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EXPERT OPINION

Applications of nanoparticles in cancer medicine and beyond: optical and multimodal *in vivo* imaging, tissue targeting and drug delivery

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Introduction: Nanotechnology has opened up the way to the engineering of new organized materials endowed with improved performances. In the past decade, engineered nanoparticles (NPs) have been progressively implemented by exploiting synthetic strategies that yield complex materials capable of performing functions with applications also in medicine. Indeed, in the field of 'nanomedicine' it has been explored the possibility to design multifunctional nanosystems, characterized by high analytical performances and stability, low toxicity and specificity towards a given cell target.

Area covered: In this review article, we summarize the advances in the engineering of NPs for biomedical applications, from optical imaging (OI) to multimodal OI and targeted drug delivery. For this purpose, we will provide some examples of how investigations in nanomedicine can support preclinical and clinical research generating innovative diagnostic and therapeutic strategies in oncology.

Expert opinion: The progressive breakthroughs in nanomedicine have supported the development of multifunctional and multimodal NPs. In particular, NPs are significantly impacting on the diagnostic and therapeutic strategies since they allow the development of: NP-based OI probes containing more than one modality-specific contrast agent; surface functionalized NPs for specific 'molecular recognition'. Therefore, the design and characterization of innovative NP-based systems/devices have great applicative potential into the medical field.

Keywords: diagnostic strategies, drug delivery, nanoparticles, optical imaging

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1. Introduction

Nanotechnology is an emerging scientific area that has created a multiplicity of intriguing and versatile submicrometer-sized materials. Nanoparticles (NPs) in particular can be prepared from a variety of materials such as metals, metal oxides, semiconductors, proteins, polysaccharides and synthetic polymers [1]. Different types of NP have been developed with peculiar physicochemical properties (such as chemical reactivity, energy absorption and biological mobility) that distinguish them from bulk materials by virtue of their size and surface characteristics [1]. In recent years, these materials have emerged as important tools in medicine, with various applications ranging from contrast agents in molecular imaging to carriers for drug delivery [2,3].

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Article highlights.

- Engineered nanoparticles (NPs) have been progressively implemented to obtain high analytical performances and stability, low toxicity and specificity towards a given cell target.
- NPs are important tools in medicine, with various applications ranging from imaging, diagnosis and delivery of therapeutic active agents.
- Recent clinical advances in optical imaging (OI) point out its potential for medical applications. Anyhow, the small organic fluorescent dyes present drawbacks such as: low fluorescence quantum yield, photobleaching, a very fast body clearance, and the lack of precise targeting properties. All these critical aspects can be overcome by the use of NPs.
- The combination of OI with other imaging systems results in deeper tissue imaging that allows accurate anatomical information. In this regard, multimodal imaging may greatly take advantage from the development of NP-based contrast agents able to combine different physical imaging properties on the same nanoplatform.
- The conjugation of targeting moieties on the surface of NPs can confer specific targeting to the imaging procedure and drug delivery.
- It is concluded that the design and characterization of innovative NP-based systems/devices containing more than one modality-specific imaging contrast agent and functionalized for specific 'molecular recognition' have great applicative potential into the medical field.

This box summarizes key points contained in the article.

