Chapter 11
Multifunctional Gold Nanocarriers for Cancer Theranostics: From Bench to Bedside and Back Again?

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11.1 Introduction

The National Cancer Institute predicts that over the next years, nanotechnology will result in important advances in early detection, molecular imaging, targeted and multifunctional therapeutics, prevention and control of cancer (National Cancer Institute 2010). Nanotechnology offers numerous tools to diagnose and treat cancer, such as new imaging agents, multifunctional devices capable of overcoming biological barriers to deliver therapeutic agents directly to cells and tissues involved in cancer growth and metastasis, and devices capable of predicting molecular changes to prevent action against precancerous cells (Baptista 2009).

Nanoparticle-based delivery systems in Theranostics (Diagnostics & Therapy) provide better penetration of therapeutic and diagnostic substances within the body

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at a reduced risk in comparison with conventional therapies (Ma et al. 2011; Praetorius and Mandal 2007). Limitations in medical practice are intimately associated with the fact that diagnostics, therapy, and therapy guidance are mostly separate from each other. It is here that theranostics unites the three stages in one single process, supporting early-stage diagnosis and treatment, overcoming some of the sensitivity and specificity of current medicines (Lammers et al. 2010, 2011; Pene et al. 2009). At the present time, there is a growing need to enhance the capability of theranostics procedures where nanoparticle-based sensors may provide for the simultaneous detection of several gene-associated conditions and nanodevices with the ability to monitor real-time drug action. These innovative multifunctional nanocarriers for cancer theranostics may allow the development of diagnostics systems such as colorimetric and immunoassays, and in therapy approaches through gene therapy, drug delivery, and tumor targeting systems (Fig. 11.1).

Nowadays the main challenge is to develop a system for molecular therapy capable of circulating in the blood stream undetected by the immune system and capable to recognize the desirable target and signal it for effective drug delivery
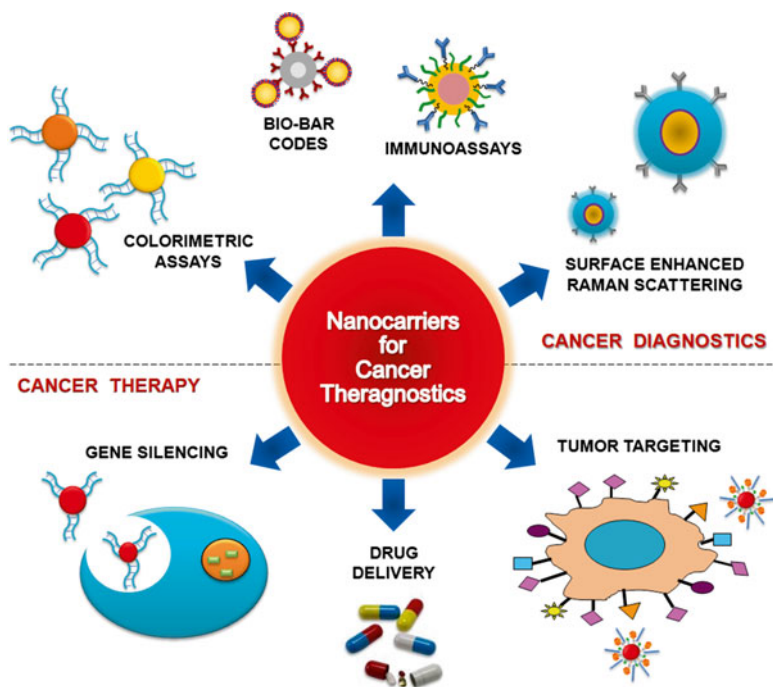

Fig. 11.1 Nanocarriers for cancer theranostics. Nanoparticles-based strategies can be used for biosensing using plasmonic nanosensors for colorimetric assays and bio-barcodes for protein detection or intense labels for immunoassays. Moreover, the use of metal surfaces to enhance the Raman scattering signal of target molecules may be used for cancer diagnostics. Engineered nanocarriers can also act as therapeutic agents via gene silencing and drug delivery systems. Some nanocarriers can be attached to specific targets for selective damage to cancer cells through tumor targeting approaches

or gene silencing. As a result, nanotechnology is playing a role in providing new types of nanotherapeutics for cancer that have the potential to provide effective therapies with minimal side effects and with high specificity (Heath and Davis 2008). The interdisciplinary and vibrant field of nanotechnology continues giving us hope of a personalized medicine as a part of cancer patient management. Gold nanoparticles (AuNPs) are one of those nanosystems that provide non-toxic carriers for drug and gene delivery applications (Nishiyama 2007; Ghosh et al. 2008a). They are versatile agents with a variety of biomedical applications including use in highly sensitive diagnostic assays (Goodman et al. 2004), thermal ablation, and radiotherapy enhancement (Hirsch et al. 2003; Hainfeld et al. 2004, 2008), as well as drug and gene delivery (Hong et al. 2006; Thomas and Klibanov 2003).

The unique characteristics of AuNPs in the nanometer range, such as high surface-to-volume ratio or size-dependent optical properties, are drastically different from those of their bulk materials and hold pledge in the clinical field for disease therapeutics (Kim 2007; Heath and Davis 2008). Nanoparticles (NPs) exhibiting these unique and broad-based optical properties, ease of synthesis and facile surface chemistry and functionalization, and appropriate size scale are generating much eagerness in clinical diagnostics and therapy. The most common applications in which gold nanocarriers have been used so far are labeling, delivering, heating, sensing, and detection (Sperling et al. 2008).

In spite of these advantages, nanoparticles show some limitations, such as their small size and large surface area can lead to particle–particle aggregation and may result in limited loading of functional components and burst release. In fact, only NPs with the appropriate size (and surface chemistry) will not be immediately recognized by our immune system and committed to removal from the organism, thus showing increased circulation times. Also, size and surface properties play an important role to avoid clearance (Sperling and Parak 2010). For example, hydrophilic nanoparticles with an effective size in the range of 10 to 100 nm are small enough to slow down activation of the mononuclear phagocyte system and are big enough to avoid renal filtration (Gil and Parak 2008). Nanoparticles with unique and broad-based optical properties, ease of synthesis and facile surface chemistry and functionalization, and appropriate size scale are generating much eagerness in biotechnology and biomedicine with particular emphasis in clinical diagnostics and therapy (Doria et al. 2012). However for the biological application of these multifunctional NPs, their functionalization with one or several biomolecules such as DNA/RNA, oligonucleotides (i.e., ssDNA/RNA, dsDNA/RNA), peptides and antibodies, fluorescent dyes, polymers, drugs, tumoral markers, enzymes, and other proteins that will introduce several functionalities and moieties is necessary. In the end the conjugation strategy is directly dependent on a numbers of factors, namely the NPs size, surface chemistry and shape, as well as the type of ligands and functional groups one desires to exploit in the functionalization (Sperling and Parak 2010).

When referring to cancer therapy, targeting and localized delivery are of utmost importance to enhance the therapeutic effect and decrease the adverse distribution to

healthy organs and tissues. Multifunctional gold nanocarriers may potentiate the development of individualized cancer therapy based on the individual's biological information within the tumor (i.e., biomolecular profiling). Gold nanocarriers can be modified with multiple cell-targeting and membrane translocating peptides, loaded with DNA/RNA and used as nanovectors (Gil and Parak 2008; Peer et al. 2007).

In this chapter, we will focus on the exciting new methods and applications of AuNPs for cancer diagnosis and therapy with particular emphasis on their use in vivo and their potential to be translated into clinical settings.

11.2   Nanodiagnostics

Nanodiagnostics can be defined as the use of nano-sized materials, devices, or systems for diagnostics purposes (Doria et al. 2007). It is a promising field as more and improved techniques are becoming available for clinical diagnostics with increased sensitivity at lower costs (Baptista et al. 2005, 2006, 2008; Doria et al. 2007).

The use of the colloidal gold color change upon aggregation is the best characterized example for diagnostic systems using AuNPs. In fact, AuNPs functionalized with ssDNA capable of specifically hybridizing to a complementary target for the detection of specific nucleic acid sequences in biological samples have been extensively used (Li and Rothberg 2004; Mirkin et al. 1996; Thaxton et al. 2006; Cheng et al. 2006; Baptista et al. 2008; Doria et al. 2007; Taton et al. 2000; Qin and Yung 2007; Sato et al. 2003, 2005; Elghanian et al. 1997).

The use of thiol-linked ssDNA-modified gold nanoparticles for the colorimetric detection of gene targets represents an inexpensive and easy to perform alternative to fluorescence or radioactivity-based assays (Storhoff et al. 2004). In 1996, *Mirkin* et al. (1996) described the use of a cross-linking method that relies on the detection of single-stranded oligonucleotide targets using two different Au-nanoprobes, each of them functionalized with a DNA-oligonucleotide complementary to one half of the given target. A mixture of gold nanoparticles with surface-immobilized non-complementary DNA sequences appears red in color and has a strong absorbance at 520 nm. When a complementary DNA sequence is added, the nanoparticles are reversibly aggregated causing a red shift in the surface plasmon absorbance to 574 nm, and the solution becomes purple in color. Consequently, these AuNPs reversibly assemble by the formation of the DNA duplex, linking the particles together. These pioneer works gave the research community the idea of an easy method to sensitize oligonucleotide–AuNP conjugates, with intense and highly tunable optical properties, ease of conjugation through the gold–thiol bond, catalytic properties, and relative biocompatibility (Thaxton et al. 2006; Cao et al. 2005).

