

# Multifunctional Gold Nanoparticles in Cancer Diagnosis and Treatment

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**Abstract:** Cancer is the second leading cause of death in the world, behind only cardiovascular diseases, and is one of the most serious diseases threatening human health nowadays. Cancer patients' lives are being extended by the use of contemporary medical technologies, such as surgery, radiotherapy, and chemotherapy. However, these treatments are not always effective in extending cancer patients' lives. Simultaneously, these approaches are often accompanied with a series of negative consequences, such as the occurrence of adverse effects and an increased risk of relapse. As a result, the development of a novel cancer-eradication strategy is still required. The emergence of nanomedicine as a promising technology brings a new avenue for the circumvention of limitations of conventional cancer therapies. Gold nanoparticles (AuNPs), in particular, have garnered extensive attention due to their many specific advantages, including customizable size and shape, multiple and useful physicochemical properties, and ease of functionalization. Based on these characteristics, many therapeutic and diagnostic applications of AuNPs have been exploited, particularly for malignant tumors, such as drug and nucleic acid delivery, photodynamic therapy, photothermal therapy, and X-ray-based computed tomography imaging. To leverage the potential of AuNPs, these applications demand a comprehensive and in-depth overview. As a result, we discussed current achievements in AuNPs in anticancer applications in a more methodical manner in this review. Also addressed in depth are the present status of clinical trials, as well as the difficulties that may be encountered when translating some basic findings into the clinic, in order to serve as a reference for future studies.

**Keywords:** gold nanoparticles, cancer, drug delivery, photothermal therapy, photodynamic therapy

## Introduction

In recent decades, a growing number of people are suffering from cancer. According to the data from GLOBOCAN 2020, there were about 19.3 million new cancer cases and 10.0 million cancer-related deaths occurred around the world.<sup>1</sup> The number of new cases and death cases from cancer is predicted to rise to 22 million and 13 million annually, respectively, by 2030.<sup>2</sup> Currently, there are many effective therapies against cancer in clinic, among which surgery, radiotherapy (RT) and chemotherapy are the most leading treatment modalities.<sup>3</sup> Although surgery is usually considered the most effective therapy at the early stage of disease, it is difficult to achieve satisfactory results at the later stage of cancer because cancerous cells have spread throughout the body.<sup>4</sup> Even some patients may undergo cancer recurrence after the operation and have short survival time.<sup>5</sup> Similar to surgery, RT is also barely efficient for metastatic tumors and may result in the recurrence of cancer. Chemotherapy is regarded as the most valuable approach for most patients with metastatic and late staged cancer, since chemotherapeutic drugs can reach every organ in the body via the bloodstream.<sup>6</sup> However, the widespread distribution of chemotherapeutic drugs might also lead to additional effects on normal tissues. Since chemotherapeutic drugs are not limited to cancer cells, large dosage and repeated administration are required for ideal results, which also leads to undesired side effects.<sup>7</sup> These drawbacks, in combination with drug resistance development and poor aqueous solubility of drugs have emerged as significant problems restricting the clinical utilization of

chemotherapeutic medications currently available.<sup>8</sup> Therefore, there is an urgent need for new strategies and devices to treat and diagnose cancer more efficiently and accurately.

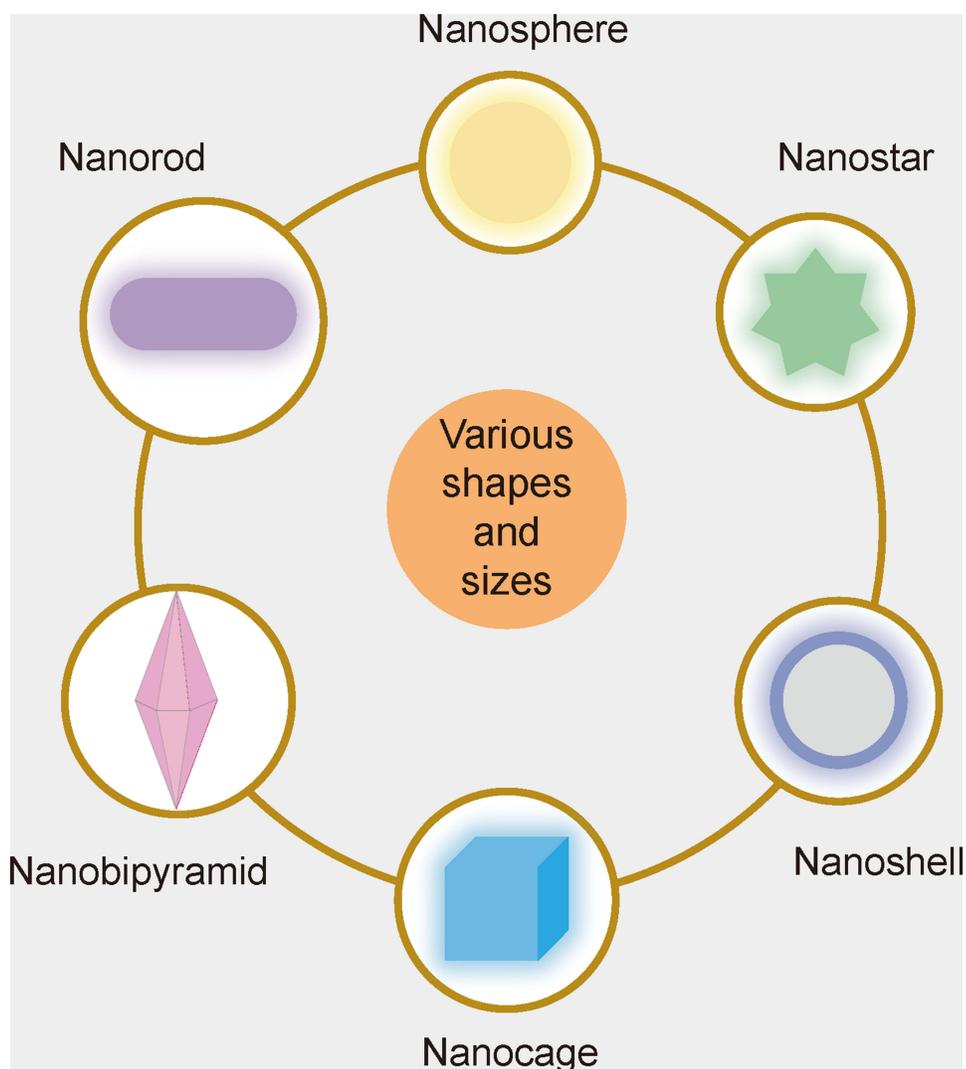
Various drug delivery systems have been developed during the last few decades to enhance the anti-tumor efficacy of chemotherapy drugs. It is feasible to deliver medications selectively into the target locations using a specialized delivery vector, hence reducing the likelihood of adverse effects. The use of nanotechnology in drug delivery systems gave reason to believe that this goal would be reached in the near future. For instance, the Food and Drug Administration (FDA) of the United States approved albumin-bound paclitaxel (*nab*-paclitaxel), a solvent-free nanoformulation of paclitaxel as a powerful therapy in patients with metastatic breast cancer in 2005. In the United States, the *nab*-paclitaxel has been approved for the treatment of many solid tumors, including breast cancer, non-small-cell lung cancer, and pancreatic cancer.<sup>9</sup> More investigations for the treatment of other solid tumors based on the *nab*-paclitaxel are ongoing. Because of their unique physicochemical features, nanoparticles (NPs) have garnered a great deal of interest in recent decades and were believed to be one of the most promising drug delivery vectors. Given that solid tumors have wider vascular cell gaps (200 nm-1.2  $\mu$ m in size) than normal tissues (<10 nm in size),<sup>10,11</sup> the NPs loading drugs may readily cross leaky tumor vascular walls and then be accumulated into tumor areas, as a result of the enhanced permeability and retention (EPR) effect.<sup>12-14</sup> More important, surface modification of NPs by introducing targeting molecules that bind to overexpressed receptors or antigens on the target cells also favors the increase in intratumoral accumulation of NPs. Both the passive (the EPR effect) and active (conjugating targeting molecules) approaches can help decrease the accumulation of available chemotherapy drugs at non-target sites, reducing unwanted side effects. Therefore, the use of nanoparticles as transport carriers brings a potential avenue for the circumvention of limitations of conventional cancer therapies.

Noble metal nanoparticles, especially gold nanoparticles (AuNPs), have drawn more and more attention in medical field, with particular emphasis on oncology in recent studies.<sup>15</sup> First of all, AuNPs have multiple geometric shapes and sizes (Figure 1), which can be controlled through simple synthetic approaches.<sup>16-18</sup> As well, many physical and chemical properties of AuNPs, such as catalytic ability, melting point, electric conductivity and color, can be controlled by changing their shape, size and even surrounding environment.<sup>19</sup> Such design flexibility for ideal performance and a specific application is difficult to be achieved in organic nanoparticles. Moreover, AuNPs have unique size- and shape-dependent optical nature, owing to the coherent oscillation of free electrons, so-called surface plasma resonance (SPR) effect.<sup>20</sup> Due to the optical property, AuNPs have been developed as ideal nano-objects, such as biosensors, imaging agents and photothermal agents for medical diagnosis, imaging and treatment, which is relatively infrequent for the other inorganic nanomaterials. Meanwhile, large surface area and high surface activity of AuNPs endow the ability of functionalization and high loading amounts. AuNPs can directly or indirectly conjugate and interact with various molecules, including drugs, nucleic acids (DNA or RNA), proteins or peptides, antibodies, targeting ligands, and other molecules (Figure 2).<sup>21</sup> The coupling possibility and diversity largely improve their biological activities and broaden the range of their potential anticancer applications. Furthermore, AuNPs have been found to be relatively stable in physiological medium because of the modification of amphiphilic materials,<sup>22</sup> and biocompatible due to inert nature of metallic gold.<sup>23</sup> All of these features have rendered AuNPs increasingly popular nano-vectors in oncology.

This review focuses on various widely utilized AuNPs applications in cancer treatment and diagnostics, including drug and nucleic acid delivery, photodynamic therapy (PDT), photothermal therapy (PTT), and X-ray computed tomography (CT) imaging, among others. In addition, possible combination therapy for full tumor suppression should be investigated further. Apart from preclinical studies and data, current breakthroughs in AuNPs in clinical trials were also thoroughly reviewed and discussed in detail. Additionally, also discussed in detail are the existing obstacles that are preventing AuNPs from receiving FDA approval, with the goal of providing a theoretical contribution to the clinical translation of AuNPs.

## AuNPs in Cancer Therapy

Due to their unique optical properties and conjugating variety, AuNPs show great potential in cancer therapy. In this section, we will discuss in detail the therapeutic applications of gold nanoparticles for malignant tumors, including drug



**Figure 1** Different sizes and shapes of AuNPs. The sizes and shapes of AuNPs can be controlled through simple synthetic methods.

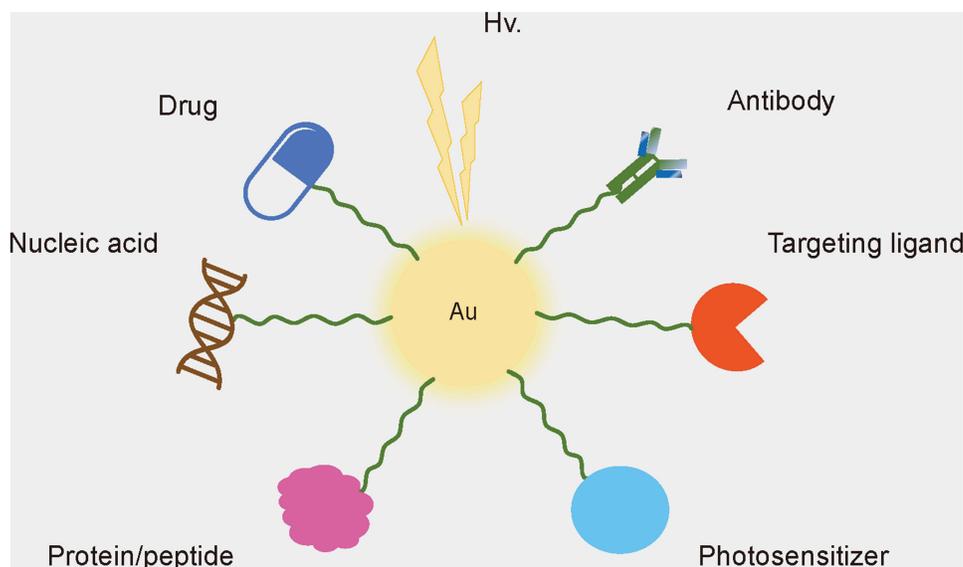
and nucleic acid delivery, PDT, PTT and possible combination therapies. Currently, these applications are still under development.