Within the set of NPs with biomedical applications, two main families can be distinguished: inorganic NPs, such as silica nanoparticles (SiNPs), gold NPs (GNPs), superparamagnetic iron oxide crystals (SPIONs), colloidal semiconductor quantum dots (QDs) and organic NPs (polymer- and lipid-based NPs) (**Table 1**). In particular, among the inorganic nanoformulations, silica-based nanomaterials have been investigated mainly because of: i) the fine size-control; and ii) a chemistry suitable for surface modification [4-6]. Based on these properties, several investigators including our group have considered SiNPs for applications as sensors [7], drug delivery systems [8] and/or bioimaging probes [9-12]. Metallic (iron oxide, gold, silver) NPs have been used for a huge number of applications in various areas of medical treatment and are emerging as tool for imaging, diagnosis, and for the delivery of therapeutic agents to tumor cells [13-15]. Metallic NPs can be used in diagnosis and therapeutics due to their unique properties of small size, large surface area to volume ratio, tunable absorption, photostability and cellular uptake [15]. Among all metallic NPs the GNPs proved to be among the safest (non-toxic) agents for biomedical applications. GNPs, based on their unique physico-chemical and plasmonic properties, which can be tuned across the vis-NIR spectral band, have been exploited for chemical and biological sensing [16], genomics and immunoassays [16], multimodality imaging, tumor targeting, and as vehicle for various therapeutics [17]. Additionally, GNPs have been used as photothermal therapeutics against cancer [17].

The intrinsic properties of SPIONs, such as inherent magnetism, broad safety margin and the availability of methods for fabrication and surface engineering, allow applications in bioimaging, drug delivery and thermal therapy [18]. SPIONs have been reported as tracers for imaging for: tumors and metastases; CNS and magnetic resonance angiography; atherosclerotic plaque and thrombosis [19]. Moreover, SPIONs can achieve a very high efficiency in drug targeting since, by locally applying an external magnetic field to the target organ, it is possible to drive the accumulation of the magnetic NPs into the site of drug action [18]. QDs are of interest since the emission wavelength of these NPs can be continuously tuned by changing the particle size, and a single light source can be used for simultaneous excitation of different-sized QDs [20]. Current and future applications of QDs include the *in vitro* and *in vivo* fluorescence imaging [20,21], the use in microarrays [22], in drug delivery [23] or as sensitizers for photodynamic therapy (PDT) [23]. Moreover, multifunctional integrated targeting, imaging and therapeutic functionalities based on QDs and fluorescence imaging system, have become effective materials for synchronous cancer diagnosis and treatment [24].

On the other hand, the organic nanoformulations currently investigated in the field of nanomedicine include: PEG, poly (glutamic acid), poly(lactic-co-glycolic acid) (PLGA), as well as N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers and polysaccharides (i.e., chitosan, cyclodextrin). In particular, PLGA has generated tremendous interest due to its excellent biocompatibility, biodegradability and mechanical strength [25]. For these reasons, the US FDA approved the use of PLGA and polylactide polymers in microspheres (to be used via parenteral administration route), implants and periodontal drug-delivery systems [26]. PLGA NPs have been used as carriers for different compounds including drugs, peptides, proteins, nucleotides and vaccines [27]. Upon surface engineering, PLGA have been assessed for targeted delivery to malignant cells with high affinity [28-31]. Finally, among the lipid-based NPs, liposomes have received a lot of attention during the past 30 years as convenient delivery vehicles for biologically active compounds [32,33]. The aqueous core can be used to encapsulate hydrophilic drugs, whereas lipophilic drugs can be incorporated into the external NP membrane. Moreover, the liposome surface can be easily functionalized to enhance their *in vivo* stability or to enable their preferential delivery. Lipidic NPs are the first nanomedicine delivery system to make the transition from concept to clinical application, and they are now an established technology platform with considerable clinical acceptance [32,33].

In the present review, we attempt to provide a general overview of the main properties of the NP-systems, either in use or under investigation, for optical bioimaging and drug delivery applications, focusing our attention on same examples of

Table 1. Relevant nanoparticles for biomedical applications.

