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ADVANCED REVIEW

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Critical parameters to translate gold nanoparticles as radiosensitizing agents into the clinic

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Abstract

Radiotherapy is an inevitable choice for cancer treatment that is applied as combinatorial therapy along with surgery and chemotherapy. Nevertheless, radiotherapy at high doses kills normal and tumor cells at the same time. In addition, some tumor cells are resistant to radiotherapy. Recently, many researchers have focused on high-Z nanomaterials as radiosensitizers for radiotherapy. Among them, gold nanoparticles (GNPs) have shown remarkable potential due to their promising physical, chemical, and biological properties. Although few clinical trial studies have been performed on drug delivery and photosensitization with lasers, GNPs have not yet received Food and Drug Administration approval for use in radiotherapy. The sensitization effects of GNPs are dependent on their concentration in cells and x-ray energy deposition during radiotherapy. Notably, some limitations related to the properties of the GNPs, including their size, shape, surface charge, and ligands, and the radiation source energy should be resolved. At the first, this review focuses on some of the challenges of using GNPs as radiosensitizers and some biases among in vitro/in vivo, Monte Carlo, and clinical studies. Then, we discuss the

Abbreviations: BPs, bipyramids; DEF, dose enhancement factor; EGF, epithelial growth factor; EPR, enhanced permeability and retention; FDA, Food and Drug Administration; GNPs, gold nanoparticles; IT, intratumoral; IV, intravenous; KeV, kiloelectron volts; kVp, kilovolts; MV, megavolts; NIR, near-infrared; NM, nanomakura; NP, nanoparticles; PEG, polyethylene glycol; RES, reticuloendothelial system; ROS, reactive oxygen species; RT, radiotherapy; TGNPs, triangular gold nanoparticles; TNF, tumor necrosis factor.

challenges in the clinical translation of GNPs as radiosensitizers for radiotherapy and proposes feasible solutions. And finally, we suggest that certain areas be considered in future research.

This article is categorized under:
Therapeutic Approaches and Drug Discovery > NA

KEYWORDS
Radiosensitizer, Clinical trial, Gold Nanoparticles, Radiobiology, Cancer therapy

1 | INTRODUCTION

Radiotherapy (RT) with other procedures like surgery and chemotherapy has been considered an effective modality to treat cancer in the clinic. Radiotherapy can be applied using an external beam or brachytherapy. The mechanisms by which both methods kill cancer cells are the same. However, radiotherapy at high doses kills both normal cells and tumor cells at the same time. In addition, some tumor cells are resistant to radiotherapy. Thus, the treatment of cancer by radiotherapy alone is not adequate due to the many adverse effects on normal cell and the high resistance of cancer cells (Jin & Zhao, 2020; Verry et al., 2019; Wu et al., 2019). The application of nanomaterials to treat and diagnose cancer have attracted interest to solve some of the shortcomings of radiotherapy. In this area, high-Z nanomaterials play critical roles as radiosensitizers to enhance the efficiency of radiation therapy (Boateng, 2017; Sancey et al., 2014). In general, radiosensitizers are materials that improve treatment efficiency with lower doses of radiation while reducing the side effects to normal tissue. Radiosensitizers can achieve these goals in different ways, such as physical, chemical, and biological mechanisms.

Over the last decade, increasing interest in improving radiation therapy efficiency has brought about developments in gold-based nanomaterials as radiosensitizers. Originally, radiation sensitization by gold nanoparticles (GNPs) was outstanding because of their interesting physical properties. High-Z nanomaterial, like gold ($Z = 79$), absorb a high percentage of x-ray energy to boost energy deposition. It has been reported that water containing GNPs can absorb 100 times more radiation than water alone (Joiner & van der Kogel, 2018; Lehnert, 2007; McDermott, 2016). Energy deposition leads to the production of free radicals, such as reactive oxygen species (ROS), in water. Due to their high chemical instability, ROS can interact with different types of biological moieties, leading to severe damage to cellular and subcellular components (Samadian et al., 2020). In comparison to physical mechanisms, chemical enhancement has not been sufficiently evaluated. Radiation scavengers have been shown to significantly reduce cell damage in different cell lines (S. Li et al., 2019; S. Li, Penninckx, et al., 2016). Moreover, radiosensitization under low-pressure oxygen and anoxic conditions leads to insignificant radiation enhancement (Jain et al., 2014). These studies strongly introduce that ROS contribute to GNP radiosensitization (Ryter et al., 2007).

Clinical trials on the applications of GNPs are currently ongoing (Schuemann et al., 2020). Despite many validation studies and controversies regarding the mechanisms and characteristics of GNPs for tumor radiosensitization, the clinical translation of GNPs needs further exploration. Although GNPs have great potential as enhancers and radiosensitizers of radiation, gold-based nanomaterials have not yet received Food and Drug Administration (FDA) approval. This review focuses on some of the challenges of using GNPs as radiosensitizers and some biases among in vitro/in vivo, Monte Carlo and clinical studies. In addition, we suggest that certain areas be considered in future research.

2 | GOLD NANOPARTICLES

GNPs are fascinating types of nanoparticles (NPs) because of their paramount potential in various areas, such as electronics, nanotechnology, and medicine. GNPs show great potential to be used as carrier in drug delivery areas, as anticancer, photothermal, antibacterial, and contrast agents in medical imaging and as potential radiosensitizers in radiotherapy (Mokammel et al., 2021). Because of the interesting properties of GNPs, they have been suggested as radiosensitizers for cancer radiotherapy (Kwatra et al., 2013). The unique attributes of GNPs, such as their high-Z ($Z = 79$), low toxicity to normal cells, high surface area to attach other materials and drugs, high penetration into tumors and cancer cells, low permeability into normal cells, and good chemical properties, have made them great candidates for

nanotheranostic studies (Y. Chen et al., 2020). Although potential radiosensitization of GNPs in the range of kiloelectron volts (keV) has been confirmed by some studies, several investigations have reported no increase in the radiosensitization at higher energies, such as in the range of megavolts (MV; K. T. Butterworth et al., 2012).

A radiosensitizer is a drug or agent that augments the anticancer effects of ionizing radiation by inducing several mechanisms, such as the generation of free radicals (H. Wang et al., 2018; Figure 1). Several studies have reported that GNPs can act as potent radiosensitizers, thus improving the therapeutic efficiency of radiotherapy against tumors (Altundal et al., 2015; Pottier et al., 2015). In addition, the therapeutic effects of GNPs can be increased through encapsulation or conjugation with other antitumor drugs (Cooper et al., 2014). Under these conditions, the anticancer drug fights the cancer cells directly, while the interaction of ionizing radiation with high-Z NPs, such as GNPs, enhances the efficiency of radiotherapy due to the release of numerous Auger electrons (Hildenbrand et al., 2018; Ngwa et al., 2014, 2017). Auger electrons with its low energy and short range, resulting in the energy deposition over a small distance in the tumor (Rosa et al., 2017). Studies revealed that the surfaces of GNPs are active and can mediate chemical pathways and reactions, thereby increasing free radicals and the generation of ROS (Rosa et al., 2017; Wunder et al., 2011).

The generated ROS can stimulate the production of superoxide by the mitochondria. This is associated with suppression of antioxidant defenses in the mitochondria and leakage/release of superoxide into the cytoplasm, resulting in the generation of hydrogen peroxide (H_2O_2). The generated radicals penetrate through membranes and induce damage to critical macromolecules such as DNA (Hei et al., 2008). These mechanisms have been found to be involved in the radiosensitization effects of GNPs (Hei et al., 2008; Taggart et al., 2016). Furthermore, GNPs have surface features that can be modified and tailored for specific applications, making GNPs the most desirable radiosensitizers for radiotherapy (Boateng & Ngwa, 2020; Cooper et al., 2014). GNPs can be coated with polyethylene glycol (PEG) or complexed with other targeting ligands and drugs for specific targeting purposes (M. Guo et al., 2017; Hainfeld et al., 2008; Kumar et al., 2013b). Studies have illustrated that PEG-coated GNPs have higher cellular uptake than bare GNPs without any ligands (M. Guo et al., 2017; Kumar et al., 2013). Preclinical, in vitro, in vivo studies and Monte Carlo simulations have shown increased dose enhancement effects with high-Z NPs (Hainfeld et al., 2008; Ngwa et al., 2012). In addition to x-ray energy, some other parameters, including the surface charge, size, shape, and concentration of GNPs, are important factors that affect the response of cells to ionizing radiation.