## AuNPs in Drug/Nucleic Acid Delivery

It has been proven that AuNPs have several fundamental properties when serving as drug delivery carriers. According to the EPR effect, the nanoscale particles can preferentially accumulate at tumor sites. In addition, surface functionalization can be easily achieved since AuNPs have negative surface charges and strong covalent affinity for thiol, amino and carboxyl. It means that AuNPs can bind with various molecules, including drugs, nucleic acids, antibodies and targeting ligands, which shows potential for active targeted delivery. These features render AuNPs promising candidates as drug carriers. Herein, we will present the utilization of AuNPs with regard to drug delivery and nucleic acid delivery.

### Drug Delivery

In 2004, Paciotti and co-workers first reported the use of colloidal gold as delivery vectors.<sup>24</sup> They conjugated tumor necrosis factor (TNF) onto the surface of AuNPs, with the aim of delivering the TNF to the tumor tissue growing in mice. It was shown that the AuNP-TNF conjugate actually possessed higher tumor accumulation and consequent lower toxicity in healthy organs when compared to native TNF.<sup>24</sup> Thereafter, the use of AuNPs as delivery tools has been explored deeply. Nowadays, it has been



**Figure 2** Diverse connecting molecules of AuNPs. AuNPs can directly or indirectly connect various molecules including drugs, nucleic acids (DNA or RNA), proteins or peptides, antibodies, targeting ligands, and other molecules for ideal biological activities and diverse medical applications.

**Abbreviation:** Hv, irradiation with light.

reported that AuNPs had the ability to deliver multiple types of anticancer molecules (Table 1), including synthetic compounds,<sup>25</sup> phytochemicals,<sup>26,27</sup> therapeutic peptides<sup>28,29</sup> and coordination compounds.<sup>30,31</sup> These anticancer molecules have cytotoxic or regulating effects on cancer cells but some drawbacks such as low solubility, short half-life, the development of drug resistance and weak tumor selectivity limit their practical applications. One of the effective approaches is to conjugate the anticancer molecules to nanoparticles, particularly AuNPs with a “hard” core.

Doxorubicin (DOX) is one of the most frequently used anticancer drugs, but it is highly likely to induce drug resistance in tumor cells. In some studies, DOX could conjugate with stabilizer-modified AuNPs through either non-covalent or covalent interactions.<sup>32–34</sup> Studies suggested that the connection favored the intracellular accumulation of the DOX in drug-resistant cancer cells, demonstrating the possibility of bypassing drug resistance in the case of conjugation (Figure 3).<sup>34</sup> The mechanism by which drug resistance could be avoided by nanoparticle-mediated conjugation may be related to different internalization mechanisms. The internalization mechanism of free DOX is different compared with the conjugated DOX that enter cells by endocytosis approach, avoiding P glycoprotein related drug resistance, as it was suggested by Wojcik et al<sup>32</sup> 5-fluorouracil (5-FU) is another powerful antineoplastic drug, whose highly polar nature limits its topical use in the treatment of skin cancer. Delivery of 5-FU by cetyltrimethylammonium bromide (CTAB)-stabilized AuNPs could gain about 2-fold higher skin permeability compared with the free 5-FU formulation and achieve 6.8- and 18.4-fold lower tumor volume compared with the negative group (Figure 4).<sup>35</sup> It indicated that attaching hydrophilic drugs to AuNPs can contribute to an increase in the skin permeability and consequent drug efficacy against skin cancer. This may have something to do with the use of stabilizer CTAB with positive electricity.<sup>36</sup> It is worth noting that stabilizers or spacers seem not to be indispensable in the structure of the conjugates. Some drugs with specific groups can directly link with AuNPs, such as methotrexate (MTX) with carboxylic groups.<sup>37</sup> Even the simplest conjugate (MTX-AuNPs) also showed enhanced cytotoxic effect against cancer cells, while free MTX exhibited reduced anticancer effect at equal doses.<sup>37,38</sup>

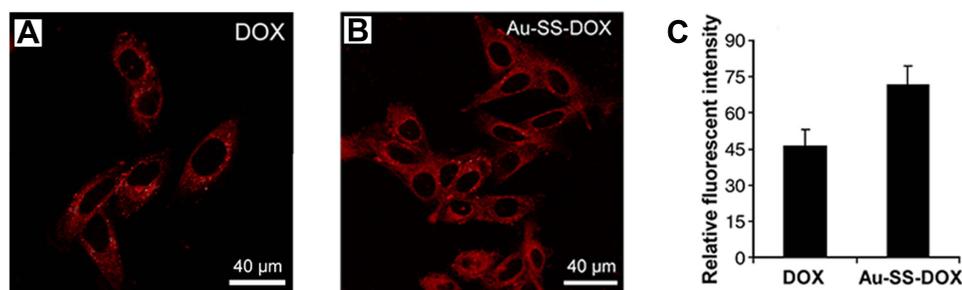
It is known that the selectivity of drugs for specific tissues and organs has become a major challenge in recent years. The development of drug delivery systems may be a crucial solution to overcome this problem. AuNPs are one of the most popular drug delivery platforms, based on their negative surface and high affinity with many functional groups, especially thiol. For active targeting, the most classic and valuable approach is still introducing targeting ligands onto the surface of AuNPs.<sup>39</sup> The working principle of this approach lies in taking advantage of the affinity of certain substrates for specific receptors. As a fact, compared with normal cells, cancer cells with a higher metabolic rate can over-express

**Table 1** Examples of Gold Nanoparticles for Various Anticancer Drug Delivery

Anticancer Drug	Modifying Molecule	Nanocomplex Name	Cell Line	Main Outcome	Anticancer Application	Reference
DOX	PEC	DOX-PEC-AuNPs	HepG2 cells	Stronger cytotoxicity compared to free DOX.	Targeted delivery of DOX to hepatocarcinoma cells	42
5-FU	PEG and FA	AuNPs-PEG-5-FU-FA	M139 and M213 cells	Higher cytotoxic effects as compared to free 5-FU and FA.	Targeted delivery of 5-FU and targeted therapy of cholangiocarcinoma cells	44
DTX	HA and GFLGC	DTX@HA-cl-AuNPs	HeLa and MCF-7 cells	Higher cytotoxicity and tumor inhibition efficacy than free DTX under near-infrared laser irradiation.	Targeted anticancer therapy in combination with laser treatment	43
LIN	CALNN and GSH	LIN-AuNPs-CALNN	MCF-7 cells	Higher antioxidant activity and anticancer activity as compared to Linalool and AuNPs alone.	Human breast cancer therapy	27
K	-	K-AuNPs	MCF-7 cells	Higher cell apoptosis, antiproliferative ability and inhibition of angiogenesis compared to pure kaempferol.	Human breast cancer therapy	26
PI	-	PI-AuNPs	HT-29 and MDA-MB-231 cells	Higher DNA disintegration in both cells and subsequent cell apoptosis compared to AuNPs and PI alone.	Human colon cancer and breast cancer therapy	29
TS265	PEG, anti-EGFR D-11 and BSA	TargetNanoTS265	A549 and HCT116 cells	Elevated tumor cytotoxicity and tumor inhibition efficacy as compared to the free TS265.	Targeted delivery of anticancer agents	30
ZnD	PEG, C225 and BSA	NanoZnD	DOX-resistant HCT116 cells	Enhanced antiproliferative potential and reduced tumor growth when compared to free ZnD.	Targeted drug-resistant cancer therapy	31

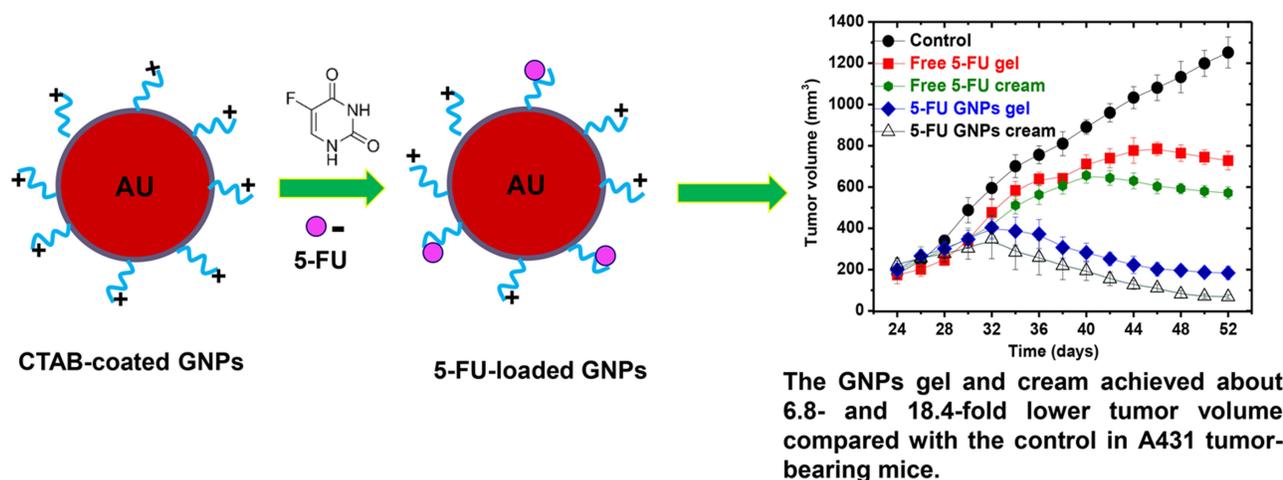
**Abbreviations:** 5-FU, 5-fluorouracil; anti-EGFR D-11, monoclonal antibody D-11 against epidermal growth factor receptor; AuNPs, gold nanoparticles; BSA, bovine serum albumin; C225, cetuximab; CALNN, Cys-Ala-Leu-Asn-Asn; DOX, doxorubicin; DTX, docetaxel; FA, folic acid; GFLGC, Gly-Phe-Leu-Gly-Cys; GSH, glutathione; HA, hyaluronic acid; K, kaempferol; LIN, linalool; PI, Boc-L<sup>D</sup>P-L-OMe; PEC, pectin; PEG, polyethylene glycol; TS265, CoCl(H<sub>2</sub>O) (phendione)<sub>2</sub>[BF<sub>4</sub>]; ZnD, [Zn(DION)<sub>2</sub>]Cl<sub>2</sub>.

certain receptors that attract and contact substances to achieve their metabolite.<sup>40</sup> The delivery carrier carrying drugs must have a selective affinity for cancer cells, so it is highly possible for drugs loaded to be selectively transported to the cancer cells with a desired dose. Based on this, several targeting molecules have been chosen, such as transferrin (Tf),<sup>41</sup> pectin (PEC),<sup>42</sup> hyaluronic acid (HA),<sup>43</sup> folic acid (FA),<sup>44,45</sup> and galactose (Gal).<sup>46</sup> Additionally, less stable oligonucleotide fragments can also be used as targeting ligands. It has been reported that by using targeting element nucleolin aptamer (AS1411), a guanosine-rich single-stranded DNA (ssDNA), both DOX and aptamer against Forkhead box M1 (FOX M1 Apt) were selectively co-delivered into cancer cells.<sup>47</sup> In return, AuNPs could also protect unstable nucleic acid fragments against degradation by nucleases.<sup>48,49</sup> Besides, some therapeutic drugs that belong to monoclonal antibodies (mAbs) are inherently targeting, such as cetuximab (C225),<sup>50</sup> trastuzumab (Tmab).<sup>51</sup> The conjugation between mAbs and AuNPs is more important for cancer treatment compared to the mAb-based monotherapy. Except for those effects mediated by mAbs alone, the conjugation can lead to other cytotoxic effects, such as oxidative stress and autophagy caused by AuNPs,<sup>52</sup> which can largely increase the overall efficacy. By loading another therapeutic drug, not only



**Figure 3** Intracellular distribution of free DOX and Au-SS-DOX in HepG2-R cells. Confocal images of cells treated with (A) free DOX and (B) Au-SS-DOX showing distribution of DOX-derived fluorescence (red). (C) Intracellular DOX fluorescence intensity in HepG2-R cells after exposure to free DOX and Au-SS-DOX for 24 hours. Reprinted from *Nanomedicine*, 8(2), Gu YJ, Cheng J, Man CW, Wong WT, Cheng SH. Gold-doxorubicin nanoconjugates for overcoming multidrug resistance. 204–211, copyright 2012, with permission from Elsevier.<sup>34</sup>

**Abbreviations:** DOX, doxorubicin; SS, disulfide linkage.



**Figure 4** Schematic diagram for AuNPs synthesis and 5-FU loading using CTAB, and growth curves of A431 tumors in C57BL/6 mice. Reprinted with permission from Safwat MA, Soliman GM, Sayed D, Attia MA. Fluorouracil-Loaded Gold Nanoparticles for the Treatment of Skin Cancer: development, in Vitro Characterization, and in Vivo Evaluation in a Mouse Skin Cancer Xenograft Model. *Mol Pharm*. 2018;15(6):2194–220.<sup>35</sup>

**Abbreviations:** 5-FU, 5-fluorouracil; CTAB, cetyltrimethylammonium bromide; GNPs, gold nanoparticles.

targeted delivery of the drug but also a combined antibody/drug effect were achieved.<sup>53</sup> These investigations demonstrated that the AuNPs modified with targeting ligands are largely useful for the targeted delivery of anticancer drugs.

