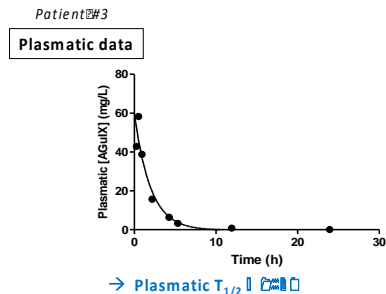
$MTD \geq 75 \text{ mg/kg}$



NANORAD Phase 1b Results August 2017 AGuIX® IV (15 to 100 mg/kg) + WBRT (10*3 Gy)		15 mg AGuIX®/kg			30 mg AGuIX®/kg			50 mg AGuIX®/kg			75 mg AGuIX®/kg		
		patient #1	patient #2	patient #3	patient #4	patient #5	patient #6	patient #7	patient #8	patient #9	patient #10	patient #11	patient #12
		NSCLC	Melanoma	NSCLC	NSCLC	Melanoma	Melanoma	Melanoma	NSCLC	Colon cancer	Melanoma	Melanoma	Breast cancer
Patient Description	Age (Years)	70	64	60	79	37	60	39	69	68	73	53	64
	Number of evaluable brain metastases	5	30	8	4	65	24	12	13	4	31	64	19
	DS-GPA score	0	0	1	0	0,5	0	0,5	2	0	0,5	1	2,5
	Extra-Cranial metastases	yes	yes	yes	yes	yes	yes	no	yes	yes	yes	no	yes
Safety and Tolerance	Complication at injection site	No	No	No	No	No	No	No	No	No	No	No	No
	Impact on Systemic response	No	No	No	No	No	No	No	No	No	No	No	No
Pharmacokinetic	Plasma half time	2h00	0h45	1h20	0h50	1h18	0h57	0h56	1h06	0h48	1h10	0h56	0h58
	Urinary excretion during first day (%)	45%	45%	55%	48%	NE	49%	70%	50%	50%	NE	88%	56%

**Safe compound:** MTD > 75 mg/kg - No grade 1/2/3 adverse event related to AGuIX®

## Pharmacokinetic results



**Short half-life**

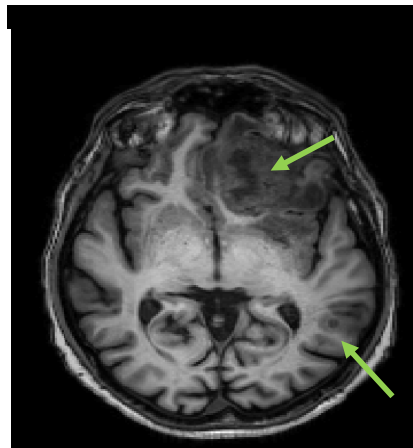
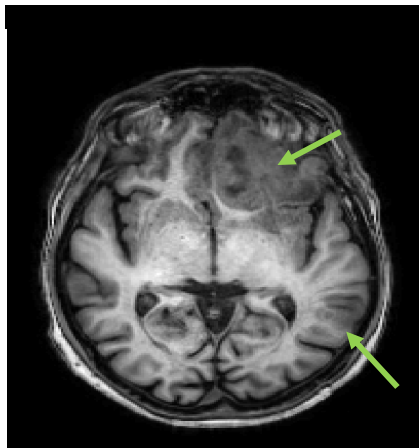
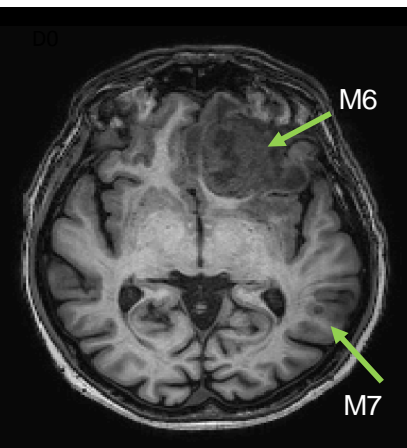
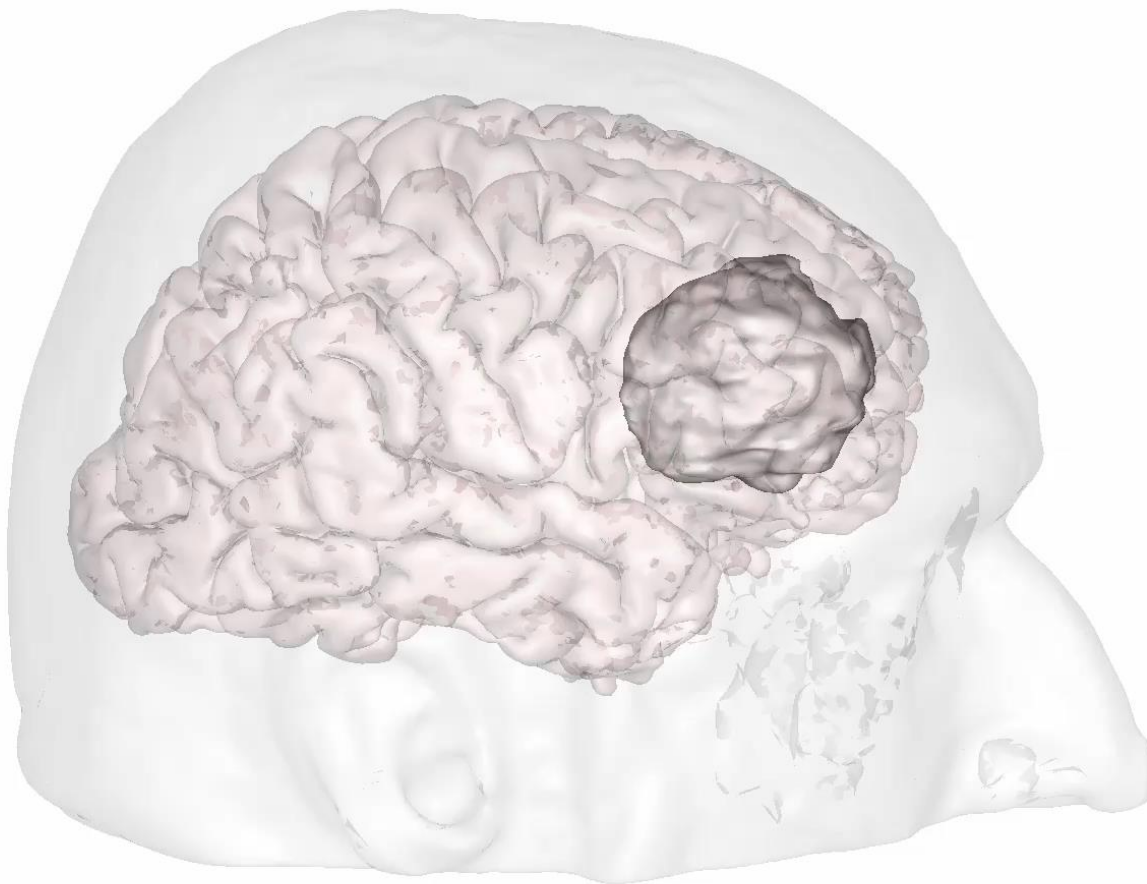
$T_{1/2}$  : 1-2h