On the other hand, in 2005 Baptista et al. introduced a non-cross linking method where thiol-linked DNA–gold nanoparticles were used in a novel colorimetric method to detect the presence of specific mRNA from a total RNA extract of yeast cells (Baptista et al. 2005). The method consists in visual and/or spectrophotometric

comparison of solutions before and after salt induced Au-nanoprobe aggregation (see Fig. 11.2)—the presence of a complementary target prevents aggregation and the solution remains red and has a strong absorbance at ±520 nm; non-complementary/mismatched targets do not prevent Au-nanoprobe aggregation, resulting in a visible change of color from red to blue and therefore a shift in the surface plasmon absorbance to 600–650 nm. This method has been successfully applied to detect eukaryotic gene expression without retro-transcription or PCR amplification steps (Conde et al. 2010b; Baptista et al. 2005); to distinguish fully complementary from mismatched sequences, with a single base mismatch, i.e., to detect common mutations within the β-globin gene (Doria et al. 2007); and in a fast and straightforward assay for *Mycobacterium tuberculosis* DNA detection in clinical samples (Costa et al. 2009; Baptista et al. 2006).

Other approaches are the use of AuNPs as a core/seed that can be tailored with a wide variety of surface functionalities to provide highly selective nanoprobes for diagnosis (You et al. 2007); the utilization of surface plasmon resonance (SPR) scattering imaging or SPR absorption spectroscopy generated from antibody conjugated AuNPs in molecular biosensor techniques for the diagnosis and investigation of oral epithelial living cancer cells in vivo and in vitro (El-Sayed et al. 2005a); the use of multifunctional AuNPs which incorporate both cytosolic delivery and targeting moieties on the same particle functioning as intracellular sensors to monitoring actin rearrangement in live fibroblasts (Kumar et al. 2007); and the employment of AuNPs in electrochemical based methods that can be coupled with metal deposition for signal enhancement (Castaneda et al. 2007).

Gold NPs have already proven to be one of the most important groups of nanomaterials for biosensing approaches. Highly sensitive and specific biosensors based on AuNPs have open up the possibility of creating new diagnostic platforms for disease markers, biological and infectious agents in the early-stage detection of disease and threats, especially in cancer (Conde et al. 2012b; Doria et al. 2012).

11.2.1 Gold Nanocarriers in Cancer Diagnosis

Cancer is the one of first leading causes of mortality in the modern world, with more than 10 million new cases every year (Hanahan and Weinberg 2000; Siegel et al. 2012). Some continue to argue that the search for the origin and treatment of this disease will continue through the next quarter of century, adding successive layers of complexity to an investigation that per se is very complex. In fact, advances in diagnosis and treatment of this disease that kills millions of people each year worldwide have not been as effective as for other chronic diseases, and only for some types of cancer there are effective methods of detection (Hanahan and Weinberg 2000). The high mortality rate in cancer is commonly attributed to the difficulties in detecting the disease at an early treatable stage. The main challenge is to find new and more effective diagnostic agents for the monitorization of predictive cell
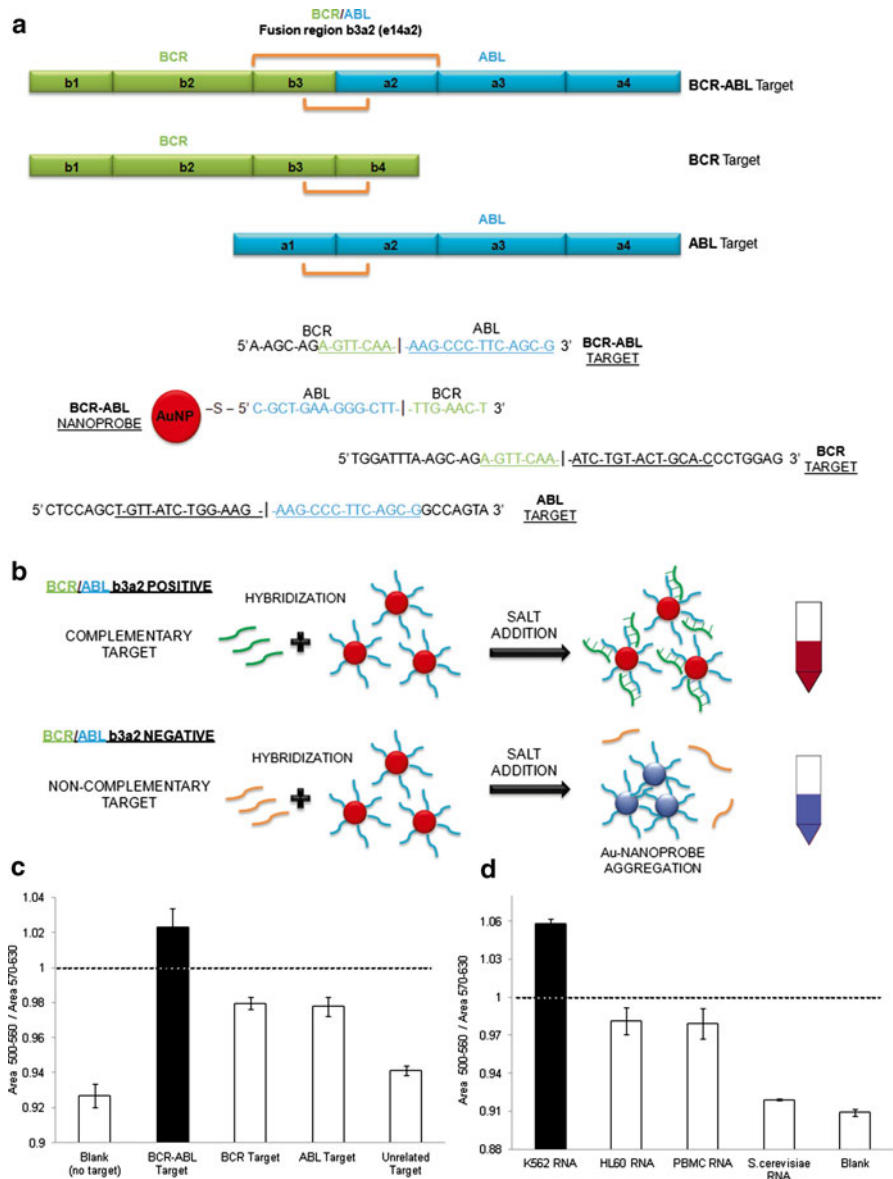
Fig. 11.2 The colorimetric assay. (**a**) Oligonucleotide probe and target sequences designed for BCR–ABL b3a2 (e14a2) junction and for BCR and ABL genes. Complementary and non-complementary target sequences were used to study the level of specific interaction between the target and the Au-nanoprobes. BCR–ABL fusion positive (100 % complementary); BCR and ABL gene sequences were used as controls (50 % non-complementary); and a completely unrelated sequence (100 % non-complementary) was used as negative control. (**b**) The assay is based on the increased stability of the Au-nanoprobes upon hybridization with the complementary target in solution, while non-hybridized Au-nanoprobes easily aggregate once the solution's ionic strength is increased. The absence of full complementarity is revealed by a change of color from *red* to *blue*

molecular changes that are involved in tumor development as the key to the efficient and ultimately triumphant treatment of cancer is early and accurate diagnosis (Etzioni et al. 2003).

It is here that nanotechnology enters the fight in the technological leap of controlling materials at nanoscale by offering a "big revolution" in new medical and healthcare diagnostic systems (Gil and Parak 2008). In fact, nanotechnology combined with biology and medicine is the most advanced technology both from an academic point-of-view and for commercial applications, producing major advances in molecular diagnostics and bioengineering (Giljohann et al. 2010; Salata 2004). AuNPs are one of those nanosystems that provide non-toxic carriers with a variety of biomedical applications including use in highly sensitive diagnostic assays (Conde et al. 2012b; Doria et al. 2012).

In reality the multiplexed marker protein assays are critical in the diagnosis of complex diseases like cancer. In fact, AuNP probes barcoded with reporter DNAs and a capture antibody have been extensively used with great promise (Stoeva et al. 2006; Son and Lee 2007). For instance, AuNP immunoassays are one of the most used nanosystems in cancer detection. Mirkin and co-workers developed an ultrasensitive method for detecting protein analytes. This facile immunoassay was used for the detection of prostate specific antigen (PSA, a valuable biomarker for prostate cancer screening) and had an almost one million-fold higher sensitivity than a conventional ELISA-based assay (Nam et al. 2003). This study was the first step to use 6 years later the AuNP bio-barcode assay probe for the detection of PSA in a clinical pilot study with 18 men who have undergone radical prostatectomy for prostate cancer. This new bio-barcode PSA assay is approximately 300 times more sensitive than commercial immunoassays (Thaxton et al. 2009).

Also, Huang et al. have reported a PSA immunoassay on a commercially available SPR biosensor. This sandwich assay with AuNPs was used for a major enhancement in sensitivity of PSA detection at clinically relevant concentrations (Huang et al. 2005). Other studies demonstrated a highly sensitive organic electrochemical transistor based immunosensor with secondary antibody-conjugated AuNPs with a low detection limit for PSA. These sensor performances were particularly improved in the lower concentration range where the detection is clinically important for the preoperative diagnosis and screening of prostate cancer (Huang et al. 2005).

