Besides surgery and chemotherapy, radiotherapy constitutes a major modality of cancer therapy. This technique consists in the deposition of a cytotoxic dose in a tumor by irradiation from an external X-ray source. Radiotherapy can be applied in combination with surgery and/or chemotherapy or alone. Despite the tremendous efforts devoted to the improvements of cancer therapy, some results remain however disappointing. For instance, the survival of patients suffering from brain cancer unfortunately has not increased. The incidence of central nervous system tumors is around 10–20 cases/year per 100,000 inhabitants. Glioblastoma is the most aggressive and the most common brain tumor (around 60% among glioma, glioma being already 70% among malignant brain tumors). The median survival is around 1 year after diagnosis, and only 9.8% of patients survive beyond 5 years.1

Although radiotherapy is presently the most efficient treatment against brain tumors, it suffers from a lack of selectivity in the killing effect, leading to numerous adverse effects in normal tissues surrounding the lesion.2,3 A better selectivity can however be achieved by microbeam radiation therapy (MRT), as demonstrated by the studies performed at ESRF.4 What distinguishes MRT from conventional radiotherapy (broad beam) is that normal tissue is more preserved despite the delivery of high doses.5,6 MRT uses arrays of narrow (∼25–100 μm) microplanar beams (peaks) separated by wider (100–400 μm center-to-center) microplanar spaces (valleys). The height of these microbeams varies from 1 to 100 μm. This geometry implies that the microbeam array dose profile displays a succession of peaks (high dose) and valleys (low dose). The ratio between the central peak and valley doses (PVDR) is an important element for the therapeutic effect of MRT. The normal-tissue toxicity of the irradiation decreases when PVDR increases. High damage of malignant tumors indeed occurs for peak entrance doses of several hundred gray (at least 1 order of magnitude higher than for conventional radiotherapy), whereas normal tissues are surprisingly preserved.5,6 The application of MRT to the treatment of rats bearing intracerebral 9 L gliosarcoma (9LGS) led for the same sparing of normal tissue to a higher tumor control than broad beam irradiation. As a result, the median survival time increased (MeST) from 19 to 21 days (sham-irradiated control rats) to 40 and 47 days using cross fired, intersecting arrays (10 mm × 10 mm) of 25 μm wide microbeams, spaced 200 μm center-to-center, and skin entrance doses of 625 Gy.7 MRT offers therefore a great potential for brain cancer therapy.

KEYWORDS: nanoparticles · gadolinium · radiosensitizer · medical imaging · theranostic agents

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since inhibition of the growth of the tumor does not induce alteration of healthy surrounding tissue. Although previous studies provided encouraging results, further improvements are still required for increasing the median survival time. In addition to the modulation of irradiation parameters and/or geometry and the use of chemotherapy, the dose enhancement in the tumor by heavy elements (high Z-elements), which are characterized by a high X-ray photon capture cross-section, constitutes an attractive strategy. Provided that the biodistribution of high Z-elements is well-controlled, it indeed makes possible the deposition of a higher uniform dose in the volume of tumor and a lower dose in normal tissue.2–4 This will be accompanied by a larger production of harmful photoelectrons and radical species. The higher energy absorption and the larger production of reactive species should contribute to a more efficient destruction of the tumor with, at the same time, a better preservation of healthy tissues.