**Urinary excretion**

**50% first Day**

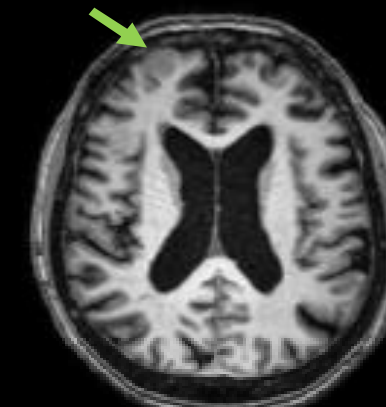
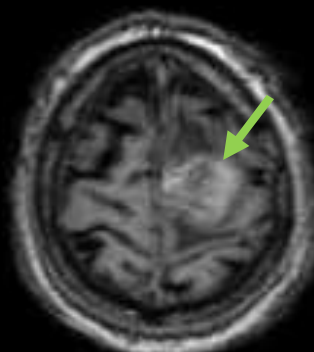
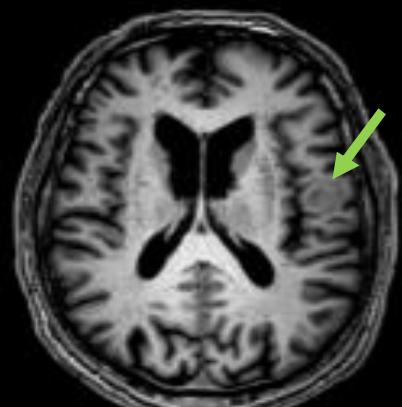
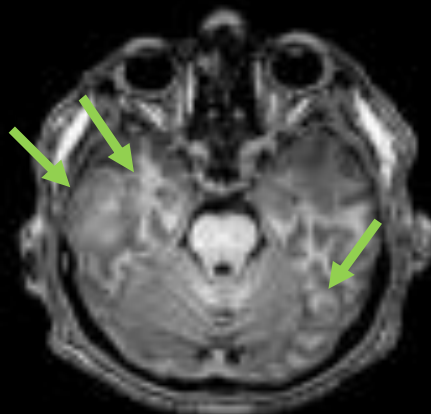
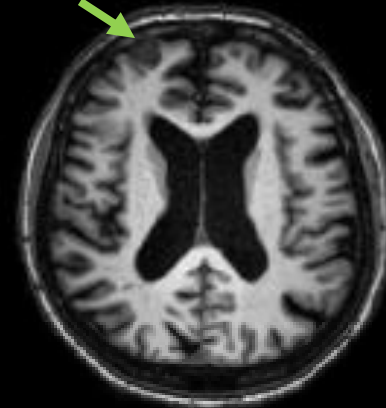
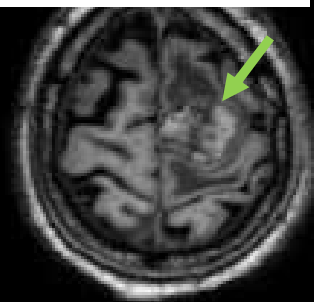
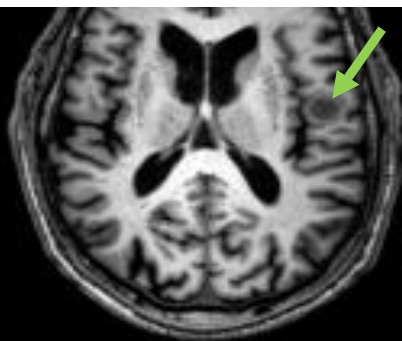
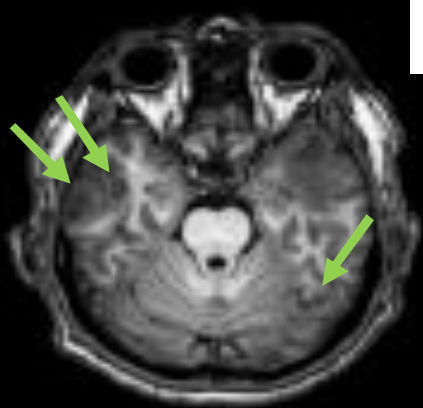
# Tumour $\beta$ AGuIX targeting & $\beta$ MRI