Fig. 11.2 (continued) due to Au-nanoprobe aggregation which is corroborated by naked eye and UV/vis spectroscopy. BCR–ABL positive: sample in the presence of complementary target (solution remains *red*); BCR–ABL negative: sample in the presence of non-complementary target (solution turns *blue*). (**c**) Spectrophotometry relative to the detection of synthetic BCR–ABL oligonucleotide target. Oligonucleotides with BCR or ABL sequence only (showing 50 % complementarity) were used as controls and an unrelated target (showing 100 % non-complementarity to the Au-nanoprobe) as negative control. (**d**) Detection of BCR–ABL in total RNA from K562 cell line, HL-60 cell line, and human PBMC (harboring 50 % complementary targets to the nanoprobe) and *S. cerevisiae* cells (100 % non-complementary). Nanoprobe aggregation as measured by ratio of area under the curve AUC500 nm-560 nm/AUC570 nm-630 nm. The *dashed line* represents the threshold of 1 considered for discrimination between positive and negative

Additionally, AuNP probes coupled with dynamic light scattering (DLS) measurements can also be used for the development of a one-step homogeneous immunoassay for the detection of free-PSA. The light scattering intensity of nanoparticles and nanoparticle oligomers is several orders of magnitude higher than proteins, making it possible to detect nanoparticle probes in the low picomolar concentration range (Liu et al. 2008).

AuNPs scatter light intensely at or near their surface plasmon wavelength region and when coupled with DLS detection can be very useful for serum protein biomarker detection and analysis. Huo et al. reported the use of citrate-AuNP to absorb proteins from the serum and form a protein corona on the nanoparticle surface. The protein corona formation and the subsequent binding of antibody to the target proteins in the protein corona were detected by DLS. Using this simple assay, the authors discovered multiple molecular aberrations associated with prostate cancer from both mice and human blood serum samples (Huo et al. 2011, 2012).

Other authors have taken a different approach to addressing the use of AuNPs in cancer biomarker detection by employing the biological applications of antibody-conjugated AuNPs in several types of cancer, such as breast cancer (Ambrosi et al. 2010; Lu et al. 2010a), pancreatic adenocarcinoma (Eck et al. 2008), cervical cancer (Rahman et al. 2005), epithelial cancer (Yang et al. 2008), liver cancer (Lan et al. 2011), prostate cancer (Lukianova-Hleb et al. 2011), and oral cancer (El-Sayed et al. 2005a).

The mechanism of selectivity and all these immunoassay's response open up a new possibility of rapid, simple, clean, easy, economically very cheap, non-toxic, and reliable diagnosis of cancer. In fact, antibody-conjugated AuNPs are one of the most used nanosystems in cancer diagnostics, being useful in molecular biosensor techniques for the diagnosis and investigation of cancer cells in vivo and in vitro. The importance of these nanosystems can be demonstrated by a significant number of companies involved in the synthesis and applications of antibody-conjugated nanoparticles, such as Magnisense SAS, Diagnostic Biosensors, LLC, Alnis Biosciences, Inc., and Invitrogen Corp (Arruebo et al. 2009).

Direct detection of cancer cells using colorimetric assays with AuNPs has been extensively used due to their simplicity and versatility, among which those based on LSPR. LSPR is the collective oscillation of the electrons in the conduction band, which is usually in the visible region giving rise to the strong SPR absorption (Daniel and Astruc 2004). These AuNPs are commonly functionalized with biomolecules (e.g., DNA, RNA, peptides, enzymes) and capable of recognizing molecular events associated with cancer development down to femtomolar level with single base discrimination resolution (Kang et al. 2010; Li et al. 2005; Medley et al. 2008). Molecular nanodiagnostics applied to cancer may provide rapid and sensitive detection of cancer related molecular alterations, which would enable early detection even when those alterations occur only in a small percentage of cells. For instance, Conde et al. present an AuNPs based approach for the molecular recognition and quantification of the *BCR–ABL* fusion transcript, which is responsible for chronic myeloid leukemia (CML). This inexpensive and very easy to perform method allows quantification of unamplified total human RNA and specific detection of the

oncogene transcript. This assay may constitute a promising tool in early diagnosis of CML and could easily be extended to further target genes with proven involvement in cancer development (Conde et al. 2010b, 2012c). The sensitivity settled by the Au-nanoprobes allows differential gene expression from 10 ng/µl of total RNA and takes less than 30 min to complete after total RNA extraction, minimizing RNA degradation (see Fig. 11.2).

Also, aptamer-conjugated AuNPs have become a powerful tool for point of care diagnostics (Mukerjee et al. 2012). Most of the common aptamer–AuNP assays are able to differentiate between different types of target and control cells based on the aptamer used in the assay, indicating the wide applicability for cancer cell detection. For instance, Liu et al. (2009) reported the use of an aptamer–nanoparticle strip biosensor for the rapid, specific, sensitive, and low-cost detection of circulating cancer cells in human blood, showing great promise for in-field and point-of-care cancer diagnosis and therapy. In another study, Medley et al. have developed a colorimetric assay for the direct detection of diseased cells and thus capable of distinguishing between cancer cells and noncancerous cells. This assay combines the selectivity and affinity of aptamers and the spectroscopic advantages of AuNPs to allow for the sensitive detection of cancer cells with both the naked eye and based on absorbance measurements (Medley et al. 2008). Another important aspect correlated with disease state in cancer patients is the presence of circulating tumor cells in the bloodstream. In order to induce optical contrast in non-pigmented cancer cells, Viator et al. (2010) attached AuNPs to a prostate cancer cell line, using optical absorption to detect such cells in a photoacoustic flowmeter designed to find circulating tumor cells in blood samples.

Another application of AuNPs in cancer is their capability to target and provide in vivo tumor detection using surface-enhanced Raman scattering (SERS). SERS has led the way in terms of use of spectroscopic methods for signal enhancement by nanostructured metal surfaces towards in vivo tracking of biomolecules trafficking (Stiles et al. 2008; Wilson and Willets 2013). SERS has been extensively used for molecular/ion detection and bioimaging applications since it minimizes photoblinking or photobleaching from conventional fluorophores, decreases signal-to-noise ratio in complex in vitro and in vivo, and usually Raman reporters are stable and yield large optical enhancements (Alvarez-Puebla and Liz-Marzan 2010, 2012a, b; Samanta et al. 2011). AuNPs covered by Raman reporters have been used for SERS to detect cancer cells in vitro and tumors in vivo (Kong et al. 2012; Qian et al. 2008b). Actually, Lin et al. (2011) described the tremendous potential of using AuNP based-SERS to obtain blood serum biochemical information for non-invasive colorectal cancer detection. Raman reporters when combined with AuNPs can elicit an optical contrast to discriminate between cancerous and normal cells and their conjugation with antibodies allowed them to map the expression of relevant biomarkers for molecular imaging (Kah et al. 2007), as well as detect and characterized circulating tumor cells. These SERS nanoparticles constitute an important tool for clinical research once they can successfully identify circulating tumor cells in the peripheral blood of 19 patients with squamous cell carcinoma of the head and neck (Wang et al. 2011).

The papers discussed earlier report the development of nanoscale devices and platforms that can be used for improved biomarker detection, such as nucleic acids (DNA or RNA) or proteins. However, the development of molecular diagnosis of cancer and at the same time the selective delivery of a specific anticancer agent by joining diagnostics and therapy (theranostics) on a single nanodevice will most definitely revolutionize the way we manage cancer (Baptista 2012).

In fact, Conde et al. recently developed a highly sensitive probe for in vivo tumor recognition with the capacity to target specific cancer biomarkers such as epidermal growth factor receptors (EGFRs) on human cancer cells and xenograft tumor models. The authors used ~90 nm AuNPs capped by a Raman reporter, encapsulated and entrapped by larger polymers and an FDA antibody–drug conjugate—Cetuximab (Erbitux®). These smart SERS gold nanoantennas present a high Raman signal both in cancer cells and in mice bearing xenograft tumors, and the Raman detection signal is accomplished simultaneously by extensive tumor growth inhibition in mice. This approach seems to be an innovative and efficient theranostics system for both tumor detection and tumor cell inhibition at the same time (Conde et al. 2014a).

Table 11.1 summarizes the latest types of AuNP-based biosensors for cancer diagnostics, according to their methodology principle. Some of the described nanosystems will most likely revolutionize our understanding of biological mechanisms and push forward the clinical practice through their integration in future diagnostics platforms. Nevertheless, very little gave successful results or went to clinical trials. Therefore new synthesis, fabrication, and characterization methods are needed for developing highly advanced AuNPs capable of use in sensitive and multiple detection methods with negligible toxicity and high sensitivity. In the future, it might be possible to apply all AuNPs properties together and evolve new chemistry for synthesis of smart materials for diagnostic applications and clinical trials.

11.3   Nanotherapy

In medical terms, a therapeutic effect is a consequence of a medical treatment of any kind, the results of which are judged to be desirable and beneficial. Conventional therapy methods in cancer involve the employment of anticancer agents that do not greatly differentiate between cancerous and normal cells (Minelli et al. 2010). Efficient in vivo targeting to heterogeneous population of cancer cells and tissue still requires better selectivity and decreased toxicity to surrounding normal cells, towards a decrease of systemic toxicity, adverse and severe side effects (Liu et al. 2007).

In another way, universally targeting cells within a tumor is not always feasible because some drugs cannot diffuse efficiently and the random nature of the approach makes it difficult to control the process and may induce multiple-drug resistance (MDR)—a situation where chemotherapy treatments fail patients owing to resistance of cancer cells towards one or more drugs (Peer et al. 2007). Consequently, nanotechnology could offer a less invasive alternative, enhancing the life expectancy and quality of life of the patient (Cuenca et al. 2006).