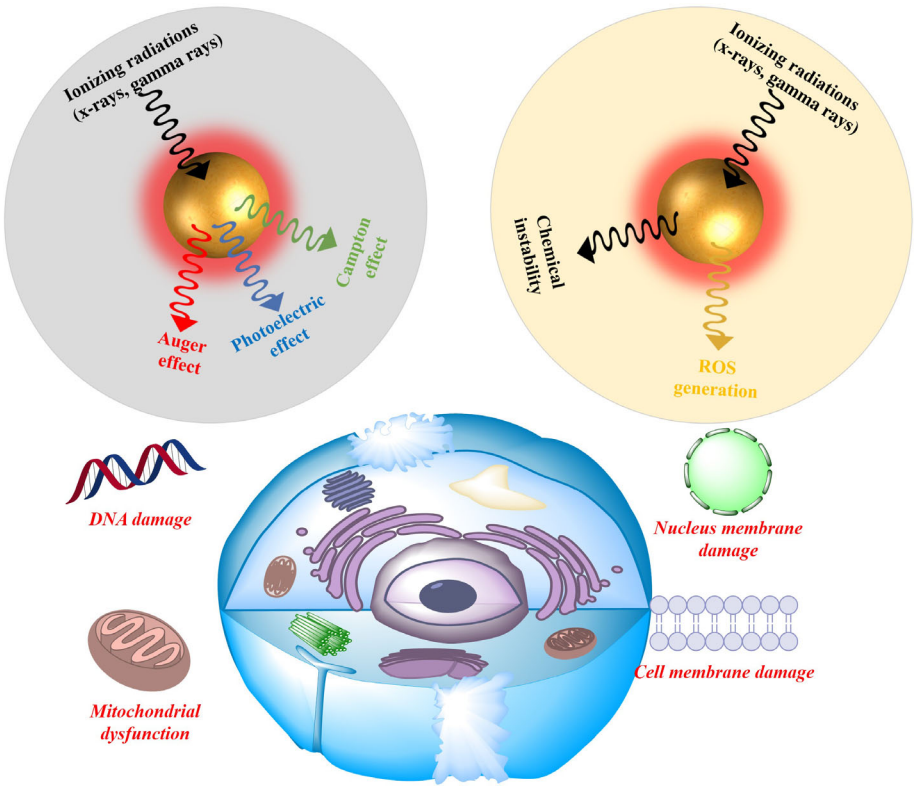


FIGURE 1 The mechanism of gold nanoparticle (GNP) radiosensitization and its effects on cells. Under radiation exposure, GNPs generate Auger electrons, reactive oxygen species (ROS), and photoelectric and Compton photons that cause cell damage.

3 | DOSE ENHANCEMENT FACTOR

Dose enhancement factor (DEF), a physical factor, is defined as the ratio of the quantity absorbed dose by equal volumes of interest with and without GNPs:

$$\mathrm{DEF} = \frac{D2}{D1},$$

where $D2$ is the quantity absorbed dose by the volume of interest with GNPs and $D1$ is the quantity absorbed dose by the volume of interest without GNPs.

The DEF is highly dependent on the properties of the GNPs, such as their size, uptake ratio, shape and concentration in the volume of interest or cell. For instance, larger-size GNPs give higher DEF, while larger GNPs lead to a lower uptake ratio. In addition, the DEF relies on radiation energy (Konefał et al., 2020; Morozov et al., 2020; Retif et al., 2015; Sung et al., 2017). Here, we review the effects of these factors on the radiosensitization of GNPs.

3.1 | Correlation between the optimum GNP size and x-ray energy with respect to the DEF

It has been reported that the radiosensitization of GNPs is a highly size-dependent factor. However, conflicting research has been reported among in vitro, in vivo, and Monte Carlo studies (Table 1).

Large GNPs have shown greater radiosensitization. Indeed, an increase in the size of GNPs is correlated with higher internal absorption of ionizing radiation. A study by Morozov et al. indicated that among various sizes of GNPs, including 12, 15, and 26, the 26-nm GNPs caused further DEF and DNA damage (Morozov et al., 2020). However, K. Butterworth et al. (2008) demonstrated that GNPs with a size of 5 nm gave a greater DEF and more DNA double-strand compared to 20-nm GNPs. In addition, according to the results of a study by Behrouzkia et al. (2019), GNPs with a diameter of 50 nm presented a higher DEF than those with a diameter of 30 or 100 nm.

Recently, Monte Carlo simulations have been considered a powerful tool to predict the radiosensitization and energy deposition of GNPs during radiotherapy. A study in Monte Carlo by Hwang et al. indicated that the dose enhancement effect has a direct relationship with NP size and concentration. However, the concentration of GNPs had a greater effect than the size. In general, this study suggested that as the concentration and diameter of the GNPs increased DEF value elevated (Hwang et al., 2017). A similar Monte Carlo study showed that the highest DEF could be achieved in low-energy photons with larger GNPs. Sardari et al. evaluated different sizes of GNPs, such as 20, 25, 30, 50, 70, 90, and 100 nm, with various x-ray energies, including 50, 95, and 250 keV and 4 MV, in Monte Carlo simulations (Keshavarz & Sardari, 2019). At GNP sizes greater than 50 nm, the in vitro and Monte Carlo data contrasted each other: increasing the size of the GNPs above 50 nm decreased the DEF in vitro but increased the DEF after the simulations.

Chithrani et al. compared the DEF for GNPs of different sizes with x-ray energies in HeLa cells. Their results revealed that 50-nm GNPs with higher uptake presented a greater DEF, giving values of 1.66 and 1.188 for 105 keV and 6 MV, respectively. They found that the DEF of the GNPs depended on NP size and x-ray energy (D. B. Chithrani et al., 2010). Liu et al. indicated that the DEF of 15-nm GNPs was contingent upon their concentration. The data of this study focused on the DEFs of GNPs at different concentrations. They reported that the DEF increased as the concentration was elevated from 0.1 to 10 mg/mL (D. B. Chithrani et al., 2010). Wang et al. showed that 49-nm GNPs gave a DEF greater than the 16-nm GNPs following irradiation of MDA-MB-231 cells. They used cell death and G2/M arrest as markers of the sensitization effects of two different sizes of GNPs. They found that GNPs with a diameter of 49 nm had a greater sensitization effect than those with a diameter of 16 nm (C. Wang et al., 2015). Hainfeld et al. evaluated the radiosensitization of 1.9-nm GNPs on EMT-6, TU-2449, and SCCVII mouse tumors following exposure to ionizing radiation at 100–250 kilovolts (kVp). Their findings indicated that the GNPs enhanced radiotherapy efficiency and improved long-term survival (Hainfeld et al., 2005, 2011, 2013). Another study by Chang et al. showed that the combination of 13-nm GNPs with 6 MV radiotherapy causes a delay in BI6I0 tumor growth plus an increase in survival compared to radiotherapy alone. They concluded that GNPs, as radiosensitizers, may have the ability to treat melanoma (Chang et al., 2008).

TABLE 1 DEF or sensitization effects of GNPs of various sizes in in vitro, in vivo, and Monte Carlo studies.

Optimum energy of the x-ray (kVp/MV)	In vitro/in vivo/ Monte Carlo	Cell line	GNP size range	Most effective size	DEF/effects	References
200 kVp	In vitro	Plasmid DNA	12, 15, 21, and 26 nm	26 nm	2.74 ± 0.61	Morozov et al. (2020)
160 kVp	In vitro	Plasmid DNA	5 and 20 nm	5 nm	1.25	K. Butterworth et al. (2008)
6 MV	In vitro	Gel-type	30, 50, and 100 nm	50 nm	1.017	Behrouzkia et al. (2019)
4–6 MV	Mote Carlo	Tumor	25, 50, 75, 100 and 125 nm	125 nm	1.011–1.047	Hwang et al. (2017)
50 keV	Mote Carlo	Tumor	20, 25, 30, 50, 70, 90, and 100 nm	100 nm	2.90	Keshavarz and Sardari (2019)
105 kVp/6 MV	In vitro	HeLa	14–74 nm	50 nm	1.66/1.18	D. B. Chithrani et al. (2010)
50 kVp	In vitro	HeLa	15 nm	15 nm	1.14–2.88	S. Liu et al. (2018)
6 MV	In vitro	MDA-MB-231	16 and 49 nm	49 nm	1.49–1.86	C. Wang et al. (2015)
100 and 250 kVp	In vivo	EMT-6, TU-2449, SCCVII	1.9 nm	1.9 nm	Increased the long-time survival	Hainfeld et al. (2005, 2011, 2013)
6 MV electron	In vivo	BI6I0	13 nm	13 nm	Delayed tumor growth	Chang et al. (2008)
6 MV	In vivo	HeLa	13.2 nm	13.2 nm	Inhibited tumor growth	Dou et al. (2016)
6 MV	In vivo	H22	8 and 50 nm	50 nm	DEF: 2.02 Inhibited tumor growth	S. Liu et al. (2018)
88 keV	In vivo	F-98 glioma cells	15 nm	15 nm	Increased lifespan	Bobyk et al. (2013)

Abbreviations: DEF, dose enhancement factor; GNPs, gold nanoparticles; KeV, kiloelectron volts; kVp, kilovolts; MV, megavolts.

