Gold-Loaded Polymeric Micelles for Computed Tomography-Guided Radiation Therapy Treatment and Radiosensitization

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ABSTRACT Gold nanoparticles (AuNPs) have generated interest as both imaging and therapeutic agents. AuNPs are attractive for imaging applications since they are nontoxic and provide nearly three times greater X-ray attenuation per unit weight than iodine. As therapeutic agents, AuNPs can sensitize tumor cells to ionizing radiation. To create a nanoplatform that could simultaneously exhibit long circulation times, achieve appreciable tumor accumulation, generate computed tomography (CT) image contrast, and serve as a radiosensitizer, gold-loaded polymeric micelles (GPMs) were prepared. Specifically, 1.9 nm AuNPs were encapsulated within the hydrophobic core of micelles formed with the amphiphilic diblock copolymer poly(ethylene glycol)-b-poly(ε-caprolactone). GPMs were produced with low polydispersity and mean hydrodynamic diameters ranging from 25 to 150 nm. Following intravenous injection, GPMs provided blood pool contrast for up to 24 h and improved the delineation of tumor margins via CT. Thus, GPM-enhanced CT imaging was used to guide radiation therapy delivered via a small animal radiation research platform. In combination with the radiosensitizing capabilities of gold, tumor-bearing mice exhibited a 1.7-fold improvement in the median survival time, compared with mice receiving radiation alone. It is envisioned that translation of these capabilities to human cancer patients could guide and enhance the efficacy of radiation therapy.

KEYWORDS: gold, nanoparticle, radiation, computed tomography, theranostic, radiosensitizer

Over the past two decades, many nanoparticle formulations have been evaluated as computed tomography (CT) contrast agents, including liposomes loaded with iodoated compounds,1-3 polymeric iodine-containing poly(ethylene glycol) (PEG)-based micelles,4 iodine-containing perfluorocarbons,5,6 bismuth sulfide nanoparticles,7 and gold nanoparticles (AuNPs).8-10 Of these, gold nanoparticles have garnered a particularly high degree of interest. This is largely due to the high mass attenuation coefficient of gold, which is ∼2.7-fold higher than iodine.11 Accordingly, it has been found that 30 nm AuNPs can attenuate 120 kVp X-rays 5.7 times more than the iodine-based agent Ultravist.12 Additional benefits of working with AuNPs include the ability to finely tune their size and shape and modify their surface with various functional groups. While other nanoparticle formulations, such as bismuth sulfide nanoparticles, may also exhibit higher X-ray absorption than iodine, it is difficult to control their size and there is a lack of chemical methods to modify their surface.10,12 Surface chemistry is important when attempting to prolong systemic circulation, a prerequisite for tumor imaging and tumor accumulation via the enhanced permeability and retention (EPR) effect.13 An extended circulation also offers an opportunity to image the reticuloendothelial system (RES), the blood pool, and in some cases the lymph system.

In addition to their use as CT contrast agents, AuNPs have also shown promise as radiosensitizers. Radiosensitization is due to the high absorbance of gold and the
resulting deposition of energy in surrounding tissues from photoelectrons (i.e., photoelectric effect), Auger electrons, and the generation of free radicals.\textsuperscript{14,15} Within the kilovoltage energy range, the radiosensitization effect is generally attributable to the photoelectric effect, while Auger electrons are hypothesized to be responsible for energy radiosensitization within the megavoltage range of radiation energies.\textsuperscript{16–19} It has been shown that AuNPs in combination with radiation treatment can lead to an increase in the number of DNA double-strand breaks compared with radiation alone.\textsuperscript{14,20–25} In one recent study it was shown that 1.9 nm AuNPs could even lead to an increase in the survival of tumor-bearing mice, compared with radiation therapy (RT) alone.\textsuperscript{9} However, because of the rapid clearance of the small nanoparticles used in this study, the tumors had to be irradiated immediately after AuNP administration. In general, rapid clearance limits tumor-specific accumulation via EPR and, thus, can limit the ability of small AuNPs to guide, via CT, the precise delivery of radiation therapy.