Table 11.1 Summary of the latest AuNP-based biosensors used in cancer diagnostics according to the type of NP, surface modification, type of cancer, and explored methodology principle

Method	Type of NP	Surface modification	Target/cells/samples	Type of cancer	Comments (reference)
Colorimetric Scanometric	40 nm spherical	dA-tailed probe applied to the strip, which contains oligo(dT)-conjugated AuNPs in dry form	Fusion genes in K562 cell line	Acute and chronic leukemia	Dry-reagent, disposable, dipstick test for molecular screening of seven chromosomal translocations associated with acute and chronic leukemia (Kalogianni et al. 2007)
	13 nm spherical	ssDNA	BCR–ABL b3a2 (e14a2) fusion transcript	Chronic myeloid leukemia	Detection and quantification of the BCR–ABL gene fusion using thiol-DNA modified AuNPs (Conde et al. 2010b)
	30 nm spherical	Thiol-terminated DNA barcodes; anti-PSA antibodies	Prostate-specific antigen (PSA) (biomarker for prostate cancer screening)	Prostate cancer	Nanoparticle based bio-barcode for PSA detection (Thaxton et al. 2009)
	15 nm spherical	Antibody anti-CA15-3–HRP (horseradish peroxidase)	CA15-3 breast cancer biomarker in human serum	Breast cancer	Enhanced AuNP based ELISA for a breast cancer biomarker detection (Ambrosi et al. 2010)
	Oval-shaped	Monoclonal anti-HER2/c-ErbB-2 antibody; S6 RNA aptamer-conjugated	SKBR-3 cells	Breast cancer	Colorimetric and highly sensitive two-photon scattering assay for highly selective and sensitive detection of breast cancer (Lu et al. 2010a)
Immuno-assays	15 nm spherical	Dithiol-PEG-COOH; F19 monoclonal antibodies	Tissues from cancerous and healthy human pancreas (patients undergoing pancreatic resection)	Pancreatic adenocarcinoma	Pancreatic adenocarcinoma (Eck et al. 2008)
	25 nm spherical	Anti-EGFR and non-specific IgG antibodies	SiHa cervical cancer cells	Cervical cancer	Optical imaging of cervical pre-cancers using AuNPs and CdSe QDs for reflectance and fluorescence imaging (Rahman et al. 2005)
	10 nm spherical	Antibody (Cetuximab)	Epidermal growth factor receptor (EGFR) in A431 cells	Liver cancer	High-performance probes based on AuNPs for detection of live cancer cell (Yang et al. 2008)
	16 nm spherical	NHS-PEG; mouse anti-human AFP antibodies (antibody-1 and antibody-2)	Liver cancer biomarker alpha-fetoprotein (AFP)	Liver cancer	One-step homogeneous detection of cancer marker using antibody–AuNP probes (Lan et al. 2011)
	60 nm spherical	PSMA (prostate specific membrane antigen); C225 (Erbitux, the antibody raised against human EGF receptor)	Living bone metastatic prostate cancer (C4-2B); human bone marrow stromal (HS-5) cells	Prostate cancer	Tunable plasmonic nanoprobes for theranostics of prostate cancer (Lukianova-Hleb et al. 2011)
	35 nm spherical	Monoclonal anti-epidermal growth factor receptor (anti-EGFR)	Nonmalignant epithelial cells (HaCaT); malignant oral epithelial cells (HOC 313 clone 8 and HSC 3)	Oral cancer	Surface plasmon resonance scattering and absorption of antibody–AuNPs in oral cancer diagnostics (El-Sayed et al. 2005a)

(continued)

Table 11.1 (continued)

Method	Type of NP	Surface modification	Target/cells/samples	Type of cancer	Comments (reference)
Surface-enhanced Raman scattering (SERS)	30 nm spherical	Mouse anti-human free PSA clone PSA-F65 and clone PSA-66	Prostate-specific antigen (PSA) in human serum	Prostate cancer	Detection of PSA with an immunoassay based on SERS and immunogold labels (Grubisha et al. 2003)
	43 nm spherical	Human serum	Human serum	Colorectal cancer	AuNP based-SERS to obtain blood serum biochemical information for non-invasive colorectal cancer detection (Lin et al. 2011)
	60 nm spherical	QSY reporter molecules; thiol-PEG-COOH; epidermal growth factor (EGF) peptide	Epidermal growth factor (EGF)	Squamous cell carcinoma of the head and neck	SERS AuNPs identified circulating tumor cells in the peripheral blood of cancer patients (Wang et al. 2011)
	35–50 nm spherical	Thiol-DNA hairpin tagged with a Raman label	BRCA1 SNPs	Breast cancer	Plasmonic nanoprobes for detection of SNPs in breast cancer BRCA1 gene (Wabuyele et al. 2010)
Electrochemical	5 nm spherical	Glutathione (GSH); primary antibodies for human interleukin-8	Interleukin-8 (IL–8) cancer biomarker in human serum	Cancer biomarker	Ultrasensitive immunosensor based on a glutathione-protected AuNP sensor surface (Munge et al. 2011)
	4 nm spherical	Antibodies for IL–6; biotinylated secondary antibody with 16–18 horseradish peroxidase labels	Interleukin-6 (IL–6) cancer biomarker in calf serum	Cancer biomarker	Inkjet printed AuNP electrochemical arrays for immunodetection of a cancer biomarker protein (Jensen et al. 2011)
	13 nm spherical	Alkaline phosphatase; poly(styrene-co-acrylic acid); TNF-α antibody	Tumor necrosis factor α (TNF-α) in human serum	Tumor necrosis factor	Electrochemical immunosensor of tumor necrosis factor based on alkaline phosphatase functionalized NPs (Yin et al. 2011)

At the moment, it is expected that the greatest gains in therapeutic selectivity will be achieved by synergistic combinations of several multicomponent targeting strategies. Currently, it is essential to develop technology for target and delivery of multiple therapeutic agents and for the simultaneous capability of avoiding biological and biophysical barriers. For example, nanoparticles can extravasate into the tumor stroma through the fenestrations of the angiogenic vasculature, demonstrating targeting by enhanced permeation and retention. These particles are able to carry multiple antibodies, which further target them to epitopes on cancer cells, and direct antitumor action, leading to cell death. Irradiation might be used to activate the nanoparticles and set up the release of their cytotoxic action (Ferrari 2005).

Due to advances in nanobiotechnology, the potential therapeutic application of gold nanocarriers represents an attractive platform for cancer therapy and has been investigated by different coworkers and used in a broad range of applications (Cuenca et al. 2006). Table 11.2 summarizes the latest progresses and general considerations for AuNP delivery and targeting in cancer therapy, according to their methodology principle, type of incubation/exposure, and target organs. In this panorama we can see that over the last 10 years, the majority (approximately 80 %) of gold nanoformulations for gene therapy, tumor targeting, and drug delivery in cancer have been tested in cell cultures and normally targeting reporter genes, such as luciferase or GFP. In the future, it is imperative to develop new therapy vehicles and extensive testing in animal models in order to develop the next-generation nanoparticle translation into the clinics. There is only one active clinical trial reporting the use of AuNPs. This phase I trial is studying the side effects and best dose of TNF-bound colloidal gold in treating patients with advanced solid tumors (clinical trial number NCT00356980), sponsored by the National Institutes of Health Clinical Center (CC) and National Cancer Institute (NCI). As it can be seen in Fig. 11.3 there is a disproportional level between nanoparticle production and their translation into clinics.

In fact, clinical trials require the coordinated effort of interdisciplinary research groups, institutes, and pharmaceuticals. Clinical-stage programs will probably have to deal with more and more human data and financial investment, before they can be viewed as a mainstream proposition for resourceful nanotherapy vehicle developers. The current generation of nanoparticles varies widely in size, chemical composition, surface charge, tissue tropism, and sensitivity that makes difficult to translate them into the manufacturing process. The community has to learn how to deal with all the data produced so far. The rules for understanding how nanoparticles interact with different organs and organisms are starting to emerge, although most of the valuable evidences have to come from animal models.

11.3.1 Gene Therapy

We are in the dawn of a new age in gene therapy driven by nanotechnology vehicles. Although there are technical challenges associated with the therapeutic application of nanoparticles, the integration of therapy with diagnostic profiling has accelerated

Table 11.2 Summary of AuNPs used in cancer therapy according to the type of NP, surface modification, type of cancer, target cells/organs/organisms, and explored methodology principle

