

Gold nanoparticle-based theranostics: disease diagnostics and treatment using a single nanomaterial

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Abstract: Nanotheranostics takes advantage of nanotechnology-based systems in order to diagnose and treat a specific disease. This approach is particularly relevant for personalized medicine, allowing the detection of a disease at an early stage, to direct a suitable therapy toward the target tissue based on the molecular profile of the altered phenotype, subsequently facilitating disease monitoring and following treatment. A tailored strategy also enables to reduce the off-target effects associated with universal treatments and improve the safety profile of a given treatment. The unique optical properties of gold nanoparticles, their ease of surface modification, and high surface-to-volume ratio have made them central players in this area. By combining imaging, targeting, and therapeutic agents in a single vehicle, these nanoconjugates are (ought to be) an important tool in the clinics. In this review, the multifunctionality of gold nanoparticles as theranostics agents will be highlighted, as well as the requirements before the translation of these nanoplateforms into routine clinical practice.

Keywords: nanotheranostics, nanomaterials, gold nanoparticle, theranostics, cancer, nanomedicine

Introduction

Theranostics is aimed toward the development of platforms capable of combining diagnostic and therapeutic agents.^{1,2} These innovative systems combined into single platforms should help overcome the troublesome differences in biodistribution and specificity of imaging molecules and therapeutic drugs.^{3,4} Theranostic approaches have been proposed for the management of several diseases/disorders, in particular for cancer diagnostics and therapeutics. Cancer therapy greatly relies on chemotherapy and radiotherapy in which most anticancer drugs are essentially taken up by cells with high proliferative rate, a characteristic of cancer cells. However, extensive proliferation is not exclusive for tumor cells, and normal tissue also suffers from chemotherapeutic action, leading to severe side effects.⁵⁻⁷ The efficacy of conventional therapies is also often limited by the natural tendency of cells to evade therapy through mutational events that lead to multidrug resistance, poor drug penetration into tumor tissues, and serious damage to normal tissues.⁸⁻¹⁰

Conventional therapy toward heterogeneous diseases is rarely targeted directly toward a specific cell type or tissue. In fact, the administration of a specific treatment to a subpopulation of patients in accordance to their molecular profile and risk of adverse effects is believed to be a more effective clinical model for improved patient outcome. By selecting patients for targeted clinical trials, based on biomarker

identification, personalized medicine is expected to reduce health costs caused by unnecessary treatment.

To overcome these issues, alternative strategies have been proposed in which nanomedicine plays a key role, such as for innovative drug delivery systems that increase the bioavailability and concentration of anticancer drugs at target site, resulting in improved therapeutic index.^{6,7,11} Combination of diagnostic and therapeutic entities into a single delivery vector using nanotechnology – nanotheranostics – constitutes a step forward for tailored early disease detection, medical treatment, and disease prevention.^{12,13} These nanoformulations must ascertain whether an individual carries a specific disease biomarker and release the appropriate therapeutic dose in a kinetically regulated manner. The design of such a system ought to include the possibility of targeted delivery^{14,15} (to minimize off-target accumulation and, therefore, minimize side effects), and the release of the imaging/therapeutic molecule should be precisely triggered in the specific cellular environment.^{16,17} The developed platform should allow a combination of different effector molecules (eg, stabilizer, therapeutic agent, targeting and imaging moieties) for maximization of theranostic effect.^{18,19}

Here, we shall address options for surface functionalization of gold nanoparticles (AuNPs) toward therapy (section “Surface functionalization of AuNPs toward therapy”), and focus on the use of AuNPs as theranostic agents, ie, imaging purposes (section “AuNPs for imaging purposes”) and therapy, eg, from drug and gene delivery vehicles to photothermal ablation agents (section “Gold nanoparticles: from conventional drug and gene delivery vehicles to photothermal ablation agents”). Current trends relating to the translation of theranostic nanomaterials from the laboratory to the clinics shall be discussed in section “Theranostic nanomaterials: from the laboratory to the clinics?”.

Several nanotheranostics platforms have been presented over the past decade, namely, metal and silica nanoparticles (NPs), liposomes, and composite NPs (Table 1).^{6,11,20,21} These platforms allow visualization and monitoring of the route taken by the formulation, providing information about delivery kinetics, intra-organ and/or intratumor distribution, and drug efficacy. Using a single NP, it is possible to tune therapy while simultaneously providing for real-time monitoring of disease progression.^{1,22} Traditional methodologies such as X-ray computed tomography (CT) and magnetic resonance imaging (MRI) do not display sufficient sensitivity to diagnose the asymptomatic phases of a disease,^{23,24} and owing to current levels of sensitivity, numerous patients entail extended unnecessary treatments. A theranostic strategy might allow treatment termination once the biomarker levels are undetectable.