### Nucleic Acid Delivery

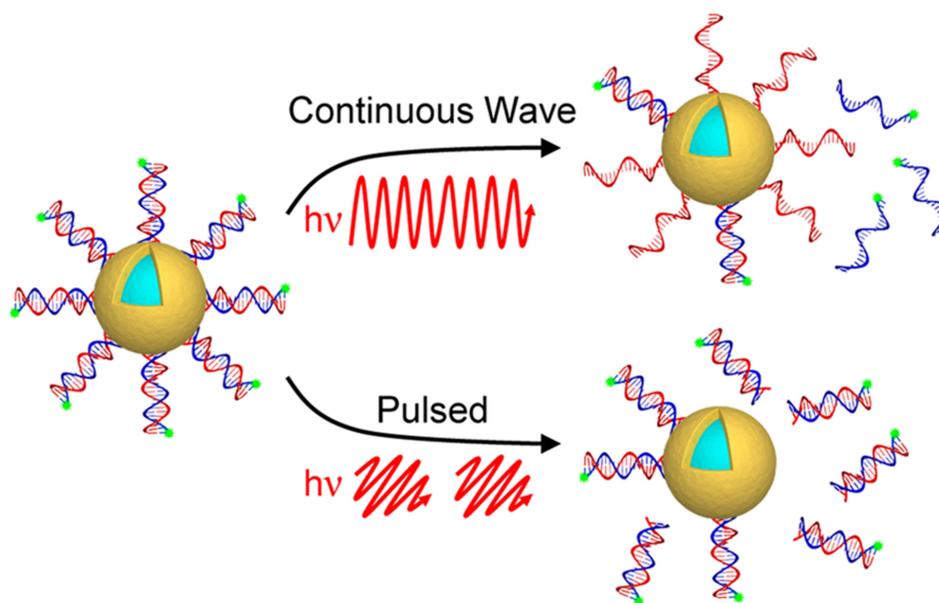
Gene therapy is an ideal method to prevent and treat cancer by using foreign DNA and RNA. Compared to small-molecule drugs, nucleic acid drugs are largely labile. On the one hand, the nucleic acid drugs are vulnerable to various environmental risks, such as enzymatic, chemical and physical degradation during gene manipulation and gene transfection.<sup>54</sup> On the other hand, such drugs as biologic agents are prone to immunogenicity and are consumed readily by innate immune cells. Therefore, ideal delivery vehicles are required to deliver such drugs into cells, to prevent nucleic acid drugs from degradation and have a better transfection effect.<sup>55</sup> Currently, viral vector systems are very popular for gene delivery but can activate the host's immune response, which reduces efficiency of future gene therapy.<sup>56</sup> Delivery of nucleic acids via non-viral vectors systems, such as gold nanoparticles, can avoid this problem. In comparison to viral vectors, surface design of AuNPs is more flexible, which aids in functionalization and biocompatibility in the body.<sup>56</sup> Moreover, AuNPs can protect nucleic acid from nuclease degradation and physical damage<sup>54</sup> and show more than 99% cellular uptake in spite of surface negative charge.<sup>57</sup>

Based on the properties above, AuNPs conjugated with nucleic acid can be used for gene silencing therapy in tumor model. For instance, Tunc et al embedded morpholino antisense oligonucleotides into a DNA-tile-AuNPs structure for treatment of breast cancer. They found that the DNA-tile-AuNPs structure delivered morpholinos and silenced the expression of *HER2* and *ER $\alpha$*  gene in breast cancer cells more effectively than the liposome-based system.<sup>58</sup> Besides, due to the photothermal effect of AuNPs, the conjugate has the ability to become a dual functional delivery nanoplatform that achieves simultaneously gene silencing and photothermal therapy.<sup>59</sup> The complex still has a good photothermal effect even after nucleic acid functionalization. The composite significantly inhibited tumor growth without overt side effects for major organs after laser exposure.<sup>60</sup> Furthermore, AuNPs can load simultaneously gene and chemotherapy drugs to achieve a synergistic effect. Huang et al prepared a multifunctional nanoplatform based on AuNPs, which co-delivered microRNA-122 and DOX, achieving triple therapy (photothermal therapy, chemotherapy and gene therapy). With the aid of polyethylene glycol (PEG) and HA, this delivery system could selectively target hepatoma carcinoma cells without toxicity to the main organs and showed a better antitumor effect than any single therapy.<sup>61</sup>

The release of DNA from the gold nanocomplex can be triggered by exogenous light. Upon laser irradiation, the heat generated by AuNPs through the photothermal effect is transmitted to the ambient DNA molecules. When the temperature reaches the threshold, the chemical linkages break, thus leading to DNA release.<sup>62</sup> Interestingly, the specific DNA release mechanisms induced by continuous wave (CW) versus pulsed lasers are different. Upon CW laser irradiation, high temperature results in dehybridization between double-stranded DNA (dsDNA) and release of nonthiolated ssDNA, while upon pulsed laser illumination, the entire DNA molecules are liberated through Au-S bond cleavage (Figure 5).<sup>63</sup> The discrepancy in release mechanism makes cell mortality rate different. In a work, an anticancer drug docetaxel (DTX) was inset into complementary dsDNA that was first attached to gold nanoshells (silica core) through the Au-thiol bond for the treatment of breast cancer. The CW laser-induced DTX release caused a significant increase in breast cancer cell death, while the pulsed laser-induced drug release resulted in unobvious cell death (Figure 6).<sup>64</sup> Accordingly, AuNPs can be used as a promising genetic drug delivery vector, achieving multifunctional anti-cancer therapy.

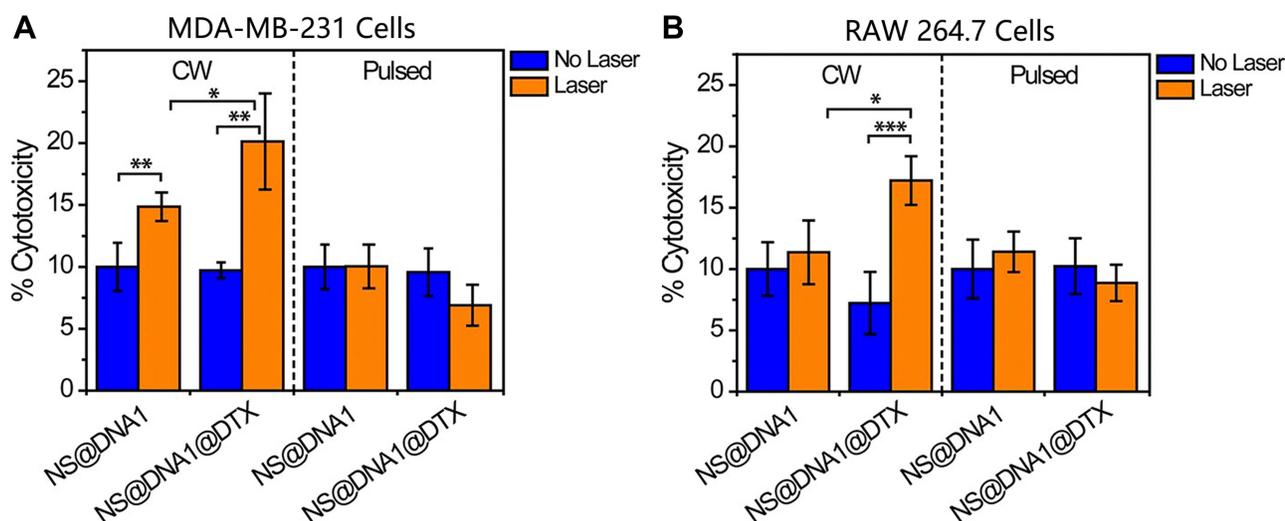
## AuNPs in Photodynamic Therapy

More than 100 years ago, photodynamic therapy has been first proposed. Nowadays, it has developed into a relatively safe and effective therapeutic modality, especially for skin diseases.<sup>65</sup> The PDT includes three important elements:



**Figure 5** NIR light-induced DNA release. CW irradiation results in dehybridization between dsDNA and release of nonthiolated ssDNA, while pulsed irradiation results in Au-S bond cleavage and release of entire DNA. Reprinted with permission from Goodman AM, Hogan NJ, Gottheim S, Li C, Clare SE, Halas NJ. Understanding Resonant Light-Triggered DNA Release from Plasmonic Nanoparticles. *ACS Nano*. 2017;11(1):171–179.<sup>63</sup>

**Abbreviation:** hv, irradiation with light.



**Figure 6** Comparison of cell viability after DTX release from a DNA host without (blue) and with (Orange) CW and pulsed lasers in (A) MDA-MB-231 and (B) RAW 264.7 cells. \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ . Reprinted with permission from Goodman AM, Neumann O, Norregaard K, et al. Near-infrared remotely triggered drug-release strategies for cancer treatment. *Proc Natl Acad Sci U S A*. 2017;114(47):12419–12424. Creative Commons license and disclaimer available from: <http://creativecommons.org/licenses/by/4.0/legalcode>.<sup>64</sup>

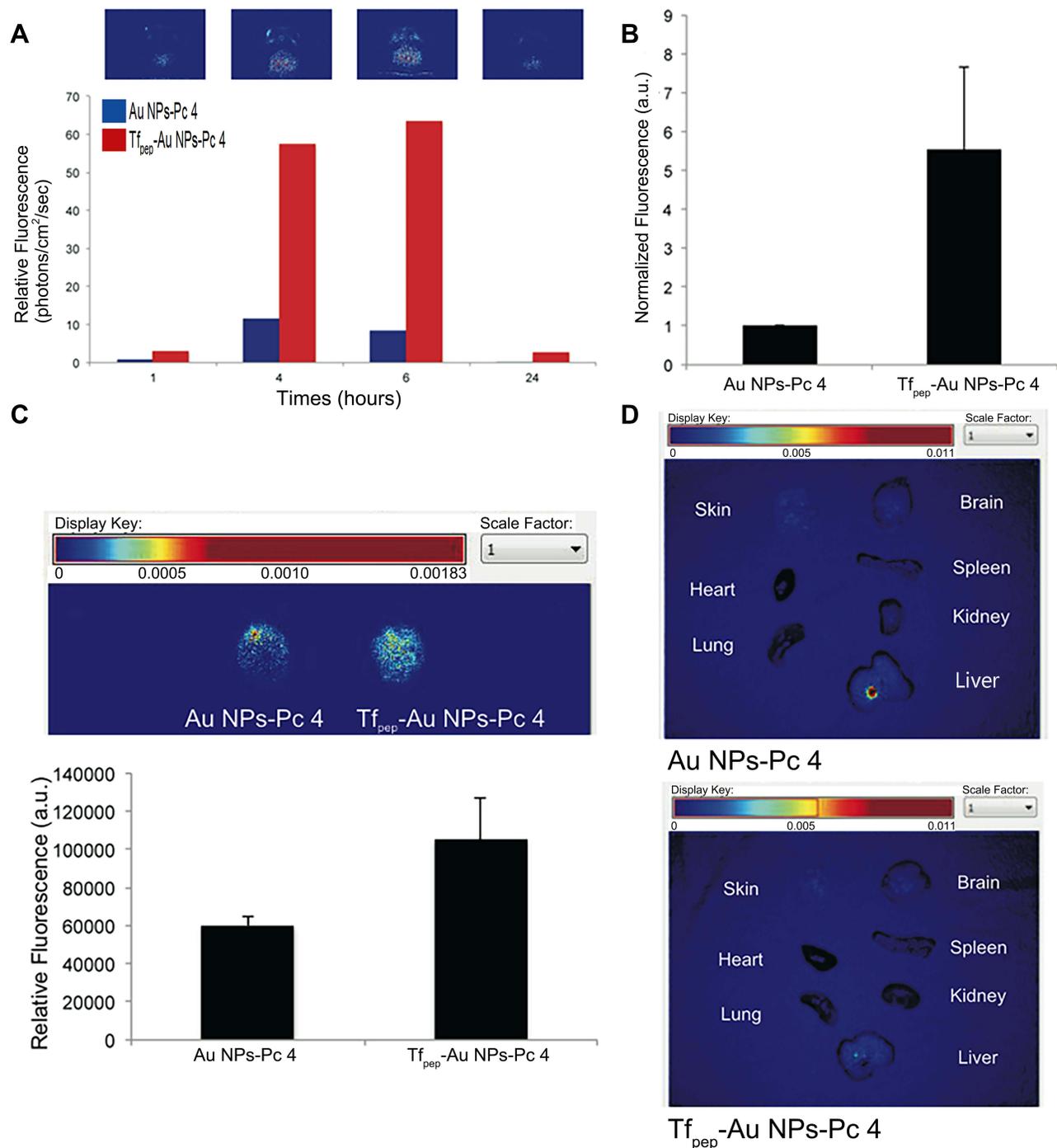
**Abbreviations:** CW, continuous wave; DTX, docetaxel; NS, nanoshell.