Method	Type of AuNP	Surface modification	Target cells/organs/organisms	Comments (reference)
Gene therapy	13 nm spherical	Thiol-ssDNA	RAW 264.7 (macrophage); HeLa cells (cervical carcinoma); NIH-3 T3 (fibroblast); MDCK cells (kidney)	In vitro intracellular gene regulation; control of protein expression in cells (Rosi et al. 2006)
	13 nm spherical	PEG-*block*-poly(2-(*N,N*-dimethylamino)ethyl methacrylate) copolymer (PEG-PAMA); siRNA	HuH-7 cells (hepatocarcinoma)	Smart PEGylated AuNPs for the in vitro delivery of siRNA and to induce gene silencing (Oishi et al. 2006)
	13 nm spherical	Thiol-siRNA	HeLa cells (cervical carcinoma)	Polyvalent RNA–nanoparticle conjugates for in vitro luciferase knockdown (Giljohann et al. 2009)
	14 nm spherical	Thiol-siRNA; naked siRNA; PEG-COOH; PEG-N₃; HIV-derived TAT peptide; RGD peptide; quaternary ammonium	HeLa cells (cervical carcinoma); freshwater polyp (*Hydra*); mice (C57BL/6J)	In vitro and in vivo RNAi triggering using hierarchical approach with three biological systems of increasing complexity (Conde et al. 2012a)
	15 nm spherical	Cationic polymers: PEI; charge-reversal PAH-Cit; MUA (mercaptoundecanoic acid)	HeLa cells (cervical carcinoma)	Charge-reversal functional AuNPs to deliver siRNA and plasmid DNA into cancer cells (in vitro) (Guo et al. 2010)
	14 nm spherical	Cy3 labeled hairpin-DNA	HCT-116 cells (carcinoma of colon)	Au-nanobeacons capable of intersecting both pathways of in vitro RNA interference, blocking exogenous siRNA and endogenous microRNA (Conde et al. 2013a)
	13 nm spherical	PEG-NH₂; siRNA; poly(β-amino esters) (PBAEs)	HeLa cells (cervical carcinoma)	Gold, poly(β-amino ester) nanoparticles that facilitate high levels of in vitro siRNA delivery (Lee et al. 2009)
	40 nm spherical	Protease-degradable poly-ʟ-lysine (PLL); siRNA	MDA-MB231-luc cells (breast cancer); LNCaP-luc cells (prostate adenocarcinoma)	Multilayer siRNA coated AuNPs using siRNA and PLL as the charged polyelectrolytes for in vitro luciferase knockdown (Lee et al. 2011)
	Au-nanospheres	Folate receptor; siRNA	Nude mice bearing HeLa cervical cancer xenografts	Near-IR light-inducible NF-kappaB in vivo downregulation through folate receptor-targeted hollow Au-nanospheres carrying siRNA recognizing NF-kappaB p65 subunit (Lu et al. 2010b)
	40 nm Au-nanoshells	PEG-NH₂; TAT-lipid; Cy3-siRNA	Mouse endothelial cells	NIR laser-induced release of siRNA from the nanoshells and in vitro GFP silencing (Braun et al. 2009)
	Spherical nucleic acid NP	siRNA	HeLa cells (cervical carcinoma); SKH-1E and C57BL/6 J mice	In vitro and in vivo topical delivery of siRNA-based spherical nucleic acid AuNP conjugates for gene regulation (Zheng et al. 2012)

Tumor targeting		pH low insertion peptide (pHLIP)	Mouse model	
Tumor targeting	Spherical	pH low insertion peptide (pHLIP)		Nanogold–pHLIP conjugates used in vivo to target tumors (Yao et al. 2013)
	6 nm spherical	Amine-terminated generation 5 (G5) poly(amidoamine) (PAMAM) dendrimers pre-functionalized with folic acid (FA) and fluorescein isothiocyanate (FI)	KB-HFAR cells (human epithelial carcinoma)	Multifunctional dendrimer-stabilized AuNPs can specifically target cancer cells expressing high-affinity FA receptors in vitro (Shi et al. 2009)
	30 nm spherical	PEGylated trastuzumab (Herceptin)	MDA-MB-361 (breast cancer) tumors	Human epidermal growth factor receptor-2 (HER-2)-targeted AuNPs that enhance the radiation response of in vitro breast cancer cells and in vivo tumor xenografts to X-radiation (Chattopadhyay et al. 2013)
	4 nm spherical	VEGF antibody (AbVF)	CLL B cells (B-chronic lymphocytic leukemia)	AuNPs enhance in vitro apoptosis in B-chronic lymphocytic leukemia (Mukherjee et al. 2007)
	60 nm spherical	PEG-COOH; malachite green isothiocyanate (MGITC); ScFv B10 (antibody fragment for human EGFR)	Tu686 and H520 cells (EGFR-positive cancer cells); nude mouse xenografted with Tu686 cells	PEGylated SERS AuNPs for in vitro and in vivo tumor targeting and detection (Qian et al. 2008a)
	Ultra-small Au-nanoclusters	Folic acid (FA); near-infrared fluorescent dye (MPA); doxorubicin (DOX)	A549 cells (lung cancer); HepG-2 (liver cancer); MDA-MB-231 (breast cancer); HTC116 (carcinoma of colon)	Cellular and in vivo studies with Au-FA-MPA and Au-FA-DOX show high affinity and anti-tumor activity to different tumors (Chen et al. 2012)
	30 nm spherical	PEGylated trastuzumab (FDA-approved humanized monoclonal antibody); radiolabeled polymer	MDA-MB-361 cells (breast cancer); athymic CD-1 mice bearing MDA-MB-361 tumors	AuNPs that enhance tumor uptake and intracellular delivery (in vitro and in vivo) while reducing the systemic exposure by evaluation of the impact of targeting and route of administration on organ distribution (Chattopadhyay et al. 2012)

Table 11.2 (continued)

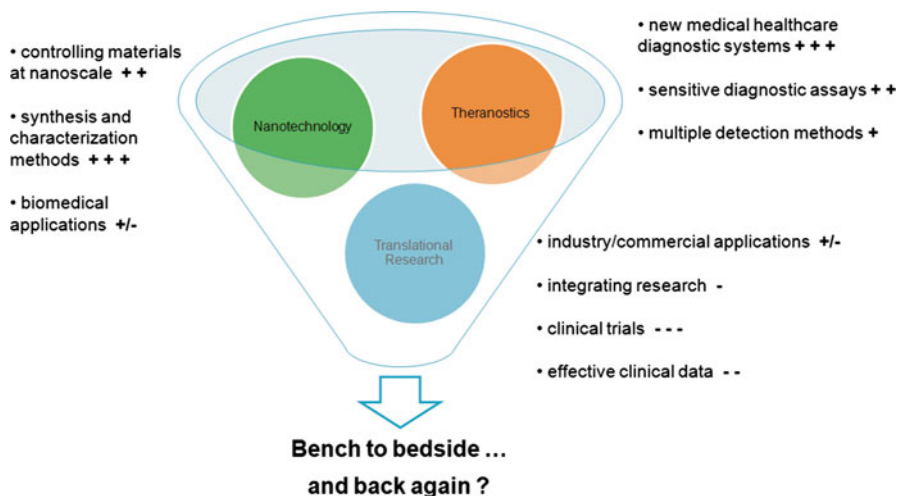| Method	Type of AuNP	Surface modification	Target cells/organs/organisms	Comments (reference)
Drug delivery	2.5 nm spherical	Hydrophobic drugs: tamoxifen (TAF) and β-lapachone (LAP); Bodipy (fluorescent probe)	MCF-7 cells (breast cancer)	Entrapment of hydrophobic drugs in AuNP monolayers with efficient in vitro release into cancer cells (Kim et al. 2009a)
	30 nm spherical	Prostate-specific membrane antigen (PSMA) RNA aptamer; doxorubicin (DOX)	LNCaP cells (prostate adenocarcinoma)	A drug-loaded aptamer—AuNP bioconjugate for in vitro imaging and therapy of prostate cancer (Kim et al. 2010)
	25 nm spherical	Thiol-PEGylated; tamoxifen (TAM)	MDA-MB-361 cells (breast cancer); MCF-7 cells (breast cancer)	Tamoxifen–poly(ethylene glycol)–thiol AuNPs that enhance potency and selective delivery for in vitro breast cancer treatment (Dreaden et al. 2009)
	180 nm spherical	PEG; oxaliplatin	A549 cells (lung epithelial cancer); HCT116, HCT15, HT29, RKO cells (all for colon cancer)	AuNPs for the improved anticancer drug delivery in vitro of the active component of oxaliplatin (Brown et al. 2010)
	13 nm spherical	Cisplatin	A549 cells (lung cancer); tumor-bearing SCID mice	In vitro and in vivo antitumoral drugs conjugated to AuNPs (Comenge et al. 2012)
	Spherical	Cyclic peptide	CCRF-CEM cells (human leukemic lymphoblasts); SK-OV-3 cells (human ovarian adenocarcinoma)	Cyclic peptide-capped AuNPs for in vitro drug delivery (Nasrolahi et al. 2012)
	40 nm Au-nanospheres	NH$_2$-PEG-COOH; doxorubicin (DOX); cyclic peptide c(TNYL-RAW), a second-generation EphB4-binding antagonist	Mice bearing Hey tumors	In vivo photothermal chemotherapy using doxorubicin-loaded Au-nanospheres that target EphB4 receptors in tumors (You et al. 2012)
	Plasmonic vesicles assembled from 14 nm spherical AuNPs	Raman reporter; PEG; hydrophobic copolymer (PMMAVP) of methyl methacrylate (MMA) and 4-vinylpyridine (4VP)	SKBR-3 cells (breast adenocarcinoma)	Self-assembled plasmonic vesicles of SERS-encoded amphiphilic AuNPs for in vitro cancer cell targeting and traceable intracellular drug delivery (Song et al. 2012)

- controlling materials at nanoscale + +

- synthesis and characterization methods + + +

- biomedical applications +/-

Nanotechnology

Theranostics

Translational Research

- new medical healthcare diagnostic systems + + +

- sensitive diagnostic assays + +

- multiple detection methods +

- industry/commercial applications +/-

- integrating research -

- clinical trials - - -

- effective clinical data - -

Bench to bedside …

and back again ?

Fig. 11.3 Disproportional level between nanoparticle production and their translation into clinics. Thousands of systems were published describing different synthesis, biofunctionalization, and characterization methods that will most likely revolutionize our understanding of chemical and biological mechanisms and push forward efficient diagnostics and therapeutic platforms. Nevertheless, very few were produced to improve a bench-to-bedside approach to translational research. Outcomes like this must be followed by extensive laboratory work, which results in improved screening procedures and a new therapy of great potential, although the final product should always be part of a two-way interaction between laboratory scientists and clinicians

the pace of discovery of new nanotechnology methods. The development of a safe, efficient, specific, and nonpathogenic vehicle for gene delivery is highly attractive (Akhtar and Benter 2007; Kim et al. 2009b).