Nanoplatform	Physico-chemical properties	Biomedical applications	Ref.
Inorganic nanoparticles			
Silica nanoparticles	Diameter from 5 to 300 nm; uniform and tunable pore size; high chemical and mechanical stability; surface functionalization and bioconjugation	Drug delivery; bioimaging; lymph node mapping; sensors	[4-12]
Gold nanoparticles	Diameter from 5 to 300 nm; tunable localized surface plasmon resonance; large absorption and scattering cross-sections; localized enhanced electromagnetic field; surface functionalization and bioconjugation	Genomics and biosensorics; immunoassays and clinical chemistry; Fluorescence Resonance Energy Transfer (FRET) technologies (e.g., for measuring protein interactions, protein conformational changes); photothermal therapy; drug delivery; optical imaging (OI); X-ray CT; MRI	[13-17]
Superparamagnetic iron oxide crystals	Iron core of 4 – 5 nm in diameter coated by either inorganic materials (silica, gold) or organic materials (phospholipids, fatty acids, polysaccharides, peptides, polymers); superparamagnetic; surface functionalization and bioconjugation	MRI; lymph node mapping, drug delivery; gene delivery; photothermal therapy	[18,19]
Colloidal semiconductor	Diameters of 2-20 nm; nanocrystals and core-shell nanocrystals based on different semiconductor materials; the band-gap energy that determines the energy of the fluorescent light is inversely proportional to the size of the quantum dot; surface functionalization and bioconjugation	Oligonucleotide array; Fluorescence Resonance Energy Transfer (FRET) technologies (i.e., for measuring protein interactions, protein conformational changes); OI of cells and tissues; lymph node mapping; photodynamic therapy; drug delivery; gene delivery	[20-23]
Organic nanoparticles			
Polymer-based nanoformulations	Diameter from 5 to 300 nm; controlled release property; surface functionalization and bioconjugation	Drug delivery; bioimaging	[25-31]
Liposomes	Diameters from 15 nm up to several μm; vesicular structures with an aqueous core surrounded by a hydrophobic membrane bilayer composed by phospholipids structure; surface functionalization and bioconjugation	Gene delivery; drug delivery	[32,33]

pre-clinical investigations in nanomedicine aimed to the generation of innovative diagnostic and/or therapeutic NP-based strategies in oncology.

2. NPs for optical imaging

Optical imaging (OI) is an emerging imaging modality with high potential for improving diseases diagnosis and treatment. The commonly used OI methods for biomedical applications include fluorescence imaging, bioluminescence imaging, optical coherence tomography, photoacustic imaging and Raman spectroscopy [34,35]. OI relies on non-ionizing radiation and uses light as the primary imaging modality to characterize local anatomy down to cellular and molecular levels. Thus, OI technologies are becoming critical tools to image structural, functional and molecular information using their unique photon absorption or scattering profiles. The most important limitation for *in vivo* OI is represented by a limited tissue penetration [36]. However, this issue can be at least partially addressed by the use of near-infrared (NIR) OI, due to reduction in scattering and minimal absorption within the NIR spectral window. Thus, NIR modalities are the OI approaches of choice to *in vivo* human studies and have dominated the field of intraoperative image-guided surgery recently [37]. In this contest, the technical advancements enabled the development of important clinical applications such as: intraoperative sentinel lymph node mapping for cancer staging [38,39], optical image guided surgery for detection of tumor margins and malignant masses [40-42], and video-angiography during surgery [43]. Several OI probes have been developed and have been used in clinical trials until now: indocyanine green (ICG), 5-aminolevulinic acid (5-ALA) and methylene blue (MB) [44]. ICG has FDA approval for clinical applications and is currently utilized in NIR fluorescence for sentinel lymph node mapping, intraoperative identification of solid tumors and angiography during reconstructive surgery [40,45]. 5-ALA has been described as a non-fluorescent pro-drug that leads to intracellular accumulation of fluorescent porphyrins in malignant gliomas, thus enabling more complete resections of

Table 2. Relevant nanoparticle-based contrast agents for optical imaging.