AuNPs have great potential to be explored as a multifunctional tool for a wide range of biological applications.^{25–27} Their unique optical properties make them excellent non-invasive imaging agents, adding to their high surface-to-volume ratio that allows them to be loaded with a plethora of molecules, such as targeting/imaging moieties and therapeutic agents (Figure 1). By integrating targeting, diagnostics, treatment, and monitoring, AuNPs overcome several constraints associated with conventional methods of diagnostics and treatment, ensuring the codelivery of imaging and therapeutic molecules at the same site, with real-time monitoring of their effects.^{14,18,28} Also, targeted molecular imaging and delivery of the therapeutic moiety, with enhanced retention at the target sites, allow reduction of the nanoconjugate concentrations in circulation and therefore reduce systemic toxicity.^{19,29}

AuNPs may work as a multimodal imaging tool by loading them with two or more contrast agents (fluorophores, MRI agents, photoacoustic agents, surface-enhanced Raman spectroscopy [SERS] reporters) to improve disease characterization and avoiding a repeated challenge on the patient immune system.³⁰ This is also valid for therapeutic agents: one can take advantage of the synergistic effects of different treatments (effective drug, photothermal therapy, and/or antisense DNA) by combining them onto a single AuNP.

This review will focus on nanotheranostics using a single agent, addressing specifically AuNPs, the need for this new model, and its advantages over conventional methodologies. A long way has been paved since the first theranostic agent – radiopharmaceutical iodine – was used for thyroid disease in the 1940s.³¹ We are now facing clinical trials of AuNPs for treatment of solid tumors: Aurimmune® (PEGylated colloidal gold particles; Phase I) (CytImmune Sciences, Rockville, MD, USA) and AuroLase® (gold nanoshell; Phase I) (Nanospectra Biosciences, Inc., Houston, TX, USA). We will also address some bottlenecks that still need to be circumvented before the most promising agents can be effectively translated to the clinical practice, eg, in vivo efficacy, stability, and toxicity.

Surface functionalization of AuNPs toward therapy

The chemical functionality and reactivity of AuNPs may be customized through surface functionalization, which modulates the solubility, stability, charge effect of the nanocomplex, and interaction with other molecules.^{14,32–36} One of the most used approaches has been the use of thiolated biomolecules to easily functionalize the particle surface.^{33,37} Such plasticity allows the modification and design of the AuNPs’

Table I Advantages and disadvantages of different nanotheranostics platforms

Type of nanoparticle	Advantages	Disadvantages
Mesoporous silica NPs	Large surface area Stability Biocompatibility Controllable porosity Surface reactivity and ease of functionalization Biodegradability	In vivo toxicity
Magnetic NPs	Large surface area Small size allows longer circulation and tissue penetration Controlled clustering Multimodal applications (targeting, diagnostics, and therapy)	Poor colloidal stability Limited biocompatibility and cytotoxicity Nonbiodegradability
Gold NPs	Large surface area Low hydrodynamic mean size Multimodal applications (targeting, diagnostics, and therapy) Suitable for photodynamic therapy Scaffold for additional agents Ease of surface modification Stability and biocompatibility	High costs for large-scale production Lack of standard protocols for translation into the clinics Nonbiodegradability
Liposomes	Flexibility of synthesis, modification, and formulation Ease of conjugation and functionalization with targeting, contrast agents, probes, and fluorophores Rapid cellular uptake and well-characterized cell internalization mechanism Controlled release Scaffold for additional agents Low immunogenicity Biocompatibility Commercially available products	Low drug-loading efficiency Batch-to-batch variation in manufacturing High production cost Limited instability and leakage of loaded materials Low solubility Rapid clearance
Dendrimers	Very precise size and shape controllability Water solubility and biocompatibility Elicit negligible immune response Easy electrostatic interaction with nucleic acids	Nonspecific cytotoxicity Limited release of the associated bioactives Rapid clearance
Hydrogels	Selective surface modification High degree of porosity and high loading capacity Controlled and sustained release into target tissues Biocompatibility and biodegradability	High production cost Instability
Quantum dots	High photo- and chemical stability Size- and structure-based tunable emission High molar extinction coefficient Low hydrodynamic mean size Scaffold for additional agents Potential for synergistic application in diagnostics and therapeutic applications. Suitable for photodynamic therapy	Toxicity Difficult surface modification (thiol groups) Particle aggregation, degradation, and removal
Carbon nanotubes	Large surface area Ultrahigh functionalization and loading capacities High penetration capacity to biological barriers Scaffold for additional agents Imaging probe on its own with high spatial resolution Suitable for photodynamic therapy	Nonbiodegradable Toxicity (oxidative stress) Lack of a standard protocol to prepare high-purity carbon nanotubes in large scale

Note: Adapted with permission from Draz M, Fang B, Zhang P, et al. Nanoparticle-mediated systemic delivery of siRNA for treatment of cancers and viral infections. *Theranostics*. 2014;4(9):1023–1030.¹²

Abbreviation: NPs, nanoparticles.

surface chemistry, thus granting control over the circulation half-time and systemic clearance of these structures in the body,³⁸ and allows the modification and tailoring of bioavailability, immunogenicity, and preferential accumulation site.³⁹ There is a wide range of stabilizers available, such as thiolated

biomolecules, polymers, dendrimers, phosphine, that may simultaneously act as therapeutic moieties (eg, microRNA, DNA, peptides, and antibodies). Polyethylene glycol (PEG) is one of the most widely used stabilizer molecules able to increase AuNPs' circulation half-life and cellular uptake, with

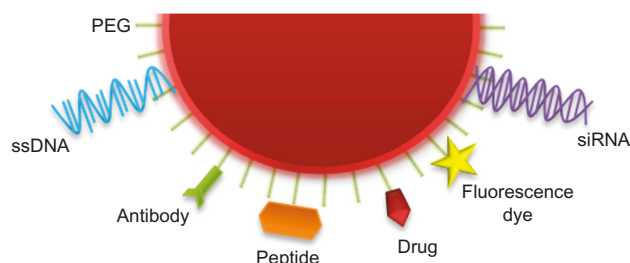


Figure 1 Representation of an AuNP for theranostics.