Gene therapy is receiving increasing attention and, in particular, small-interference RNA (siRNA) shows importance in novel molecular approaches in the knockdown of specific gene expression in cancerous cells. In fact, this non-viral-vector-mediated delivery of therapeutic siRNAs is highly desirable and constitutes an important challenge to gene therapy (Castanotto and Rossi 2009; Li et al. 2002; Soutschek et al. 2004).

In fact, antisense DNA (Fichou and Ferec 2006; Toub et al. 2006) and RNA interference (RNAi) via the use of small-interfering RNA (Fire et al. 1998; Baker 2010; Milhavet et al. 2003; Wall and Shi 2003) have emerged as powerful and useful tools to block gene function and for sequence-specific posttranscriptional gene silencing, playing an important role in downregulation of specific gene expression in cancer cells. Thus, one drawback of using naked siRNAs is that they show extremely short half-lives, weak protection against action by RNAses, poor chemical stability, and common dissociation from vector (Hannon and Rossi 2004). In fact, the major obstacle to clinical application is the uncertainty about how to deliver therapeutic RNAs (e.g., miRNA and/or siRNA) with maximal therapeutic impact. AuNPs have shown potential as intracellular delivery vehicles for antisense oligonucleotides

(e.g., miRNA and/or siRNA) with maximal therapeutic impact. AuNPs have shown potential as intracellular delivery vehicles for antisense oligonucleotides (Rosi et al. 2006) and for therapeutic siRNA by providing protection against RNAses and ease of functionalization for selective targeting (Giljohann et al. 2009; Whitehead et al. 2009). For example, Mirkin and coworkers reported the use of polyvalent RNA–AuNP conjugates that are readily taken up by cells and that the particle bound siRNA can effectively regulate genes in the context of RNA interference (Giljohann et al. 2009).

Several other studies using engineered NPs modified with siRNA have demonstrated a cytoplasmic delivery system of siRNA and efficient gene silencing using AuNPs (Giljohann et al. 2009; Lee et al. 2008, 2009; Guo et al. 2010). However, almost all nanoconjugates using siRNA have exclusively been tested in cell cultures targeting only reporter genes.

Recently, Conde et al. provided evidence of in vitro and in vivo RNAi triggering via the synthesis of a library of novel multifunctional AuNPs, using a hierarchical approach including three biological systems of increasing complexity: in vitro cultured human cells, in vivo freshwater polyp (*Hydra vulgaris*), and in vivo mice models (Conde et al. 2012a). The authors developed effective conjugation strategies to combine, in a highly controlled way, specific biomolecules to the surface of AuNPs such as (a) biofunctional spacers: poly(ethylene glycol) (PEG) spacers used to increase solubility and biocompatibility; (b) cell penetrating peptides (CPPs) such as TAT and RGD peptides, a novel class of membrane translocating agents named CPPs that exploit more than one mechanism of endocytosis to overcome the lipophilic barrier of the cellular membranes and deliver large molecules and even small particles inside the cell for their biological actions; and (c) siRNA complementary to a master regulator gene, the protooncogene c-myc, were bond covalently (SH-siRNA) and ionically (naked/unmodified siRNA) to AuNPs (Conde et al. 2012a).

However, efforts to target siRNA-nanoparticles to organs are less advanced. Some organs need smaller and novel NPs to access different kinds of tissue. Another challenge, which also requires novel materials, is the endosomal release of siRNA, once it is transported across the cell membrane. Most of the described systems also get trapped by the lysosomes and their siRNA cargo gets compromised. Probably the endosomal escape or siRNA accessibility to form the RNA-induced silencing complex (RISC) requires substantially more circulation time.

While the AuNPs for gene therapy discussed thus far employ the load of siRNA to nanoparticles, AuNPs have also shown potential as intracellular delivery vehicles for antisense oligonucleotides (ssDNA, dsDNA) by providing protection against intracellular nucleases and ease of functionalization for selective targeting (Whitehead et al. 2009; Giljohann et al. 2009). So far, several strategies for gene delivery systems have been developed, including mixed monolayer protected AuNPs (Rana et al. 2012; Ghosh et al. 2008a), polymer–AuNPs complexes (Thomas and Klibanov 2003; Ghosh et al. 2008b), and dsDNA and ssDNA functionalized AuNPs (Conde et al. 2010a; McIntosh et al. 2001).

Recently, Conde et al. developed a new theranostic system capable of intersecting all RNA pathways: from gene specific downregulation to silencing the silencers,

i.e., siRNA and miRNA pathways. The authors reported the development AuNPs functionalized with a fluorophore labeled hairpin-DNA, i.e., gold nanobeacons, capable of efficiently silencing single gene expression, exogenous siRNA, and endogenous miRNAs while yielding a quantifiable fluorescence signal directly proportional to the level of silencing (Conde et al. 2013a).

From a synthetic point of view the differences between DNA–AuNPs and siRNA–AuNPs are still unclear. From the papers reported so far the siRNA and DNA–gold nanoconjugates are almost from the same size and charge and show similar efficiencies. Nevertheless siRNA–AuNP conjugates require functionalization with thiol-PEG molecules to achieve equivalent stability to DNA–AuNPs, which may have something to do with the capacity loading of the different molecules due to singular hydrophobicity and/or hydrophilicity, molecular weight, and charge density properties between RNA and DNA (Gary et al. 2007). However, this fact needs additional clarification.

Further research into the fundamental mechanisms of in vivo gene therapy using nanodevices could unveil new dimensions of nanoparticle-mediated gene silencing that will have profound implications for understanding gene regulation, and which could also affect the development of functional genomics and therapeutic applications. One of the most important issues that are still unclear is how biocompatible AuNPs will be following intravenous injection, in particular when the ultimate destination is the cytoplasm and/or nucleus inside cells. Future in vivo work will need to cautiously consider the accurate option of chemical modifications to incorporate into the nanoparticles to avoid off-target effects. Though the nanoparticles' potential against cancer is still in need of further optimization and characterization, it is now time to start translating these promising platforms to the clinical settings towards widespread effective therapy strategies in the fight against cancer.

11.3.2   Tumor Targeting

Nanoparticles are excellent tumor-targeting vehicles because of a unique inherent property of solid tumors. Numerous tumors present with defective vasculature and poor lymphatic drainage, due to the rapid growth of solid tumors, resulting in an enhanced permeability and retention effect. This effect allows nanoparticles to accumulate specifically at the tumor site (Minelli et al. 2010; Gil and Parak 2008). Once the tumor is directly connected to the main blood circulation system, multifunctional nanocarriers can exploit several characteristics of the newly formed vasculature and efficiently target tumors. Tumor cells are supplied by blood capillaries that perfuse the cells of the tissue where nanocarriers can passively accumulate or anchor through targeting moieties to biomarkers overexpressed by tumor cells (Conde et al. 2012b).

Shi et al. developed a simple system with multifunctional amine terminated poly(amidoamine) (PAMAM) dendrimers, folic acid (FA), and fluorescein isothiocyanate functionalized in gold nanoparticles. This approach can specifically target cancer cells expressing high-affinity FA receptors in vitro (Shi et al. 2009).

Further work in tumor targeting was reported in a subcutaneous model of colon cancer, where it was demonstrated that systemically delivered AuNPs (size, approximately 33 nm) conjugated to tumor necrosis factor (TNF) accumulated in tumors (Paciotti et al. 2004). This study outlines the development of a colloidal gold nanoparticle vector that targets the delivery of TNF to a solid tumor growing in mice.

Mukherjee et al. studied B-chronic lymphocytic leukemia (CLL) which is characterized by apoptosis resistance. They found induction of significantly more apoptosis in CLL B cells by co-culture with an anti-VEGF antibody. To increase the efficacy of these agents in CLL therapy they focused on the use of AuNPs, by attaching VEGF antibody to the nanoparticle's surface. The AuNP–VEGF antibody treated cells showed significant downregulation of anti-apoptotic proteins (Mukherjee et al. 2007).

In cancer research, colloidal gold can be used to target tumors and provide detection using SERS (surface-enhanced Raman spectroscopy) in vivo. These AuNPs are surrounded with Raman reporters which provide light emission that is over 200 times brighter than quantum dots (Cai et al. 2008; Kneipp et al. 2006). It was found that the Raman reporters were stabilized when the nanoparticles were encapsulated with a thiol-modified polyethylene glycol coat and gave large optical enhancements. This allows for compatibility and circulation in vivo. When conjugated to tumor-targeting ligands, these conjugated SERS nanoparticles were able to target tumor markers such as EGFR, which is sometimes overexpressed in cells of certain cancer types (El-Sayed et al. 2005a, 2006), and then detect the location of the tumor on human cancer cells and in xenograft tumor models (Qian et al. 2008a). Qian et al. described biocompatible and nontoxic nanoparticles for in vivo tumor targeting and detection based on PEGylated gold nanoparticles and SERS. These conjugated nanoparticles were able to target tumor biomarkers such as EGFRs on human cancer cells and in xenograft tumor models (Qian et al. 2008a).