Although many chemicals containing high Z-elements (e.g., iodinated molecules initially conceived as contrast agents for X-ray imaging) have been developed and employed, the results were not satisfying because the amount of radiosensitizers in the tumors is too insufficient and tumor-to-normal tissue high Z-element ratio remains too low.5 Because of their reduced dimensions, molecules are indeed characterized by a too rapid clearance and by an extravasation from normal blood vessels, which impede a preferential accumulation in solid tumors, which is essentially based on an enhanced permeability and retention (EPR) effect.10–15 The intense research activity devoted to nanomedicine that exploits the progress of nanoscience and nanotechnology for medical applications showed that multifunctional nanoparticles exhibit numerous assets for advantageously replacing molecular drugs.16–20 On the basis of a physical and chemical point of view, the multifunctional particles appear very well suited for achieving image-guided therapy for at least three reasons.21–29 The first one lies in their size, which can be sufficiently large for avoiding their extravasation from normal vessels and sufficiently small for loading the tumor by passive accumulation (EPR effect) since solid tumors are irrigated by porous vessels.10–13 The leakage from these abnormal vessels is easily observed for nanoparticles, whose size ranges between 1 and 100 nm. The second reason rests on the possibility to gather, despite the small volume of the nanoparticles, a large range of properties that can be accurately tuned by a convenient choice of components. Nanoparticles can therefore be designed for combining several medical imaging techniques (magnetic resonance imaging (MRI), computed tomography (CT), nuclear medicine imaging (single-photon emission computed tomography (SPECT), positron emission tomography (PET), and/or near-infrared fluorescence (NIRF)) or for associating imaging and therapeutic activity.21–23 The latter can be realized either by drug delivery24,25 or by an interaction between harmless nanoparticles and external physical stimuli.26 Finally the behavior of the particles (for instance, colloidal stability in biological fluids, biodistribution, and clearance) can be adapted by the control of their size but also by a post-functionalization of the nanoparticles. Choi et al. demonstrated that quantum dots (QDs) are renally cleared when their hydrodynamic diameter does not exceed 5–6 nm.30,31 However renal clearance was also observed for larger nanoparticles (8–10 nm) possessing a different surface chemical composition.32 Multifunctional nanoparticles containing high Z-elements should therefore appear as an attractive alternative to molecular radiosensitizers. Although many papers are devoted to the capacity of gold and platinum nanoparticles to enhance the dose effects of X-ray beams, no study has been yet performed on gadolinium-based nanoparticles (GBNs).33–35 Despite a smaller value, the atomic number of gadolinium (Z = 64) is sufficiently high for inducing a dose enhancement of X-rays.36–38 Moreover GBNs are attractive because they can be easily followed up by MRI.39,40 The possibility to monitor the biodistribution by MRI is indeed crucial for optimizing the effect of radiotherapy since the irradiation can be triggered only when the gadolinium content deduced from MR images is both high in the tumor and low in the surrounding healthy tissue. In order to exploit the attractive features of Gd element and of multifunctional nanoparticles, we developed in this study GBNs for image-guided microbeam radiation therapy and evaluated their potential in the case of the gliosarcoma mouse model (9 L gliosarcoma, 9LGS).

RESULTS AND DISCUSSION

The potential of hybrid nanoparticles composed of a gadolinium oxide core and a fluorescent and hydrophilic polysiloxane shell for medical imaging (MRI, fluorescence imaging) and neutron capture therapy was previously revealed.41 In order to limit the presence of inactive matter for radio-enhancement, the thickness of the polysiloxane shell was reduced by decreasing the amount of polysiloxane precursors (tetraethyl orthosilicate (TEOS), aminopropyltriethoxysilane (APTES)). In contrast to previous studies, the molar Si to Gd ratio was fixed to 4 instead of over 10.32,39,41,42 Initially the encapsulation of each gadolinium oxide core aims at both conferring additional characteristics (fluorescence, high colloidal stability) and ensuring the preservation of the core.43 In order to anticipate a less efficient protective effect of the polysiloxane shell owing to the reduction of its thickness, DTPA derivatives were grafted onto the polysiloxane shell for capturing gadolinium ions, which could be eventually released. DTPA is indeed well known for its ability to form gadolinium chelates, which are widely used as contrast agents for clinical
diagnosis in MRL. Elemental analysis performed by ICP-MS after a thorough purification of the nanoparticles revealed that the encapsulation of the oxide core and the postfunctionalization of the shell by DTPA were carried out with relatively high yields since Si to Gd and DTPA to Gd molar ratios are close to the initial ratios (3.5 vs 4 and 0.87 vs 1.2, respectively). The reduction of the amount of polysiloxane shell precursors led, as expected, to a decrease of the hydrodynamic diameter ($D_h = 2$ nm, while $D_h > 8$ nm when Si/Gd > 10) (Figure 1a). Despite the difficulty in observing these ultrasmall nanoparticles, HR-TEM experiments confirmed that the diameter of gadolinium oxide nanoparticles embedded in a polysiloxane shell does not exceed 2 nm (Figure 2a and b). The cores have a size distribution with an average size of 1.1 nm and a mean standard deviation of 0.6 nm (Figure 2a). Although the contrast of particles is blurred by that of the supporting carbon film, a gray halo is visible around Gd$_2$O$_3$ cores due to the presence of the polysiloxane shell (Figure 2b).