When designing a treatment plan, radiation oncologists must take into account several critical factors including the mapping of true tumor margins, which can sometimes be challenging to define using current imaging techniques. Therefore, a more accurate definition of tumor boundaries would facilitate more precise delivery of radiation therapy and as a result decrease normal tissue exposure to undesirable radiation.\textsuperscript{26–28} With this goal in mind, it is envisioned that long-circulating AuNPs that appreciably accumulate in tumors via EPR can be used to guide RT planning and treatment, through improved contrast-enhanced delineation of tumor boundaries via CT, thus minimizing energy deposition in surrounding healthy tissues. In addition, AuNP-mediated radiosensitization can also directly increase the radiation dose received by the tumor, thus providing a second complementary mechanism by which the overall therapeutic index can be increased.

In this study, we describe the development of a multifunctional micelle that simultaneously exhibits long circulation times, achieves appreciable tumor accumulation, generates CT image contrast, and serves as a sensitizer for radiation therapy in cellular and animal models at sublethal radiation doses. Specifically, using a microemulsion synthesis method, we have been able to prepare gold-loaded polymeric micelles (GPMs), with tunable hydrodynamic diameters ranging from 25 to 150 nm. The GPMs are formed using the amphiphilic diblock copolymer poly(ethylene glycol)-b-poly(ϵ-caprolactone) (PEG-b-PCL) and have tightly packed clusters of 1.9 nm AuNPs incorporated within the hydrophobic core (Figure 1). We first evaluated the ability of GPMs to enhance double-stranded DNA breaks \textit{in vitro} in response to radiation. Next, we assessed whether GPMs are capable of generating contrast for CT blood pool and tumor imaging. Finally we investigated whether the radiosensitization in cells translated to an improvement in survivability in murine tumor xenograft models.

**RESULTS AND DISCUSSION**

**Characterization of 1.9 nm AuNPs.** Hydrophobic AuNPs were prepared with dodecanethiol as a capping agent. Transmission electron microscopy (TEM) was used to characterize the mean core size of the individual hydrophobic AuNPs. TEM images showed a uniform distribution of AuNPs with a core size of 1.93 ± 0.16 nm (Supp. Figure S1). Purity was confirmed via UV–vis spectroscopy (Supp. Figure S2).

**Synthesis and Characterization of GPMs.** GPMs were prepared by encapsulating 1.9 nm AuNPs within the diblock copolymer PEG-b-PCL, using a microemulsion method. These GPMs were soluble in aqueous solutions owing to the hydrophilic PEG corona of the diblock copolymer. Following synthesis and purification of the GPMs, six different sizes (25–150 nm, Figure 2) were collected using differential centrifugation, as confirmed by dynamic light scattering (DLS). The DLS measurements demonstrate particle measurements with a low polydispersity index for all GPM fractions (<0.1). TEM was used to determine the morphology of the GPMs and the packing of AuNPs within the hydrophobic core. TEM micrographs revealed a narrow distribution of spherical GPMs with tightly packed clusters of AuNPs contained within the hydrophobic core of the micelles (Figure 2). The TEM micrographs also revealed a low polydispersity, correlating well with the DLS measurements. The zeta potential of the various GPM formulations was near neutral. A summary of the GPM physical-chemical properties is provided in Supp. Table S1.

**Evaluation of GPMs as a Radiosensitizer.** GPMs with a hydrodynamic diameter of approximately 75 nm were selected for a more detailed evaluation as radiosensitizing agents. This size was selected because it was...
produced in significantly higher yields than the other sizes and was thus more amenable for in vivo testing.

To evaluate the radiosensitization effects of the 75 nm GPMs in vitro, HT1080 human fibrosarcoma cells were irradiated (4 Gy) or mock irradiated in the presence or absence of GPMs and analyzed for double-strand breaks (γ-h2ax staining) (Figure 3). Immunofluorescent images revealed very low levels of γ-h2ax foci (observed as bright fluorescent spots) in unirradiated cells, regardless of the presence of GPMs. In contrast, high levels of γ-h2ax foci were observed within the nuclei of cells that received radiation treatment, with a noticeably higher number of double-strand breaks in cells that were treated in the presence of GPMs. Quantitatively, there were very few γ-h2ax foci per unit area in unirradiated controls, as expected, and there was no statistically significant difference between cells incubated in the presence or absence of GPMs. However, when cells were irradiated, the number of γ-h2ax foci increased and a statistically significant difference was observed between cells irradiated in the presence and absence of GPMs (p < 0.05). Compared to cells receiving radiation only, the cells that were irradiated in the presence of GPMs exhibited roughly a 2.2 times higher density of DNA double-strand breaks. Furthermore, clonogenic survival assays revealed a decrease in survival of HT1080 cells irradiated in the presence of GPMs compared to those receiving irradiation alone (Figure 3). A statistically significant difference in survival (p < 0.05) was observed for radiation doses of 4 and 6 Gy. Using the linear-quadratic model to assess the enhancement of radiation effects, it was estimated that GPMs produced a sensitizer enhancement ratio of approximately 1.2, which is consistent with previous studies that utilized AuNPs as a radiosensitizer.22,29