Nanoplatform	Optical properties	Ref.
Quantum dots	High photoluminescence quantum yield; high molar extinction coefficients; broad absorption and narrow emission spectra; large distinction between the excitation and emission spectra	[24]
Silica nanoparticles	Tunable intensity profile across the NIR spectrum; large pseudo Stokes-shifts	[54]
Upconversion nanoparticles	Large anti-Stokes shift; NIR excitation; high signal-to-noise ratio; high photostability	[56]
Polymer-based NPs and lipid-based NPs embedding organic NIR dyes	Protection of the NIR dyes against chemical and/or biological degradation, improvement of their photophysical properties	[57]

contrast-enhancing tumor, leading to improved progression-free survival in patients with malignant glioma [46]. More recently, the use of MB has been reported for intraoperative NIR fluorescence imaging of a paraganglioma, allowing the identification of an otherwise undetectable lesion [47]. Anyhow, the small organic fluorescent dyes present drawbacks such as low fluorescence quantum yield, which results in reduced brightness, photobleaching, a very fast body clearance, and the lack of precise targeting properties. All these critical aspects can be overcome by the use of nanostructured probes. Indeed, the performances of NP-contrast agents, when compared with conventional OI methods, are characterized by: i) increased contrast efficiency [44]; ii) increased circulation (blood residence) time [3]; iii) possibility of combining different functions (diagnosis and therapy) [48]; iv) improved tumor penetration [2,49]; v) multi-spectral capabilities [21] and; vi) multi-modal detection, as NPs provide a versatile carrier system to simultaneously load modality-specific contrast agents [48]. So far, a wide range of fluorescent NPs suitable for OI have been designed and tested in preclinical studies [50] and the characteristics of the most relevant NP types are summarized in **Table 2**. Among the different types of fluorescent NPs, the main distinctive property of QDs in comparison with traditionally used organic dyes, is their outstanding resistance to photobleaching. QDs have also a high photoluminescence quantum yield and high molar extinction coefficient, especially in the UV-VIS wavelength range. They can exhibit a broad absorption with narrow and symmetric emission spectra spanning from VIS to NIR depending on QDs composition and size [51]. A possible drawback of QDs is related to their composition; since they typically contain highly poisonous heavy metal, there is some concern about their possible toxicity. In this context, biodegradable organic NPs together with silica (that is recognized as safe by FDA) NPs are now receiving increasing attention as OI probes [10,12,52]. In this respect, we have recently reported a synthetic strategy for the development of silica-PEG core-shell nanostructures doped with a donor–acceptor couple, able to display a tunable intensity profile across the NIR spectrum [12,53,54]. It is worth mentioning that recently tiny dye-doped SiNPs, called Cornell dots (C dots), have entered clinical investigation in melanoma patients [9,55]. These NPs consist of NIR fluorescent molecules encapsulated in a silica core and surrounded by a silica shell, which is functionalized with both cyclic RGD peptide and radioactive iodine. Its applications in melanoma include real-time, intraoperative detection and imaging of nodal metastases, differential tumor burden and lymphatic drainage patterns. It is of interest the evidence that this multimodal nanoplatform has advanced to the clinical translation by combining key benefits of NIR fluorescence imaging (enhanced sensitivity/contrast) with those of positron emission tomography (PET), (depth penetration, quantitation). By exploiting NIR fluorescence imaging the authors were able to elucidate sub-millimetre structures of metastatic disease in small local/regional nodes that PET could not, though PET enabled deeper penetration and quantitation [9,55].

Upconversion NPs are emerging as a new class of OI contrast agents. They are extremely interesting particularly for their ability to emit anti-Stokes shifted light (upconversion) with relatively high brightness, thus providing low imaging background and deep tissue penetration [56]. Also, polymer-encapsulated organic NPs have attracted increasing attention for optical properties and outstanding performance as imaging agents [57]. In this context, we have shown that incorporation of the fluorescent probe Cy5.5 into PLGA-NPs engineered with an anti-CD20 antibody (Rituximab) allowed to visualize the CD20-positive tumor mass by OI [31]. As newly emerging optical nanoprobes, surface-enhanced Raman scattering (SERS) tags have been gaining great interest in the application of biomedical imaging and phototherapies [58,59]. In particular, SERS tags combine metallic (Au or Ag) NPs and specific organic Raman reporter molecules, such as organic dyes. A protective shell together with antibodies or ligands is also employed for targeting, which endows the SERS tag with specific targeting ability and biocompatible properties. Fluorescent-SERS dual mode tags showed great potential for bioimaging due to the combined advantages of intuitive, fast imaging of fluorescence and multiplex capability of SERS technique [60].