The presence of polysiloxane is confirmed by nanoprobe EELS spectra comparing the Si-L$_{2,3}$ - Gd-N$_{4,5}$ ionization edges from GBN before and after synthesis of the polysiloxane network (Figure 2c). After usual background subtraction, the spectrum of the nanoparticles after hydrolysis condensation of polysiloxane precursors exhibits a Si-L$_{2,3}$ edge with a shape similar to that of a SiO$_2$ reference (Figure 2d). However the visualization of the core shell structure is very difficult to observe and requires calculation from TEM phase-contrast imaging at low spatial resolution (Figure 2e).

Arrows in Figure 2e point out isolated particles where the dense Gd$_2$O$_3$ core appears darker than the lighter surrounding polysiloxane shell. Projected potential calculations based on the atomic structure of crystalline, cubic Gd$_2$O$_3$ and vitreous silica provide an image of a core (diameter: 1 nm) embedded in a polysiloxane shell (thickness: 0.5 nm) (Figure 2e, upper part of the inset). This result is in accordance with the diameter obtained by photon correlation spectroscopy (PCS). For comparison the lower part of the inset of Figure 2e displays the projected potential calculations applied to TEM phase-contrast imaging at low spatial resolution of gadolinium oxide cores before encapsulation.

The grafting of DTPA confers to the nanoparticles a high colloidal stability at pH 7.4, although the zeta potential is close to 0 mV (3.0 mV). It must be pointed out that the colloidal stability is preserved in aqueous solution containing a high concentration of sodium chloride since no large agglomerate was detected by PCS. After NaCl addition to a GBN colloid, the hydrodynamic diameter, which was equal to 2 nm in water, increased to 4.2 nm (Figure 1b). These results indicate that the high colloidal stability of the aqueous GBN solution is ensured by a process that does not rest on the electrostatic repulsion; otherwise, precipitation would be observed in saline solution.

The in vitro MRI and synchrotron radiation computed tomography (SRCT) experiments showed that the presence of GBNs in aqueous solutions induces an enhancement of the contrast, which increases with the gadolinium concentration (Figure 3).

The ability of GBNs to behave as efficient positive contrast agents for MRI was assigned both to the high longitudinal relaxivity ($r_1$ ($r_1 = 9.4$ s$^{-1}$ mM$^{-1}$ at 60 MHz) and to the value of the transverse to longitudinal relaxivities ratio ($r_2/r_1 = 1.13$). These features can be exploited for monitoring the behavior of GBNs in 9LGS-bearing rats after an intravenous injection of an aqueous GBN colloid ([Gd$^{3+}$] = 40 mM, $V = 1.4$ mL).

MRI clearly shows that the nanoparticles are present in the tumor since a strong positive contrast appears in the right hemisphere of the brain a few minutes after the injection. The delineation of the tumor is clearly visible and can be observed for 45 min at least (Figure 4).

This visual observation is corroborated by the intensity variation of the MR signal in the right hemisphere (i.e., the hemisphere containing the tumor) compared to that in the left hemisphere. In other words, the contrast enhancement (EHC), which reflects the gadolinium content in the imaged tissue, is higher in the right hemisphere (RH) than in the left one (LH) after intravenous injection of GBN (EHC(RH)/EHC(LH) > 1, Table 1). Moreover this ratio significantly increases by a factor of 1.68 when the delay between the image acquisition and the injection varies from 5 to 20 min ([EHC(RH)/EHC(LH)]$_{20}$/[EHC(RH)/EHC(LH)]$_5$ = 1.68, Table 1). As expected, the differences are more pronounced when the comparison is performed between the contrast enhancement of smaller regions of interest located in tumor (GS) and in normal tissue (NT) (Figure 5 and Table 1).

![Figure 1](image_url)
Figure 5 depicts the temporal evolution of the MRI signal. It clearly shows that the gadolinium content in the normal tissue (left hemisphere) increases until 5 min and decreases afterward, while the content in the tumor reaches a plateau. Consequently, these results suggest that the amount of nanoparticles is more and more important in the right hemisphere (and peculiarly in the tumor) than in the left hemisphere, between 5 and 30 min after injection. To determine the particle distribution in the brain, ex vivo elemental analyses by ICP-MS were performed separately on the right and left hemispheres for different delays between injection and euthanasia (Figure 6). The amount of gadolinium in the right hemisphere (i.e., containing the tumor) is larger than in the left hemisphere even 5 min after the intravenous injection (Figure 6 and Table 1). The difference in gadolinium content between the hemispheres is assigned to the presence of a denser vasculature around the tumor and a disrupted blood brain barrier, which are responsible for a greater blood volume and an enhanced permeability and retention effect, respectively.10–15,18,19