Although cancer therapies are improving, some formulations are not reaching with high efficiency tumor cells or tissues, and countless doubts remain over the efficacy of those that do. To efficiently target a cancer cell, either a circulation cell or a cell from the primary tumor or one hidden within a population of normal cells represents an exceptional challenge. In fact, there are a lot of limitations for tumor targeting as some nanocarriers can also target normal proteins which are not exclusively expressed by the cancer cell. Targeting specific cells may be completely different to target the organ. The most important aspects that the researchers need to take into account are the specificity of the nanoparticle to the target molecules, as well as toxicological and immunological effects (Schroeder et al. 2012). For example, the combination of small size nanoparticles with their special thermal, imaging, drug/gene carrier, or optical characteristics with the specific and selective recognition abilities of antibodies will definitely produce a hybrid product that shows versatility and specificity.

Actually, Conde et al. (2013b) reported the evaluation of the inflammatory response and therapeutic siRNA silencing via RGD-nanoparticles in a lung cancer mouse model. This study reported the use of siRNA/RGD gold nanoparticles capable of targeting tumor cells in two lung cancer xenograft mouse models, resulting in successful and significant *c-Myc* oncogene downregulation followed by tumor growth inhibition and prolonged survival of the animals. This delivery system can achieve translocation of siRNA duplexes directly into the tumor cell cytoplasm and

accomplish successful silencing of an oncogene expression. Actually, RGD/siRNA–AuNPs can target preferentially and be taken up by tumor cells via αvβ3 integrin-receptor-mediated endocytosis with no cytotoxicity, showing that they can accumulate in tumor tissues overexpressing αvβ3 integrins and selectively deliver *c-Myc* siRNA to suppress tumor growth and angiogenesis (Conde et al. 2013b).

Therefore, multifunctional nanocarriers have the potential to join numerous therapeutic functions into a single platform, by targeting specific tumor cells, tissues, and organs.

11.3.3   Drug Delivery

The vast majority of FDA approved used drugs exhibit a short half-life in the blood stream and a high overall clearance rate. In fact, the major obstacles/limitations in drug delivery are cytoplasmic and systemic delivery of the drug, renal clearance, target site accumulation after administration, and heterogeneous vascular perfusion and diffusion. Actually, these small drug molecules usually diffuse rapidly into healthy tissues and are dispersed consistently within the body. As a consequence, just a small amount of the drug can reach the target site, which often leads to side effects. These obstacles usually occur with drugs that exhibit a narrow therapeutic index, such as anticancer biomolecules, immunosuppressive agents, as well as antirheumatic medicines. Poor drug delivery and accumulation at the target site frequently lead to significant limitations, such as multi-drug resistance, which leads many cancers to develop severe resistance to chemotherapy drugs (Ehdaie 2007).

Nanocarriers can be used to optimize the biodistribution of drugs to diseased organs, tissues, or cells, in order to improve and target drug delivery (Han et al. 2007b).

It is important to realize that the nanoparticle-mediated drug delivery is feasible only if the drug distribution is otherwise inadequate. These cases include drug targeting of difficult, unstable molecules (proteins, siRNA, DNA), delivery to the difficult sites (brain, retina, tumors, intracellular organelles), and drugs with serious side effects (e.g., anti-cancer agents). The performance of the nanoparticles depends on the size and surface functionalities in the particles. Also, the drug release and particle disintegration can vary depending on the system (e.g., biodegradable polymers sensitive to pH). An optimal nanodrug delivery system ensures that the active drug is available at the site of action for the correct time and duration, and their concentration should be above the minimal effective concentration (MEC) and below the minimal toxic concentration (MTC) (Han et al. 2007a; Langer 2000).

AuNPs are also being investigated as vehicles for drug delivery such as paclitaxel (Gibson et al. 2007). *Gibson* et al. describe the first example of 2 nm AuNPs covalently functionalized with the chemotherapeutic drug paclitaxel. The administrations of hydrophobic drugs require molecular encapsulation and it is found that nanosized particles are particularly efficient in evading the reticuloendothelial system. This approach gives a rare opportunity to prepare hybrid particles with a well-defined amount of drug and offers a new alternative for the design of nanosized drug-delivery systems (Kim et al. 2009a; Hwu et al. 2009; Gibson et al. 2007) (see Fig. 11.4).
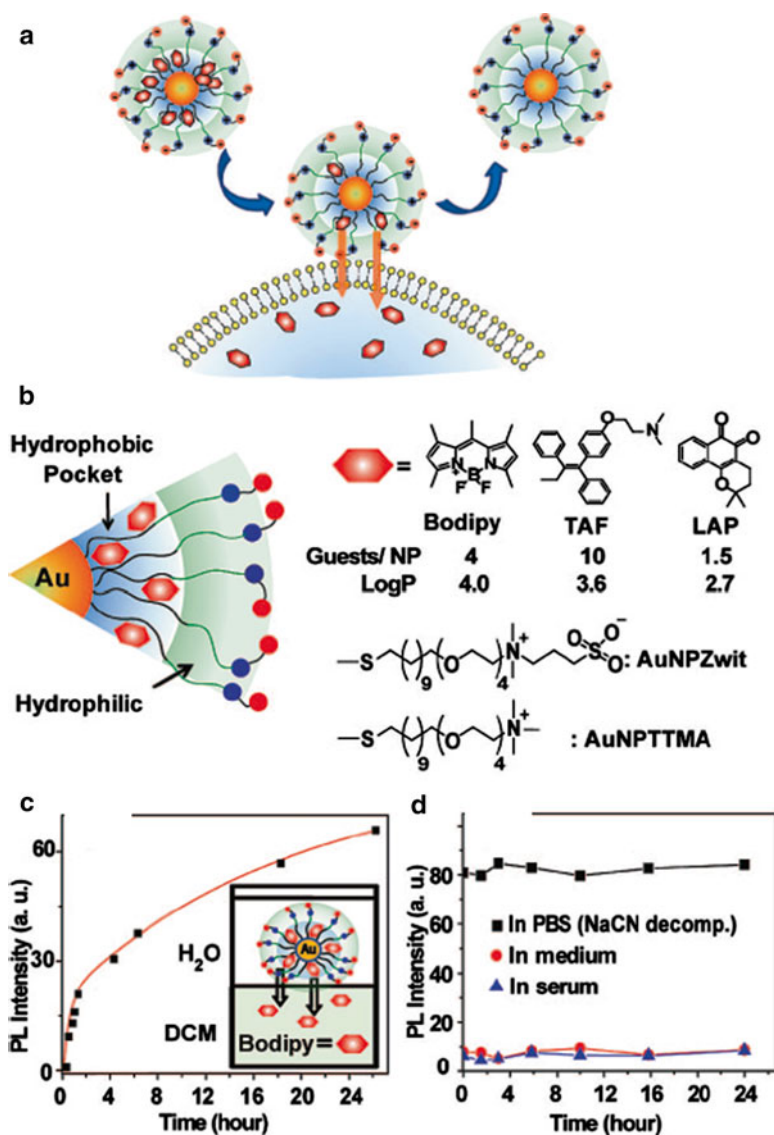
Fig. 11.4 Drug delivery. Entrapment of hydrophobic drugs in nanoparticle monolayers with efficient release into cancer cells. (**a**) Delivery of payload to cell through monolayer–membrane interactions. (**b**) Structure of particles and guest compounds: Bodipy, TAF, and LAP, the number of encapsulated guests per particle, and log P of the guests. (**c**) Release of Bodipy from AuNPZwit Bodipy in DCM-aqueous solution two-phase systems (λex 499 nm, λem 517 nm). (**d**) PL intensity of AuNPZwit-Bodipy in cell culture medium and 100 % serum, indicating little or no release relative to AuNPZwit-Bodipy in PBS after NaCN induced release of guest molecules (λex 499 nm, λem 510 nm). Reproduced with permission from Kim et al. (2009a). Copyright 2013, American Chemical Society

Nanotechnology has provided over the last years novel and powerful systems that may be used for the treatment of human diseases. However the majority of products, reagents, and drugs being used for the development of these nanoscale systems have to be approved by the main supervising agencies, such as the FDA and EMA (Baptista 2009).

Thus far, some limitations for the correct design and application of nanoparticles, such as pharmacokinetics, biodistribution, and side effects of the nanotherapy and safety profile of nanoparticles before and after conjugation and toxicity, need to be clarified to validate efficient clinical appliance (Baptista 2009).

11.4 NanoToxicity

The AuNPs have a proclivity in vivo and in vitro to bioaccumulate within various types of cells with a special affinity for macrophage-type cells (both histiocytes and blood phagocytic cells) and reticuloendothelial cells throughout the body. They also produce varying degrees of bioaccumulation in such tissues as lymph nodes, bone marrow, spleen, adrenals, liver, and kidneys (Lasagna-Reeves et al. 2010; Chen et al. 2009; Dobrovolskaia et al. 2008).

Research shows that nanoparticles can stimulate and/or suppress the immune responses and that their compatibility with the immune system is largely determined by their surface chemistry. In fact, the influence of size, solubility, and sur-
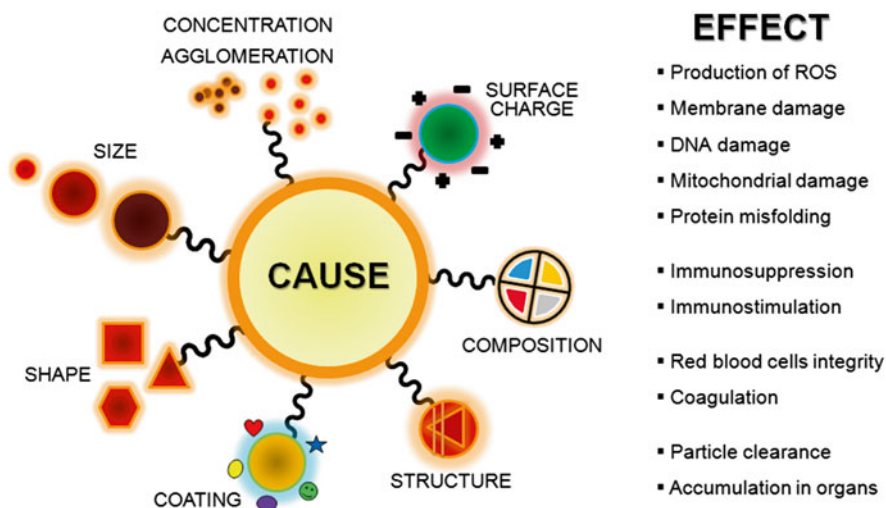

Fig. 11.5 Nanotoxicity: causes and effects. Nanoparticles biocompatibility/effects and their use in biological applications can be influenced by size, shape, solubility, composition, and surface charge and modification/chemistry

face modification on the biocompatibility of nanoparticles and their use in biological applications are well known (Dobrovolskaia and McNeil 2007) (see Fig. 11.5).