Besides NPs made by an intrinsically fluorescent material, the entrapment of NIR-emitting organic dyes into NPs is versatile approach to optimize the OI performances of a fluorescent nanoprobe. With this approach the nanoparticle matrix can shield the doping dyes against chemical and/or biological degradation and frequently improves the resistance to photobleaching and the emission properties [50]. A similar behavior was shown for the encapsulation of the NIR fluorescent dye

IR-783 within the human serum albumin that reduced the dye photobleaching significantly [61].

3. Nanoplatforms for multimodal OI

The current imaging modalities vary in sensitivity, resolution and quantitative capabilities, with each modality offering its own unique benefits and intrinsic limitations. Obviously, multi-modality imaging – combining different imaging modalities – has the potential to overcome the limitations of a single imaging modality, and therefore, is a central task for current and future clinical imaging research [62]. As already discussed, the main limitation of OI is the relatively small penetration, due to the scattering and absorption of light in tissues [36]. Thus, the combination of OI with other imaging modalities, such as MRI, PET, CT and/or photoacustic, results in deeper tissue imaging providing accurate anatomical information. In this regard, multimodal imaging may greatly take advantage from the development of multifunctional NP-based contrast agents able to combine different physical imaging properties on the same nanoplatform (**Table 3**). Moreover, the conjugation of recognition moieties on the surface of multifunctional nanomaterials confers specific targeting properties to the imaging procedure (**Figure 1**) and may allow concomitant therapeutic applications [48] (**Figure 2**).

For instance, as outlined in **Figure 1**, NP-based multimodal contrast agents offer several applications for multimodality imaging in oncology surgery. While OI allows for the intra-surgery discrimination of tumor margins, pre-surgery analyses with multimodal NPs that combine OI and MRI or PET modalities offer high spatial resolution and assessment of the depth of tumor penetration, lymph node involvement and presence of distant metastatic diseases [63-65]. Further increment in imaging performance has been obtained by the design of trimodality imaging probes combining contrast agents for OI, MRI and PET [66-68]. In recent years, the emerging development of multimodal NP carrying therapeutic agents has received considerable attention for imaging-guided therapy (**Figure 2**) that combine the possibility to improve the targeting specifically to the diseased tissue and also to monitor the efficacy of the treatments [18,19,69]. The clinical area that certainly benefits the most from the combination of imaging and treatment is cancer research. Several studies have reported multifunctional and multimodal NPs applied in pre-clinical and clinical settings for: i) delivery of therapeutic agents to tumor cells [70,71] and ii) assessment of the cancer therapeutic response [72]. Increasing evidences have also demonstrated the feasibility and potency of utilizing multifunctional and multimodal NPs for CNS diseases by using specific targeting moieties able to cross the blood brain barrier and, thus, allowing the delivery of therapeutic agents to the brain [73,74] beside the guide surgical resection/ablation of brain tumors [67]. Finally, in the context of cardiovascular diseases, image-guide therapy has been applied for: i) intramyocardial injection of therapeutics guided by multimodal imaging [75]; and ii) for early and accurate evaluation of the response to pro-angiogenic therapy in preclinical diabetic stroke models [76].