Dou et al. used GNPs in the size range of 3–50 nm to improve tumor radiotherapy efficiency in a mouse model study. Their results demonstrated that GNPs with a size of 13 nm remarkably improved survival and inhibited tumor growth. These data suggested that 13-nm GNPs may have functional applications as adjuvants for clinical x-ray therapeutics (Dou et al., 2016). Liu et al. showed that 8- and 50-nm GNPs could prevent tumor growth and proliferation through the amplification of apoptosis via radiation therapy, with DEF values of 1.93 and 2.02, respectively. Elsewhere, 50-nm GNPs gave a greater radiosensitization effect for transplanted hepatocarcinoma (S. Liu et al., 2018). In another study, Bobyk et al. injected 15-nm GNPs into rats before irradiation with 88 keV x-rays. They observed a higher survival rate, going from 23.3 days to 41.6 ± 3.2, while that in the group treated with GNPs alone remained unchanged (23.3 ± 0.7 days). Notably, GNP uptake was observed in both normal and tumor tissues (Bobyk et al., 2013).

4 | GNP PARAMETERS AND CELLULAR UPTAKE

All of the characteristics of GNPs, including their shape, surface charge, size, and coating, can affect their interactions for cellular uptake. Notably, radiosensitization by GNPs is highly dependent on the cellular uptake and concentration of GNPs in the cell. Accordingly, the effects of these features on GNP cellular uptake are explained further.

4.1 | GNP size and uptake

Size is an important factor for the biological features and powerful application of GNPs in radiotherapy. The impact of this parameter on the distribution and cellular uptake has been investigated (Her et al., 2017; Schuemann et al., 2016).

Jiang et al. evaluated the uptake of GNPs (size <10 nm) with different ligands (zwitterionic, anionic, and cationic) by HeLa cells. They found that the percentage of GNPs with zwitterionic and anionic ligands in the cell diminished with increasing NP size, while the percentage of GNPs with cationic ligands increased by enlarging the NP size (Y. Jiang et al., 2015). In addition, Chithrani et al. indicated that the cellular uptake of GNPs was powerfully size dependent and that HeLa cells showed the highest uptake when the GNPs were 50 nm in size (B. D. Chithrani et al., 2006). Jiang et al. demonstrated that 40- to 50-nm GNPs had the highest concentration and effectiveness in HeLa and SK-BR cells. Even though all NPs within the 2–100 nm size range were found to be effective on cellular death, but 40- and 50-nm NPs showed the greatest effects. Their findings indicated that GNP design should be considered for medical applications such as medical imaging and therapeutics (W. Jiang et al., 2008). Huo et al. reported that GNP size had a great influence on uptake and penetration into tumors. They found that the 50-nm NPs were more effective than the 100-nm NPs in all in vitro, ex vivo, and in vivo assays with MCF-7 breast cells. Furthermore, the 50-nm GNPs showed excellent penetration into cell monolayers and deep accumulation in spheroid cells ex vivo and tumor xenografts in vivo (Huo et al., 2013).

Another interesting study by Ma et al. indicated that the accumulation of GNPs in LC3 cells was highly size dependent and that the amount of 50-nm GNPs in the cells was vastly different than that with 25- and 10-nm GNPs. They concluded that GNPs with a size of 50 nm had the most substantial effect on the autophagy rate (Ma et al., 2011). Yue et al. demonstrated that U87 cells had the highest uptake efficiency of 40- and 50-nm GNPs compared to 13-nm GNPs in vitro. They found that the large GNPs (40 and 50 nm) had greater potential than the 13-nm GNPs as carriers of siRNA for the delivery to cells (Yue et al., 2017). The results of Enea et al. indicated that the primary rat hepatocytes (PRH) and HepaRG cellular uptake of GNPs was size dependent. Their data showed that higher internalization of 15-nm GNPs in PRH cells would induce more toxicity compared to that in HepaRG cells (Enea et al., 2021). In contrast, a study done by Lu et al. showed that the uptake of 20-, 40-, and 70-nm GNPs was similar. Their findings showed that the cellular uptake rate was not size dependent. In addition, they concluded that the penetration of GNPs into spheroids may depend on certain factors, including diffusion and transcellular movement. Hence, the process of cellular uptake in 3D cultures was more complicated than that in monolayer models (Lu et al., 2020). The data of another study by Xia et al. indicated that GNP size was a critical factor for cellular uptake and biological distribution in vivo, which should be considered in future studies. Various sizes of GNPs in the range of 5–50 nm were used with HepG2 cells, whereby the 50-nm GNPs showed the highest uptake (Xia et al., 2019).

M. Liu et al. (2017) proposed that not only internalization of GNPs is critical but also the clustering state and intracellular trafficking of NPs are essential for designing sophisticated treatment strategies. Moreover, in this scenario, the real-time monitoring of NPs provides valuable results. They used plasmonic and correlative fluorescence imaging to monitor DNA-decorated GNPs. They reported that the endocytosis of GNPs follows multiple pathways including the early stage: GNPs in the single NP state while during the vesicular transport and maturation cluster formation occurs (Figure 2). Overall, the findings of these studies revealed that an optimal, smaller size of GNPs would maximize their effective accumulation in the tumor tissue (Table 2).

4.2 | GNP shape and uptake

A better clarification and understanding of cellular uptake are required when examining the interactions of GNPs with cells and their membranes. The shape, the size, surface charge as well as surface coating of nanostructures can affect their interactions with cells (Langille et al., 2012). As such, GNPs have shape-dependent physically and chemically (Millstone et al., 2009). In last decade, different GNPs with different shapes have been fabricated and designed, such as clusters, spheres, stars, cages/cages, core-shells, and rods (F. Chen et al., 2021; Sarfraz & Khan, 2021; Figure 3).

To better comprehensive of the interaction between cells and GNPs with different shapes would support developments in radiosensitization, uptake, and drug delivery. Here, we have compared and summarized some studies on the cellular uptake of GNPs with various shapes. It has been reported that the cellular uptake of spherical GNPs by HeLa cells is greater than the uptake of rod-shaped GNPs. Nambara et al. performed a compared the cellular uptake rate of triangular gold nanoparticles (TGNPs) with spherical NPs of a similar surface area by HeLa and RAW264.7 cells. Their results indicated that TGNPs with large sizes had higher levels of uptake by RAW264.7 and HeLa cells, but when the NPs were smaller, the uptake of the spherical GNPs was higher. They highlighted that the uptake rate of GNPs was shape dependent (Nambara et al., 2016; Yue et al., 2017). A study by Asrin et al. indicated that gold nanostars showed