Figure 6 shows the decrease of the gadolinium concentration when the delay between injection and euthanasia increases. This decrease is more striking in the left hemisphere, as confirmed by the increase of the ratio of gadolinium content in the right and left hemispheres between 5 and 20 min after the intravenous injection of GBN (\([r_{Gd(RH)} / r_{Gd(LH)}]_{5} / [r_{Gd(RH)} / r_{Gd(LH)}]_{20} = 1.74\), Table 1). It must be pointed out that the value of this ratio, which is determined by ICP, is, as expected,
very similar to that determined from MR images ([EHC(RH)/EHC(LH)]_20/[EHC(RH)/EHC(LH)]_5 = 1.68, Table 1). This similarity indicates that MRI is very well suited for guiding radiotherapy since a rapid, precise, and reliable localization of GBN can be established by this noninvasive medical imaging technique. The decrease in the left hemisphere reflects the removal of the nanoparticles from the blood system. The clearance seems to be performed via renal excretion since the presence of GBN is attested only in the kidneys, bladder, and urine by the techniques (MRI and SRCT) permitted by the imaging multimodality of the GBN (Figure 7).

The ICP-MS analysis of the urine indicates that up to 30% of gadolinium can be removed by urine during the first hour after intravenous injection. The renal clearance of GBN, which is favored by their small hydrodynamic diameter, leads to the elimination of particles in excess and should avoid any long-term toxicity.30–32 The slower decrease of the gadolinium content in the right hemisphere confirms that a significant part of the nanoparticles are trapped in the tumor, this accumulation being probably driven by the EPR effect. The accumulation of GBN in the tumor volume attested by MRI and ICP appears favorable for radiotherapy.

For evaluating the dose enhancement effect of the GBN, 9LGS-bearing rats were exposed to MRT 5 and 20 min after intravenous injection. The survival of these rats was compared to the survival of untreated rats and of MRT-only treated rats. The irradiated-only animals had a median survival time (MeST) of 47 days, which corresponds to an increase in lifespan (ILS) of 147% when compared to untreated controls of the present study (MeST for untreated rats = 19 days) (Figure 8).

The MeST was extended to 90 days by performing MRT 20 min after intravenous injection of GBNs (ILS = 373%), while it was shortened to 34 days by reducing...
the delay between nanoparticle injection and MRT to 5 min (ILS = 78%). Remarkably, 4 rats in 8 were still alive 100 days after the tumor implantation in the group of animals that were irradiated 20 min after injection of GBNs. The lifespan of these survivors has been multiplied by a factor 5 (ILS = 373%). This experiment obviously evidences a high radiosensitizing effect of gadolinium-based nanoparticles. The efficiency of MRT can be therefore improved by a GBN delivery by intravenous injection prior to the irradiation. However it appears that the delay between the administration of the nanoparticles and the irradiation is a key parameter since a beneficial effect on the survival is observed when the MRT is performed 20 min after injection, while the irradiation performed 5 min after injection is, on the contrary, detrimental for the survival (Figure 8). The MeST shortening observed when MRT was performed 5 min after the intravenous injection is not due to an intrinsic toxic effect of the nanoparticles or of the X-ray microbeams since the lifespan of the rats treated only by MRT or by MRT 20 min after intravenous injection increased. The difference observed between these three treatments has then to be interpreted on the basis of the radiosensitizing effect of GBNs only. In this context, the contrasted observations are certainly related to the difference in the distribution of GBNs within the brain, as highlighted by MRI and ICP-MS experiments. Precisely, even if the amount in the right hemisphere (tumor bearing) is slightly lower 20 min than 5 min after the intravenous injection, the survival of rats irradiated 20 min after injection is longer. This is due to a greater difference in the gadolinium content between the hemispheres that results from a greater particle elimination in the same left hemisphere (Figure 6). Therefore, although the amount of nanoparticles in the tumor is an important parameter, the amount of nanoparticles in healthy tissue is probably still more crucial because healthy tissue can be seriously altered by the dose enhancement of X-rays induced by the presence of particles. This clearly indicates the benefit that can be derived from the monitoring of therapeutic agents by medical imaging.