AuNPs are generally considered to be benign. However, the size similarity of AuNPs to biological matters could provide "camouflage" to cellular barriers, leading to undesired cellular entry which might be detrimental to normal cellular function (Connor et al. 2005).

Pan et al. (2007) recently conducted a systematic investigation of the size-dependent cytotoxicity of AuNPs against four cell lines. They found that AuNPs 1–2 nm in size displayed cell-type dependent cytotoxicity with high micromolar IC50s. In contrast, AuNPs 15 nm in size were nontoxic to cells at concentrations 60-fold higher than the IC50 of the smaller AuNPs. These results seemed to confirm size dependent toxicity of AuNPs (Kim et al. 2009a; Visaria et al. 2006; Paciotti et al. 2004; El-Sayed et al. 2005b; Huang et al. 2008), an inference that has hitherto been somewhat ambivalent. On the other hand, it was found that the presence of sodium citrate residues on AuNPs impaired the viability in the alveolar type-II cell lines A549 and NCIH441. Interestingly, the presence of an excess of sodium citrate on the surface of NPs not only reduced the in vitro viability of A549 and NCIH441 cell lines but also affected cellular proliferation and increased the release of lactate dehydrogenase (marker for apoptotic cell degradation) (Uboldi et al. 2009).

Although AuNPs are generally considered as highly biocompatible, previous in vitro studies have also shown that cytotoxicity of AuNPs in certain human epithelial cells was observed (Freese et al. 2012; Rothen-Rutishauser et al. 2007).

Now the most urgent questions rise up. Are the gold nanoparticles cytotoxic or biocompatible? And how can the gold nanoparticles be designed to avoid these effects?

There does not seem to have a simple answer. Even though there is not any general mechanism for making nanoparticles universally "non-toxic" to all living cells and all organisms, there are important findings that can be applied for increasing nanoparticle biocompatibility and reducing cytotoxic interactions in vivo and in vitro.

Using the lowest nanoparticle dose to get the desired response for the shortest period of time, in general, seems to promote biocompatibility as well as coating a nanoparticle of the outer coating completely covers the nanoparticle reactive surface (a non-continuous covering, the presence of cracks, roughness, or interruptions could lead to complement or antibody attachment, or dissolution of the coating by cell digestion) and cannot be removed and utilized by the living cell (Bellucci 2009).

It is essential to test nanoparticle/biological interactions experimentally and modify the nanoparticles for best biocompatibility with the cell in order to eliminate some obstacle, like the peroxidation of membrane lipids, the generation of reactive oxygen species, the acute and chronic release of pro-inflammatory factors, modification in genetic cellular function, and the possibility of nanoparticles becoming inactive/unavailable during filtration or passage through pores and fenestrations (Sun et al. 2005) due to size, inflexibility of the nanoparticle core, or protein adsorption and agglomeration (Bellucci 2009).

When interpreting nanoparticle interactions with cells and organisms, it is important to remember that living systems may appear normal and be capable of growth and function, but they may be genetically altered in subtle ways following nanoparticle exposure, which can produce serious consequences at some time in the distant future. Conversely, other cells that seem to be damaged may, in time, recover from nanoparticle exposure and function normally in the absence of the nanoparticles (Bellucci 2009).

In conclusion, the only weapon that we have to insure that these new materials are well designed and safely used is to question and test each new nanoparticle to make sure that it has been designed for safety (with maximum biocompatibility) during handling, use, and disposal. Evaluating the biocompatibility of nanomaterials is imperative. In fact, it is important to carefully characterize the biocompatibility and safety of the nanomaterials if they are to be used for medical purposes. Despite the major scientific advances made in the field of molecular and cell biology and biotechnology, the basic concepts of regulatory toxicology have hardly changed over the past decades (Dobrovolskaia and McNeil 2007). Actually, the vast majority of studies report the biocompatibility of nanomaterials only through the study of cell viability. Almost no importance is given when testing nanomaterials in the detection of genetic damages (DNA strand breaks and the formation of nuclear abnormalities), or in identifying protein markers of toxicity, or measuring the level of oxidative stress. For example, when using gene silencing technologies, the function of specific genes and proteins in toxicity pathways could be identified, once DNA-damage response (DNA repair, cell-cycle regulation, and apoptosis) encompasses gene-expression regulation at the transcriptional and post-translational levels.

In fact, Conde et al. reported a gold-nanobeacon system (Conde et al. 2013a) used for gene therapy that was extensively evaluated for the genotoxic, cytotoxic, and proteomic effects after incubation in cancer cells (Conde et al. 2014b). The exposure was evaluated by two-dimensional protein electrophoresis followed by mass spectrometry to perform a proteomic profile and MTT assay, glutathione-S-transferase assay, micronucleus test, and comet assay to assess the genotoxicity. An assessment of genome-related toxicity revealed no significant DNA damage increase, as well as no potential mutagenic or clastogenic consequences to the cell (Conde et al. 2014b).

11.5   Conclusions and Future Perspectives

Over the last decade, thousands of different gold nanocarriers were developed and published. Almost 20 % of these papers were published in 2010 alone. It is indisputable that the use of gold nanocarriers has been gaining momentum as vectors for therapy and diagnostic strategies, combining the AuNPs' ease of functionalization with numerous biomolecules, high loading capacity, and fast uptake by target cells.

Here, we have reviewed part of this exciting progress and research advances within the context of multifunctional gold nanocarriers for cancer theranostics.

Despite the significant efforts towards the use of gold nanocarriers in biologically relevant research, more in vivo studies are needed to assess the applicability of these materials as delivery agents. In fact, only a few went through feasible clinical trials. Nanoparticles have to serve as the norm rather than an exception in the future conventional cancer treatments. Future in vivo work will need to carefully consider the correct choice of chemical modifications to incorporate into the multifunctional gold nanocarriers to avoid activation off-target, side effects, and toxicity. Moreover the majority of studies on nanomaterials do not consider the final application to guide the design and functionalization of NP. Instead, the focus is predominantly on engineering materials with specific physical or chemical properties.

Although great effort has been applied to the detection of microorganisms and/or virus using gold biosensors, only a few were used for the detection of chronic diseases, such as cancer. The lack of specific and unique DNA/RNA markers and the complexity of the intricate regulation pathways have made the development of probes rather difficult. Particularly in cancer, future trends need to be focused on the detection of microRNAs and circulating DNA biomarkers or the recognition of circulating tumor cells followed by a detection step. Since most of the nanodiagnostic systems report DNA sequence analysis/detection, the great majority ignored the important genomic and transcriptomic information when detecting messenger RNA and microRNAs, which are considered to be excellent biomarkers for the early diagnosis of cancer.

To improve medicine, scientific discoveries must be translated into clinical applications. Such discoveries typically begin at "the bench" with basic/fundamental research in which scientists study disease at a molecular or cellular level and then progress to the clinical level, which is the patient's "bedside." Scientists and clinicians are increasingly aware that this bench-to-bedside approach to translational research is really a two-way interaction. However, an additional effort should appear towards the development of new clinical strategies.

Moreover, the systems discussed here are each of them unique in many aspects. Some of the nanoformulations have very well-defined structures, whereas some are highly heterogeneous. Some have a wide range of size, charge, and surface. This raises the important question about the reliability of their production protocols. The great majority of the nanosystems described here present or will present additional challenges in the scale-up of the manufacturing process. Besides, central concerns about nanoparticle's biodistribution and the proper considerations of safety for the patients. Therefore, the establishment of safe regulatory approval nanoformulations turns to be essential.

For that reason, it is imperative to learn how advances in nanosystem's capabilities are being used to identify new diagnostic and therapy tools driving the development of personalized medicine in oncology; discover how integrating cancer research and nanotechnology modeling can help patient diagnosis and treatment; recognize how to translate nanotheranostics data into an actionable clinical strategy; discuss with industry leaders how nanotheranostics is evolving and what the impact is on current research efforts; and last but not least, learn what approaches are proving fruitful in turning promising clinical data into treatment realities.

Although all studies described here provide a baseline level of data in support of the effectiveness and safety of nanomaterials, we wonder how useful the data generated will be in successfully predicting and preventing scientists from jeopardizing the safety of the future patients.

With chemists, biologists, and materials scientists working together with clinicians and engineers, but especially with "translational innovators," new solutions to crucial nanobiomedical problems will hopefully be found.

Competing Interests
The authors declare that they have no competing interests.

Authors' Contributions
JC conceptualized the manuscript and wrote the draft. FT, PVB, and JMF contributed in the draft and concept of the paper. All authors contributed in the revision process. All authors read and approved the final manuscript.

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