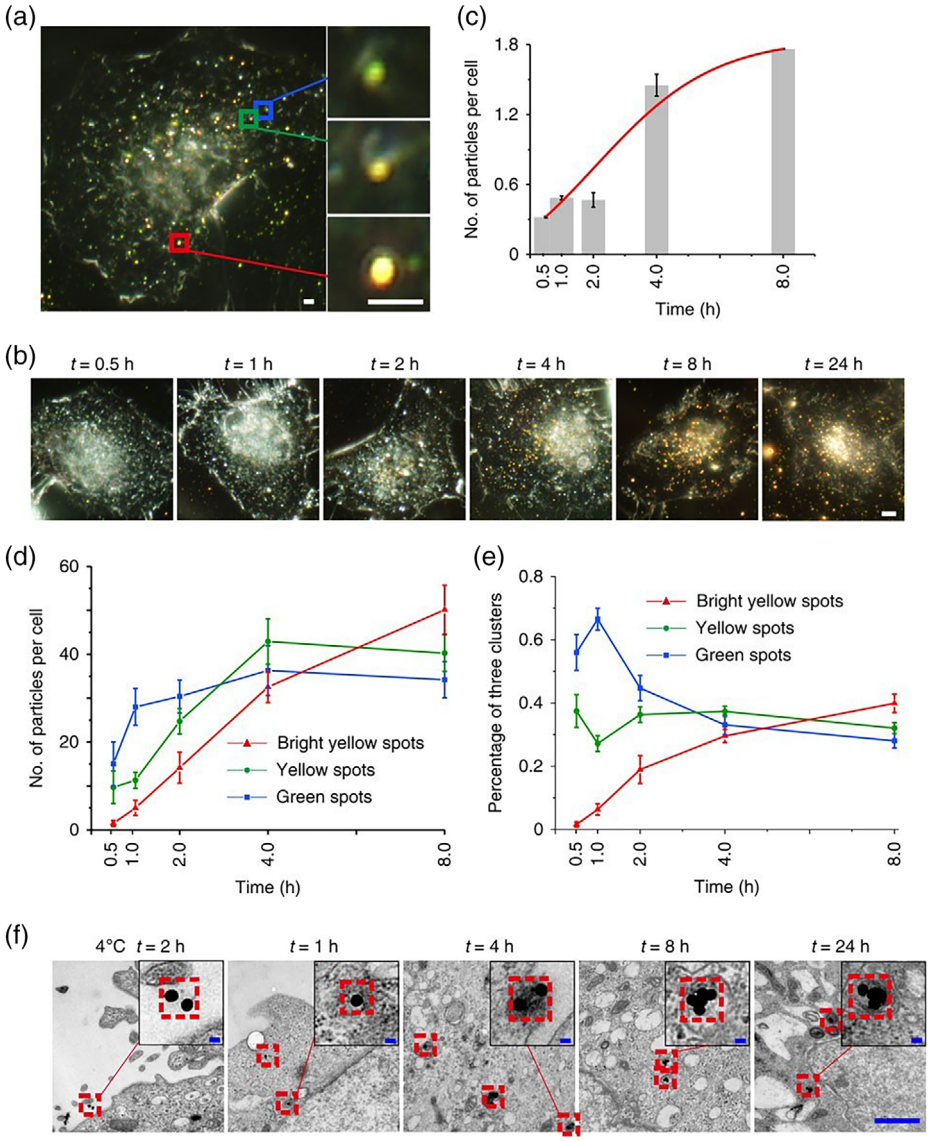


FIGURE 2 Cell entry of DNA-decorated gold nanoparticles (GNPs). (a) Dark-field microscopy image of large clusters (bright yellow spots in red rectangle), small clusters (yellow spots in green rectangle), and single particles (green spots in blue rectangle). Scale bar, 2 μm. (b) Dark-field microscopy image of time evolution of DNA-decorated GNPs incubated with HeLa cells. Scale bar, 2 μm. (c) Time-dependent inductively coupled plasma atomic emission spectrometry analysis, (d) averaged GNPs population, (e) percentages of different clustering states of DNA-decorated GNPs in cells over time (t), and (f) transmission electron microscopy image of time evolution of DNA-decorated GNPs incubated with HeLa cells. Scale bar, 2 μm. Reproduced with permission from M. Liu et al. (2017).

the highest cellular uptake compared to the other structures investigated. In their study, various gold nanostructure shapes, including stars, cages, hollows, rods, and spheres, were investigated with MCF-7 cells (Pakravan et al., 2021).

Yue et al. explored the effects of NP shape on U87 cell uptake. They compared the uptake rate of 50-nm spheres shape to 40-nm stars. Their results showed that the cellular uptake of the spherical NPs increased at a higher rate than that of the nanostars. They reported that the uptake efficiency of the 50-nm spheres after 24 h was 1.6 times higher than that of the 40-nm stars (Yue et al., 2017). A new study by Bandyopadhyay et al. compared five types of gold nanostructures, including nanorods, tetrahexahedra, nanomakura, bipyramids, and spheres, on glioblastoma-astrocytoma cells. The findings indicated that the highest cell uptake rate was observed with the nanomakura shape, with 20% cell death. The other GNPs showed the same trend in uptake, which might show that NPs with nanomakura shape are taken up at a higher rate versus other shapes. These data suggested that size plays an unaccountable role in the cytotoxicity and uptake rate in glioblastoma-astrocytoma cells. For instance, small spherical GNPs (15 nm)

TABLE 2 Correlation between gold nanoparticles (GNPs) size and uptake rate from in vitro studies.

In vitro/in vivo	Cell line	Range of GNP sizes	Size for maximum uptake	References
In vitro	HeLa	<10 nm	2 nm for GNPs with zwitterionic and anionic surfaces/6 nm for GNPs with a cationic surface	Y. Jiang et al. (2015)
In vitro	HeLa	10–100 nm	50 nm	B. D. Chithrani et al. (2006)
In vitro	HeLa, SK-BR-3	2–100 nm	40 and 50 nm	W. Jiang et al. (2008)
In vitro/in vivo	MCF-7	50 and 100 nm	50 nm	Huo et al. (2013)
In vitro	LC3	10, 25, and 50 nm	50 nm	Ma et al. (2011)
In vitro	U87 cells	13, 40, and 50 nm	40 and 50 nm	Yue et al. (2017)
In vitro	HepaRG/PRH	15 and 60 nm	15 nm	Enea et al. (2021)
In vitro	A549	15, 40, and 70 nm	Not statistically significant	Lu et al. (2020)
In vitro	HepG2	5–50 nm	50 nm	Xia et al. (2019)

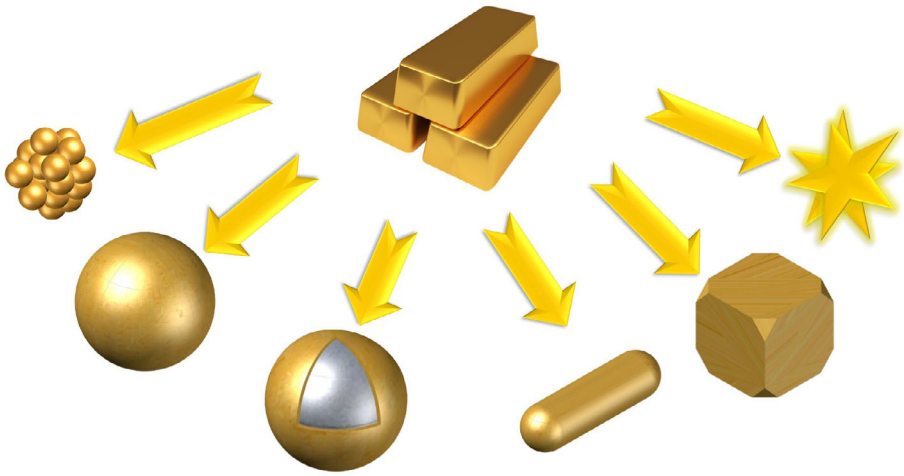


FIGURE 3 Gold nanostructures with different shapes.

demonstrated the same uptake and cytotoxicity as the large bipyramids (650/270 nm). Therefore, it was concluded that shape is a critical parameter and feature for cellular uptake (Bandyopadhyay et al., 2018).

Steckiewicz et al. investigated the uptake and toxicity of GNP rods, GNP stars, and GNP spheres on human osteoblast, osteosarcoma, and pancreatic duct cell lines. The findings indicated that the uptake of the GNPs was shape dependent. GNP stars with the highest uptake were the most cytotoxic type of NPs tested, while the GNP spheres had a modest anticancer potential as well as lower cellular uptake (Steckiewicz et al., 2019). In 2019, Jeong Lee et al. synthesized three different shapes of GNPs (nanospheres, nanostars, and nanorods) to evaluate the effects of shape on uptake by HepG2 cells. The results revealed the uptake level order of nanospheres > nanorods > nanostars (Y. J. Lee et al., 2019). All of these studies suggest that their results are in contrast with those of other studies.

4.3 | GNP surface charge and uptake

Surface charge affects the cellular internalization and radiosensitization of GNPs. It has been shown that electropositive NPs have more efficient cellular uptake than electronegative NPs. This is due to the favorable electrostatic interactions between the positively and negatively charged groups cell membrane (Fytianos et al., 2015; Verma & Stellacci, 2010). Saha et al. (2013) illustrated that the surface coating of GNPs had a powerful influence on their uptake into HeLa cells and MCF10A cells. In another study, Karolina et al. observed that positively charged GNPs presented higher uptake than neutral and negative particles into MDA-MB-231 and MCF-7 cells. In addition, they reported that the surface charge was a key factor in the uptake and transportation of GNPs to cells (Valente et al., 2020).