4. NPs for drug delivery

The development of drug delivery systems able to improve the bioavailability, tissue uptake and pharmacokinetics of therapeutic agents is central to biomedical research and pharmaceutical industry. In this respect, clinical results suggest that NPs carrying therapeutic agents can exhibit enhanced efficacy, while simultaneously reduce side effects, owing to properties such as cell/tissue targeted delivery and active cellular uptake [3,77]. By itself, injected NPs preferentially accumulate in tumor tissues due to the enhanced permeability and retention effects and therefore are ideally suited for the delivery of chemotherapeutics in cancer therapy [78]. Among the examples of NP carrying chemotherapeutics listed in **Table 4** approved by FDA, we can mention: Genexol-PM, a polymeric micellar formulation of paclitaxel [79] and Doxil, a liposomal formulation of doxorubicin [80], both of which are used as first-line treatment of different tumors. Genexol-PM has significantly improved the maximum tolerated dose of paclitaxel, allowing delivery of higher doses without additional toxicity [79]. Doxil has dramatically prolonged doxorubicine circulation time and enhanced drug release at the tumor site [80]. Nanotherapeutics are rapidly progressing and are being implemented to solve several limitations of conventional drug delivery systems. While some nanomedicine-based drug delivery systems have already been marked and others are in clinical trials (**Table 4**), most are in the stage of preclinical development. Advantages and disadvantages of the different types of nanocarriers are summarized in **Table 5**.

To improve the therapeutic index (the ratio of the toxic to the therapeutic dose) of drug formulations, reducing their toxicity to normal tissues, a second generation of NPs for drug delivery includes surface functionalities that enable the specific 'molecular recognition' of the target tissue or active/triggered release of the payload at the site of the disease [81]. The engineering of a targeting ligand onto the NP surface result in localized drug delivery, which should translate into greater efficacy and reduced drug side effects [82,83]. Cancer treatment stands to benefit from targeted drug delivery, as tumor cells express many molecules on their surface that distinguish them from normal cells [84]. In this context, targeted polymeric NPs are emerging as an important class of therapeutic, among these, BIND-014 has been the first targeted polymeric NP for cancer chemotherapy to reach clinical development [85]. BIND-014 is a polymeric NP containing the chemotherapeutic docetaxel and is targeted to recognize the prostate-specific membrane antigen, a tumor antigen expressed on prostate cancer cells and on the neo-vasculature of most non-prostate solid tumors. Initial clinical data in patients with advanced solid tumors indicate that BIND-014 displays an improved pharmacokinetic and pharmacological profile in comparison with docetaxel [86,87].

Table 3. Example of nanoparticle-based multimodal contrast agents for multimodal optical imaging.

Nanoparticle	Imaging modalities	Applications	Ref.
Iron oxide	Magnetic resonance/positron emission tomography (PET)/optical	Imaging of sentinel lymph nodes	[66]
Graphene	PET/optical	Image-guided photodynamic cancer therapy	[70]
Upconversion nanoparticle	CT/magnetic resonance/optical	Image-guided cancer therapy	[71]
Dendrimer-based nanoparticle	Magnetic resonance/optical	Image-guided therapy of stroke	[76]
Polymeric nanoparticle	Magnetic resonance/optical	Imaging for tumor detection	[100]
Chitosan nanoparticle	PET/optical	Imaging for tumor detection	[101]
Upconversion nanoparticle	Single-photon emission CT/CT/magnetic resonance/optical	Tumor angiogenesis imaging	[102]
Reduced graphene oxide-iron oxide nanoparticle	Photoacustic/magnetic resonance/optical	Image-guided photothermal cancer therapy	[103]
Dendrimer-based nanoparticle	Magnetic resonance/optical	Image-guided surgery of brain tumors	[104]
Plant viral nanoparticle platform tobacco mosaic virus	Magnetic resonance/optical	Imaging of atherosclerotic plaques *in vivo*	[105]