Stability of GPMs in Serum. Prior to evaluating GPMs as an imaging and radiosensitizing agent in living subjects, the stability of 75 nm GPMs was evaluated in fetal bovine serum (Supp. Figure S3). Upon incubating the GPMs with 100% serum for 24 h at 37 °C, there was no difference in the size of the GPMs as determined by DLS, and no visible precipitates were observable in the solution. Moreover, no evidence of leaching of AuNPs from the micelle or alteration in the micelle structure was observed in TEM images following incubation in serum (Supp. Figure S4), suggesting that GPMs are sufficiently stable for in vivo studies. It should be noted that all of the micelle samples (i.e., all size fractions) also appear to be stable in PBS at pH 7.4 for months with no observable changes in hydrodynamic diameter or structure, as determined by DLS and TEM, respectively.
GPM Pharmacokinetics. Long circulating particles that are able to avoid rapid clearance from the bloodstream via glomerular filtration and the RES are necessary for EPR-driven tumor accumulation. Therefore, if GPMs are to be used to help delineate tumor margins and guide radiation therapy, it is necessary for them to exhibit a long circulation half-life. It was determined that the circulation half-life of 75 nm GPMs is ∼1 h during the early distribution phase and 8.7 h during the elimination phase (Figure 4). This long circulation time is likely governed by the dense hydrophilic PEG coating present on the micelle.

The ability of GPMs to generate contrast in vivo was validated in mice. Images acquired 30 min postinjection demonstrated enhancement of the great vessels and minor branches such as the renal vessels and interlobular vessels (Figure 4). Furthermore, the cardiac chambers were readily visualized, demonstrating the potential use of GPMs as a blood-pool contrast agent. After 24 h there was residual enhancement of the heart and great vessels, indicative of the long circulation time of the GPMs. The administered dose of GPMs (650 mg Au/kg) was well within the range of clinically approved intravenous contrast agents. For example, ioxilanol (Visipaque) is typically administered at doses ranging from 300 to 1200 mg of I/kg body weight. Additionally, the dose utilized in this study was lower than what was used in prior studies that employed gold as a radiosensitizer (i.e., >1350 mg Au/kg).9,26

The biodistribution of GPMs was evaluated at 48 h and at 1 week postinjection by performing an inductively coupled plasma–optical emission spectroscopy (ICP-OES) analysis of gold content within the heart, kidneys, lungs, spleen, liver, feces, and urine (Supp. Table S2). As expected, the largest fractions of gold were observed in the liver and spleen. All organs examined showed a marked reduction in gold accumulation between the two time points evaluated. Specifically, ICP-OES findings revealed a 28% reduction of gold in the liver and a 47.5% reduction of gold in the spleen. Evaluation of gold content within feces and urine suggests that the primary route of clearance was biliary excretion. It is interesting to note that at the 48 h time point ∼67% of the total injected dose was not accounted for in the organs analyzed or feces.

It is presumed that a significant fraction of the injected gold was removed through biliary excretion within the first 24 h. Feces within the first 24 h were not collected. Upon visual inspection, no other organs appeared to exhibit any obvious signs of gold uptake, which is typically accompanied by a dark purplish hue, with the exception of the skin, which did have some discoloration.

Toxicity Analysis. The intravenous injection of GPMs (650 mg Au/kg) into healthy mice led to no signs of illness, change in activity, or weight loss (Supp. Figure S5). Notably, the amount of gold administered was well below the LD50, which was previously reported to be 3.2 g Au/kg.6 A toxicological analysis of mice 1 day and 1 week following the administration of GPMs (650 mg Au/kg) revealed normal blood chemistry, compared to saline-injected controls (Supp. Table S3).