However, to the bare surface charge of GNPs, the types of ligands and proteins on their surface are also critical factors affecting gold NP uptake and penetration into cells (Y. Jiang et al., 2015). Surface change and modification of gold NPs protects the NPs from aggregation and helps with cellular uptake. For instance, Fernandez et al. reported that GNPs conjugated with zwitterionic ligands had more significant uptake compared to those with PEGylated structures (Fernández et al., 2015). Another structure consisting of polyvinyl alcohol conjugated with GNPs with a negative surface charge had high cellular uptake. These data suggest that the surface charge and ligand are other important properties of GNPs that regulate cellular uptake (Fytianos et al., 2015). Thus, the bare surface charge and type of ligand should be considered in the design of GNPs for applications as radiosensitization agents in radiotherapy. Furthermore, cell type has been reported to be an important factor for the internalization of GNPs in living cells. Q. Xia et al. showed that the uptake of GNPs into HepG2 cells was higher than that in normal L02 cells when the particle size of GNPs increased from 5 to 50 nm. Therefore, they suggest that cell type is important when assessing cell uptake (Xia et al., 2019).

4.4 | GNP surface modification and conjugation

GNP surface modification plays an important role in the interesting properties for various biomedical purposes, including diagnosis, theranostics, and suitable cell uptake. To achieve these goals, GNPs have been synthesized with polymers, surfactants, ligands, drugs, proteins, peptides, and oligonucleotides to attain target specificity (Mahato et al., 2019). However, for therapeutics and imaging in combination with radiotherapy, it is necessary to choose the right surface modification that increases the absorption of GNPs and help of with blood circulation time and cellular uptake (Cao-Milán & Liz-Marzán, 2014; J. Song, Wang, et al., 2016). The surface modification techniques of GNPs can be categorized into two main groups: physical interactions or chemisorption and chemical interactions of chemisorption. The physical interactions including ionic attraction or electrostatic interactions and hydrophobic attraction are spontaneous and relatively simple process but the stability of the interactions is low and sensitive to various environmental conditions, such as pH, salts concentration, and ionic strength. On the other hand, the chemical interactions (e.g., thiol–noble metal surface interaction and N-hydroxy-succinimide-ethyl N-[3-dimethylaminopropoyl] carbodiimide reaction) are more stable with low sensitivity to the environmental conditions, while required multistep and chemical steps (Jazayeri et al., 2016; Yüce & Kurt, 2017).

In addition to surface modification/conjugation, targeting tactics have significant impacts on therapy outcomes. GNPs use in cancer cells can use either active targeting or passive targeting methodologies. Active targeting is achieved by conjugating GNPs with specialized compounds or ligands that seek for and bind to specific receptors on cancer cells. Cancer cells' enhanced endocytic absorption and vascular leakage near tumors facilitate passive targeting by increasing NP accumulation, extending their circulation duration, and decreasing their cytotoxicity in tumor tissues. Chitosan, cytotoxic medicines, PEG, radioactive elements, and proteins are all examples of common surface modifications. Most PEGylated NPs congregate in and around blood vessels in vivo, limiting their uptake by cells. In order to selectively sensitize tumors to ionizing radiation, it is crucial to create and alter the GNP surface (Kumar et al., 2013b; J. Li, Zhang, et al., 2016; Yasui et al., 2014; X.-D. Zhang et al., 2012).

Protein recovery and depollution are just two of the many uses for chitosan, a synthetic natural cationic polymer with unusual properties. In addition, it can regulate medication release, is biocompatible and biodegradable, and is sensitive to chemical alterations. A subset of the biomedical sciences can make use of these pharmacological characteristics. (Fathy et al., 2018; Muxika et al., 2017; Younes & Rinaudo, 2015). In vivo research confirmed that intravenous (IV) chemotherapy medications control metastases to distant organs and had additive antitumor effects when combined with radiation therapy focused on a smaller area. Use of both approaches is necessary to solve the problems of radioactive tolerance and sublethal damage healing that arise from hypoxia (Chemoradiotherapy for Cervical Cancer Meta-Analysis Collaboration, 2008; Pearcey et al., 2002). For instance, Cisplatin plays a key role in cancer chemotherapy (C. Guo et al., 2014; Koutcher et al., 2011). However, the clinical applications of these nanostructures are limited by their significant systemic toxicity.

Although surface modifications can improve GNP stability and address aggregation and flocculation, irradiation is less effective when GNPs are not concentrated preferentially in tumor regions. Cancer cells cannot be targeted individually with this kind of passive approach. The size of the NPs core and the surface density of the capping molecules are two primary limits of the passive targeting and surface modification techniques (J. Song, Wang, et al., 2016). Therefore, there is still work to be done to improve the therapeutic efficacy of clinical radiation by raising the particular tumor

concentration of GNPs. The effectiveness of local GNP absorption and concentration in radiosensitization of cells. There appear to be certain regions within and around the nucleus that are unusually reactive to GNPs, according to a few studies. In order to get GNPs to accumulate in the nucleus, one must employ active targeting. In contrast to passively depending on enhanced permeability and retention (EPR) effects, active targeting relies on the bonding between specific ligands and receptors overexpressed on the surface of cancer cells (Rosenblum et al., 2018; Sinha et al., 2006).

There are three broad categories of active targeting materials based on the substrates they are designed to interact with: carbohydrates on the cell surface, cellular antigens for antibodies, and cell surface receptors. Folate, for example, is well suited for cancer targeting due to its long half-life, lack of immunogenicity, cancer cell selectivity, and ease of conjugation chemistry (Khoshgard et al., 2014; Samadian et al., 2016). Khoshgard et al. assessed cell damage after being exposed to orthovoltage x-rays (120–250 kVp) and megavoltage rays (cobalt-60) and compared the internalization of folate-conjugated GNPs to that of nonfolate-conjugated (PEGylated) GNPs. Researchers found that the folate-conjugated GNPs performed better than the PEGylated GNPs in terms of internalization into HeLa cells, cancer cell death rates, and dose-effective fractions (DEFs) over a range of irradiation intensities. They concluded that GNPs can promote cell death when exposed to orthovoltage x-rays and that using folate nanoconjugates as receptor targeting would improve the specificity of this killing impact (Khoshgard et al., 2014). Active targeting strategies for surface-modified GNPs have also made use of other surface modifiers such as trastuzumab, EGF, and glucose (Hu et al., 2015; L. Song, Falzone, et al., 2016).

Using GNPs that have been coated with antibodies can be another active targeting technique. According to research by Cai et al., trastuzumab-conjugated GNPs labeled with 177Lu (Trastuzumab-GNPs-177Lu) are an effective and relatively safe way to treat breast cancer (Cai et al., 2017). Some research has shown that nuclear targeting is an important method of active GNP targeting. According to research by C. Yang et al., GNPs coupled with nuclear targeting peptides can enter cell nuclei and improve the efficacy of radiation therapy. Their findings demonstrated a fourfold increase in cell mortality and an increase in DNA damage (Yang et al., 2014).

5 | ROUTES OF ADMINISTRATION

There are two main methods to administer GNPs. These administration routes are the (1) IV and (2) direct intratumoral (IT) routes that have advantages and disadvantages (Table 3). When appropriately sized GNPs are administered intravenously, particles accumulate in the tumor through the EPR route. However, other organs also take in some particles. Another route is the IT injection of particles, which increases the density of the GNPs in the tumor cells and enhances the effectiveness, but this route results in heterogeneous distribution (Ruoslahti et al., 2010; Sadauskas et al., 2009).