Note: AuNPs loaded with a plethora of moieties allow AuNPs to act as molecular sensors for disease diagnostics, as therapeutic agents by themselves due to their photothermal conversion capacity, and also as vehicles for delivering other effective therapeutics and imaging agents to specific locations.

Abbreviations: AuNP, gold nanoparticle; PEG, polyethylene glycol.

reduced immunogenicity.⁴⁰ Bifunctional PEGs with a thiol molecule in one extremity and a different functional group on the other extremity (eg, amine, carboxylic, biotin, azide) have also been used to allow further functionalization with other biomolecules (Table 2).

The surface of an AuNP can also be modified simply using ionic coupling methods, in which the ligand exhibits an opposite charge to that of the AuNP or of the capping agent.^{41,42} Ionic coupling procedures are quicker and do not require preactivation of molecules but originate more unstable constructs, and control over the biomolecule orientation is hindered.⁴³ The widely known biotin–streptavidin interaction can also be used for the surface modification of AuNPs. Although noncovalent, the functionalization of the AuNP with avidin or biotin usually requires a covalent immobilization of these molecules (either by direct functionalization using a thiolated molecule or through EDC coupling). Hydrophobic interactions, while not being covalent interactions, usually require a covalent attachment using a thiol coupling.⁴⁴

Considering the ease of surface modification, coupled with their optical properties, AuNPs are good candidates for the development of theranostics systems. For instance,

doxorubicin (DOX) can be loaded to PEG-coated hollow gold nanospheres (HAuNS) both inside and outside HAuNS, increasing the DOX payload compared to solid AuNPs. Using near-infrared (NIR) laser, DOX is released in the region of interest, inducing cancer cell death.⁴⁵ Nanorods functionalized with DOX may be further conjugated to a cRGD peptide for tumor targeting and pH-sensitive controlled drug release.⁴⁶ Increased cytotoxicity of PEG-AuNPs functionalized with oxaliplatin has been demonstrated, when compared to free oxaliplatin, in A549 lung epithelial cancer cells and several colon cancer cell lines (HCT116, HCT15, HT29, and RKO), possibly due to enhanced cellular uptake.⁴⁷

Gold nanoparticles as theranostic agents

Smartly designed NPs have already made their way to the clinics, eg, Doxil® (Janssen Biotech Inc, Horsham, PA, USA), Abraxane® (Celgene Corporation, Berkeley Heights, NJ, USA) – therapeutic application; Resovis® (Bayer Schering Pharma AG, Berlin, Germany) and Feridex® (Bayer Healthcare Pharmaceuticals, Wayne, NJ, USA) – imaging agents, but the development of an equivalent able to combine both strategies is still underway. Indeed, several platforms have already been proposed integrating therapeutic strategies (ie, chemo-, genetic-, immunotherapy, photothermal ablation) and imaging agents to monitor NP fate, eg, MRI, CT, and photoacoustic tomography (PAT).^{48–50}

Nanomaterials, such as AuNPs, exhibit remarkably different properties from the bulk material. Owing to the spatial confinement of the conduction electrons, unique optical properties arise (localized surface plasmon resonance [LSPR]) with strong impact on sensing and therapeutic applications.^{51–54} The intensity and frequency at which the LSPR occurs may be tailored through the variation of the metal composition, size, shape, and the dielectric surrounding.^{55,56} The light extinction induced by the LSPR

Table 2 Common covalent immobilization strategies for AuNP functionalization

Coupling reaction	Functional group at AuNP surface	Functional group on ligand	Linkers/spacers	Examples of biomolecules coupled
Thiol	Direct coupling	Thiol	–	Thiolated DNA ^{28,64} Thiolated PEG ¹¹⁶ Thiolated fluorophores
EDC/NHS	Carboxylic/phosphate or amine	Amine or carboxylic/phosphate	Bifunctional PEG/DNA chain harboring a thiol and a(n) amine/carboxylic group	Proteins ¹¹⁷ Antibodies ¹⁴ Fluorophores ¹¹⁸
Maleimide	Thiol/maleimide	Maleimide/thiol	Homo-bifunctional thiolated PEG or hetero-bifunctional PEG harboring a maleimide	Peptides ¹¹⁹ DNA ¹²⁰ Chemotherapeutic agents ¹²¹

Abbreviations: AuNP, gold nanoparticle; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; NHS, N-hydroxysuccinimide; PEG, polyethylene glycol.

comprises both absorption and scattering processes.⁵⁷ The scattering component of the extinction band is proportional to the particle size, while the absorption component is inversely proportional to the particle size.⁵³ AuNPs' spectral behavior may also be explored in other regions of the spectrum. When an AuNP absorbs light, it can decay (in a nonradiative manner) to a lower energy level through the generation of heat. This particular phenomenon can be applied in photothermal therapy. Not only the LSPR is highly tunable, but also its extinction coefficient for laser absorption can be five orders of magnitude higher than those of conventional organic dyes used in photothermal procedures.⁵³ Moreover, AuNPs are known to be resistant to photobleaching.⁵⁸

Although in an early stage of technological development, AuNPs-based diagnostic and therapeutic approaches have already been proposed to carry a drug or imaging agents directly targeted to a specific tissue and/or cell type. Generally, particulates between 10 nm and 60 nm in diameter tend to passively accumulate in tumor tissues due to the enhanced permeability and retention (EPR).⁵⁹ EPR occurs due to changes to tumors' endothelia, where the leaky vasculature allows extravasation of NPs to the extracellular milieu and decreased lymphatic drainage results in local accumulation. Yet, although NPs tend to naturally accumulate in tumors, functionalization allows proper recognition of selective cellular target – active targeting.