GPMs as a CT Contrast Agent. The ability of GPMs to accumulate within tumors at sufficient levels to provide CT contrast was confirmed in mice bearing HT1080 flank tumors. Axial tumor slices of three different tumor-bearing mice were analyzed precontrast as well as 30 min, 24 h, and 48 h postcontrast (Figure 5). The variation of signal enhancement from slice to slice was accounted for by normalizing the signal to the corresponding paraspinal muscles for each slice. In the precontrast image, the tumor on the flank of the mouse located between the thigh and paraspinal muscles is not clearly delineated. At the 30 min time point, there is no qualitative or statistical difference in contrast enhancement within the tumor compared to the precontrast image. However, at 24 and 48 h postcontrast, the tumor is revealed as a hyperintense heterogeneously enhancing region with well-defined margins. These enhancements in tumor contrast were statistically different from both the precontrast and 30 min time points. This result is likely due to the...
extravasation of GPMs out of leaky vasculature and accumulation within the tumor owing to the EPR effect. This distinction between tumor and normal tissue can help in the design of radiation treatment of cancer by enabling visualization of regional tumor margins and spread, to help localize and maximize radiation doses to malignancies while minimizing exposure of normal tissue. Notably, within the tumor margins, the contrast enhancement was somewhat heterogeneous, likely due to variations in the ability of GPMs to penetrate far beyond the vascular wall.

To demonstrate the importance of a long circulating platform for effective extravasation and accumulation in tumors, 1.9 nm control AuNPs were also administered to tumor-bearing mice and imaged 30 min, 24 h, and 48 h postcontrast. At each time point, no visible tumor contrast enhancement was observed, compared with precontrast images. This is likely because >90% of the particles are cleared within the first 30 min.8

To quantitatively determine the amount of GPMs and 1.9 nm AuNPs delivered to the tumor, as well as other organs, the liver, spleen, lung, heart, kidneys, and tumor were harvested 48 h postinjection and the gold content was analyzed by ICP-OES (Figure 6). Mice injected with GPMs had the highest levels of gold in the liver and spleen and only modest levels of gold in the heart, lungs, and kidneys. In contrast, mice injected with 1.9 nm AuNPs had higher levels of gold within the kidney, lower uptake in the liver and spleen, and very modest uptake in the heart and lungs. This difference in organ distribution is expected since the mechanism of elimination differs for both formulations. In general neutrally charged particles with hydrodynamic sizes smaller than ∼6 nm are cleared from the systemic circulation via glomerular filtration and excreted in the urine, whereas particles greater than ∼6 nm are primarily cleared by the RES system.31 This disparity in elimination was also supported by in vivo CT imaging. Following GPM administration, the spleen and liver gradually brighten over the course of 48 h (Supp. Figure S6), whereas mice injected with AuroVist exhibit very bright contrast within the kidneys and bladder at early time points of CT imaging and gradually return to baseline at 24 h postinjection. With respect to tumor delivery, mice injected with GPMs displayed a statistically significant 6-fold increase in gold accumulation (6.2 ± 1.2% ID/g) compared to mice injected with AuroVist (1.0 ± 0.1% ID/g). Upon adjusting for tumor volume, the average concentration of gold within the tumor was calculated to be 0.57 ± 0.1 mg/mL and 0.14 ± 0.01 mg/mL for mice injected with GPMs and AuroVist, respectively. In general, the sensitivity for Au detection using CT imaging is estimated to be around 0.5 mg/mL.21 However, as a result of the heterogeneous distribution of GPMs within the tumor, some regions likely have gold concentrations well above this lower detection limit. Furthermore, these tumor concentrations were well above the 0.1 mg Au/mL needed for a radiosensitization effect in vitro. Importantly, this circulation-mediated increase in nanoparticle delivery was sufficient to provide CT contrast prior to tumor radiation therapy. This demonstrates the importance of having a long circulating platform since the improvement in delivery has the ability to