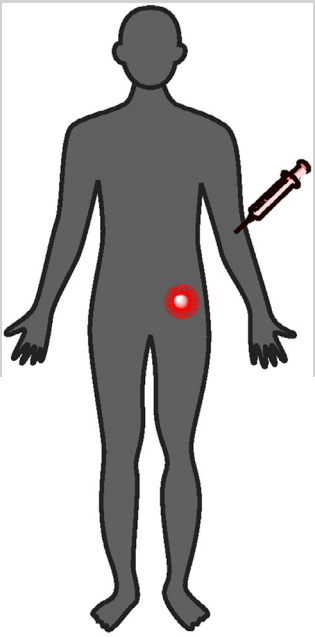
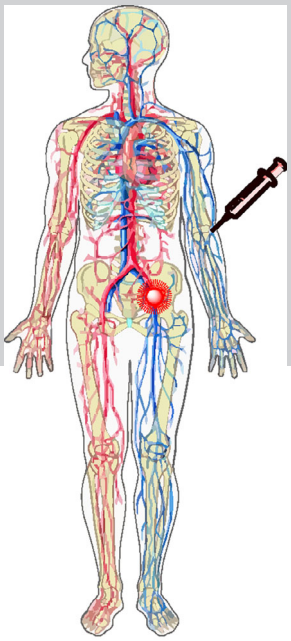
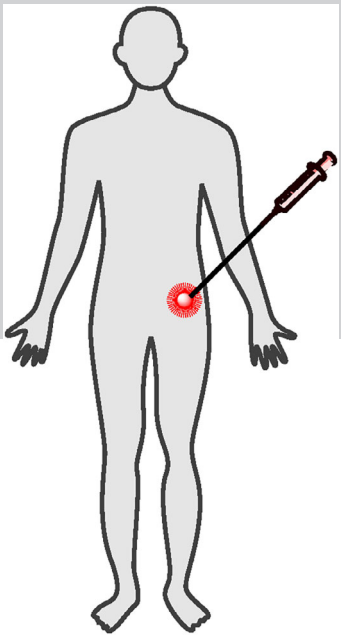
Smilowitz et al. showed that IV-injected GNPs have a greater curative and treatment effect than direct IT injection of GNPs when combined with radiation therapy. They found that IV injection of GNPs make easily reach tumor cells than direct GNP infusion into the tumor area (Smilowitz et al., 2018).

There is some concern related to the accumulation and chronic toxicity of GNPs in the spleen and liver after IV injection (Ruoslahti et al., 2010; Sadauskas et al., 2009). Sadauskas et al. showed that only 9% of the 40-nm GNPs that were trapped in mouse livers after IV injection were cleared within 6 months. To reduce spleen and liver accumulation, as mentioned above, some strategies have been used, such as coating and decorating surface of NPs with polymers including PEG derivatives (Owens & Peppas, 2006). Huang et al. suggested that IT injection of GNPs for thermal ablation treatment would be the preferred procedure of administration compared to IV. Nonetheless, this delivery route was not studied by Huang et al. and is based on the claim that NPs will be effectively retained at the tumor site (X. Huang et al., 2011). In addition, it is not clear whether intramuscular administration will overcome the challenge of rapid NPs uptake and clearance by the reticuloendothelial system (RES), like the liver and spleen, as a consequence of the redistribution of the NPs from the tumor to systemic circulation. Therefore, scientists have favored suggesting IV injection.

6 | BIOLOGICAL EFFECTS OF GNPs WITH RADIOTHERAPY IN VITRO AND IN VIVO

The biological response to GNPs remains unclear. It has been reported that the biological response to GNPs during radiotherapy is an important physical effect (dose enhancement). Some cellular and molecular effects include DNA

TABLE 3 Routine formulation administration routes.

	Intravenous		Intratumoral
	 Nontargeted or Systemic	 Tumor targeted	
Advantages	Practical	• Practical • Higher tumor uptake • Lower systemic cytotoxicity • Higher internalization ability • Higher DEF in radiotherapy • Lower clearance rate and prolong the blood circulation time • Lower accumulation by other healthy organs • Higher specificity for cancer cells	• Very high initial tissue concentration locally • Gradual absorption into the blood • Immediate access of the agent to tumor-draining lymph nodes • Requires a low dosage • Low side effects • High therapeutic index • Low cytotoxicity
Disadvantages	• Low therapeutic index • High toxicity • Low DEF in radiotherapy • Repeated dosing • Low the blood circulation time • Low internalization to cells • Low tumorous cells uptake • High clearance rate • Accumulation by other healthy organs • Lower specificity • Inefficient NP/drug diffusion	• Off-tumor toxicity • Relatively high dose required • Low percentage of GNPs reaches tumorous cells • Complicated surface targeting/conjugation	• Invasive • Technical • Drug toxicity in surrounding tissues • Heterogeneous agent distribution

damage, oxidative stress, cell cycle arrest, and defects in signaling, all of which are dependent on the shape, concentration, and size of the GNPs (K. T. Butterworth et al., 2012; Choudhury et al., 2013; Rosa et al., 2017). Here, we summarized some studies to investigate the biological responses to GNPs with and without radiotherapy (Table 3). It is important to mention that GNPs are used as both targeted and nontargeted agents with radiotherapy (Muddineti et al., 2015).

A study by Martínez-Rovira et al. showed that 1.9-nm GNPs combined with ionizing radiation induced some molecular damage, for instance, in the DNA, protein and lipid spectral regions, leading to cell death in F98 and U87-MG glioma cell lines. F98 glioma cells showed more sensitivity to GNPs, even without irradiation. However, even in combination with radiation, U87-MG cells seemed to be less sensitive to GNPs than F98 cells (Martínez-Rovira & Prezado, 2015). Another study by Her et al. indicated that GNPs sensitized the MDA-MB-231 and MDA-MB-436 cell lines to radiation. Based on their in vitro results, the PEG-GNP complex enhanced the uptake rate in both cell lines when combined with radiation, a larger number of DNA double-strand breaks were observed compared to PEG-GNPs or radiation alone. Taken together, the GNPs radiation-sensitizing effects through both physical and biological pathways (Her et al., 2016).

Kim et al. indicated that GNPs with a size of 180 nm enhanced the sensitivity of hypoxic tumor cells (CT26) to 8 Gy of single-dose radiation. The results demonstrated that the combination of GNPs with radiotherapy promoted ROS generation in hypoxic tumor cells, leading to apoptosis. Hence, GNPs have sensitizer potential when used in combination with radiotherapy (Kim et al., 2016). Another in vitro study by Zhang et al. showed that GNPs with sizes of approximately 20 and 28 nm boosted cell damage under megavoltage radiotherapy. They observed that the GNPs induced ROS generation and cell arrest in the G2/M phase as well as apoptosis in LS180 cells (X. Zhang et al., 2018).

Joh et al., in an in vivo study, observed that the combination of GNPs with radiation therapy promoted DNA damage in brain tumors and enhanced the survival of mice bearing tumors. Furthermore, delayed tumor growth was observed. Their data suggested that GNPs can cross the blood–brain barrier to penetrate the tumor and enhance the radiation effects (Joh et al., 2013). An in vitro and in vivo study by Chattopadhyay et al. showed that targeted GNPs sensitized MDA-MB-361 cells to radiation. Their findings using the c-H2AX immunofluorescence test showed that the DNA damage in the combination therapy group was greater than that the single treatment groups. In contrast, the viability of the cells treated with x-ray alone versus GNPs and the x-rays were not significantly different. The in vivo data from this study indicated that tumor growth was slow and that the tumor size following treatment with a combination of GNPs and radiation was half that of the tumor size in the group treated with irradiation alone after 4 months (Chattopadhyay et al., 2013).

Recently, Janic et al. explored the effects of GNPs in combination with radiotherapy in in vitro and in vivo studies. They used 4- and 14-nm GNPs to treat MCF7 breast cancer cells and observed that the GNPs enhanced the radiation-induced DNA damage and cytotoxicity. They also used a xenograft mouse model to demonstrate that the enhancement after combination therapy was size dependent. The survival log-rank test revealed that the 14-nm GNPs had greater toxicity than the 4-nm GNPs when used in combination with radiotherapy (Janic et al., 2021).

Due to the EPR effect, which allows for the preferential delivery of drugs to tumors, and the ability of NPs to be programmed to sustainably release therapeutic agents even in conditions that could benefit from the combination therapy, nanomedicine has an advantage over conventional medicine. The EPR phenomenon is related to macromolecule or NP propensity to preferentially assemble at locations with higher vascular permeability, such as solid tumor tissue (Matsumura & Maeda, 1986). When injected intravenously, for instance, tumor accumulation, IT dispersion, and uptake into tumor cells depend on the NPs design, specifically their size, shape, and surface characteristics or functionalization (Perrault et al., 2009). Size has been shown to have a substantial impact on how NPs interact with tumor capillaries during transit (Decuzzi et al., 2009). One of these studies found that smaller gold NPs (6 nm) tend to be excreted by the kidneys shortly after IV administration, whereas larger NPs tend to accumulate in the RES. In addition, Perrault et al. investigated the ability of sub-100-nm NPs to target tumors in nude mice injected with MDA-MB-435 xenografts (Perrault et al., 2009). According to their findings, intravenously delivered 100-nm NPs accumulated in tumors the most, 4.3 times more than 60- and 80-nm NPs, 9 times more than 40-nm particles, and 38 times more than 20-nm particles. Recent studies also suggest that particle shape has a key effect on how well NPs perform in vivo (Champion et al., 2007; Geng et al., 2007). Particularly, particle transport properties, cell–particle interactions, and drug release kinetics are affected by shape and shape-related form factors, such as aspect ratio or edge geometry, with disk-shaped NPs having longer half-lives in circulation (Tan et al., 2013).