AuNPs for imaging purposes

AuNPs are extremely versatile since they may act as an imaging probe on their own and/or be easily functionalized with suitable imaging agent, overcoming some of the technique's limitations. In fact, the idea of using multiple modalities has become popular, and recently, hybrid imaging instruments have become available, with the first to appear being a fusion of positron emission tomography (PET)/CT instrument, followed by PET/MRI, and, more recently, ultrasound/CT. These techniques may clearly benefit from multimodality, since a single agent could avoid the administration of multiple doses.^{30,60} The choice of imaging modality must be carefully considered since each one has its own advantages and limitations – eg, modalities with the highest sensitivity have relatively poor resolution.⁶⁰ Improvement in these systems might allow the observation of tissues not only on its basic anatomic configuration but also on the molecular level. Moreover, real-time noninvasive monitoring of theranostic NPs enables a rapid decision on whether the treatment regimen is effective in a given patient. Herein, the focus shall be

on AuNPs, and for further insights into other systems, please refer to additional reading.^{61–63}

AuNPs as fluorescence modulators

AuNPs and other noble metal NPs are known to modulate fluorescence of fluorophores in their vicinity,^{64–66} including quenching due to energy transfer or charge transfer. As the distance between a NP and a fluorophore increases, quenching by the NP decreases. Modulation of fluorescence has been used for the development of biosensing approaches and tracking of nanoformulations in living systems. One such example suitable for theranostics is the so-called gold nanobeacons (Au nanobeacons), ie, AuNPs functionalized with an effector ssDNA in a hairpin configuration tagged with a suitable fluorophore.^{14,28} Upon hybridization to the specific target (eg, mRNA or miRNA), the hairpin opens, quenching by the AuNP ceases to exert its influence, and fluorescence is observed, thus allowing to track the silencing effect within cells and tissues. This simple nanotheranostics platform may be easily applied to any RNA sequence (Figure 2).

Traditional fluorophore quenching may be substituted by SERS.⁶⁷ In fact, noble metal NPs, such as gold and silver, are known to promote SERS of adsorbed dyes on NP surfaces, which has been widely used for biosensing in complex media.^{68,69}

These optical properties (high extinction coefficient with possibility of heat generation or scattered light, fluorescence modulation, and SERS) may be explored in a wide range of theranostics applications, which may also profit from the ease of AuNP surface modification with no observable toxicity.¹⁹

Computed tomography and magnetic resonance imaging

CT is often the first approach for the detection of the region of pathology within tissues and organs. As for MRI, CT does not have the needed sensitivity to target molecular events or even to diagnose abnormal cellular events, which require contrast agents, such as gadolinium (Gd) in MRI and iodine contrast in CT.^{70,71} Although routinely used in the clinics, iodinated and Gd-based (to a lower extent) contrast agents exhibit side effects, namely, CT-associated life-threatening contrast-induced nephropathy and MRI-associated nephrogenic systemic fibrosis.⁷¹ For instance, contrast enhancement in CT is usually based on low-molecular-weight compounds that show rapid clearance, where AuNPs appear as an interesting alternative.^{70,72–74} In fact, acetylated dendrimer-entrapped gold nanoparticles (AuDENPs) were used for

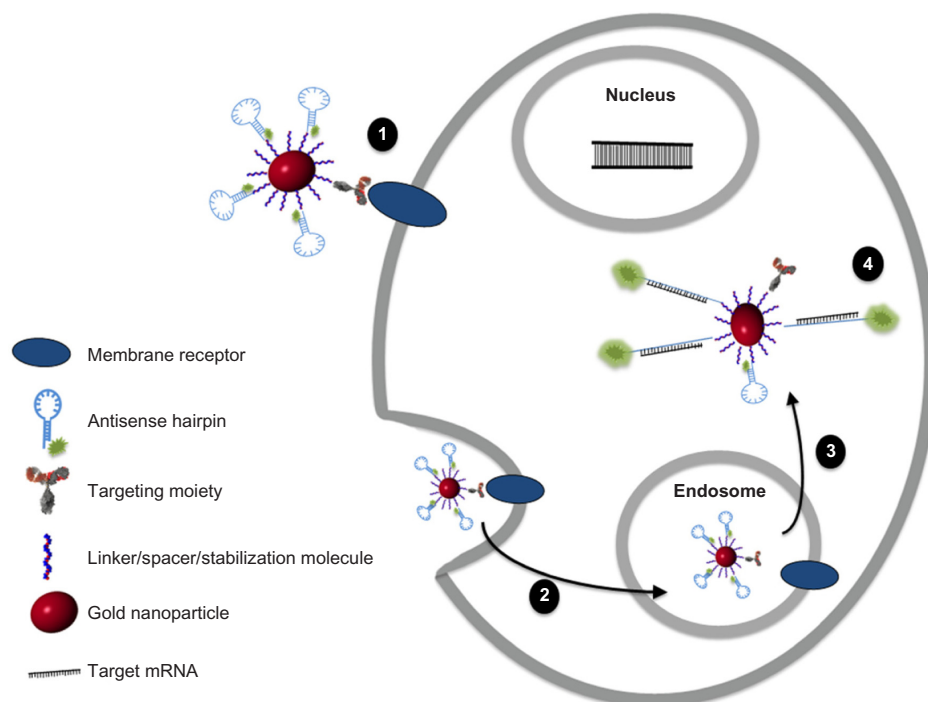


Figure 2 Au nanobeacons as a theranostic approach for specific gene silencing with real-time monitoring of the inhibition process.

