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Gadolinium-based nanoparticles as sensitizing agents to carbon ions in head and neck tumor cells

Anne-Sophie Wozny[a,b,1], Marie-Thérèse Aloy[a,1], Gersende Alphonse[a,b], Nicolas Magné[a,c], Marc Janier[d], Olivier Tillement[e], François Lux[e], Michael Beuve[f], Claire Rodriguez-Lafrasse[a,b,*]

[a]*Univ Lyon, Université Lyon 1, UMR CNRS5822/IN2P3, IPNL, PRISME, Radiobiologie Cellulaire et Moléculaire, Faculté de Médecine Lyon-Sud, Oullins cedex, France*
[b]*Hospices Civils de Lyon, Centre Hospitalier Lyon-Sud, Pierre Bénite, France*
[c]*Département de Radiothérapie, Institut de Cancérologie de la Loire Lucien Neuwirth, St Priest en Jarez, France*
[d]*Univ Lyon, Université Lyon1, CNRS, LAGEP UMR 5007, IMTHERNAT, Hôpital Edouard Herriot, Lyon, France*
[e]*Univ Lyon, Université Lyon 1, Institut Lumière Matière, UMR 5306 CNRS, Villeurbanne cedex, France*
[f]*Univ Lyon, Université Lyon 1, UMR CNRS5822/IN2P3, IPNL, PRISME, PHABIO, Villeurbanne, France*

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Abstract

Hadrontherapy presents the major advantage of improving tumor sterilization while sparing surrounding healthy tissues because of the particular ballistic (Bragg peak) of carbon ions. However, its efficacy is still limited in the most resistant cancers, such as grade III-IV head and neck squamous cell carcinoma (HNSCC), in which the association of carbon ions with gadolinium-based nanoparticles (AGuIX®) could be used as a Trojan horse. We report for the first time the radioenhancing effect of AGuIX® when combined with carbon ion irradiation in human tumor cells. An increase in relative biological effectiveness (1.7) in three HNSCC cell lines (SQ20B, FaDu, and Cal33) was associated with a significant reduction in the radiation dose needed for killing cells. Radiosensitization goes through a higher number of unrepaired DNA double-strand breaks. These results underline the strong potential of AGuIX® in sensitizing aggressive tumors to hadrontherapy and, therefore, improving local control while lowering acute/late toxicity.

Key words: Gadolinium nanoparticles; Radiosensitization; Residual double strand breaks; Head and neck squamous cell carcinoma (HNSCC); Carbon ion irradiation

Nanoparticles (NPs) containing high-Z elements are promising candidates for enhancing radiotherapy efficiency. The interaction of radiation with metals produces secondary particles (photoelectrons, Auger, Compton electrons, etc.),[1,2] depending on the beam energy, which leads to the neighboring generation of reactive oxygen species and, subsequently, to a local dose enhancement.[3] After the pioneering work of Hainfeld[4] using gold NPs (GNPs), a large number of reports confirmed the advantage of metallic NPs in association with radiotherapy to overcome tumor radioresistance.[5,6] Gadolinium-based nanoparticles (GBNs) proved to be efficient radiosensitizers in different *in cellulo* and *in vivo* tumor models.[7] This effect should be theoretically observed at the K-edge of gadolinium (around 50 keV); however, surprisingly, we[8] and others[7,9,10] observed an enhancement of photon effects in different cellular models at energies between 220 kV and 6 MV (clinical energy). In particular, we demonstrated the radiosensitizing effect of a first generation of GBNs (DTPA as chelator) combined with 250 kV photon radiation in radioresistant cellular and animal models of head and neck squamous cell carcinoma (HNSCC).[8] One of the major criticisms of GBNs is their lack of specific targeting, despite tumor enrichment by the enhanced permeability and retention (EPR) effect, and the potential risk of toxicity in healthy tissues when combined with conventional radiotherapy.

Hadrontherapy with protons or carbon ions ($^{13}C^{+6}$) has been demonstrated to target tumors based on its high-energy delivery

Competing interests: F. Lux, and O. Tillement have to disclose the patent WO2011/135101. O. Tillement has to disclose the patent WO2009/053644. These patents protect the AGuIX® nanoparticles described in this publication.