a photosensitizer (PS), light with an appropriate wavelength and molecular oxygen ( $O_2$ ). The original PS molecule can be activated easily by light to transform into an excited singlet state. However, the unstable excited singlet PS easily loses spare energy in the form of heat production or emission of light to turn into an excited triplet state. Compared to the excited singlet state, the excited triplet state is more stable, so that it has sufficient time to undergo photochemical reactions in the presence of  $O_2$  to produce reactive oxygen species (ROS) such as singlet oxygen ( $^1O_2$ ).<sup>66</sup> The overproduction of ROS triggers oxidative stress, causing cell death by cell apoptosis or necrosis. The PDT takes advantage of this working principle to damage diseased cells. However, most of the PSs are low water-soluble and tend to aggregate in physiological media, largely affecting the quantum yield of  $^1O_2$ . Another drawback of PSs is their non-specific biodistribution in vivo, which may cause unwanted systematic side effects. Hence, to obtain the ideal PDT result, it is crucial to ensure the stability and tumor selectivity of the PS molecules in vivo.

Conjugating the PSs on the AuNP surface is an effective method to overcome the above drawbacks. It has been reported that incorporating hydrophobic porphyrin derivatives to the AuNPs that were first modified with hydrophilic PEG chains rendered it possible to obtain a higher aqueous solubility.<sup>67,68</sup> Meanwhile, the water-soluble, porphyrin-containing conjugate was shown to produce higher  $^1O_2$  compared to free porphyrin derivatives, thus achieving increased PDT results in cell model.<sup>67,68</sup> A similar effect could also be achieved in the case of conjugation of AuNPs with meso-tetrahydroxyphenylchlorin (mTHPC), a -hydrophobic second-generation photosensitizer.<sup>69</sup> Modifying hydrophilic molecules onto the AuNPs bound to hydrophobic drugs is of great significance to improve the solubility and stability. This strategy has enabled higher death rates of cancer cells compared to free PS molecules. For some water-soluble second-generation PSs such as methylene blue (MB)<sup>70</sup> and 5-aminolevulinic acid (5-ALA),<sup>71</sup> they fortunately bypass the problem associated with solubility of first-generation PSs. However, the hydrophilic nature limits the ability to cross lipophilic cell membranes, consequently decreasing the intracellular accumulation of the water-soluble PSs and PDT efficacy. It was confirmed that the 5-ALA when bound to AuNPs grafted with targeting moiety Arg-Gly-Asp (RGD) peptide, could significantly increase its intracellular amount and exhibit the stronger photodynamic cytotoxicity than free 5-ALA.<sup>72</sup> This result can be attributed to the integrin (specific receptor of the RGD peptide) mediated endocytosis effect, which thus causes the increase in intracellular 5-ALA amount and PDT efficacy. Different from the 5-ALA, the MB can easily cross the cell membrane in spite of the hydrophilicity due to the special structure of plane benzene ring.<sup>73</sup>

Targeted delivery of PS molecules using AuNPs for PDT is an important strategy for the selective treatment of malignant tumors. Dixit and co-workers constructed a nanocomplex (Tf<sub>pep</sub>-coated PEGylated AuNPs) to selectively

deliver photodynamic prodrug phthalocyanine 4 (Pc 4) to glioblastoma cells. Interestingly, they found that the efficiency of Pc 4 delivery and PDT was superior over untargeted (AuNPs-Pc 4) NPs, confirming the potential of the Tf<sub>pep</sub>-AuNPs for targeted delivery to glioblastoma cells with high Tf receptor expression level (Figure 7).<sup>74</sup> In other studies, authors used the same PEGylated Au nanostructures to load Pc 4. By conjugating the targeting molecules to AuNPs, selective



**Figure 7** Tf<sub>pep</sub>-Au NPs-Pc 4 accumulate in orthotopic brain tumors in vivo. **(A)** Representative in vivo fluorescence hotmap images of a mouse implanted with an orthotopic glioma injected with Tf<sub>pep</sub>-Au NPs-Pc 4 over time. Bar graph quantifies uptake of Tf<sub>pep</sub>-Au NPs-Pc 4 versus Au NPs-Pc 4. **(B)** Mice were analyzed for free Pc 4 fluorescence (RFU) 6 hours post injection. **(C)** Brains of glioma mice were excised and examined for ex vivo fluorescence (hotmap) after 6 hours. **(D)** Fluorescence imaging 24 hours post injection of ex vivo organs from mice injected with Au NPs-Pc 4 or Tf<sub>pep</sub>-Au NPs-Pc 4. Reprinted with permission from Royal Society of Chemistry. Dixit S, Novak T, Miller K, Zhu Y, Kenney ME, Broome AM. Transferrin receptor-targeted theranostic gold nanoparticles for photosensitizer delivery in brain tumors. *Nanoscale*. 2015;7(5):1782–1790.<sup>74</sup>

**Abbreviations:** Au NPs, gold nanoparticles; Pc 4, phthalocyanine 4; Tf<sub>pep</sub>, transferrin.

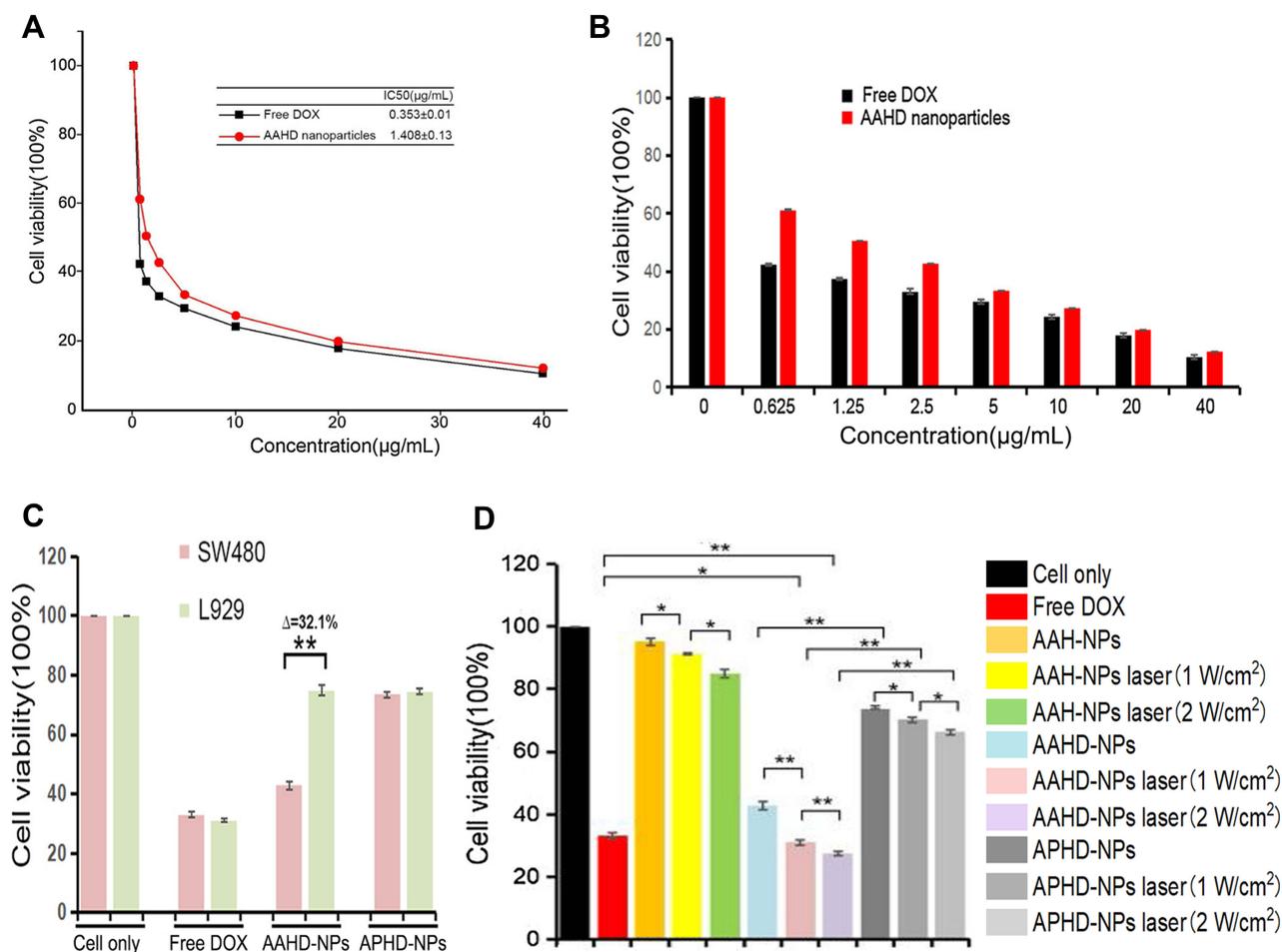
delivery of the Pc 4 into the cancer cells was achieved.<sup>75,76</sup> Savarimuthu et al synthesized AuNPs conjugated photosensitizer protoporphyrin IX (PpIX) equipped with FA, which is efficiently taken up by Vero and HeLa cells due to the presence of the FA and thus exhibit superior phototoxicity compared to the Au-PpIX electrostatic and covalent complexes.<sup>77</sup> In 2018, Calavia et al prepared Pc-AuNPs with lactose as a Pc-releasing nanosystem, where the targeting ability of the lactose towards galectin-1 receptors overexpressed on breast cancer cells was assessed in vitro. It was shown that the lactose-Pc-AuNPs possessed higher cytotoxicity in comparison with the control Pc-sPEG-AuNPs.<sup>78</sup> In several studies, small AuNPs (<10 nm) and photosensitizer verteporfin are co-encapsulated into different organic nanocarriers via different methods, whose surfaces then were modified with triphenylphosphonium, a cationic mitochondrial targeting moiety.<sup>79,80</sup> Upon X-ray radiation, the nanocomplexes generated cytotoxic  $^1\text{O}_2$  within the mitochondria, leading to the alteration of mitochondrial membrane potential and increase in the level of cell apoptosis, but with greatly reduced radiation doses and radiation side effects.<sup>79,80</sup> In conclusion, AuNPs bound to the PS and targeting warheads have great application prospects in terms of targeted PDT.