***AGuIX<sup>®</sup> specific uptake in metastases: proofs of tumor targeting with MR imaging***



- AGuIX Uptake in tumours after nano-Drug IV injection
- MRI Enhancements of the metastases
- Some Persistence +1 week

***AGuIX<sup>®</sup> specific uptake in metastases***

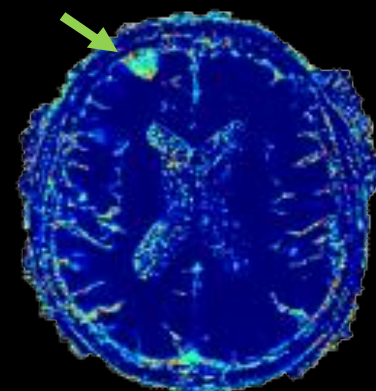
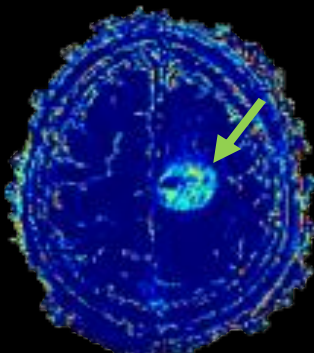
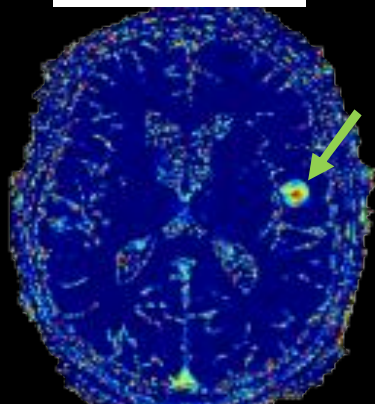
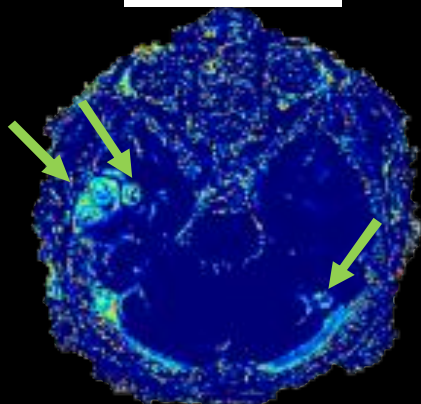


**Melanoma**  
patient #7

**Lung cancer**  
patient #8

**Colon Cancer**  
patient #9

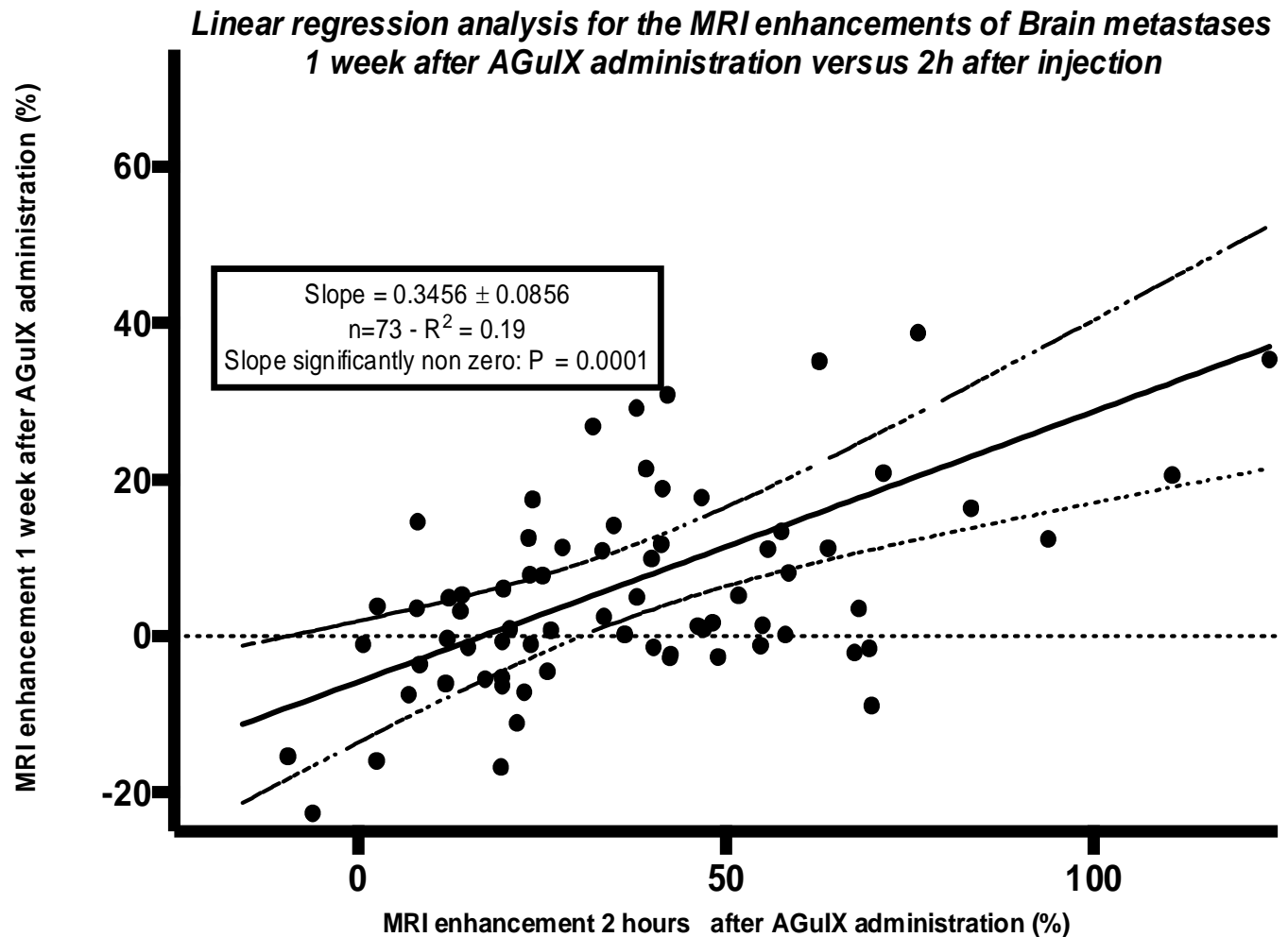
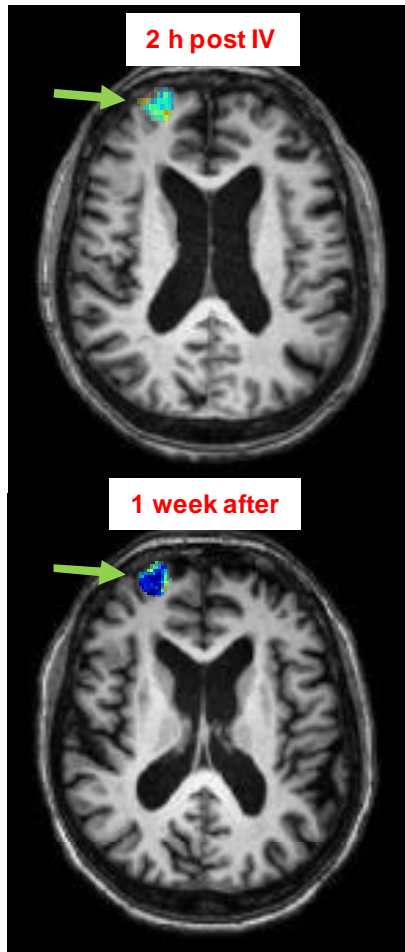
**Breast Cancer**  
patient #12