Figure 6. ICP-OES analysis of gold distribution at 48 h following the administration of GPMs or AuroVist. The percent injected dose per gram of tissue was calculated by measuring the concentration of gold in excised organs via ICP-OES. The asterisk indicates statistical significance (p < 0.05).
the predetermined threshold volume (1300 mm$^3$). The stereotactic radiation. Mice were monitored for tumor
mice receiving GPMs, which enabled CT-guided trast enhancement was visible within the tumors of
GPMs 24 h prior to radiation treatment. Notably, con-
radiation therapy (6 Gy) alone or were injected with
receiving GPMs. The next two groups either received
the unirradiated controls with one of the two groups
four groups (n = 7 per group). The first two groups were
the unirradiated controls with one of the two groups
receiving GPMs. The next two groups either received
radiation therapy (6 Gy) alone or were injected with
GPMs 24 h prior to radiation treatment. Notably, con-
trast enhancement was visible within the tumors of
mice receiving GPMs, which enabled CT-guided stereotactic radiation. Mice were monitored for tumor
growth and were sacrificed when the tumors reached
the predetermined threshold volume (1300 mm$^3$). The survival time was measured from the time of radiation
(or mock irradiation). Mice that received GPMs prior to
radiation therapy exhibited a statistically significant
(p < 0.05) improvement in median survival (68 days),
compared to mice treated with radiation alone
(38 days) (Figure 7). It should be noted that all mouse
groups appeared to tolerate GPMs very well over the
unirradiated controls with one of the two groups
receiving GPMs. The next two groups either received
radiation therapy (6 Gy) alone or were injected with
GPMs 24 h prior to radiation treatment. Notably, con-
trast enhancement was visible within the tumors of
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groups appeared to tolerate GPMs very well over the
of the three mice had complete remission with no palpable tumor, while the third mouse had a palpable static tumor. With respect to the unirradiated groups, GPMs alone had no effect on tumor growth compared to untreated controls. These results suggest that the EPR-dependent accumulation of GPMs within tumors can guide and enhance the efficacy of radia-
tion therapy.

CONCLUSION

Gold nanoparticles present a promising platform for therapeutic and imaging (theranostic) applications because of their unique physical-chemical properties, their ability to be easily functionalized, and their safety profile. Gold has been used in medical practice throughout history and continues today as a treatment for rheumatoid arthritis.$^{32}$ Numerous animal studies suggest that AuNPs are also very well tolerated.$^{33–38}$ In fact, several AuNP formulations have even entered clinical trials for cancer treatment, including CYT-6091 and AuroShell. In this study 1.9 nm AuNPs were encapsulated within the biocompatible and biodegradable polymer PEG-b-PCL, forming gold-loaded polymeric micelles. An anticipated benefit of this GPM formulation over pegylated, solid AuNPs of similar size is that it is easy to incorporate other anticancer and/or other metallic nanoparticles into the micelle core,$^{39–41}$ if additional functionality is desirable. Moreover, we believe that the presence of many small AuNPs (1.9 nm) may allow for more rapid dissolution and excretion, compared with a single large AuNP. It was previously reported that with 40 nm solid AuNPs there is only a 9% fall in the content of gold in the liver from day 1 to 6 months.$^{42}$ In addition, many studies report inefficient clearance and a persistent accumulation of AuNPs within the reticuloen-
thelial system.$^{43–45}$ In contrast, we observed more than a 28% fall in gold content within the liver between day 2 and day 7. These results are very promising, although a more complete analysis must still be performed to study additional and later time points before a definitive con-
clusion can be drawn.

In summary, we showed that GPMs were capable of enhancing radiation-induced DNA double-strand breaks in a cell culture model, consistent with prior work with solid AuNPs.$^{14,15,20–25,29}$ Further, because of their extended clearance half-life, GPMs exhibited improved EPR-dependent accumulation in murine tumor xenografts, compared to individual 1.9 nm AuroVist nanoparticles. The higher levels of GPM accumulation in the tumor provided clear and quantifiable improve-
ment in CT contrast. The combination of CT-guided radiation therapy and gold-mediated radiosensi-
tization led to a statistically significant increase in the mean survival time of tumor-bearing mice com-
pared with mice receiving radiation alone. Accurate delineation of tumor boundaries and tumor-specific radiosensitization is important for radiotherapy, due
METHODS
Synthesis of 1.9 nm Gold AuNPs. Dodecanethiol-capped AuNPs were prepared according to the procedure described by Brust et al.,\(^4\) using a two-phase reduction of tetrachloroaurate (HAuCl\(_4\)) by sodium borohydride (NaBH\(_4\)) in the presence of an alkanethiol. Briefly, 30 mL of an aqueous solution of 30 mM hydrogen tetrachloroaurate (HAuCl\(_4\)) was mixed with 50 mM tetracyclammonium bromide in 80 mL of toluene. The solution was stirred until the HAuCl\(_4\) solution transferred into the organic phase. Then, 0.84 mM dodecanethiol was added to the solution while stirring (HAuCl\(_4\)) by sodium borohydride (NaBH\(_4\)) in the presence of the HAuCl\(_4\) solution transferred into the organic phase. Then, bromide in 80 mL of toluene. The solution was stirred until the concentration of injected sample. Blood samples (10 \(\mu\)L) were saved for ICP-OES for the determination of the gold content ratio was calculated as the ratio of the mean inactivation efficiency/100)). Each point on the survival curve represents the mean surviving fraction from at least three replicates. The survival curves were fitted to a linear-quadratic equation: surviving fraction = exp(\(−\frac{\gamma D°}{D^2}\)). The sensitization enhancement ratio was calculated as the ratio of the mean inactivation doses, defined as the dose at which there is 37% survival, with and without GPMs.