## AuNPs in Photothermal Therapy and in Combined Therapy

Beyond PDT, photothermal therapy is another attractive light-activated application of AuNPs for cancer treatment. It is reported that AuNPs have the ability to absorb light to produce localized high temperatures (>43 °C), based on the SPR effect of free electrons, ultimately resulting in thermal damage for cancer cells.<sup>81–83</sup> Importantly, AuNPs can change their absorption and scattering cross sections and spectrums (from visible to near-infrared (NIR) region) by changing their size, shapes and adjacent environment.<sup>84,85</sup> For instance, the transition efficiency of rod-shaped AuNPs can be tuned by changing their aspect ratio of length to diameter.<sup>86</sup> A similar effect can also be achieved in shell-shaped AuNPs by changing the relative ratio of core radius and shell thickness.<sup>87,88</sup> All of these characteristics endow AuNPs with outstanding advantages over other nanomaterials that are also used as photothermal agents. However, PTT using visible light is usually limited to superficial tumors due to their short wavelengths and consequent weak tissue penetration ability. By contrast, the NIR region is more important since the long wavelengths allow deeper light penetration into living tissues with less tissue damage.<sup>55,89</sup>

Hyperthermia involves cancer cell death through two mechanisms, namely cell apoptosis and necrosis. Generally, hyperthermia induces cell apoptosis by irreversible mitochondrial damage and ROS overproduction.<sup>90</sup> Some investigations, however, have found that the hyperthermia can also induce cell necrosis by cell membrane damage and protein denaturation.<sup>91,92</sup> Markovic et al thought that the mechanisms of thermal damage of cancer cells were apparently related to induced oxidative stress and depolarization of mitochondrial membrane, eventually resulting in mixed apoptotic/necrotic cancer cell death.<sup>93</sup> In fact, using a single PTT usually cannot completely eradicate the tumor owing to the intratumoral uneven and uncontrollable heat distribution; the surviving cancer cells fast develop resistance to thermal stress, jointly leading to tumor metastasis and recurrence.<sup>94,95</sup> To further improve the efficacy of cancer treatment, PTT has been developed to combine with other therapies, such as chemotherapy, PDT and RT.<sup>11,96,97</sup>

With regard to the combination of PTT and chemotherapy, many types of gold nanocomplexes exhibited strikingly enhanced cytotoxicity and tumor inhibition effect in comparison with a single application of PTT or chemotherapy.<sup>98–101</sup> A good example is the AuNPs modified with aptamer AS1411 and hairpin DNA loading with DOX, which were used for targeted and synergistic chemo-PTT.<sup>102</sup> Upon 808 nm NIR laser illumination (1 or 2 W/cm<sup>2</sup>), the viability of colon cancer cells was found to dramatically decrease when compared to separate application of PTT and chemotherapy (Figure 8).<sup>102</sup> The NIR laser not only generated the photothermal effect of gold nanomaterials, but also accelerated the release of chemotherapeutic drugs from AuNP-based nanocomplexes, enhancing the synergistic treatment effect at the target site.<sup>103</sup> In addition, a high concentration of glutathione (GSH)<sup>104</sup> and a weak acidic condition<sup>105</sup> are also responsible for a controlled release of the loaded drugs. Importantly, the combination therapy effectively avoided side effects caused by high doses of chemotherapeutic agents.<sup>106</sup> Even under sub-therapeutic doses, combined therapy also shows a better antitumor efficacy.<sup>107,108</sup> The synergistic effect between hyperthermia and drugs may be attributed to the following mechanisms. First of all, high temperatures can delay the repair pathways of DNA damage induced by chemotherapeutic agents, stabilizing chemotherapy-caused DNA damage response.<sup>109</sup> Moreover, hyperthermia can increase the permeability of tumor vasculature and blood flow, which increases the accumulation of chemotherapeutic



**Figure 8** CCK-8 assay of different formulations. **(A & B)** Cytotoxicity and IC50 of free DOX, AAHD-NPs. **(C)** The cell viability of SW480 or L929 cell treated with different NPs. **(D)** Cytotoxicity of chemotherapy and PTT effect by a 808 nm laser irradiation (1 W/cm<sup>2</sup> or 2 W/cm<sup>2</sup>) for 10 min. \*P < 0.05. \*\*P < 0.01. Reprinted from *Biomed Pharmacother*, 130, Zhang Y, Zhou L, Tan J, Liu J, Shan X, Ma Y. Laser-triggered collaborative chemophotothermal effect of gold nanoparticles for targeted colon cancer therapy. 2020;10492. Creative Commons license and disclaimer available from: <http://creativecommons.org/licenses/by/4.0/legalcode>.<sup>102</sup>  
**Abbreviations:** AAH, gold nanoparticles modified with AS1411 and DNA; AAHD, gold nanoparticles modified with AS1411 and DNA loading with doxorubicin; APHD, gold nanoparticles modified with DNA loading with doxorubicin; DOX, doxorubicin; IC50, half-inhibitory concentration; NPs, nanoparticles; PTT, photothermal therapy.

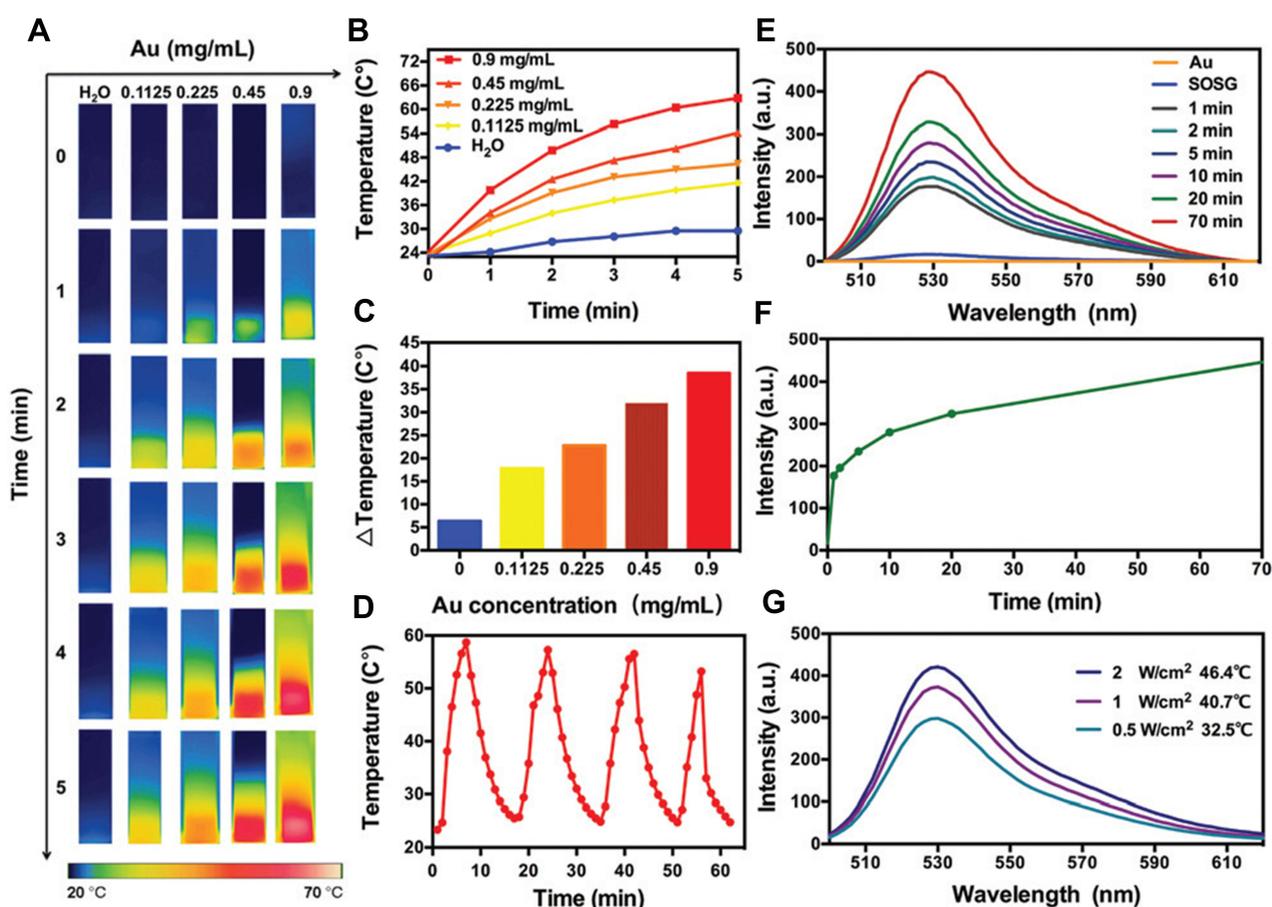
drugs within tumor tissue.<sup>110,111</sup> Furthermore, hyperthermia can inhibit the expression of drug resistance-related transporters, mainly P-glycoprotein (P-gp) through high expression of heat shock factor-1 (HSF-1) trimers, and then provide the possibility of overcoming multidrug resistance (MDR).<sup>112</sup> The gold nanocomplex in combination with drugs can merge dual therapeutic functions simultaneously under laser irradiation, thus giving rise to synergistic therapeutic effects.

Another interesting aspect is that PTT can be combined with PDT to achieve a synergistic anticancer treatment. As mentioned above, PTT alone cannot completely annihilate tumors. Likewise, a single PDT may do not attain a satisfactory result. For one thing, the PDT treatment usually uses light with limited wavelengths (<700 nm), which are difficult to penetrate deep tumor tissue.<sup>113</sup> For another thing, ample <sup>1</sup>O<sub>2</sub> generation requires sufficient oxygen supply within tissue, but the hypoxic state of tumor microenvironment often makes the PSs ineffective in the area.<sup>114</sup> Fortunately, it has been corroborated that a combination of PTT and PDT could complement one another to provide better therapeutic effects than a single PTT or PDT.<sup>115–117</sup> The reason may be that hyperthermia caused by PTT can increase oxygen amount within tumor tissue via boosting blood flow to improve the generation of <sup>1</sup>O<sub>2</sub>, and in turn <sup>1</sup>O<sub>2</sub> generation caused by PDT can enhance cytotoxicity induced by PTT.<sup>118</sup> However, combined PTT/PDT treatment often requires two kinds of different wavelength lights, which causes complexity of treatment and limits clinical

transformation. Hence, it is necessary to develop a novel PTT and PDT agent that can be activated by a single wavelength light. For this purpose, Liu's group designed captopril-stabilized Au nanoclusters ( $\text{Au}_{25}(\text{Capt})_{18}$ ) as the PTT and PDT agent and chose 808 nm NIR light as the single light source. They found the  $\text{Au}_{25}(\text{Capt})_{18}$  had a quick temperature increasing rate (within 5 min) and continuous  $^1\text{O}_2$  generation (at least 1 hour) under NIR laser exposure (Figure 9).<sup>118</sup> The results suggested that modified AuNPs can be used as a single agent which performs concurrently PTT and PDT under single wavelength light irradiation.

Furthermore, a collaborative effect also can be achieved by the application of both PTT and RT together. The combination of PTT and RT promotes an increase in cancer cell lethality, even at a low nanoparticle concentration.<sup>97</sup> Additionally, heating the tumors with NIR laser to 48 °C before RT could reduce the X-ray dose required to control the tumor, thus protecting adjacent normal tissues from ionizing radiation-associated damage.<sup>119</sup> On the other hand, using mild PTT after RT was able to inhibit the self-repair of damaged DNA induced by RT by down-regulating the expression of DNA repair proteins, thus improving radiosensitive efficiency.<sup>120</sup> Accordingly, the dual PTT/RT therapy was indeed more effective than the use of PTT alone or RT alone.