CONCLUSION

In conclusion, the combination of GBNs and MRT improves the survival of rats bearing aggressive brain tumors. The efficacy of this binary therapeutic system mainly rests on the difference of gadolinium content between tumor and healthy tissue. Since the latter depends on the delay between irradiation and injection, the possibility to follow up GBNs by MRI constitutes an interesting asset for determining the most suitable moment for irradiation. Gadolinium-based nanoparticles obviously exhibit the properties required for the theranostic agents: contrast enhancement of medical imaging (MRI, CT), therapeutic activity (dose enhancement of X-ray microbeams), and a safe behavior after intravenous injection (passive accumulation in the tumor, renal clearance). Their use for image-guided radiotherapy, the first step toward personalized therapy, can be therefore envisaged.

MATERIALS AND METHODS

Chemicals. Gadolinium chloride hexahydrate (99%, Nano-H, France), sodium hydroxide (99%, Sigma Aldrich), diethylene glycol (>99%, SDS), tetraethyl orthosilicate (Si(OCH2CH3)4, TEOS, 98%, Aldrich), (3-aminopropyl)triethoxysilane (H3N(CH2)3-Si-(OC2H5)3, APTES, 99%, Aldrich), diethylentriaminopentacetic dianhydride (DTPADA, 98%, Aldrich), triethylamine (TEA, 99%, Aldrich), anhydrous dimethylsulfoxide (DMSO, Aldrich), and...
analyze the Si-L$_{2,3}$ and Gd-N$_{4,5}$ ionization edges with electron temperature for weeks without alteration. Stirred at 180°C under vigorous stirring. Afterward, the solution was heated and stirred at 180°C for 3 h. A transparent colloid of gadolinium oxide nanoparticles was obtained and can be stored at room temperature for weeks without alteration.

**Coating of Gadolinium Oxide Cores by a Polysiloxane Shell.** The silane precursors (APTES (10.1 mL) and TEOS (6.4 mL)) and hydrolysis solution (aqueous Et$_3$N in DEG (0.1 M of TEA, 10 M of water)) were sequentially and alternatively added to a 400 mL DEG solution of the Gd$_2$O$_3$ nanoparticles ((Gd] = 45 mM) under stirring at 40°C. The Si to Gd molar ratio is fixed to 4, and the proportion of the polysiloxane shell precursors is 60% APTES/40% TEOS. The whole addition of silane precursors and hydrolysis solution is performed in six steps. Each step consists in the addition to the colloid of a portion of the silane precursors mixture (5% for the first step; 5% for the second step, 15%; and for the other ones, 20%) followed by the addition of the hydrolysis solution mixture (for the first step, 5%; for the second step, 15%; and for the other ones, 20%). The delay between both additions is one hour. After the last addition, the final mixture was stirred for 48 h at 40°C.

**Dilution of the Polysiloxane Shell by DTPADA.** To stabilize the particles in a biological environment and to capture released Gd$^{3+}$ ions, nanoparticles were functionalized by hydrophilic and chelating molecules, DTPADA. The grafting of DTPADA onto the polysiloxane shell of the nanoparticles is rendered possible by the presence of amine groups since APTES is hydrophilic and chelating molecules, DTPADA. The grafting of DTPADA onto the polysiloxane shell of the nanoparticles is rendered possible by the presence of amine groups since APTES was used for the synthesis of the shell. A 100 mL amount of crude GBN colloid (vide supra) was mixed with anhydrous DMSO (20 mL) containing DTPADA (DTPADA/APTES molar ratio 1.1, i.e., 4.25 g of DTPADA) and stirred for 1 h.

**Purification of GBNs.** To transfer nanoparticles from DEG to water, nanoparticles were precipitated in 500 mL of acetone. After centrifugation, the supernatant was removed. The white powder was washed with an ethanol/acetone mixture (v/v 85/15) by three cycles of dispersion–centrifugation (10 min at 3500 rpm). The supernatant was removed after each centrifugation. Then the white powder was dispersed in water. The purification of nanoparticles was performed by tangential centrifugation in Vivaspin (5 kDa). A typical procedure consists in centrifugation of 20 mL of aqueous colloid until the volume was 10 mL. Afterward, the GBN aqueous suspension, which is retained by the membrane, was diluted by the addition of deionized water (10 mL), while the filtrate containing the impurities was removed. The cycle “tangential centrifugation–dilution” was repeated 10 times.

**Size Measurement and Structural Characterization.** Direct measurement of the size distribution of the nanoparticles was performed with the size analyzer NanoS PCS from Malvern Instruments. Measurements were directly taken on the colloid after surface modification of the nanoparticles.