*Corresponding author at: Laboratoire de Radiobiologie Cellulaire et Moléculaire, PRISME UMR CNRS5822, Faculté de Médecine Lyon-Sud, BP12, 69921 Oullins cedex, France.
E-mail address: claire.rodriguez-lafrasse@univ-lyon1.fr (C. Rodriguez-Lafrasse).

[1] Should be considered as first co-author.

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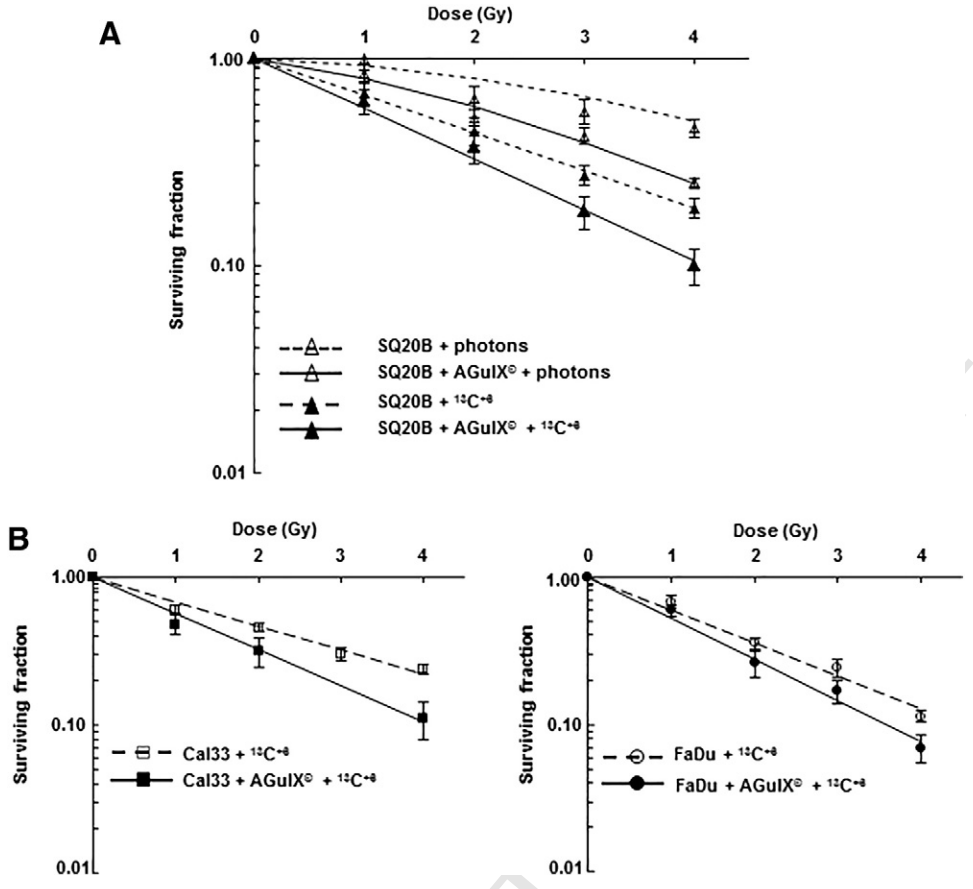

Figure 1. Radiosensitizing effect of AGuIX® in SQ20B (**A**), Cal33 and FaDu cells (**B**) irradiated with $^{13}C^{+6}$ or photons.

Table 1
Radiobiological parameters for HNSCC cell lines irradiated with photons or carbon ions in the presence or not of 0.8 mg/ml AGuIX®.