The size and form of the NPs also affect intracellular uptake and intratumor diffusion once they have entered the tumor. Perrault et al. found that the permeation of NPs within the tumor is highly dependent on the overall size of the NP, with larger NPs appearing to stay near the vasculature and smaller NPs rapidly diffusing throughout the tumor matrix, in a systematic investigation of the effect of NP size (10–100 nm) on passive targeting of tumors in vivo (Perrault et al., 2009). The research effectively showed that PEGylated GNPs must have a diameter of less than 100 nm in order to travel across the tumor and away from the vasculature. The NPs are then smaller and more easily diffuse across the interstitial space of the tumor. In addition, it is now well acknowledged that smaller particles are absorbed

more effectively by non-phagocytic cells if particle surface characteristics are favorable. By localizing and penetrating breast cancer cells, multicellular tumor spheroids, and tumors in mice, ultra-small GNPs smaller than 10 nm have been shown by K. Huang et al. (2012) to have distinct benefits over NPs bigger than 10 nm.

In addition to size and shape, target accumulation, IT diffusion, and cellular absorption are greatly influenced by the surface characteristics or functionalization of NPs like GNPs. Without taking into account the biological difficulties of in vivo distribution, surface functionalization studies were primarily used in the design of the first generation of nanomaterials to evaluate in vitro absorption and cytotoxicity (Hauck et al., 2008). The second generation of NP design placed a strong emphasis on surface modification to confer stealth and actively target the NPs in order to significantly increase their concentration in the target volume. Choi et al. explored the mechanism of active targeting in solid tumors using transferrin-containing GNPs in one notable study, and they demonstrated that targeted GNPs are more effective at penetrating solid tumor cancer cells than their nontargeted analogs, which mostly rely on the EPR (Choi et al., 2010). In general, active targeting takes use of the surface receptors that are (over)expressed on cancer cells by offering targeting ligands that can interact with these receptors. Proteins, aptamers, and tiny molecules like vitamins, peptides, or carbohydrates are among the ligands that have been studied for active targeting (Kang et al., 2010; Table 4).

7 | RESULTS OF CLINICAL TRIALS

The Aurimune (CYT-6091) Phase I study was launched in 2006 and 2007, which used tumor necrosis factor (TNF)-conjugated PEGylated GNPs with a size of 27 nm for unspecified solid tumors and 10 specified cancer types. The company responsible for this study was Cytimmune Sciences. Their findings revealed that TNF-conjugated-PEGylated GNPs induced TNF-α release and anticancer effects, although its clinical use should be limited due to serious adverse effects, such as hypotension and septic shock-like syndrome (Verhoef et al., 2007).

However, this TNF-conjugated-PEGylated GNP increased TNF's antitumoral activities with reduced side effects in animal model (mice; Goel et al., 2008). Patients with advanced and metastatic solid tumors participated in a Phase I clinical trial (NCT00356980) to determine the maximum tolerable dose of CYT-6091 and its potential side effects. The results suggested that CYT-6091 would be effective at killing off malignancies. Previous studies have shown that

TABLE 4 Cellular and molecular effects of GNPs in radiotherapy in vitro and in vivo.

GNP size	Radiation dose and source	In vitro/in vivo	Cell line(s)	Effect(s)	References
1.9 nm	5, 10, and 20 Gy (90 kVp)	In vitro	F98, U87-MG	Decreased cell viability	Martínez-Rovira and Prezado (2015)
50 nm	2 Gy (225 kVp)	In vitro	MDA-MB-231 and MDA-MB-436	Cell cycle arrest and increased DNA double-strand breaks	Her et al. (2016)
180 nm	8 Gy (6 MV)	In vitro	CT26	Increased cell death, ROS, and apoptosis	Kim et al. (2016)
19.7 ± 2.8 nm and 27.8 ± 1.8 nm	2, 4, 6, 8, and 10 Gy (6 MV)	In vitro	LS180	Increased ROS and decreased cell viability and cell cycle arrest	X. Zhang et al. (2018)
23 nm	20 Gy	In vivo	U-251	Increased DNA damage and survival	Joh et al. (2013)
30 nm	11 Gy (6 MV)	In vitro and in vivo	MDA-MB-361	Increased DNA damage and decreased cell viability in vitro; increased survival in vivo	Chattopadhyay et al. (2013)
4 and 14 nm	15 Gy (160 kVp)	In vitro and in vivo	MDA-MB-231	DNA damage, cytotoxicity and delayed tumor growth	Janic et al. (2021)

Abbreviations: GNPs, gold nanoparticles; kVp, kilovolts; MV, megavolts; ROS, reactive oxygen species.

CYT-6091 was three times more hazardous than the same dose of TNF-α (Goel et al., 2009). Although no data from a 2007 early Phase I research (NCT00436410) investigating CYT-6091 distribution in patients with primary or metastatic cancer having surgery have been published, they are expected in the near future.

During 2017, researchers tried out NU-0129 in yet another clinical experiment. NU-0129 is a spherical nucleic acid coated 13-nm GNP that may pass the blood–brain barrier. The Bcl2L12 gene, which is involved in apoptosis resistance and is overexpressed in most human glioblastomas, can be targeted with the use of this complex. By injecting NU-0129, Jensen et al. in 2013 demonstrated that expression of Bcl2L12 in glioblastoma may be suppressed, resulting in a slowing of tumor growth in xenografted mice without adverse consequences. To this end, in 2017, a Phase I safety trial of NU-0129 was initiated to assess the drug's potential benefits for individuals with recurrent glioblastoma multiforme. The trial in the clinic is ongoing, and the findings have not been released (Kumthekar, 2019; Libutti et al., 2010).

Another 150-nm complex, AuroShell, is composed of silica NPs that have been coated with a very thin layer of gold. Three separate clinical trials were conducted using it between 2006 and 2016. This complex's anticancer action is predicated on the enhancement of hyperthermia efficiency by near-infrared (NIR) light. Following NIR light absorption by the silica core, heat is generated by the relaxing of gold electrons that can be exploited for thermal ablation of cancer. Mice with PC-3 prostate cancers that were treated to NIR irradiation via a combination of AuroShell and an NIR laser showed a 35°C increase in body temperature, compared to a 14°C increase in control mice (NIR laser only). Nanospectra Biosciences Inc. launched three clinical investigations after collecting this preliminary results. Nano-object NIR benefit was assessed in two pilot studies, one for patients with refractory and/or recurring head and neck malignancies (NCT00848042) and another for patients with primary and/or metastatic lung tumors (NCT00848043). (NCT01679470). The third study is now enrolling patients to evaluate AuroShell's efficacy in the targeted ablation of neoplastic prostate tissue using NIR irradiation (NCT02680535). These investigations started in 2006, but their findings have not been made public (Jensen et al., 2020).

Note that none of the above studies have been conducted using GNPs as radiosensitizers. Recently, increasingly complex GNPs have been developed, enabling the use of chemotherapy drugs (Stern et al., 2008) or tumor cell (Fang et al., 2017; Yang et al., 2018), or specific cell substructure targeting (Aliru et al., 2016; Fang et al., 2017). With this method in mind, it is made clear that each team is utilizing a gold nano-object of a predetermined size, coating, and form. As a result, sharing information and drawing meaningful conclusions remains challenging. This radiosensitization effect is the best illustration of how little we know about its underlying mechanisms. Although elucidating this process is essential prior to clinical investigations, its precise nature is still up for discussion in the scientific community (Falk, 2017).

