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

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
AGulX®
Preclinical Multimodal Nanoparticles
Laboratory batches of ≈50 g

Theragnostic Nanoparticles
(MRI-SPECT/PET-fluorescence-Therapy)

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

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

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**Injection IV:** 80 μL at 40 mM in Gd - Male c57BI/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 $CD \approx 6 \, \mu\text{mole} \, \text{Gd}$

**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.

<table>
<thead>
<tr>
<th>AGuiX® / µmol (Gd)</th>
<th>Diarrhea</th>
<th>Lethargy</th>
<th>Closed eyes</th>
<th>Difficulty to wake up after anesthesia</th>
<th>CSS Clinical state score</th>
<th>Death</th>
<th>% weight variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+3.2 %</td>
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<tr>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>+5.4 %</td>
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<tr>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>+0.8 %</td>
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<tr>
<td>&gt;10 CD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>+0.5 %</td>
</tr>
</tbody>
</table>

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
\( Z = 64 \)
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...).

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 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.
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: \( \text{SER}_{2\text{Gy}} \approx 2 \)  

Real concentration of Gd after cell washing is around 0.1 mg/ml  
Curve shapes present high \( \alpha/\beta \) ratio (also with ESRF’s observations \( \approx 30 \))  
Similar to hadrons effects … ? Complex damages… ?
# 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**

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*Ross Berbeco – Boston*

<table>
<thead>
<tr>
<th>Dose</th>
<th>KV% Survival without NPs</th>
<th>KV% Survival with NPs</th>
<th>MV% Survival without NPs</th>
<th>MV% Survival with NPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Gy</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

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High radiosensitizing effect

\[ \text{SER}_{4\text{Gy}} \approx 1.5 \]

for both the kV and MV irradiations
In *Vitro* experiments of dose-enhancing effects of AGuIX®

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Radiation / Energy</th>
<th>Cell line</th>
<th>Gd-AGuIX®</th>
<th>Biological effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. Elleaume <em>et al.</em></td>
<td>31 to 80 keV Synchrotron ESRF</td>
<td>Rat malignant glioma F98</td>
<td>13.3 mM&lt;sup&gt;+&lt;/sup&gt; - 5 h (washing or not)</td>
<td><strong>SER&lt;sub&gt;4Gy&lt;/sub&gt; 1.45 - 2.10</strong></td>
</tr>
<tr>
<td><em>UJF/CEA - Grenoble</em></td>
<td></td>
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</tbody>
</table>
| K. Butterworth *et al.*| 200/250 kV         | Human Prostate Cancer DU145 & PC3 | 0.1-5 mM<sup>+</sup> - 1 h | SF<sub>4Gy</sub> 1.17 - 2.50  
SF<sub>4Gy</sub> 1.25 - 1.33 |
| *Queen’s University - Belfast* |                |                            |           |                    |
| R. Berboco *et al.*    | 200/250 kV         | Human Cervix carcinoma HeLa | 0.5 mM<sup>+</sup> - 1 h | **SER<sub>4Gy</sub> 1.6 (DEF 1.46)** |
| *Harvard MS - Boston*  |                    |                            |           |                    |
| C. Rodriguez *et al.*  | 200/250 kV         | Human Head Neck carcinoma SQ20B & stem cells | 0.4-0.6 mM<sup>+</sup> - 1 h  
0.6 mM<sup>+</sup> - 1 h | **SER<sub>2Gy</sub> 1.22-2.14**  
**SER<sub>2Gy</sub> 1.4** |
| *HCL - Lyon*           |                    |                            |           |                    |
| M. Dutreix *et al.*    | 660 keV            | Human Glioblastoma U-87 MG | 0.5 mM - 1 h | γ-H<sub>2</sub>AX + 80% |
| *Institut Curie - Paris* |                   |                            |           |                    |
| H. Elleaume *et al.*   | 1.25 MeV Cobalt - CEA | Rat malignant glioma F98 | 13.3 mM<sup>+</sup> - 5 h | **SER<sub>4Gy</sub> 1.45 - 1.55** |
| *UJF/CEA - Grenoble*   |                    |                            |           |                    |
| R. Berboco *et al.*    | 6 MV MV Linear Accelerator | Human Cervix carcinoma HeLa | 0.5 mM<sup>+</sup> - 1 h | **SER<sub>4Gy</sub> = 1.6 (DEF 1.44)** |
| *Harvard MS – Boston*  |                    |                            |           |                    |
| M. Barberi *et al.*    | 6 MV MV Linear Accelerator | Human Glioblastoma U-87 MG | From 0.01 to 0.5mM<sup>+</sup> - 24 h | **SER<sub>4Gy</sub> 1.1 - 1.5** |
| *CRAN – Nancy*         |                    |                            |           |                    |
| G. Blondiaux *et al.*  | Neutron Cyclotron Orleans, France | Mouse Lymphoma EL4 | 0.05-0.3mM - 1 h | **SER<sub>4Gy</sub> > 2 (estimation)** |
| *CERI - Orleans*       |                    |                            |           |                    |
| S. Lacombe *et al.*    | Ions He<sup>2+</sup> beam Chiba, Japan | Ch. Hamster ovary carcinoma CHO<sup>a</sup> | 1 mM – 6 h | **SER<sub>4Gy</sub> = 1.14** |
| *Univ. Paris sud - Orsay* |                |                            |           |                    |
| Lacombe *et al.*       | C<sup>6+</sup> beam Chiba, Japan | Ch. Hamster ovary carcinoma CHO<sup>a</sup> | 1 mM – 1 h | **SER<sub>4Gy</sub> = 1.5** |
| *Univ. Paris sud - Orsay* |                |                            |           |                    |
| C. Rodriguez *et al.*  | C<sup>6+</sup> beam Germany | Human Head Neck carcinoma SQ20B | 0.3<sup>a</sup>-0.6<sup>a</sup> mM – 1 h | **SER<sub>2Gy</sub> 1.33 – 1.59** |
| *HCL - Lyon*           |                    |                            |           |                    |

<sup>c</sup>) AGuIX-DTPA; <sup>d</sup>) 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)

Last points
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

Survival (%)

0 10 20 30 40 50 60 70 80 90 100 110

Days

0 25 50 75 100 125

Irradiation only
no particles IV

Irradiation & particles
20 min after IV

Irradiation & particles
5 min after IV

No treatment

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
colour damages

No need of specific irradiations
contentional 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<sub>1</sub> 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

Ionizing radiation
primary or secondary species

Auger electrons

« ejected »-electron
Propagation to neighbour High Z species

Nano particle effect

Auger shower propagation

Distance between two Gd neighbour ≈ 1 nm in 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...

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 → H₂O₂
H₂O₂ 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 H₂O₂…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)

Eric Deutsch (Frédéric Law- Lung)

Claire Rodriguez (Shady Korb – Head & Neck)
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Et al. !