		$\alpha(Gy^{-1})$	$\beta(Gy^{-2})$	SF2	D_{10}(Gy)	RBE
Photons	SQ20B	0.07	0.03	0.74	7	
	SQ20B + AGuIX®	0.19	0.04	0.58	5.1	1.37
	SQ20B	0.42		0.43	5.5	1.27
	SQ20B + AGuIX®	0.56		0.33	4.1	1.7
$^{13}C^{+6}$	Cal33	0.38		0.47	6.1	1.14
	Cal33 + AGuIX®	0.56		0.32	4.1	1.7
	FaDu	0.51		0.36	4.5	1.33
	FaDu + AGuIX®	0.64		0.28	3.6	1.66

SF2, survival fraction at 2Gy; D_{10}, dose of radiation corresponding to 10% of survival; RBE, Relative Biological Effectiveness at 10% survival.

(Bragg peak) at the end of the course.[11] This specific characteristic affords a limited energy deposition in surrounding healthy tissues as well as a massive transfer of energy within the tumor. Furthermore, $^{13}C^{+6}$ exhibits a higher relative biological effectiveness (RBE) compared with photons because a higher local dose is delivered along the particle tracks, leading to complex, unrepairable DNA damage and cell death.[12–14]

Thus, combining hadrontherapy with GBNs may be of particular interest for amplifying the local energy deposition in radioresistant tumors, such as grade III-IV HNSCC (35% survival at 5 years), which relapse even after carbontherapy.[15]

Table 2
Isobolographic analyses of radiation and AGuIX® in three HNSCC cell lines.

	Photon irradiation	D (Gy)	Isobolographic Analyses	
			50% survival	10% survival
SQ20B		1	Syn	Syn
		2	+	+
		3	+	Syn
		4	Syn	Syn
	$^{13}C^{+6}$ irradiation			
SQ20B		1	+	Syn
		2	Syn	Syn
		3	Syn	Syn
		4	Syn	Syn
Cal33				
		1	+	+
		2	+	Syn
		3	NA	NA
		4	Syn	Syn
FaDu				
		1	+	+
		2	+	+
		3	+	+
		4	Syn	Syn

Syn: synergistic effect; Ant: antagonistic effect; +: additive effect.