**Conformation of sizes was obtained from high-resolution transmission electron microscopy (HRTEM) performed at CLYM (Centre Lyonnais de Microscopie) with a JEOL 2010F transmission electron microscopy (HRTEM) performed at the biomedical beamline ID17 of the European Synchrotron Radiation Facility (Grenoble, France).

**SRT Imaging.** SRT Imaging of phantoms containing GBNs at various gadolinium concentrations and in vivo imaging were performed at the biomedical beamline ID17 of the European Synchrotron Radiation Facility (Grenoble, France). The SRT images were obtained using a monochromatic X-ray beam tuned slightly above the gadolinium K-edge energy (50.239 keV). The acquisition was performed during a rotation of the sample around a vertical axis by means of a rotation stage. The attenuation was measured with a cryogenically cooled, high-purity germanium detector. The cross-sectional imaging and MRT Experiments. All operative procedures related to animal care strictly conformed to the Guidelines of the French Government with licenses 380532 and A3818510002. All experiments were performed under anesthesia with the following parameters: 5% isoflurane for induction and 1% isoflurane of xylazine/ketamine (645/5.4 mg kg$^{-1}$) for maintenance.

**Brain Tumor Inoculation.** The animal care used included mating and an operation performed on the right hemisphere after the sacrifice of the animals. The animals were sacrificed by decapitation after inhalation of isoflurane 2% in O$_2$/N$_2$ (25%/75)%.

**MR Imaging.** Images were acquired before and after injection using a 7 T Bruker imaging system (BioSpec; Bruker, Erlangen, Germany) equipped with a 400 mT/m gradient. Anesthesia was induced with 3–4% isoflurane and maintained with 1.5–2% isoflurane in a mixture of O$_2$/N$_2$ (25%/75)%.

**Functionalization of GBNs with T$_2$-Weighted Magnetic Resonance Imaging (MR).** A typical protocol consisted in a T$_2$-weighted Turbo-RARE SE sequence (TE = 36 ms, TR = 4200 ms, field of view = 2.56 cm, matrix = 256 × 256, slice thickness = 0.65 mm), T$_2$-weighted FLASH images (TE = 3.6 ms, TR = 86.07 ms, field of view = 2.56 cm, matrix = 256 × 256, slice thickness = 0.65 mm), and a dynamic series of transverse slices centered on the rat brain, obtained with a T$_2$-weighted MSME sequence (TR/TE = 113.4/10.6 ms, slice thickness = 1.5 mm, field of view = 5 cm, matrix = 256 × 256).

**Intravascular injection of GBN ([Gd] = 40 mM) was performed during the fourth repetition of the dynamic acquisition.** The duration was about 60 s. The measurement method of enhancement after injection of gadolinium on MR imaging was performed as follows. Regions of interest (ROIs) with the same area were drawn, and the signal in this area on each image was measured. Positive enhancement of the signal (EHC) in each area was calculated as (EHC(RH)/EHC(LH)), where EHC was the signal intensity value measured at each time after injection, and EHC was the signal value before injection. To compare the positive enhancement of the signal in healthy tissue and tumor, the enhancement of a ROI in the right hemisphere (containing the tumor) is divided by the enhancement of a ROI with an identical area but located in the left hemisphere (normal tissue) for a definite time (EHC(RH)/EHC(LH)), with t = 5 and 20 minutes, please see Table 1). The ROIs of RH and LH have the same area. They cover the right and the left hemispheres, respectively. The ROIs of GS and NT have the same area, which is smaller than the area of the ROIs of the RH and LH. They cover a small region of the tumor (GS) and of the normal tissue in the left hemisphere (NT), respectively. The temporal evolution of the EHC(RH)/EHC(LH) ratio is obtained by dividing the EHC(RH)/EHC(LH) ratio calculated for the image recorded 20 min after the injection of GBN by the one calculated 5 min after the injection (i.e., [EHC(RH)/EHC(LH)]$_{20}$/[EHC(RH)/EHC(LH)]$_{5}$). The temporal evolution of the EHC(RH)/EHC(LH) ratio was obtained in a similar way (EHC(GS)/EHC(GS))$_{20}$/EHC(GS)/EHC(GS))$_{5}$).