Building on the hard lessons learned from the first two generations third-generation NPs is being made to give more precise control over biodistribution in vivo and disease-responsive drug release (Grieneisen & Zhang, 2011; Kumar et al., 2013a). Recent studies show the development of a third-generation platform for RT applications called the AuRad™ (Kumar et al., 2013a). The multifunctional gold nanorod for RT is made with size and surface functionalization as two of its most important features. The size is chosen so that it will stay in the bloodstream longer, take up more tumor cells, and be cleared in a controlled way. PEGylation, on the other hand, makes the NPs stay in the bloodstream longer when they are given intravenously. This gives the NPs enough time to gather in the tumor in high concentrations. The hetero-bifunctional PEG with amine, carboxyl, and methoxy ligands also makes a flexible nanoplatform that can be used to attach different molecules, like fluorophores, peptides, drugs, and radiolabels. Experiments done on this gold nanorod imaged well for optical tracking in vitro and in vivo systems. Fluorescence imaging of cells incubated with GNPs shows that the fluorescent NPs were strongly taken up by the cells. Notably, there was no damage to the shape of the cells, which suggest that the NPs were not harmful. Overall, the data from the current review of the literature show that active targeting would be a more effective way to get GNPs to the target to improve RT. Other third-generation NPs, such as biogenic GNPs and drug-loaded gold plasmonic NPs for treating multidrug-resistant cancer and other uses, can also be used to deliver drugs (S.-M. Lee et al., 2014; Pandey et al., 2013; Tripathi et al., 2015).

The Indian Government authority approved the GNP-based Nano-Ayurvedic (Nano Swarna Bhasma) for female invasive breast cancer Stage IIIA or IIIB. It has demonstrated safe and provided enhanced survival (Khoobchandani et al., 2020). Although this study showed that GNPs have great radiosensitizing potential when used in combination with radiotherapy, administering GNPs directly to patients as radiosensitizers requires more development studies.

8 | CONCLUSION, CURRENT LIMITATIONS, AND SUGGESTIONS

Over the past decade, many multidisciplinary researchers have been concentrating on GNPs as a nanotheranostic agent in medicine. Although some scientists have reported the extraordinary effects of GNPs as radiosensitizers in in vitro and in vivo studies, such compounds have not yet received FDA approval for clinical radiotherapy aims. Intensive preclinical research has expanded our understanding of GNPs radiosensitization beyond physical dosage enhancement. Several biological and chemical processes and clinical translation hurdles have been presented. Recent research has focused on using GNPs' synthetic adaptability to create multifunctional nanoplatforms and broadening the clinical usage of GNPs radiosensitizers. Safety and in vivo efficacy issues continue despite advances. Radiosensitization mechanisms, especially at therapeutically relevant radiation energy, must be understood to address these concerns. Particle and radiation-related factors' effects on radiosensitization and GNP's in vivo fate must be examined. These findings will assist design and producing clinically suitable GNPs radiosensitizers. In these conditions, the clinical translation of GNPs-based radiosensitizers looks unlikely, because this field is very young compared to other nanoplatforms and many possibilities and improvements remain. Interdisciplinary research is needed to speed up GNPs radiosensitizers clinical use. Perhaps some notes and limitations that we have reviewed in this article should specifically be considered in subsequent in vivo studies.

Monte Carlo studies showed an increase in DEF with increasing GNP size, while the DEF in biological systems and in vitro studies decreased as the GNPs became larger than 40–50 nm. In addition, in vivo, the size that has been used has been less than 50 nm. The conclusion is that the uptake of GNPs is important for radiation dose enhancement. The second division was that even though the cell uptake is shape dependent, in some comparative studies, there was no agreement on the suitable shape for the design of GNPs. A study reported that spherical GNPs would offer better uptake than GNP stars, but in another study, this claim was rejected. Further studies should compare different GNP shapes and cell internalization. Third, it has been reported that the uptake of GNPs is cell dependent. Therefore, each cell line may have a specific uptake level. Moreover, most studies have shown that GNPs with a negative surface charge would present better cell penetration than nontargeted GNPs of the same size with a positive surface charge.

In addition, targeted GNPs with specific ligands have shown lower cytotoxicity. Although the physical enhancement of GNPs in radiotherapy is clearly understood, the cellular and molecular mechanisms of GNP radiosensitization in vivo are still unknown. Thus, we recommend and suggest further molecular techniques and tests in future in vivo studies. Notably, we commented on some GNP features, but energy is also critical for the potential radiosensitization of GNPs during radiotherapy. In addition, although the DEF of GNPs was observed in both kVp and MV, many studies have shown that GNPs at low energy (kVp) would give a greater DEF compared to high energies Megaelectron Voltage (MeV). This is due to the higher energy absorption by GNPs at the kVp level, indicating that GNPs may be suitable for radiotherapy of superficial tumors. Hence, the DEF is dependent on both the number of GNPs and the source of energy. However, for most clinical radiotherapy applications, MeV are used. In addition, most of these studies have been performed with a single high dose, which is not possible in the clinic.

It seems that toxicity is the most important challenge that must be addressed. Several studies have suggested that some parameters such as the cytotoxicity, size, efficacy, biodistribution, half time, and physiological response of GNPs are needed to be clarified and investigated.

Dosage, duration, and route of administration are also highly correlated with harmful effects. More work at the cellular and molecular levels is required to determine the likely toxicity mechanism of GNPs in vivo. Long-term data is scarce, and most of the conclusions drawn in this field are based on a relatively small number of observations covering a relatively little time period. Therefore, more extensive in vivo research over extended periods of time is required. There is not yet a standard method for determining the toxicity of GNPs. This brings up even another cause for concern. The lack of a uniform detection process and evaluation criteria leads to inconsistent findings and different interpretations. It is crucial to develop standardized criteria and methods for toxicity assessment of NPs to enable comparison of data across studies and speed up the clinical translation of GNPs.

In summary, several nanostructures based on GNPs have been tested in human clinical trials (NCT00356980, NCT00436410, NCT00848042, NCT02755870, NCT02680535, etc.). Despite the generally good and optimistic nature of these trial study results, the most majority of such trials have failed or are constrained to Phase I development. There have been no successful late-stage clinical or commercial launches of GNP-based products. There are ongoing, suspended, and completed trials. To date, there are fewer GNP-based agents in clinical trials compared to other nanocomplex formulations, such as liposomes and polymeric micelles. Though the results of several research have been encouraging, there are still important issues that must be addressed. The toxicity of GNPs is a problem that must be

addressed effectively. Although it has no known chemical activity, gold nonetheless carries some chemical toxicity due to its status as a noble metal.

Furthermore, almost little was known on the chemical mechanism through which GNPs interacted with their targets. The toxicological implications of such a mechanism are as important as the therapeutic/diagnostic ones. To enhance cancer management, however, a comprehensive knowledge of NPs-target interactions and potential pathways is essential. Furthermore, GNP uptake by target cells needs to be extensively investigated in a variety of cellular types. Knowledge of the process of delivery is essential for optimizing the drug's intracellular quantity and overall therapeutic efficacy. NP uptake by cells is typically attributed to endocytosis, but this is not always the case, especially for GNPs. Cell uptake of GNPs and their potential for toxicity can be affected by a number of factors. These include particle size, shape, surface charge, coating, examined cell type, and so on. However, there is still no consensus on or explanation for how GNPs are taken up by cells.

Based on the evidence presented here, it is clear that there is still a long way to go and much more work to do before GNP-based goods are widely adopted in the real world. The promise of GNPs in cancer research, however, gives us hope for the eventual development of GNP-based, combination radiation cancer treatments that are both more effective and more precise. We also anticipate a significant role for GNP-based systems in the future, both in terms of early tumor diagnostics and tumor treatment at various stages, with the ultimate goal of avoiding the usual limitations of conventional tumor therapies. In addition, the radiosensitizing activities of GNPs during irradiation have not been studied, as shown by recent clinical trials. However, when combined with lasers (photothermal therapy) and drug delivery, the outcomes were impressive. In conclusion, we recommend that future in vivo investigations involving GNPs consider the optimal size, shape, concentration, surface charge, ligands, and x-ray energies.

AUTHOR CONTRIBUTIONS

Kave Moloudi: Visualization (equal); writing – original draft (equal). **Ali Khani:** Visualization (equal); writing – original draft (equal). **Masoud Najafi:** Visualization (equal); writing – original draft (equal). **Rasool Azmoonfar:** Visualization (equal); writing – original draft (equal). **Mehdi Azizi:** Visualization (equal); writing – original draft (equal). **Houra Nekounam:** Visualization (equal); writing – original draft (equal). **Mahsa Sobhani:** Conceptualization (equal); writing – review and editing (equal). **Sophie Laurent:** Writing – review and editing (equal). **Hadi Samadian:** Conceptualization (equal); project administration (equal); writing – original draft (equal).

CONFLICT OF INTEREST STATEMENT

The authors have declared no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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