Evidenced by MRI, up to 30% of the targeted dose is maintained in the tumor after 1 week

### AGuIX® persistence in tumors

*Particles are present in the metastases and can interact with ionizing radiation for several days after intravenous administration*



Good radiation sensitizers must be specific to tumor cells to maximize effects on the tumor and reduce damage to normal healthy cells. MRI confirms preferential uptake of AGuIX® nanoparticles in BM compared to normal brain tissue, and its ability to selectively accumulate in tumor tissue while sparing normal tissue.

# Tumour responses

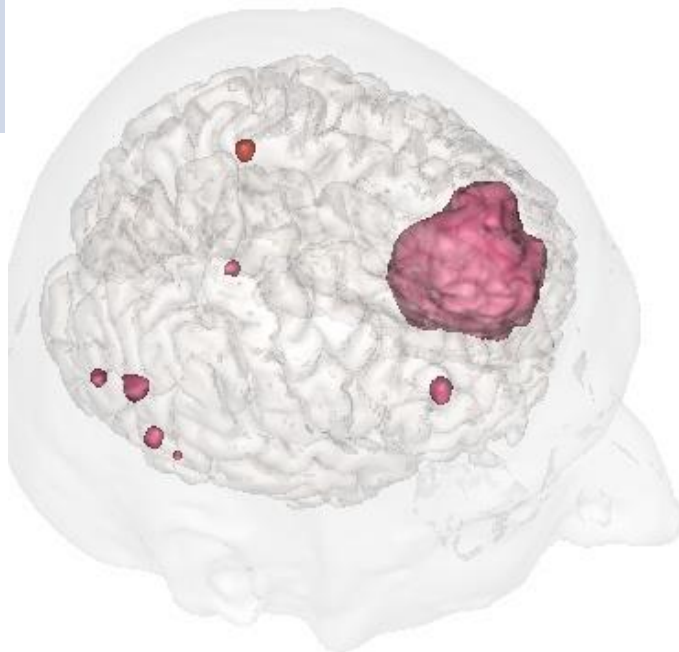
***Clinical responses: first evidences of AGulX<sup>®</sup> therapeutic effects***