**SRCT Imaging.** SRCT Imaging of phantoms containing GBNs at various gadolinium concentrations and in vivo imaging were performed at the biomedical beamline ID17 of the European Synchrotron Radiation Facility (Grenoble, France). The SRCT images were obtained using a monochromatic X-ray beam tuned slightly above the gadolinium K-edge energy (50.239 keV). The acquisition was performed during a rotation of the sample around a vertical axis by means of a rotation stage. The attenuation was measured with a cryogenically cooled, high-purity germanium detector. The cross-sectional

**Conformation of sizes was obtained from high-resolution transmission electron microscopy (HRTEM) performed at CLYM (Centre Lyonnais de Microscopie) with a JEOL 2010F microscope equipped with a Gatan Digi-PEELS energy-loss spectroscopy (EELS). Drops of colloidal solutions were deposited on dedicated TEM carbon grids and observed after natural drying.

**Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Analysis.** Determination of gadolinium content in colloids, in the right and left hemispheres of the brain and in urine, was performed by ICP-MS analysis (Agilent 7500ce). Before measuring Gd concentration, samples of colloidal solution were diluted in 0.5 M HNO$_3$ (1:2500, v/v) and in HNO$_3$ (2%, 2 ppb in; 1:50, v/v). The determination of gadolinium content in right and left hemispheres of the brain and in urine required their dissolution in aqua regia. The resulting solutions were diluted in HNO$_3$ (2%, 2 ppb in, 1:3000 v/v for brain and 1:3000 v/v for urine).
images obtained by SRCT experiments were calculated from the numerous projections measured at small angular intervals. The data processing was performed under IDL software called SNAKRE code (University of Pennsylvania, Philadelphia).

In vitro imaging of drug-encapsulated nanoparticles was also performed at the ID17 biomedical beamline at the European Synchrotron Radiation Facility (Grenoble, France). MRT uses X-rays emitted tangentially to the ring from relativistic electron bunches circulating in a storage ring. The wiggler source produces a white spectrum of photons that extends after filtration (Be (0.5 mm), C (1.5 mm), Al (1.5 mm), and Cu (1.0 mm)) from 50 to 350 keV (mean energy of 90 keV). The quasi-laminar beam is spatially fractionated into an array of microbeams by using an adjustable multislit collimator positioned 41.7 m from the photon source and 100 cm upstream from the head of the animals. Upstream from the multislit collimator, the dose rate within a homogeneous field of 10 mm × 10 mm was approximately 90 Gy s⁻¹ m⁻². Downstream of the multislit collimator, the peak entrance dose within the microbeam was ∼72 Gy s⁻¹ m⁻².

**In Vivo Irradiation Methods.** Ten days after tumor inoculation, the animals were positioned prone on a Kappa-type goniometer (Huber, Germany) in front of the X-ray source, on a homemade acrylic frame. Rats were anesthetized by intraperitoneal injection of a mixture of xylazine/ketamine before placement in a vertical stereotatic holder screwed to the beam and received a lateral irradiation, from their anatomically right side to their left. Then, a 90° angle was applied to the motorized goniometer, and the second irradiation was performed in the anatomically anteroposterior direction. The field of irradiation was fixed at 10.5 mm height and 8 mm width, and was centered at the theoretical center of the tumor (i.e., 3.5 mm lateral to the bregma, 6 mm deep in the skull in the right hemisphere). The microbeams were 50 μm wide with an on-center distance fixed at 211 μm. The total irradiation procedure lasted about 2 min. Animal immobility during exposure was checked on three control video screens located in the control hut. A series of 27 rats was divided into untreated rats (n = 4), MRT-treated rats without GBN injection (n = 7), treated rats with a 5 min delay between GBN injection and MRT (n = 8), and treated rats with a 20 min delay between GBN injection and MRT (n = 6). These delays between drug injection and MRT were chosen according to results obtained by MRT. The spatial configuration of the microbeams was checked by radiochromic films (Gafchromic, HD-810).

**Survival Analysis.** The rats were followed up at the animal facility after the irradiation. At a later tumor stage, rats were euthanized by intracardiac injection of pentobarbital sodium less than 1 day before their anticipated death as judged by clinical signs. Some of them were found dead. The time between implantation and death was recorded as survival time (one day was added for euthanized rats). The median survival time (MeST) postimplantation was calculated, and Kaplan–Meier survival data were plotted versus time after tumor implantation. The increase in lifespan in percent (ILS) characterizes the difference between median survival time for treated and untreated rats divided by the median survival time for untreated rats.

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**REFERENCES AND NOTES**