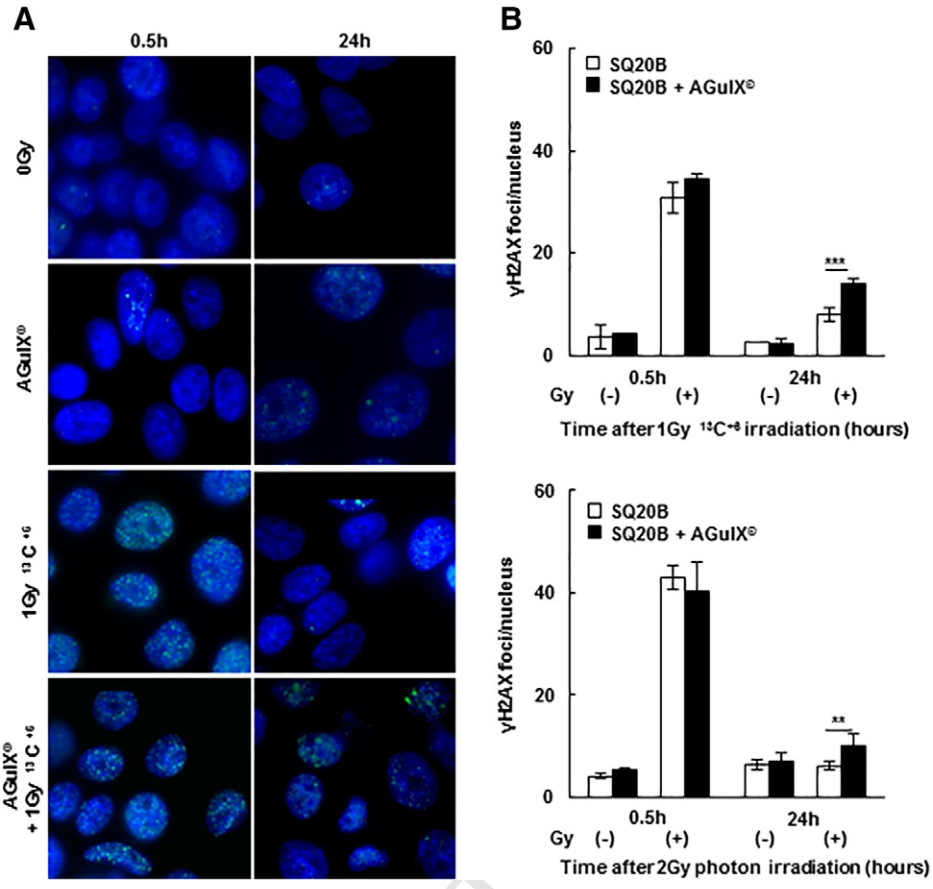

Figure 2. Increase in residual DSBs in SQ20B cells after the combination of irradiation with AGuIX®. (**A**) γ-H_2AX images at 0.5 and 24 h following $^{13}C^{+6}$ irradiation+/−AGuIX®. (**B**) Quantification of γ-H_2AX foci after $^{13}C^{+6}$ or photon irradiation+/−AGuIX®. Values represent the mean ± SD of two duplicate experiments (**, $P < 0.005$; ***, $P < 0.001$ vs irradiated SQ20B cells).

In this work, we used second-generation GBNs containing DOTAGA as a chelator (named AGuIX®), which are currently being tested in a clinical trial (NCT02820454). We present the first evidence of an additive or synergistic effect between AGuIX® and $^{13}C^{+6}$ irradiation in HNSCC cell lines and a better efficiency compared with AGuIX® and photons, which suggest a promising approach for the management of aggressive cancers located near organs at risk.

Methods

The GBNs (AGuIX®) synthesized by NH TherAguix (Lyon, France) are composed of a polysiloxane shell surrounded by covalently bound DOTAGA (Gd) ((1,4,7,10-tetraazacyclododecane-1-glutaric acid-4,7,10-triacetic acid)-Gd).[7] The experiments were performed within the interdisciplinary research program of GANIL (P1022-H).

Three human-derived HNSCC cell lines (SQ20B, FaDu, and Cal33) were cultured as described previously.[8] Cells were incubated for 1 h with 0.8 mg/ml AGuIX® in DMEM and immediately irradiated (or not, in control flasks) in fresh complete medium.

Photon irradiation (250 kV) was performed at the Lyon-Sud University on an X-Rad320 irradiator (Precision X-Ray Inc., North Blanford, CT), and 75 MeV/n $^{13}C^{+6}$ irradiation was performed at GANIL (Caen, France), as described previously[12] and in Supplementary Materials.

Clonogenic survival curves and dose–response interactions between radiation and AGuIX®, as evaluated using an isobolographic analysis,[16] are both described in Supplementary Materials. The relative biological effectiveness (RBE) was calculated as the ratio of doses that induced 10% survival for the photon irradiation (radiation reference) relative to $^{13}C^{+6}$+/−AGuIX® or with photons + AGuIX® (Supplementary Materials).

To assess DNA double-strand breaks (DSBs), the number of initial (0.5 h) and residual (24 h) γ-H_2AX foci was quantified after AGuIX® treatment combined or not with irradiation of 1 Gy $^{13}C^{+6}$ (biological equivalent dose) or 2 Gy photons (Supplementary Materials). Statistical significance was tested using Student's *t* test.

Results

A dose–response (0.3 to 2 mg/ml) study after 4 Gy photon irradiation indicated 0.8 mg/ml AGuIX® concentration as the best compromise between radiosensitization and the absence of cytotoxicity over the 120 h that followed treatment (data not shown).