Notes: 1) Target cell recognition, 2) Au nanobeacon internalization, 3) endosome escape, 4) gene silencing with concomitant enhancement of fluorophore emission.

Abbreviation: Au, gold.

in vitro and in vivo CT imaging of a human lung adenocarcinoma cell line (SPC-A1 cells). Intratumoral and intraperitoneal administration of acetylated AuDENPs allowed the detection of cancer cells under X-ray in xenograft tumor models. In vitro assays show that acetylated AuDENPs are biocompatible and do not affect cell viability, making them good CT contrast agents.⁷⁵

Effective examples of AuNPs for MRI analysis include an innovative vector for improving MRI contrast agents currently used for clinical diagnosis.^{72,76} Moreover, Gd-loaded AuDENPs can be used for dual-mode CT/MRI.⁷⁷ For instance, Alric et al⁷² functionalized AuNPs with diethylenetriaminepentaacetic acid (DTPA) to chelate Gd ions (Gd^{3+}). A total of 50 Gd ions per particle increases the system's stability, allowing the application as contrast agent for both MRI and X-ray CT in vivo. Owing to their size, these nanoconjugates can also be used as sensitizers for radiotherapy.⁷² Similarly, nanoconjugates formed by silica layer-coated gold nanorods to which folic acid (FA) was covalently anchored (AuNR-SiO₂-FA) were shown to be selectively taken up by MGC803 cells, subsequently enhancing their sensitivity to X-ray radiation. This way, these particulates may become a good candidate for X-ray CT imaging associated to tumor targeting. These same nanoconjugates could be eventually used for photothermal therapy.⁴⁹

Another example is the Co@Pt-AuNP platform that presents strong magnetism and high stability, resulting in a good MRI contrast agent to monitor the progression of A β protofibrils involved in Alzheimer's.⁷⁸

Photoacoustic tomography

AuNPs may also be used in PAT, a noninvasive modality combining high sensitivity of optical imaging with high resolution of ultrasound imaging.⁷⁹ Contrast agents for PAT are photoabsorbing agents that, when excited at their resonance wavelength, convert light into heat (photothermal conversion) correlated to the generation of acoustic waves (due to thermoelastic expansion), which can then be detected by an ultrasound transducer.⁸⁰ PAT has been slowly overcoming the limited resolutions of techniques dependent solely on ultrasonic diffraction. When used in the NIR of the spectrum, PAT is not constrained by the optical diffusion limit, which allows the reconstruction of images with improved spatial resolution, deep penetration depth, and excellent image contrast when compared to conventional optical imaging.⁸¹ Gold nanorods, due to their cylindrical shape, present characteristic SPR toward the NIR, and are of extreme use for PAT.^{82,83} PAT molecular imaging in deep tissue has also been improved by using reporter-gene products.^{84–86} Recently, Taruttis et al⁸⁷ demonstrated real-time imaging of siRNA in

liposome–AuNR hybrids within tumors through multispectral optoacoustic tomography, an upgrade of PAT.

Gold nanoparticles: from conventional drug and gene delivery vehicles to photothermal ablation agents

AuNPs' high surface area makes them proper systems for efficient vectorization of high payloads of drugs,⁴⁵ including siRNAs or microRNAs.^{18,28,88} A wide range of molecules may be used for active targeting toward effective nanotheranostics, from hormones to cytokines, antibodies, and peptides (Table 3).

Another efficient way to reduce side effects is via systems that release their payload in a unique environment, as response to either internal or external stimuli – smart agents (triggered release). These systems may respond to changes in the pH or even to specific protease activity within the tumor surroundings.^{89,90} However, owing to AuNPs' intrinsic characteristics, light and heat have been the most commonly explored actuator of drug release.^{91,92} As mentioned earlier, when AuNPs absorb light, it can be dissipated as heat, which may be used for the controlled release of drugs functionalized or adsorbed to the AuNPs' surface.⁹³ In addition, the photothermal effect might increase blood perfusion in the tumor region, further increasing the delivery of therapeutics.⁹⁴ AuNPs' shape and size can be tuned at will (eg, nanospheres, nanorods, nanocages, or nanoshells), whose spectral behavior is directly correlated with a shift in the LSPR to the NIR region (650–900 nm), thus allowing deeper tissue penetration of light. Indeed, AuNP-mediated hyperthermia shows particular promise in animal studies, and early clinical testing is currently underway.⁹⁵