# Clinical benefits in +90% patients (10p/11p)

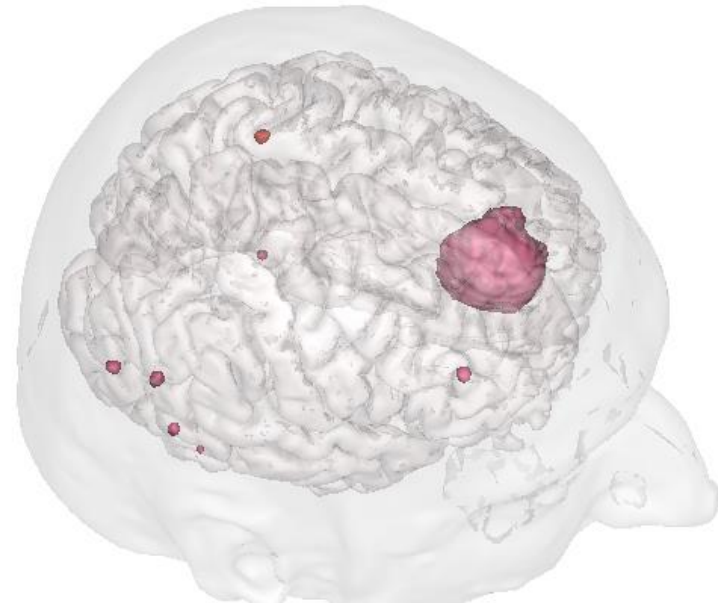
NANORAD Phase 1b <i>Results August 2017</i> <i>AGuIX® IV (15 to 100 mg/kg) + WBRT (10*3 Gy)</i>		15 mg AGuIX®/kg			30 mg AGuIX®/kg			50 mg AGuIX®/kg			75 mg AGuIX®/kg		
		patient #1	patient #2	patient #3	patient #4	patient #5	patient #6	patient #7	patient #8	patient #9	patient #10	patient #11	patient #12
		NSCLC	Melanoma	NSCLC	NSCLC	Melanoma	Melanoma	Melanoma	NSCLC	Colon cancer	Melanoma	Melanoma	Breast cancer
Evolution of Target Lesions	Sum of the longest diameter at D0	12.0 mm	40.9 mm	60.5 mm	25.9 mm	36.5 mm	26.5 mm	56.1 mm	43.9 mm	58.5 mm	31.6 mm	23.5 mm	27.5 mm
	Shrinkage at D28 (%)	-25%	-1%	-26%	/	46%	13%	-6%	-3%	-17%	-8%	-8%	-22%
	Shrinkage at D100 (%)	/	-67%	-33%	/	/	/	NE	NE	-28%	/	<i>in progress</i>	<i>in progress</i>
Best Objective CNS Response (RECIST V1.1)		SD	SD	PR	NE	PD	SD	SD	<i>SD-in progress</i>	<i>SD-in progress</i>	SD	<i>SD-in progress</i>	<i>SD-in progress</i>
OS from MBM diagnosis / from onset of WBRT (weeks)		19.7/10.6	33.4/24.0	36.1/20	7.0/1.3	34.9/4.7	45.0/15.6	20.7/12.7	46.1/16.6	23.4/18.6	15.7/7.1	14.0/8.4	78.7/7.0
Clinical benefits		Yes	Yes	Yes	NE	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes

**Inclusion MRI (D0)**

Patient 13  
 NSCLC  
 epidermoid carcinoma



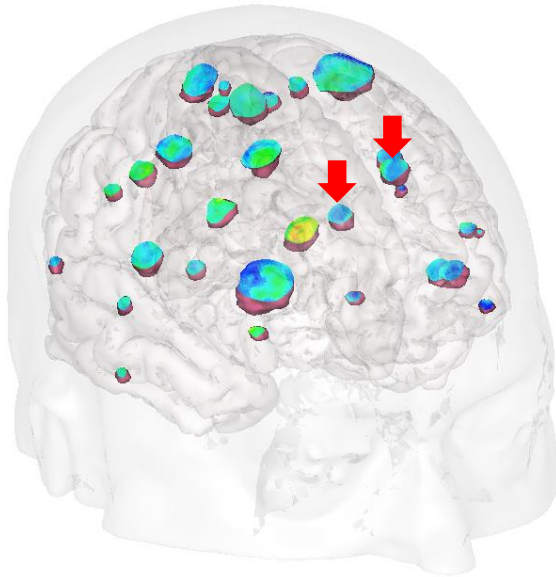
**3 months post treatment MRI (D100)**



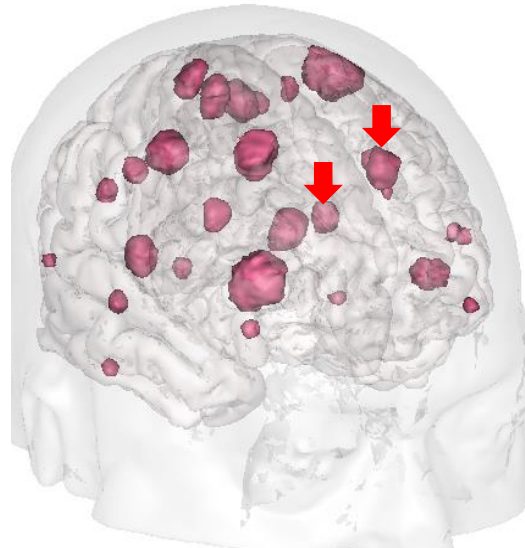
**Reduction of the tumour volume by a factor 3**

# Clinical responses

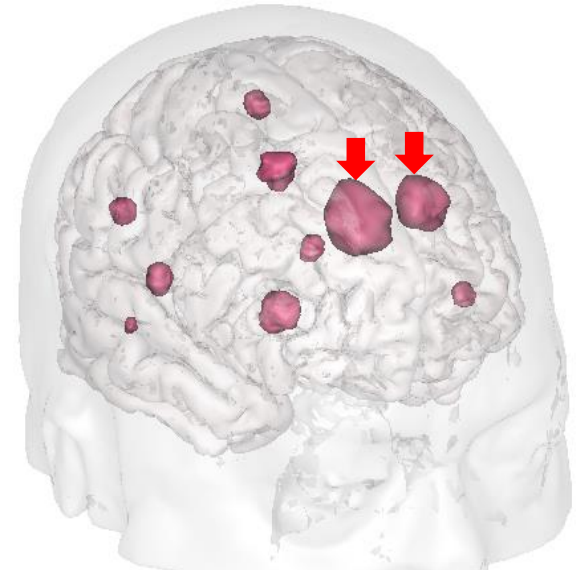
Inclusion MRI (D0)