Quantification of Blood Clearance via ICP-OES. Approximately 6-week-old female nu/nu nude mice (n = 3) were used for the GPM blood clearance experiments. The GPMs were injected retro-orbitally at a dose of approximately 100 ppm in 200 \(\mu\)L of injected solution. Prior to injection, an aliquot of the GPM solution was saved for ICP-OES for the determination of the gold concentration of injected sample. Blood samples (10 \(\mu\)L each) were collected from each animal using the tail-nick method at 1 min, 10 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 24 h, and 48 h postinjection.

Contrast-Enhanced in Vivo CT Imaging and Biodistribution Analysis. Approximately 6-week-old female nu/nu nude mice (Charles River Laboratory, Charles River, MA, USA) were maintained in accordance with the Institutional Animal Care and Use Committee of the University of Pennsylvania. Mice were anesthetized using isoflurane, and HT-1080 cells were injected subcutaneously into the back left flank (2 \(×\) 10\(^6\) cells in 0.1 mL of PBS). Tumors were grown until the mean diameter was approximately 7–8 mm, and precontrast cone beam CT imaging was performed using an SARRP (Gulmay Medical, Inc.). CT imaging was conducted at 50 kVp (0.5 mA), and 1440 projections were used to reconstruct the cone-beam images using the algorithm provided by the manufacturer. Immediately following the precontrast image acquisition, either GPMs or 1.9 nm AuroVist gold nanoparticles (Nanoprobes, Yaphank, NY, USA) were intravenously injected into the HT1080 tumor-bearing mice (N = 3 for each group). Using isoflurane to anesthetize the mice, both contrast agents were administered by retro-orbital injection (650 mg/kg Au in 0.2 mL). Postcontrast images were collected 30 min, 24 h, and 48 h postinjection with the same imaging parameters used for precontrast images. After 48 h, the animals were sacrificed and the tumors, livers, spleens, kidneys, hearts, and lungs were harvested. Tissue samples were thoroughly washed with PBS and blotted dry to minimize the contribution of any nanoparticles remaining in the bloodstream.
were weighed and digested in HNO₃ overnight at 70 °C. Following the overnight digestion, HCL was added to dissolve the gold. Samples were diluted with Millipore water and analyzed for gold content using ICP-OES.

**Radiology** Twelve nude mice (females) were randomized into four groups of three animals per group receiving 650 mg Au/kg or sham-injected with phosphate-buffered saline. Animals were weighed and observed regularly for clinical signs for up to 1 week postinjection. Animals were euthanized by CO₂ 1 day and 1 week after intravenous gold injections, and 0.3 mL of blood was removed from the right ventricle immediately after the cessation of breathing. Blood chemistry analytes included blood urea nitrogen, phosphate, albumin, globin, gamma-glutamyl transpeptidase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, cholesterol, calcium, creatine, glucose, and phosphorus.

**In Vivo Radiation Therapy.** Approximately 6-week-old female nu/nu nude mice were anesthetized using isoflurane, and only (intravenous injection of GPMs (650 mg/kg Au in 0.2 mL) 24 h group received 6 Gy RT only; the second groups received an bearing mice were split into four groups of seven each: the first of blood was removed from the right ventricle immediately through a 17 mm diameter collimator. Mice were monitored for tumor growth and were sacrificed when the tumor volume reached 1300 mm³. Tumor volumes were calculated assuming an elliptical tumor shape (1/2 length × width)14,15. Survival time to this end point was calculated from date of treatment.

**Conflict of Interest:** The authors declare no competing financial interest.

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**Supporting Information Available:** Tables S1 to S3 and Figures S1 to S7. This information is available free of charge via the Internet at http://pubs.acs.org.

**REFERENCES AND NOTES**