Triple therapy seems more necessary for complete tumor inhibition, as evidenced by the fact that its therapeutic efficacy is obviously superior to that of any monotherapy or dual therapy. Although various dual therapeutic therapies



**Figure 9** Photothermal and photodynamic effects of  $\text{Au}_{25}(\text{Capt})_{18}$ . (A) Thermal images of  $\text{Au}_{25}(\text{Capt})_{18}$  aqueous solution at different Au concentrations under laser irradiation (808 nm, 2  $\text{W}/\text{cm}^2$ , 5 min); also, DI water under the same condition was set as the control. (B) Temperature curves in (A). (C) Temperature changes in (A). (D) Photostability evaluation by measuring the temperature curves of  $\text{Au}_{25}(\text{Capt})_{18}$  aqueous solutions after 4 heating-cooling cycles under repeated laser irradiation (2  $\text{W}/\text{cm}^2$ , 5 min for each). (E) Generation of  $^1\text{O}_2$  by  $\text{Au}_{25}(\text{Capt})_{18}$  via recording the fluorescence emission spectra at 530 nm under laser irradiation at different time points (1  $\text{W}/\text{cm}^2$ , 1–70 min). (F) Fluorescence intensities with irradiation time increasing in (E); (G) Generation of  $^1\text{O}_2$  by  $\text{Au}_{25}(\text{Capt})_{18}$  via recording the fluorescence intensities at 530 nm under laser irradiation with different power densities for 5 min. Reprinted with permission from Royal Society of Chemistry, Liu P, Yang YV, Shi L, et al. Concurrent photothermal therapy and photodynamic therapy for cutaneous squamous cell carcinoma by gold nanoclusters under a single NIR laser irradiation. *J Materials Chem B*. 2019;7(44):6924–6933.<sup>118</sup>

**Abbreviation:** SOSG, singlet oxygen sensor green.

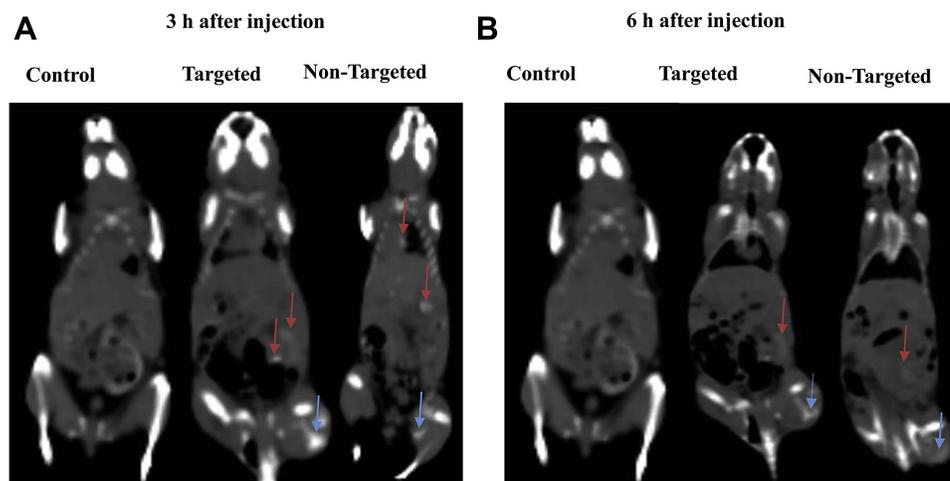
(chemotherapy/PTT, PTT/PDT, PTT/RT) based on AuNPs have been vigorously developed, the triple therapy of AuNP-based nanoplatfoms is still in an infancy stage. Recently, several multifunctional nanoplatfoms based on copper sulfide nanoparticles,<sup>121,122</sup> bismuth sulfide nanoparticles,<sup>123</sup> and especial AuNPs have been developed for triple-modality cancer therapy.<sup>124–126</sup> The outcomes of these studies demonstrated the great potential of triple therapy to eradicate tumors compared to monotherapy and dual therapy. In 2018, Xu et al reported a pH-responsive DOX and 5-ALA co-loaded gold nanorods (AuNRs) for enhanced breast cancer treatment through combined chemo/photothermal/photodynamic therapy.<sup>127</sup> This complex nanosystem was constructed by first decorating AuNRs with mercaptopropionylhydrazide (MPH) and thiol-terminated monomethoxy PEG (mPEG-SH) via Au-S bonds, and subsequently linking onto MPH molecules with chemotherapeutic agent DOX and pro-photosensitizer 5-ALA through acid-labile hydrazone bonds, termed as AuNRs-MPH<sup>-ALA/DOX</sup>-PEG. The cumulative release amounts of DOX and 5-ALA from AuNRs-MPH<sup>-ALA/DOX</sup>-PEG were higher in phosphate buffer solution (PBS) at 37 °C at pH 5.0 than at pH 7.4 (eg 58% vs 3% of DOX, 71% vs 6% of 5-ALA within one day). In vitro and in vivo studies showed that the resulting GNRs-MPH<sup>-ALA/DOX</sup>-PEG could kill human breast cancer MCF-7 cells and inhibit tumor growth without overt side effects more efficiently, respectively, through a superadditive antitumor effect.<sup>127</sup> Previously, a novel multifunctional nanoplatfom based on gold nanocages (AuNCs) with NIR stimuli-responsive DOX and photosensitizer indocyanine green (ICG) release was also developed for simultaneous chemo/photothermal/photodynamic therapy of breast cancer.<sup>128</sup> In another work, Xu et al reported the preparation and in vitro and in vivo evaluation of a new nanocomposite based on HA modified AuNCs (AuNCs-HA) for synergistic RT/PTT/PDT of breast cancer. In agreement with results of reports on chemotherapy/PTT/PDT, the AuNCs-HA exhibited complete tumor growth inhibition, compared to each monotherapy or dual therapy.<sup>129</sup> These studies provide new avenues for triple therapy of malignant tumors, which represents a feasible and simple strategy for constructing ideal cancer-aimed nanoplatfoms that integrate multiple treatment functionalities.

## AuNPs in Cancer Imaging

Imaging technologies can provide anatomical information of tissues with accuracy and specificity, which is indispensable for clinicians to diagnose diseases. Many studies have reported AuNPs have promising potential applications in cancer detection, especially in the fields of CT imaging. Therefore, AuNPs-based CT imaging technology will be elaborated in detail in this part.

Nowadays, CT imaging has become a widely used diagnostic tool due to its high efficiency and fairly low cost. The X-ray-based CT technology can take advantage of differences in X-ray attenuation to reconstruct 3D images of subject tissues.<sup>130</sup> Tissues with different densities will present different X-ray attenuation, particularly between healthy and diseased tissues.<sup>131,132</sup> Thus imaging and distinguishing the interface between two neighboring tissues or imaging soft tissues in contact with physiological fluids such as blood is very challenging unless using contrast agents.<sup>133</sup> The most commonly used CT contrast agent currently remains iodine-based compounds, such as iohexol.<sup>39</sup> However, short imaging times caused by rapid renal clearance and side effects at iodine concentrations required for imaging limit their use.<sup>133,134</sup> Hence, it is necessary to develop novel materials as CT contrast agents. This need has driven the development of AuNPs as promising alternatives. Due to higher atomic number and electron density of gold (79 and 19.32 g/cm<sup>3</sup>), AuNPs exhibit a higher X-ray absorption coefficient than iodine-based contrast agents.<sup>133</sup> Importantly, AuNPs have prolonged vascular retention time due to high molecular weight and increased intratumoral accumulation for enhanced CT imaging.<sup>135,136</sup> These favorable characterizations, in combination with good biocompatibility make the AuNPs an ideal contrast candidate for CT imaging.

In a study, PEGylated hollow AuNPs (mPEG@HAuNPs) with a diameter of 63.4 nm were chosen as contrast agents for CT imaging studies in vivo. The results showed a sustained contrast enhancement at the tumor area in the mPEG@HAuNPs group. Comparatively, no inherent contrast created from the same doses of iohexol (0.6 mg/g) at the tumor site was observed at 12 h post-injection.<sup>137</sup> Moreover, AuNPs can be multi-functionalized through modification on their surface, taking advantage of the interaction between sulfur and gold atoms. By exploiting this characteristic, it is possible to link multiple targeting moieties on the surface, which can contribute to the selectivity of the contrast agents towards specific tissues. For instance, the FA was conjugated onto AuNPs through a cysteamine (Cys) linking, to target against human nasopharyngeal KB cancer cells. The authors found that the FA-Cys-AuNPs conjugates led to a 2.03-times increase in the attenuation of X-ray intensity in comparison to AuNPs alone (Figure 10).<sup>138</sup> Likewise, Beik et al also



**Figure 10** Coronal CT images of nude mice after intravenous injection of AuNPs and FA-Cys-AuNPs at 3 hours (A) and 6 hours (B) (blue arrows indicate the tumor site and red arrows indicate other sites). The injection of the AuNPs (either non-targeted or targeted) leads to an enhanced CT contrast of the tumor area, and 3 h post injection shows a maximum CT enhancement of the tumor area. Reprinted from *Int J Biochem Cell Biol*, 114, Khademi S, Sarkar S, Shakeri-Zadeh A, et al. Targeted gold nanoparticles enable molecular CT imaging of head and neck cancer: an in vivo study. 105554, Copyright 2019, with permission from Elsevier.<sup>138</sup>

conjugated the FA to AuNPs for targeted CT imaging of KB cells. They found the FA-modified AuNPs had benefits of image contrast enhancement as well as radiation dose reduction.<sup>139</sup> Hence, the AuNPs modified with FA can be used not only for targeted CT imaging to detect cancer cells overexpressing FA receptors but also for X-ray-based RT where RT needs to increase the radiation dose of X-rays.

Multimode imaging is essential for clinicians to identify diseased tissues as it can offer more accurate and ample imaging information than single imaging modalities. By combining other contrast-enhancing agents with gold nanomaterials, it is possible to exploit novel systems that provide contrast for CT imaging along with other imaging approaches, such as magnetic resonance imaging (MRI)<sup>140</sup> and NIR fluorescence imaging.<sup>141</sup> Ge's group developed a PEG modified nano-system (named as PEG-LnAu NPs) incorporating 10 nm AuNPs and lanthanide ions ( $Gd^{3+}$  and  $Yb^{3+}$ ) for CT/MR dual mode imaging. The multifunctional nanoplatform (PEG-LnAu NPs) showed good performance in both MRI and CT imaging and could be used as a positive-contrast agent for dual mode imaging.<sup>142</sup> Kuhn's team reported a versatile nano-system loading two imaging enhancing elements (gold and magnetic iron oxide NPs) for tri-modal (MR, CT and intravascular ultrasound (IVUS)) imaging. In vitro results showed that the nanoprobe has concentration-dependent contrast-enhancing ability for MR, CT and IVUS imaging, without toxicity to cells even at a maximum concentration of 100  $\mu g/mL$ .<sup>143</sup> Moreover, the use of FA modified AuNPs loaded with other imaging materials for targeted tumor dual mode imaging has also been successfully reported.<sup>141,144</sup>

## AuNPs in Clinical Trials

As described above, AuNPs do show potentially useful properties in many preclinical studies. Nevertheless, there are few examples associated with AuNPs being under clinical trials, and, till now, no AuNP-containing formulations are used successfully in clinical practices. Table 2 exhibits a list of clinical trials of AuNPs for disease treatment and diagnosis to date.

Aurimune (CYT-6091) is the most typical example of AuNPs used in clinical testing for cancer treatment. The nanocomplex is a PEGylated 27-nm AuNP containing recombinant human tumor necrosis factor alpha (rhTNF $\alpha$ ). TNF $\alpha$  is a potent anticancer agent, but its extreme side effects largely limit its clinical use.<sup>145</sup> Here, by conjugating the rhTNF $\alpha$  onto the colloidal AuNPs to deliver the rhTNF $\alpha$  to tumor sites, it is favorable to ameliorate the systemic toxicity of TNF $\alpha$ .<sup>24</sup> Moreover, the PEG coating is introduced to the surface of colloidal gold particles, prolonging circulation time in blood and accumulation of colloidal gold particles in the tumor sites. Nowadays, its Phase I clinical trial (NCT00356980) has been successfully completed and published, where clinical safety profile of this drug was demonstrated. The trial results showed that the CYT-6091 at the dose tested (50–600  $\mu g/m^2$ ) was safely tolerated and a maximum tolerated dose was not reached even at the highest dose (600  $\mu g/m^2$ ). No severe side effects occurred except that first two patients

**Table 2** List of Clinical Trials of Gold Nanoparticles for Disease Treatment and Diagnosis<sup>154</sup>

Product Name	Component/ Material	Research Purpose	Application	Phase/Status	Clinical trials.gov Identifier	Reference
Aurimune (CYT-6091)	PEGylated colloidal AuNP-bound rhTNF $\alpha$	To study the side effects and best dose of the CYT-6091 in treating patients with advanced solid tumors	Treatment of advanced solid tumors	Phase I, completed	NCT00356980	<sup>146</sup>
		To study TNF in patients undergoing surgery for primary cancer or metastatic cancer	Treatment of primary or metastatic cancer	Early phase I, completed	NCT00436410	-
AuroShell	Silica-gold core shell nanoparticle coated with PEG	To study the adverse effects of the AuroShell in treating patients with refractory or recurrent head and neck cancer	AuroLase therapy of refractory or recurrent head and neck cancer	Pilot study, completed	NCT00848042	-
		To evaluate the efficacy of AuroLase therapy in subjects with primary or metastatic lung tumors	AuroLase therapy of primary or metastatic lung tumors	Pilot study, terminated	NCT01679470	-
		To determine the efficacy of using MRI/US fusion imaging technology to direct focal ablation of prostate tissue using nanoparticle-directed laser irradiation	Thermal ablation of prostate tissue	Extension study, recruiting	NCT04240639	-
				Phase I, completed	NCT02680535	-
Silica-Gold Nanoparticles	Silica-Gold Nanoparticles	To evaluate the safety and feasibility of two delivery approaches for treatment of coronary atherosclerosis	Photothermal therapy of atherosclerosis	Not applicable, completed	NCT01270139	<sup>158</sup>
Nanosensors	Gold nanoparticles and carbon nanotubes	To prove the diagnosis of Parkinson's disease from exhaled breath	Distinguish Parkinson's disease	Observational, completed	NCT01246336	-
		To prove the diagnosis of GC from exhaled breath and seek the interrelationship among breathomics, metabolomics and transcriptomics	Identifying GC	Observational, completed	NCT01420588	<sup>156</sup>
	Gold nanoparticles coated with organic ligands	To evaluate the diagnostic performance of a novel electronic nose for the detection of PAH	Diagnosis of PAH	Not applicable, completed	NCT02782026	-
CNM-Au8	Gold nanocrystal	To evaluate the safety, tolerability, and pharmacokinetics of CNM-Au8 in healthy male and female volunteers	Treatment of relapsing multiple sclerosis	Phase I, completed	NCT02755870	-

(Continued)

Table 2 (Continued).