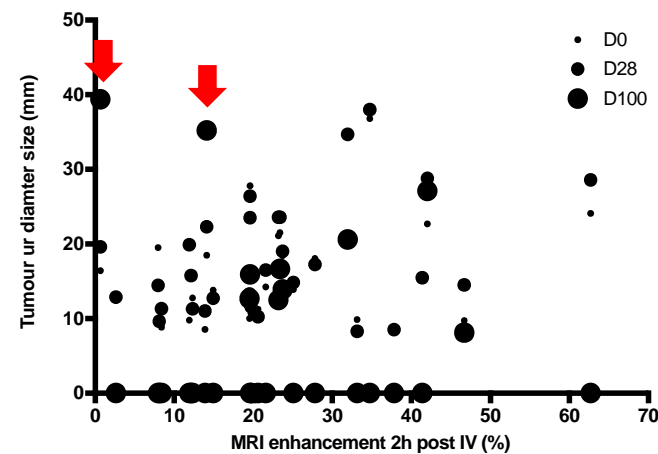
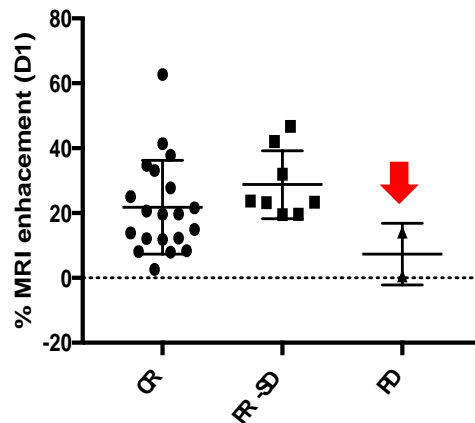
1 months post-treatment MRI (D28)



3 months post-treatment MRI (D100)