Combination of imaging platforms with hyperthermia is currently being developed in which a single particle is used to image tumors and later accurately and efficiently ablate them. Yushen et al developed a microcapsule of AuNP-poly(lactic acid) with surface deposition of graphene oxide. The assembly was used for in vivo NIR photothermal therapy, suitable as contrast agent for both ultrasound and CT imaging. Together, AuNPs and graphene oxide coating allowed improved photothermal conversion, with the final outcome being total tumor remission due to the performed laser ablation treatment.⁷³ However, the challenge of using gold nanorods in this type of therapy is the observation that after an intense and prolonged period under laser illumination, gold nanorods might lose stability, being reshaped to gold nanospheres, thus losing response to NIR frequencies.⁹⁶

Although gold nanorods tend to be considered the “gold standard” in photothermal therapy, colloidal gold nanospheres have more frequently been used as theranostic agents. Their main disadvantage is the absorbance peak, situated in the visible region, whereas the low toxicity of nanospheres is one of the biggest advantages, since geometry plays a considerable role in acute cell toxicity. Hyperthermia success of AuNPs is determined by several factors, namely, sufficient depth penetration (up to 1 cm in the NIR) and low energy absorption by healthy tissues (to reduce collateral damage). For example, Melancon et al conjugated hollow gold nanoshells (HAuNS) displaying an average diameter of 30 nm with cetuximab (CET), an antiepidermal growth factor receptor (EGFR) therapeutic antibody. CET-HAuNS selectively bound to cells overexpressing EGFR (A431 cells) that, upon irradiation with NIR laser, led to elevation of temperature, inducing cell death by photothermal ablation. Moreover, labeling of HAuNS with gamma emitter.⁹⁷ In allowed monitoring tissue distribution of CET-HAuNS in vivo, revealing increased uptake in tumors expressing EGFR.⁹¹

SERS has also been used for tracking these multimodal NPs.⁶⁹ Of particular interest is the work presented by Song et al⁹⁸ in which an amphiphilic AuNP was coated with a Raman reporter BGLA (2-(4-(bis(4-(diethylamino)phenyl)(hydroxymethyl)phenoxy)ethyl 5-(1,2-dithio-lan-3-yl)pentanoate). The particle was made stealth by PEG incorporation and pH-sensitive (due to PMMAVP grafts – hydrophobic copolymer of methyl methacrylate [MMA] and 4-vinylpyridine [4VP]). DOX was also included in this intricate system. Incorporation of HER2 antibody provided for specific targeting. Cellular binding, uptake, and intraorganelle disruption of the SERS-encoded pH-sensitive plasmonic vesicles were assessed.⁹⁸ This unique system is an example of a promising platform for targeted theranostic applications of AuNPs.

Theranostic nanomaterials: from the laboratory to the clinics?

One of the first agents developed as a simultaneous targeting moiety and therapeutic agent was Herceptin® (trastuzumab; Hoffman-La Roche Ltd, Basel, Switzerland), a humanized antibody capable of targeting and blocking the overexpression of HER2 protein used for the treatment of HER2-positive metastatic breast cancer.^{99,100} Similar approaches have followed, but most failed to deliver due to stand-alone limitations of these agents.¹⁰¹ Such failures have increased the costs of R&D without corresponding delivery to the market. As seen earlier, recent advances in nanotechnology have paved the way for the development of theranostics

Table 3 Types of theranostic agents using AuNPs: their sizes, detection method, and therapeutic effect

Nanoconjugate	Therapeutic agent	Type of nanoparticle	Particle size	Imaging	Therapy	Short description	References
PTX@PEG-AuNP	Paclitaxel	Nanospheres	20–40 nm	Fluorescence (Rhodamine B)	Drug delivery	PEG@AuNPs, PTX, and rhodamine B linked beta-cyclodextrin for theranostics in vitro. PTX is released using intracellular glutathione.	50
DOX-PSMA-AuNP	Doxorubicin	Nanospheres	65 nm	X-ray CT	Chemotherapy	RNA aptamers bind PSMA, allowing specific imaging of prostate cancer cells expressing PSMA.	122
Au@DTDTPA-Gd	–	Nanospheres	2.4 nm	MRI, X-ray CT	Radiotherapy	Easy detection of Au@DTDTPA-Gd by MRI and X-ray CT; radiosensitizer for radiotherapy.	72
Au@PLA-(PAH/GO) ₂	–	Spherical microcapsules	1.5 µm	US, X-ray CT	PTT	Contrast agent to simultaneously perform US and X-ray CT, both in vivo and in vitro.	73
CET-HAuNS	Cetuximab	Hollow nanospheres	35 nm	PET, PAT	Photothermal ablation	Excellent colloidal stability, high absorption coefficient and efficient photothermal effect in NIR.	91,97
AuNS	–	Nanoshell microcapsules	2.3 µm	US	PTT	AuNS can induce photothermal ablation of HeLa cells via exposure in NIR.	123
DOX@PEG-HAuNS	Doxorubicin	Hollow nanospheres	52 nm	PET, optical imaging MRI	Thermal ablation, drug release	DOX released by photothermal conversion.	45,91
SPIO@AuNS	–	Nanoshell	80–90 nm		PTT	Superparamagnetic iron oxide-silica core with Au nanoshell for MRI (T2-weighted images).	91,124,125
siRNA-HAuNS	siRNA	Hollow nanospheres	40 nm	PET, PAT	PTT, radiotherapy	siRNA released by NIR irradiation through photothermal therapy.	91,126
AuNS@Fe ₃ O ₄ @SiO ₂ -ICG	HER2	Nanoshell	Silica core: 60 nm Au shell: 74 nm Fe ₃ O ₄ nanoparticles: 10 nm	Optical imaging, MRI	PTT	ICG on AuNS@Fe ₃ O ₄ @SiO ₂ increases fluorescence of ICG. Conjugation with anti-HER2 targeted breast cancer cells.	127
Anti-HER2-AuNS@SiO ₂	HER2	Nanoshell	Silica layer: 10 nm Silica core: 120 nm	OCT	PTT	Photothermal therapy induces cell death via nanoshell absorption of NIR light.	128
Anti-EGFR-AuNS	EGFR	Hollow nanoshell	Au shell thickness: 5–35 nm	Optical imaging: TPL	PTT	Conjugation with anti-EGFR for targeting of cancer cells; cell death by photothermal therapy.	95,129
Anti-EGFR-AuNR	–	Nanorods	50 nm × 13 nm (length/diameter)	X-ray CT	PTT	High X-ray absorption and spectral bandwidth. Single iv to destroy human xenograft tumor in mice.	27
PEG-AuNR	–	Nanorods	47 nm × 13 nm (length/diameter)			Gene silencing.	82
siRNA-AuNR	siRNA	Nanorods	72 nm × 35 nm (length/diameter)	Light scattering	Gene therapy	Higher cytotoxicity compared to free cisplatin.	130
Cisplatin@AuNR	Cisplatin	Nanorods	29.1 nm × 8.8 nm (length/diameter)	TEM	Thermal ablation, D	Target cancer cells; drug release by hyperthermia promotes thermo-chemotherapy.	82,131
DOX@AuNR	Doxorubicin, folate receptor	Nanorods	50 nm × 10 nm (length/diameter)	Cy3 luminescence	Thermo-chemotherapy, hyperthermia	In vitro and in vivo application of CET-PEG-AuNR to NIR absorption imaging and photothermal therapy for epithelial carcinoma.	132
CET-PEG-AuNR	Cetuximab	Nanorods	Ratio (longitudinal/transverse): 4.0	Optical imaging	PTT		