Product Name	Component/ Material	Research Purpose	Application	Phase/Status	Clinical trials.gov Identifier	Reference
C19-A3 GNP	Human C19A3 proinsulin peptide coupled to gold	To study the risk of C19A3 GNP administration and the feasibility of delivering C19A3 GNP via microneedles	Treatment of type I Diabetes	Phase I, unknown	NCT02837094	-
NU-0129	A Spherical Nucleic Acid Gold Nanoparticle	To evaluate the safety of intravenous NU-0129 in patients with recurrent GBM or GS	Treatment of recurrent GBM or GS	Early phase I, completed	NCT03020017	-
CD24-Gold Nanocomposite	Gold nanoparticles conjugated to CD24	To introduce a novel diagnostic and prognostic approach in early detection of cancer stem cells in salivary gland tumors	Diagnosis of salivary gland tumors	Observational, completed	NCT04907422	-
GNP and PepGNP-Dengue	Gold nanoparticle mounted with/ without Dengue virus peptides	To test the safety of 2 doses of a T-cell priming specific cocktail of Dengue virus peptides mounted on a gold nanoparticle	Prevention of Dengue	Phase I, recruiting	NCT04935801	-
GNP and PepGNP-SARS-CoV-2	Gold nanoparticle mounted with/ without Coronaviruses peptides	To investigate the safety of 2 doses of a T-cell priming specific cocktail of Coronaviruses peptides mounted on a gold nanoparticle	Prevention of Coronavirus SARS-CoV-2 Infection COVID-19	Phase I, recruiting	NCT05113862	-

**Abbreviations:** CD24, cluster of differentiation 24; COVID-19, coronavirus disease 2019; GBM, glioblastoma; GC, gastric cancer; GNP, gold nanoparticles; GS, gliosarcoma; PAH, pulmonary arterial hypertension; PEG, polyethylene glycol; PepGNP, gold nanoparticle mounted with peptides; rhTNF $\alpha$ , recombinant human tumor necrosis factor alpha; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF, tumor necrosis factor.

experienced controllable fevers. As for efficacy, although it was not the overarching objective of this study, one patient underwent a partial response and other four patients displayed stable disease.<sup>146</sup> The encouraging results obtained here have initiated the Phase II clinical study to further demonstrate its clinical efficacy.

Auroshell is another example of AuNPs under clinical trials for cancer treatment. Auroshell is a silica-gold core shell nanoparticle with a total diameter of 144–150 nm, which is developed by Nanospectra Sciences Inc to thermally ablate the localized tumors.<sup>147</sup> In Aurotherapy, the Auroshell particles accumulate in the tumor tissues via the EPR effect and then are activated by an external NIR laser, without effect to adjacent normal tissues. Therefore, photothermal cancer therapy is an effective and highly selective approach with minimal side effects. These efforts build on many previous testing.<sup>148–150</sup> Preclinical biosafety of the Auroshell particles has been assessed in a series of *in vitro* and *in vivo* studies, where the particles were well tolerated and toxicity was not observed after intravenous infusion.<sup>151</sup> Furthermore, an initial clinical trial based on the Auroshell particles was performed in 22 patients with prostate cancer, whose results also suggested excellent tolerability in human beings. It was found that only 2 adverse events (an itching and a burning sensation of the epigastrium) were judged to be associated with the particle infusion.<sup>152</sup> After confirming safety profile of the Auroshell particles, researchers further assessed the feasibility and safety of the AuroShell-mediated laser ablation in combination with MR/ultrasound fusion imaging in 16 low- or intermediate-grade prostate cancer men.<sup>153</sup> Corresponding with previous findings, a patient suffered from transient epigastric pain during the particle infusion. Except for this, no

other adverse events occurred. With respect to treatment efficacy, the tumor ablation zones were free in 62.5% of patients at 3 months and 87.5% of patients at 12 months.<sup>153</sup> These clinical results revealed that the AuroShell-directed PTT is a relatively safe and feasible modality for the targeted treatment of prostate tumors. Now this company is recruiting participants with prostate cancer for another clinical study (NCT04240639), with the aim of the focal ablation of prostate tissue via Auroshell particle directed laser irradiation.<sup>154</sup>

Besides, several other gold nanoparticle-based agents have also completed preliminary clinical studies or are under clinical testing. Khoobchandani et al investigated an intervention of green nanotechnology in preclinical and clinical studies for human metastatic breast cancer therapy. In this work, authors have succeeded in clinical translation, from cells, animals to humans, of AuNP-based Nano-Ayurvedic drug: Nano Swarna Bhasma, which is composed of AuNPs and mangiferin (a phytochemical).<sup>155</sup> Another pilot clinical trial (NCT01420588) related to AuNPs has been performed and published. Researchers developed a gold nanomaterial-based nanosensor and assessed the feasibility of a new avenue involving a breath test with the sensor for identifying gastric cancer from other gastric diseases.<sup>156</sup> The completion of the pilot study has provided great confidence in the success of an ongoing multicenter clinical trial.

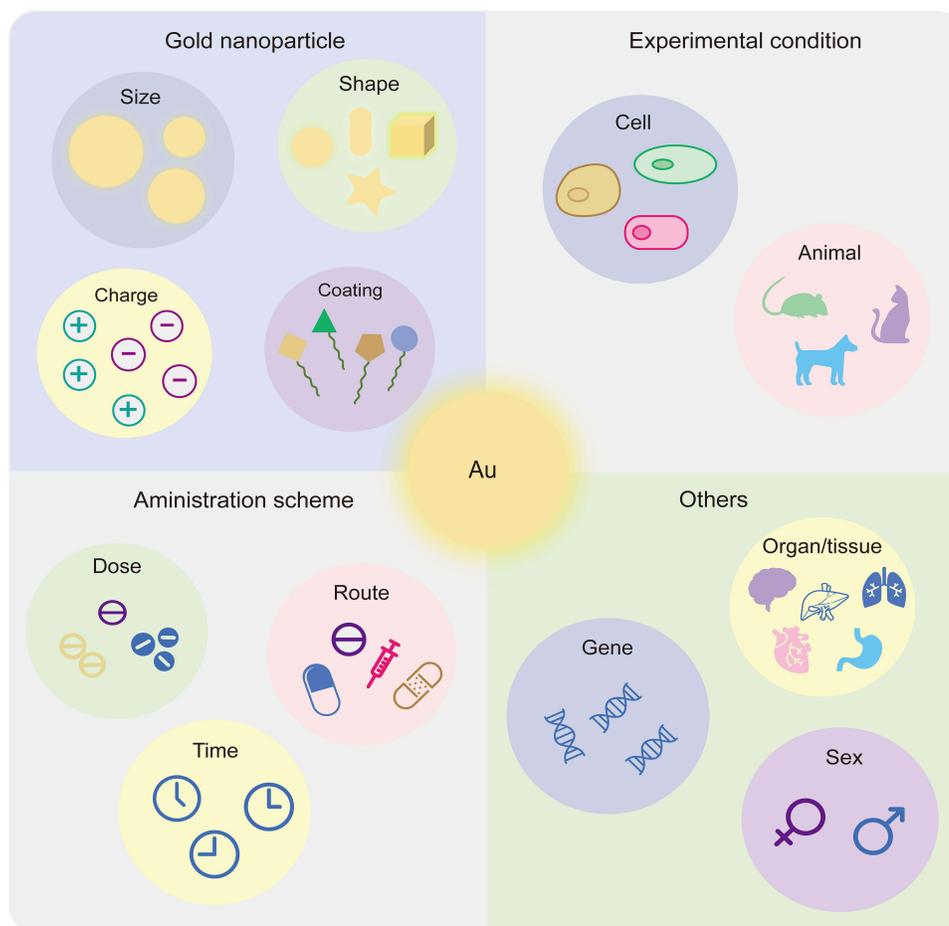
Moreover, due to the excellent photothermal conversion efficiency, AuNPs show great promise in other fields other than oncology. Pang et al developed a hydrogel eye patch that was composed of AuNR core and palladium shell and assessed safety and effectiveness in animal and human tests. It was found that the hydrogel eye patches could heat up spontaneously in response to various visible light, thus producing more tears to relieve the dry eye and moisturize the eyelid skin.<sup>157</sup> In another example, the safety and feasibility of two delivery approaches for treatment of coronary atherosclerosis were evaluated (NCT01270139). The first is a PTT method using silica-gold NPs and the second is a magnetic navigation approach with delivery of silica-gold iron bearing NPs. The obtained results showed that both delivery approaches have an acceptable level of safety for clinical practice.<sup>158</sup>

## Challenges of AuNPs in Clinical Applications

Although the preclinical and initial clinical studies are encouraging, there are still several important issues that need to be fully clarified prior to clinical use of AuNPs. It is reported that toxicity is the most important problem amongst them. Despite several studies indicating that AuNPs were relatively low toxic due to chemical inertness of metal gold,<sup>159–162</sup> the toxicity produced by AuNPs have been demonstrated in multiple cell and animal models. Many factors, no doubt, are able to largely affect their biodistribution in vivo and eventual toxicity, such as fundamental features of particles (eg, particle size, shape, surface charge, and coating), experimental conditions (eg, cell and animal model tested, assessed duration), administration scheme (eg, administration route, dose, time and times) and so forth (Figure 11). Thereby, the results may be diverse and even contradictory sometimes. This, along with the heterogeneity among individual tissues and cells, makes it intricate and challenging to utterly comprehend their interplay with the living organisms. Hence, toxicity profile of AuNPs and other reasons decelerating clinical translation of the AuNPs will be described fully in this part.

Particle size has been reported to impact toxicity of AuNPs, wherein smaller particles were observed to be more toxic than the larger ones.<sup>163,164</sup> This may be attributed to the fact that small nanoparticles cross the cell membrane and the nucleus pore more easily, thus favoring the intracellular ROS generation and DNA damage.<sup>165,166</sup> However, at a more early time point, Chen et al found that AuNPs of 8–37 nm induced severe disease in BALB/C mice after the AuNPs were injected intraperitoneally, while AuNPs of 3, 5, 50, and 100 nm did not exhibit deleterious effects.<sup>167</sup> The results may be related to urinary elimination and excretion since particles smaller than 5.5 nm can be removed rapidly and efficiently through urinary system from the body.<sup>168</sup>

Particle shape is equally thought to be an important factor in affecting AuNP toxicity. Comparative toxicity analysis among various shaped AuNPs has already been established. Nevertheless, opinions differ in the shape effect of nanoparticles on cells. In the view of Patibandla et al, AuNRs have more deleterious effects on zebrafish than spherical AuNPs.<sup>169</sup> They attributed the toxicity of AuNRs to CTAB coating, which is an essential but toxic surfactant for the synthesis of AuNRs.<sup>170</sup> Thus, the toxicity of AuNRs can be improved by coating them with alternative biocompatible materials, such as phosphatidylcholine and PEG,<sup>171,172</sup> underlining the impact of surface coating materials on toxicity. However, Tarantola et al pointed out that spherical AuNPs are more toxic than rod-shaped particles due to the larger surface area ratio of spherical particles and thus higher intracellular gold content.<sup>173</sup> In other studies, it was observed that non-spherical (star/flower-shaped) AuNPs had relatively stronger toxicity than



**Figure 11** Factors influencing the toxicity of AuNPs. Most factors are able to influence toxicity of AuNPs, including fundamental features of the particles (eg, particle size, shape, surface charge, and coating), experimental conditions (eg, cell and animal model tested, assessed duration), administration scheme (eg, administration route, dose, time and times) and so forth.

spherical AuNPs.<sup>174,175</sup> They attributed this outcome to the larger specific surface area presented by non-spherical AuNPs than spherical AuNPs. Higher is the internalization, more is the harmful substances carried into cells and severer is the cell damage. However, in another study, spherical and rod-shaped nanoparticles were observed to be more toxic than star-, flower- and prism-shaped AuNPs.<sup>176</sup>

It is equally important to consider the impact of surface charge on the toxicity of AuNPs. It was reported that positively charged particles were more toxic than negative or neutral counterparts.<sup>177,178</sup> The toxicity of cationic AuNPs may be as a result of the presence of electrostatic interaction of positive NPs with a negative cell surface, thereby increasing cellular uptake or membrane disruption. The outcome is either beneficial or unintended, which depends on the research purpose and concrete application. Cho et al provided a different example of the effect of cationic AuNPs on two different cells. They found that a positively charged AuNP-dendron conjugate (PCD-AuNP) is no toxic towards two cells used even at the highest dose tested (40  $\mu\text{g}/\text{mL}$ ), but there is a visible attachment of the PCD-AuNPs on the monkey kidney Vero cell surface.<sup>179</sup> The specific mechanism requires to be further researched and elucidated.