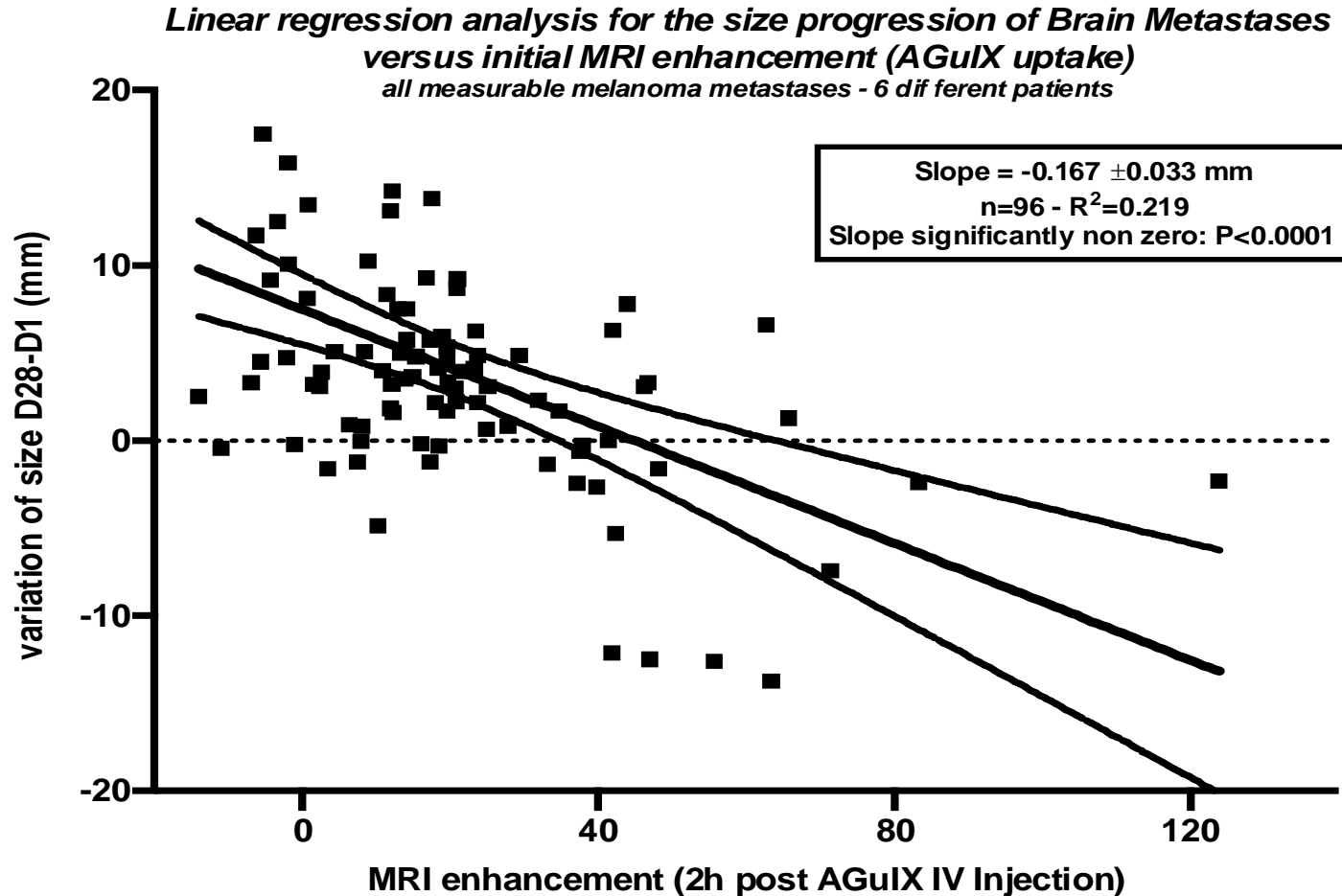


Tumour response and AGuIX MRI enhancement D1

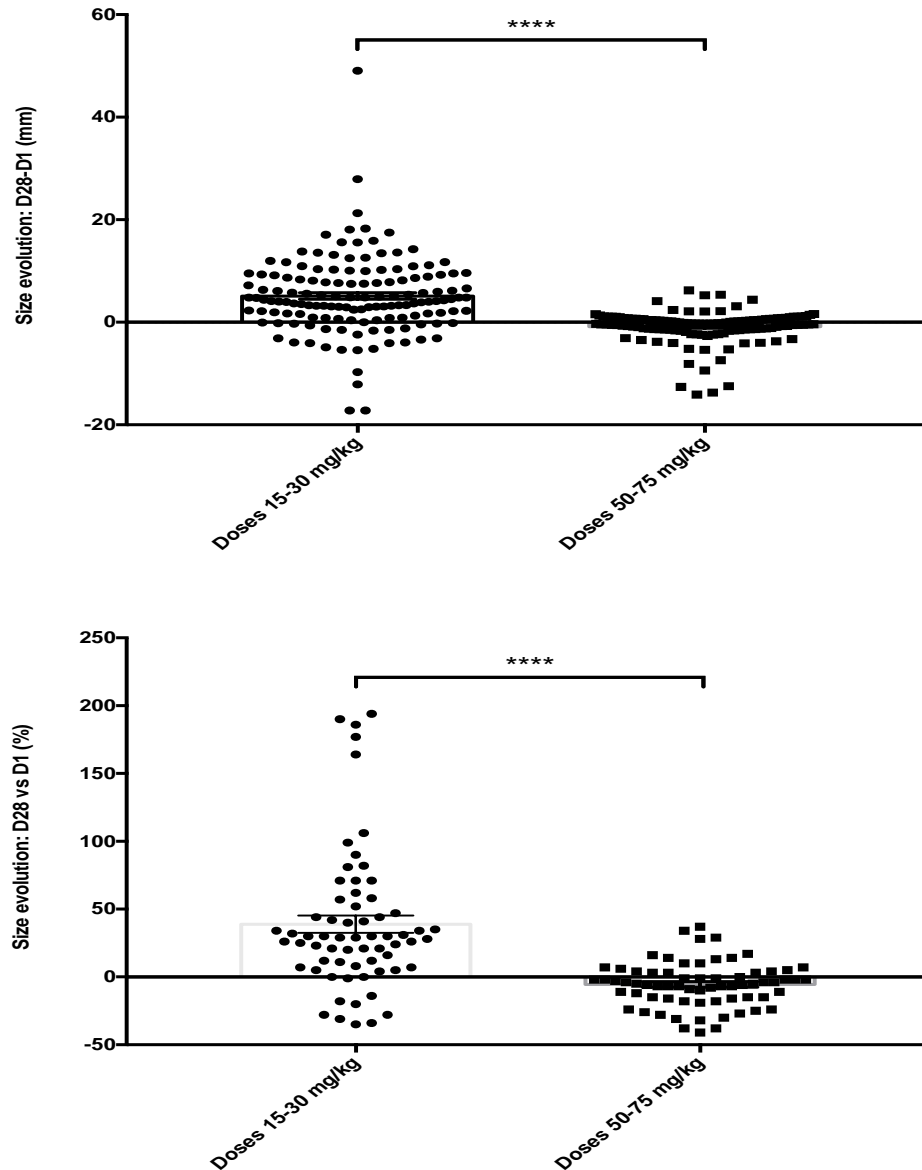


The two Progressive tumours (PD) have a very low AGuIX uptake

# Dose effect response between metastases: Correlation with individual metastasis progression and AGuIX<sup>®</sup> uptake