AuNR-DOX-cRGD	Doxorubicin	Nanorods	45 nm × 10 nm (length/diameter)	PET, optical imaging, CT	Chemo- and photothermal therapy	High cellular uptake; combined cancer therapies and multimodality imaging.	46
AuNR@SiO ₂ -DOX	Doxorubicin	Nanorods	60 nm × 15 nm (length/diameter) Silica shell: 30 nm Mesopores: 5 nm	TPL	Thermo-chemotherapy, hyperthermia	DOX loaded in mesopores and released by low-power-density laser; hyperthermia induced by high-power-density laser.	133
AuNR-SiO ₂ -FA	Folate receptor	Nanorods	46 nm × 18 nm (length/width)	X-ray CT	PTT, radiotherapy	Folic acid promotes selective targeting, enhanced therapy, and strong X-ray attenuation.	49
Fe ₃ O ₄ @SiO ₂ @AuNR@mSiO ₂ -FA	Folate receptor	Nanorods	Silica layer: 8 nm SPION core: 90 nm Silica layer: 10 nm Mesoporous silica shell: 5 nm Size of composite: 200 nm Ratio: 2 (length/width)	MRI	PTT	Increased cellular uptake in KB cells and hyperthermia-induced cytotoxicity.	48
MMP-AuNR	MMP substrate peptide	Nanorods		Cy5.5 fluorescence	PTT	Cy5.5-MMP substrate is attached to AuNRs. MMP enzyme, produced by cancer cells, degrades MMP substrate and Cy5.5 produces fluorescence.	134

Abbreviations: AuNRs, gold nanoparticles; AuNR-SiO₂, silica layer-coated gold nanorod; CET, cetuximab; CT, computed tomography; D, drug delivery; DTDTPA, diethylenetriaminepentaacetic acid; DOX, doxorubicin; EGFR, epidermal growth factor receptor; FA, folic acid; HAuNS, hollow gold nanospheres; MMP, matrix metalloproteinase; MRI, magnetic resonance imaging; NIR, near-infrared; PAT, photoacoustic tomography; OCT, optical coherence tomography; PEG, polyethylene glycol; PET, positron emission tomography; PTT, photothermal therapy; PSMA, prostate-specific membrane antigen; PTX, paclitaxel; SPIO, superparamagnetic iron oxide; TEM, transmission electron microscopy; TPL, two-photon luminescence; US, ultrasound.

agents for a sustained, controlled, and targeted delivery of therapeutics coupled with the capability to follow in real-time distribution to tissues and organs, thus allowing therapy evaluation.⁶³ These systems have been designed keeping in mind the selectivity for the most appropriate and effective therapy with fewer side effects.^{102,103} Despite the cumbersome task at hand, several nanosystems have been put forward for approval by the US Food and Drug Administration (FDA). Nanomedicines to make it to the market (worth US\$33 billion) include Doxil® (Janssen Biotech) (Caelyx® in Europe), Myocet® (Sopherion Therapeutics Inc, Princeton, NJ, USA), DaunoXome® (Galen US Inc, Souderton, PA, USA), DepoCyt® (Pacira Pharmaceuticals Inc, San Diego, CA, USA), Abraxane® (Celgene Corporation), Genexol-PM® (Samyang Biopharmaceuticals Corporation, Jongno-gu, Seoul, Korea), and Oncaspar® (Enzon Pharmaceuticals Inc, Bridgewater, NJ, USA).¹⁰⁴