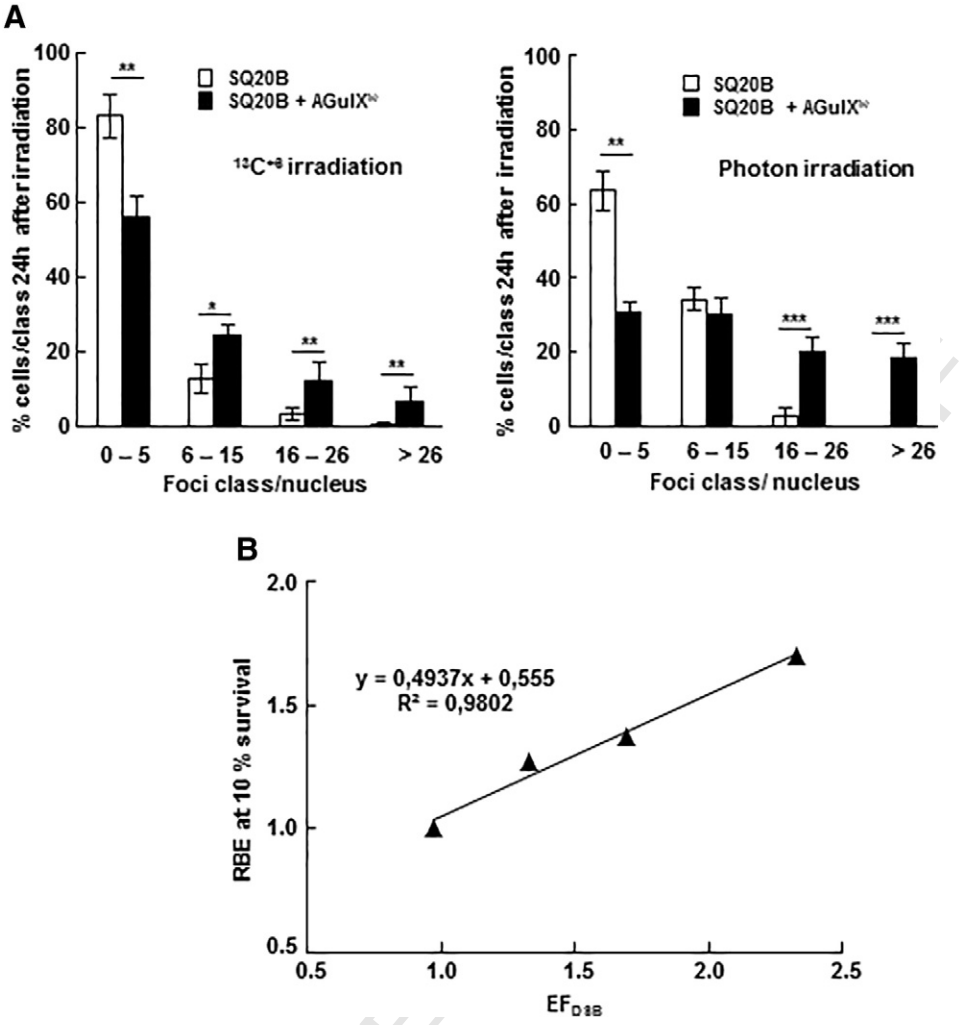

Figure 3. Distribution of cells based on the number of γ-H_2AX foci/nucleus after irradiation with (**A**) $^{13}C^{+6}$ or (**B**) photons+/−AGuIX®. Values represent the mean ± SD of two duplicate experiments (*, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$ vs irradiated SQ20B cells). (**B**) Correlation between RBE and EF_{DSB} in different experimental conditions.

To investigate the radiosensitizing effect of AGuIX®, cell survival curves were established for the radioresistant SQ20B cells in response to photon or $^{13}C^{+6}$ radiation+/−AGuIX® (Figure 1, *A*). Radiosensitivity parameters were deduced from the linear quadratic equation (Table 1). $^{13}C^{+6}$ irradiation induced a higher level of clonogenic cell death compared with photons, as assessed by a recorded RBE of 1.27. The association of AGuIX® with photon irradiation clearly decreased cell survival (RBE, 1.37). The presence of AGuIX® induced an increase in the α-parameter, while the β-parameter remained nearly constant. Interestingly, $^{13}C^{+6}$ irradiation alone (RBE, 1.27) led to a curve that was superimposable with photon + AGuIX®. The decrease in cell survival was even more important for the combination of AGuIX® + $^{13}C^{+}$ irradiation (RBE, 1.7). The SF_2 and D_{10} parameters were significantly decreased compared with that observed for AGuIX® + photons. The radiosensitizing effect of AGuIX® + $^{13}C^{+6}$ was confirmed in two other HNSCC cell lines, Cal 33 and FaDu (Figure 1, *B* and Table 1). Isobolographic analyses of survival curves in the three cell lines (Table 2) revealed a synergistic (at least additive) effect of AGuIX® at 50% and 10% survival, regardless of dose and type of radiation used.