Furthermore, Bahamonde et al found that mice and rats responded differently to PEGylated AuNPs exposure, where mice experienced robust macrophage response and no fatality was seen, whereas abundant rats suddenly died within hours of administration.<sup>180</sup> The results indicated AuNPs have species-specific differences in terms of toxicity, even amongst closely related groups. Ginzburg et al observed a synergistic increase in toxicity of PEG-stabilized AuNPs in the presence of the surfactant, while the components separately were no toxic.<sup>181</sup> These results demonstrated the importance of identifying strategies for choosing safe NP/additive combinations. Additionally, Schwartz et al reported that 10-nm

AuNPs had potential nephrotoxicity when were co-administrated with cisplatin, paraquat and 5-aminosalicylic acid due to their interactions with drugs, whereas AuNPs themselves were not hepatotoxic or nephrotoxic when alone administered.<sup>182</sup> It is very clear that the dose at which particles are administered is critical for toxicity, since many drugs that are beneficial at low doses may exhibit harm at high doses. The administration route and assessed duration are also crucially important for toxicity.<sup>183–185</sup> However, these conditions are diverse in different studies, and they are not always fully reported in the literature. Therefore, it is suggested that authors reported the employed conditions in as much detail as possible, which helps readers to comparatively analyze.

Although plentiful researchers have been attempting to figure out the toxicity profile of AuNPs *in vivo*, it is still difficult to draw consistent and meaningful conclusions from these reports owing to the variations in many parameters and conditions.<sup>186</sup> As a result, the contradictory results were obtained in different studies with different experimental designs. Moreover, many studies merely considered toxicity evaluation for particles, without involving the possible toxicity mechanisms.<sup>187</sup> Thereby there is an urgent demand for deeper studies to reveal the potential toxicity mechanism of AuNPs *in vivo*, not only at the cellular level but also at the molecular level. Furthermore, most results are built on short-term observation data, and long-term data are relatively scarce. Thus further long-term *in vivo* investigations should be implemented for more representative data. Lastly, no standardized method that is universally appropriate for toxicity testing of various types of AuNPs to date is also a matter of concern.<sup>188</sup> The lack of a unified detection method and evaluation standard leads to the presence of conflicting results and varying interpretations. Therefore, it is recommended to establish uniform standards and methods for toxicological testing of nanoparticles, to facilitate comparisons of data among different studies and clinical translation of AuNPs.

Last but not least, it is worth considering the trade-off between underlying benefit and cost of production when designing new multifunctional formulations. The addition of new functionality, such as targeting and imaging contrast enhancement not only means an increase in patient survival time and available clinical benefit, but also additional cost and complexity of synthesis and purification.<sup>189</sup> The production and conjugation of some ligands for targeting is greatly expensive and inefficient.<sup>190</sup> Even though the cost related to adding imaging contrast materials may be relatively lower than adding targeting ligands, the costs would be likely to be shrunk partly and the benefits more profound, if the nanomaterial itself had both therapeutic and imaging abilities, such as AuNPs.<sup>189</sup> Hence, the functionality of the materials themselves and design cost are also major concerns when constructing novel formulations.

## Conclusion and Outlooks

Cancer is still a largely complex and serious disease. In addition to exploiting new therapeutic drugs, taking full advantage of available chemotherapy drugs is another feasible strategy. At present, the use of nanotechnology has had a crucial impact on many fields of science, including medicine, biology, physics and chemistry. Due to unique pathophysiological phenomenon of solid tumors and unique physicochemical properties of AuNPs, the use of AuNP-based delivery systems provided the potential for improved cancer treatment and diagnosis. In previous studies, AuNPs have been used as delivery carriers for various agents, especially cytotoxic drugs, unstable nucleic acid drugs, and hydrophobic/hydrophilic photosensitizers for PDT. In addition, AuNPs have been used for cancer phototherapy, such as PTT by using their light absorption properties. To complement their respective disadvantages and further improve the efficacy of cancer treatment, the AuNPs have been developed as a promising nanoplatform that integrates multiple treatment functionalities, like chemotherapy/PTT, PTT/PDT, PTT/RT, chemotherapy/PTT/PDT, and RT/PTT/PDT. In preclinical research, AuNPs have also been used as biological imaging contrast agents, particularly CT imaging, which helps clinicians recognize tumor conditions and choose applicable therapeutic strategies.

In addition to positive preclinical research, a series of AuNP-based nanoproducts (NCT00356980, NCT00848042, NCT01270139, NCT01246336, NCT02755870, NCT03020017, etc) have been used in clinical trials. These results achieved in the clinical studies are mostly positive and very encouraging. However, most of them are limited to early phase I or phase I clinical studies, and to date, no AuNP-based products have actually entered the late phase of the clinical process or have been successfully on the market. Some clinical trials are recruiting, withdrawn, and even terminated. Compared to other nano-formulations, like liposomes and polymeric micelles, the number of AuNP-based

products in clinical trials is still relatively small. When basic preclinical studies are being conducted, more studies are required to devote to pushing basic experimental studies of AuNPs towards clinicalization.

Although the results achieved from many studies are very encouraging, there are several critical issues that deserve to be taken seriously into account. One important problem is that toxicity of AuNPs requires to be addressed properly. In spite of chemical inertness, gold is, after all, a noble metal and has inherently chemical toxicity to a certain degree. Introducing functional moieties such as stabilizing materials and biocompatible materials seems to reduce toxicity of AuNP core to some extent, but it is worth noting that a number of surface modifications may also cause unwanted side effects. Except for the nanoparticle core, some toxicity may be attributed to these modifying materials. Further research should be required to quantify the trade-off between treatment/diagnosis benefit and toxicity after functional modifications. It is equally important to consider whether and how the functional substances affect the biodistribution and consequent side effects. Similarly, if we want to avoid the plague of immunogenicity *in vivo*, surface modification technology requires to be further optimized. Another possible effect on biosafety is biodistribution of AuNPs, because AuNPs can accumulate in the spleen and liver, and other sites, hence showing toxic effects in these organs. There is need for further knowledge to understand the biodistribution profile of AuNPs.

Moreover, molecular interactions between gold nanoparticles with targets were scarcely reported. Such interactions include not only therapeutic/diagnostic effects but also toxicological aspects. A full understanding of nanoparticle-target interactions and potential mechanisms is greatly important in nanotechnology for improved cancer management. Furthermore, the specific mechanism that AuNPs are internalized by target cells also needs to be fully studied in diversified cell models. A comprehensive understanding of the intake mechanism is crucial for improved intracellular drug amount and eventual therapeutic effect. Although most of the current literature attributes the internalization mechanism of nanoparticles to endocytosis, not all AuNPs are suitable for this case. In agreement with the toxicity, the uptake mechanism of AuNPs by cells may depend on a variety of factors, such as particle size, shape, surface charge, coating, cell model tested and so on. However, so far there is no unified conclusion and explanation about the internalization mechanism of AuNPs in the academic circle.

For biomedical applications, especially in clinical practice, a requirement for the production of AuNPs with long-time stability is essential. Possible aggregation of AuNPs during preparation and use processes may pose further threats to the lives of patients. To avoid this, improved and reproducible manufacturing technologies are indispensable for large-scale production of AuNPs with high stability. In addition, there is still a need for adequately cost-effective AuNP-based systems. Adding additional functions means not only the increase in clinical benefit, but also elevated cost and complexity of synthesis and purification. The high cost places a financial burden on the cancer patients themselves and their families. Hence, in future work, more studies should be performed to develop newly budget-friendly functional AuNP-based products.

Taking all aspects into account, there is still a long way and there is more work to be done before AuNP-based products are successfully on the market. Nevertheless, existing evidence on potential of nanogold in cancer research field allows us to believe that we can develop more efficient and accurate cancer therapies based on AuNPs in the future. We also believe that, in the future, AuNP-based systems will play a more important role in early tumor diagnosis and tumor treatment in different stages, with the hope of circumventing the common drawbacks of currently available tumor therapies in clinic.

## Abbreviations

<sup>1</sup>O<sub>2</sub>, singlet oxygen; 5-ALA, 5-aminolevulinic acid; 5-FU, 5-fluorouracil; AAH, gold nanoparticles modified with AS1411 and DNA; AAHD, gold nanoparticles modified with AS1411 and DNA loading with doxorubicin; anti-EGFR D-11, monoclonal antibody D-11 against epidermal growth factor receptor; APHD, gold nanoparticles modified with DNA loading with doxorubicin; Au<sub>25</sub>(Capt)<sub>18</sub>, captopril-stabilized Au nanoclusters; AuNCs, gold nanocages; AuNPs, gold nanoparticles; AuNRs, gold nanorods; BSA, bovine serum albumin; C225, cetuximab; CALNN, Cys-Ala-Leu-Asn-Asn; CD24, cluster of differentiation 24; COVID-19, Coronavirus Disease 2019; CT, computed tomography; CTAB, cetyltrimethylammonium bromide; CW, continuous wave; Cys, cysteamine; DOX, doxorubicin; dsDNA, double-stranded DNA; DTX, docetaxel; EPR, enhanced permeability and retention; FA, folic acid; FDA, Food and Drug Administration;

FOXMI Apt, Forkhead box M1; Gal, galactose; GBM, Glioblastoma; GC, gastric cancer; GFLGC, Gly-Phe-Leu-Gly-Cys; GNP, gold nanoparticles; GSH, glutathione; HA, hyaluronic acid; HSF-1, heat shock factor-1; hv, irradiation with light; Hv, irradiation with light; IC50, half-inhibitory concentration; ICG, indocyanine green; IVUS, intravascular ultrasound; K, kaempferol; LIN, linalool; mAbs, monoclonal antibodies; MB, methylene blue; MDR, multidrug resistance; mPEG@HAuNPs, PEGylated hollow AuNPs; mPEG-SH, thiol-terminated monomethoxy polyethylene glycol; MPH, mercaptopropionylhydrazide; MRI, magnetic resonance imaging; mTHPC, meso-tetrahydroxyphenylchlorin; MTX, methotrexate; NIR, near-infrared; NPs, nanoparticles; NS, nanoshell; O<sub>2</sub>, oxygen; P1, Boc-L<sup>D</sup>P-L-OMe; PAH, pulmonary arterial hypertension; PBS, phosphate buffer solution; Pc 4, phthalocyanine 4; PCD-AuNP, AuNP-dendron conjugate; PDT, photodynamic therapy; PEC, pectin; PEG, polyethylene glycol; PepGNP, gold nanoparticle mounted with peptides; P-gp, P-glycoprotein; PpIX, protoporphyrin IX; PS, photosensitizer; PTT, photothermal therapy; RGD, Arg-Gly-Asp; rhTNF $\alpha$ , recombinant human tumor necrosis factor alpha; ROS, oxygen species; RT, radiotherapy; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SOSG, singlet oxygen sensor green; SPR, surface plasma resonance; ssDNA, single-stranded DNA; Tf, transferrin; Tf<sub>pep</sub>, transferrin; Tmab, trastuzumab; TNF, tumor necrosis factor; TS265, CoCl(H<sub>2</sub>O) (phendione)<sub>2</sub>[BF<sub>4</sub>]; ZnD, [Zn(DION)<sub>2</sub>]Cl<sub>2</sub>.

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## Disclosure

The authors report no conflicts of interest in this work.

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