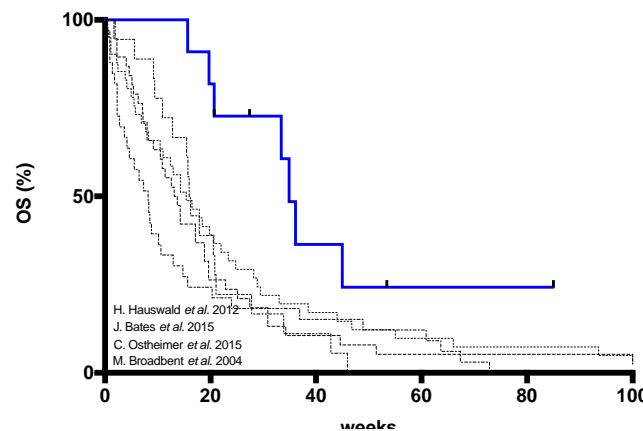
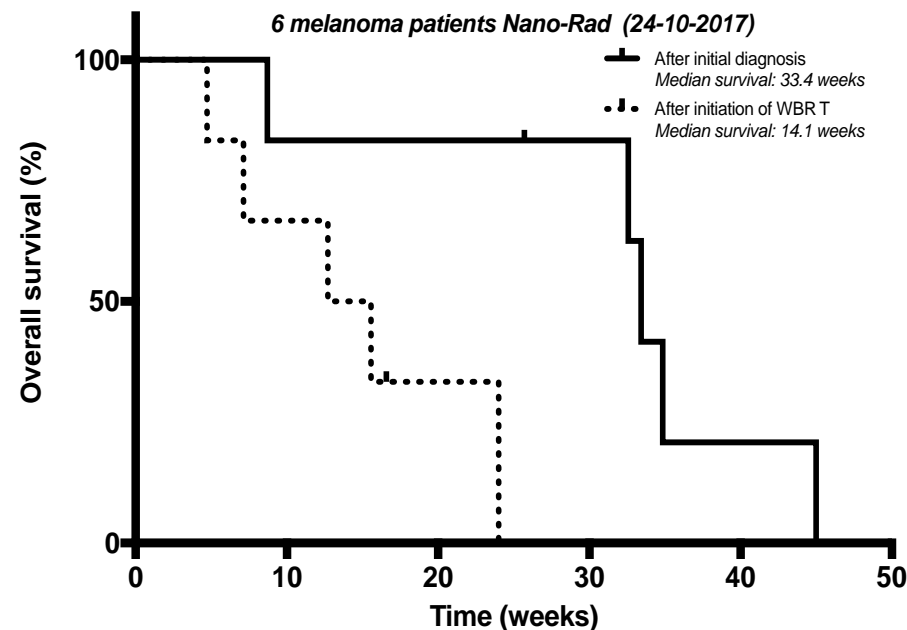
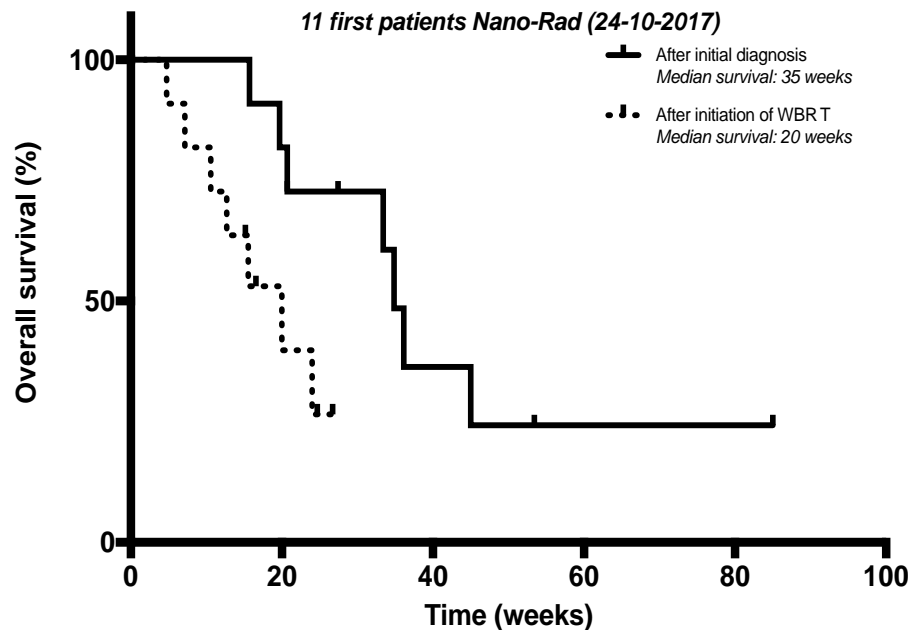


Metastases present better responses to treatment  
when associated with higher AGuIX<sup>®</sup> uptakes



Metastases present better responses to treatment when associated with **higher AGuIX<sup>®</sup> uptakes**

# Kaplan-Meier Survival curves (partial results 24-10-2017)



Overall Survival curves at these first steps seem encouraging

# NANORAD

## Intermediate results after 12 patients

### *Safety excellent*

*no side effect, MTD +75 mg/kg and 100 mg/kg in progress*

### *Tumour targeting and AGulX uptakes*

*melanoma, NSCLC, Colon carcinoma, breast cancer*

### *AGulX persistence in tumor*

*+1week*

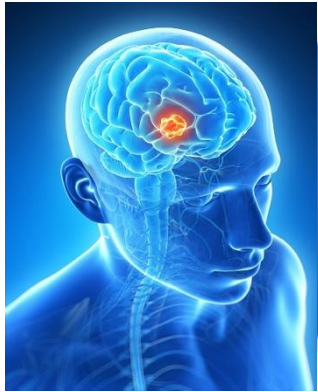
### *MRI T1 contrast agent*

*high efficiency even 2h post injection*

### *Clinical response*

*First evidences of AGulX radiosensitive effects in human*

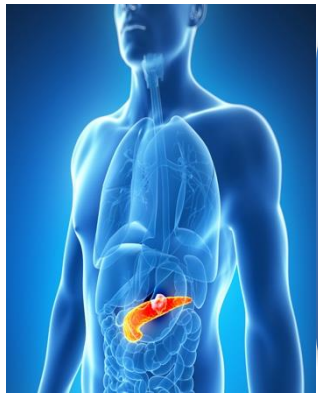
## Clinical strategy : two parallel clinical approaches



### CNS

Resistant tumors in  
healthy sensitive  
brain

- Multiple Brain Metastases (WBRT)
- Oligometastases (SRT)
- Spine Metastases (SRT)
- Glioma (3DCRT or IMRT)



### Body

Radioresistant tumors  
in sensitive OAR  
environment

- Cervical (IMRT and IGBRT)
- Pancreas (SBRT)
- Lung (SBRT)
- Head and Neck (IMRT)
- Prostate (IMRT or SBRT)



# Acknowledgements

## Patients.

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