The initial and residual DSBs were estimated from the quantification of γH_2AX foci (Figure 2). The combination of photon or $^{13}C^{+6}$ radiation with AGuIX® did not significantly increase the number of initial DSBs at 0.5 h compared with irradiation alone (Figure 2, *B*). In contrast, a significant increase in residual foci at 24 h was measured in cells treated with AGuIX® + $^{13}C^{+6}$ ($P < 0.001$) and at a lower level after AGuIX® + photons (14 ± 1 and 10 ± 1 foci/cell, respectively) compared with cells irradiated only with $^{13}C^{+6}$ or photons (8 ± 0.3 and 6 ± 0.39, respectively). As a consequence, the enhancement factor for DSBs (EF_{DSB} = residual foci after tested condition/residual foci after photons) increased from 1.34 after $^{13}C^{+6}$ irradiation to 2.33 after AGuIX® + $^{13}C^{+6}$. Figure 3, *A* shows an increased percentage of cells exhibiting a high number of foci/nucleus (greater than 16) for AGuIX® + $^{13}C^{+6}$. A relationship between EF_{DSB} and RBE was evidenced in the different experimental conditions (Figure 3, *B*).

In this study, we demonstrated for the first time that AGuIX®, a new generation of GBNs displaying DOTAGA as chelator and synthesized for clinical use,[17] increased the killing effect of $^{13}C^{+6}$ or photon irradiation in three HNSCC radioresistant cell lines. A synergistic (at least additive) effect of AGuIX® was observed regardless of cell line or source of radiation. Interestingly, for carbon ion radiation, a synergistic effect was observed at 4Gy, which is the dose that is used for hadrontherapy of HNSCC.[15] The sensitizing effect of AGuIX® to photon radiation has been previously published using different tumor models.[9,10,18–21] Nevertheless, the enhancement of the $^{13}C^{+6}$ irradiation effects was only demonstrated for DTPA-GBNs in CHO cells[22] and Gold-NPs in HeLa cells.[23] Interestingly, the cell survival curves in response to $^{13}C^{+6}$ alone or AGuIX® + -photons were very similar, the predominance of the α-parameter indicating the presence of complex and directly lethal lesions.[8,10] Schuemann et al[6] modeled the very high increase in the local dose (ionizations) around the nanoparticle, which was quite comparable to that observed along the tracks of $^{13}C^{+6}$. Although AGuIX® has not been localized in nuclei but observed free in the cytosol or sequestered in lysosomes[20] (Simonet, personal communication), the combination of AGuIX® with photons and, even to a greater extent with $^{13}C^{+6}$ radiation, led to increased residual DSBs and percentage of cells with a high number of foci, thus confirming the generation of complex DNA lesions. These results are in agreement with those of Kotb et al[24] in melanoma and Detappe et al[10] in pancreatic tumor cells for AGuIX® + photon treatment. Conversely, Štefančíková et al[21] did not find significant differences in U87 glioblastoma cells. Different cellular models and experimental conditions may explain these discrepancies.

The sensitizing effect of AGuIX® regardless of $^{13}C^{+6}$ irradiation is not currently explained, as the probability of direct interactions between NPs and $^{13}C^{+6}$ is relatively weak. A better hypothesis relies on the interaction with AGuIX® of the electron cascades produced along the $^{13}C^{+6}$ trajectories. Current work by our team is aimed at modeling this phenomenon using NanOx®.[25]

In conclusion, the association of AGuIX® + $^{13}C^{+6}$ radiation afforded a higher efficacy regarding HNSCC cell killing compared with AGuIX® + photons. A strategy combining AGuIX®, which accumulates in tumors via the EPR effect, with carbontherapy (focused beam and high RBE) may provide a complementary approach in most resistant tumors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nano.2017.07.015.

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