The overall nanomedicine market was evaluated in US\$53 billion (2009) and is expected to be worth a staggering \$100 billion in 2014.¹⁰⁵ Several other FDA-approved nanomedicines have since reached the market, the last one to be Marqibo® (Spectrum Pharmaceuticals, Irvine, CA, USA) (FDA approval in 2012), which consists of a vincristine sulfate liposome injection for treatment of adult patients with Philadelphia chromosome-negative (Ph⁻) acute lymphoblastic leukemia in second or greater relapse or patients whose disease has still progressed despite two or more antileukemia therapies. Magnetic NPs are also making their way to clinical trials, and some formulations have been clinically approved for therapeutic applications and imaging (eg, Feridex® IV and Endorem® [Bayer Healthcare Pharmaceuticals] for liver and spleen imaging, Lumiren® (Guerbet SA, Villepinte, France) and Gastromark® (Advanced Magnetix Inc, Rochester, IN, USA) for bowel imaging).¹⁰⁶ NanoTherm® (Magforce AG, Berlin, Germany), licenced in Europe since 2010 for the treatment of brain tumors, is an iron oxide NP administered to provide hyperthermia treatment localized to a tumor, sparing adjacent healthy tissue.¹⁰⁷ A recent report shows that 247 nanomedicine products are approved or are in various stages of clinical study.¹⁰⁸ Several more nanomedicines are to receive regulatory approval, but not all nanomedicines are available to the consumer.¹⁰⁹ Almost 200 patents regarding NP-based drug delivery systems were registered between 2009 and 2012 in the World Intellectual Property Organization (WIPO), clearly indicating that these nanosystems may actually compete with conventional pharmaceuticals in the future.¹¹⁰

The ultimate goal in the R&D of a nanomedicine is its successful translation from bench to bedside. As mentioned

earlier, there are still significant challenges, such as 1) scalability, 2) batch-to-batch reproducibility, 3) lack of complete information concerning the interaction between nanotheranostics and tissues (in vivo – eg, internalization and biodistribution), 4) effective increase of therapeutic indexes, 5) reluctance of the pharmaceutical industry to invest in nanotheranostics, 6) regulatory issues and safety guidelines from authorities and agencies (eg, FDA and European Medicines Agency [EMA]) that have to be addressed before nanotheranostics exert their full impact on the clinics.¹⁰⁶ The health benefits of nanotheranostics structures depend on their reproducibility and mostly differentiation from available products concerning improved efficacy, safety, and pharmacokinetics.^{106,111} Among these issues, research still needs to fully address nanotoxicology, in particular effects arising from the chronic administration.¹¹² Furthermore, nanomedicine regulation still presents gaps, such as standardization, safety, and risk guidelines. Several key points need to be addressed in the near future before widespread use of nanomaterials, and nanotheranostics in particular: reference nanomaterials; uniformity of standards for and/or working definitions of nanomaterials; improvement to the current definitions of nanomaterial, for the purpose of regulation (namely, the difference between nanomaterial and nanoscale material); development of toxicology tests and physicochemical characterization studies for nanomaterials. These issues are key to ensure that nanomedicines and nanotheranostics platforms do not face commercialization bottlenecks due to lack of standards and parameters that support rational preclinical characterization.¹¹² Nevertheless, FDA, in accordance with the Nanotechnology Characterization Laboratory (NCL), is gathering relevant information for the resolutions of some of the issues mentioned earlier. And EMA is ready to follow suit. By the conduction of transitional studies that will help nanomedicine developers to bring their academic work to the market, NCL gained the knowhow on which approaches are most effective in characterizing the properties of nanomedicines. For instance, NCL established more than 40 protocols to characterize NPs' physicochemical properties, in vitro immunogenicity, and cytotoxicity, together with the standard absorption, distribution, metabolism, excretion, and toxicity profiles, using animal models.^{113,114} Most of the reports available in the literature fail to deliver data on in vivo evaluation of nanomedicines and of nanotheranostic materials in particular. Existing data refer either to therapeutics or to diagnostics, leaving theranostics outside the scope of detailed testing.^{3,102,115}

Final remarks

The use of nanomaterials for biosensing and diagnostics is making its way into the clinics, and industry is grasping the novelty and the amazing performance provided by these platforms and strategies to boost their market position. In what therapeutics is concerned, nanomedicines are entering a decisive phase of clinical trials, and plenty of nanoformulations and radical strategies and delivery platforms will not make through the strenuous process of validation and certification before settling into the clinical setting. What is more, these new nanoscale formulations have been strongly focused on anticancer therapeutics, where the risk-to-gain ratio clearly favors the development and assessment of nanomaterials.

Nanotheranostics is following these two vectors of development very close, taking advantage of hurdles and obstacles removed by the isolated counterparts. One should note that nanotheranostics is more than the addition of parts; a synergy between diagnostics and therapeutics is in place that could not be attained via the isolated effect of either strategy. Perhaps because of this great advantage, validation and characterization are even more complex and are still waiting for suitable tools to ascertain the real value in the clinics. Nanotheranostics is moving forward fast and focused, and one ought to be ready to make the best of these powerful tools in the coming years.

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Disclosure

The authors report no conflicts of interest in this work.

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