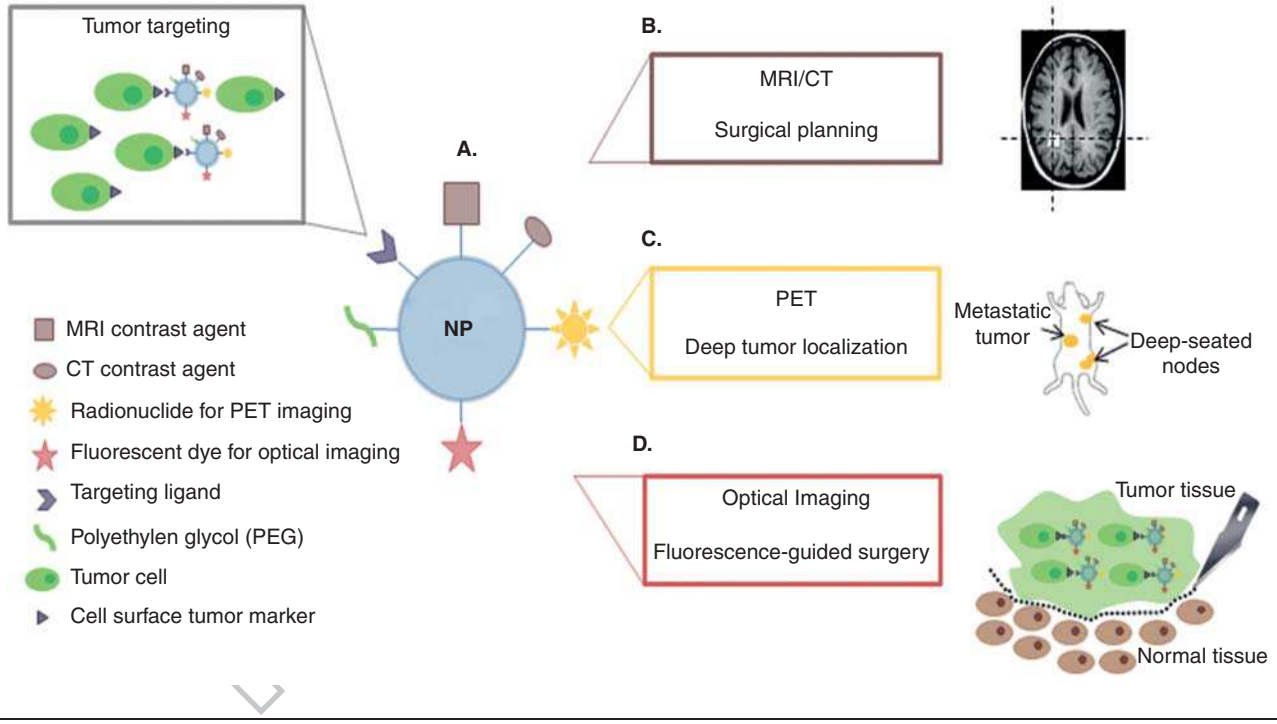

Figure 1. Multifunctional and multimodal NPs for tumor molecular imaging. Schematic representation of the structure of multimodal NPs and their applications in imaging. (A) Multiple functionalities such as magnetic, X-ray absorber, radionuclide, optical dye and targeting moiety can be integrated into a single NP system, which acts as a multimodal molecular imaging platform. **(B)** MRI and CT provide valuable preoperative information for surgical planning (i.e., assessment of anatomical extent of the primary tumor and of the regional lymph node metastasis). **(C)** PET has the advantage of detecting deep-seeded cancer metastasis. **(D)** Fluorescence-guided surgery aids surgeons in the intraoperative identifications and removal of malignant lesions.

CT: Computed tomography; NP: Nanoparticle; PET: Positron emission tomography.

CALAA-01 is another notable example of targeted NP for cancer therapy, which has been evaluated in a Phase I clinical trial for the treatment of patients with solid tumors [88]. It is a polymeric NP that encapsulates small interfering RNA and is functionalized with human transferrin protein (Tf), to target the Tf receptor overexpressed on the surface of most cancer cells. It is to note that all the NPs mentioned so far are made by soft materials; the advancement of hard carriers is

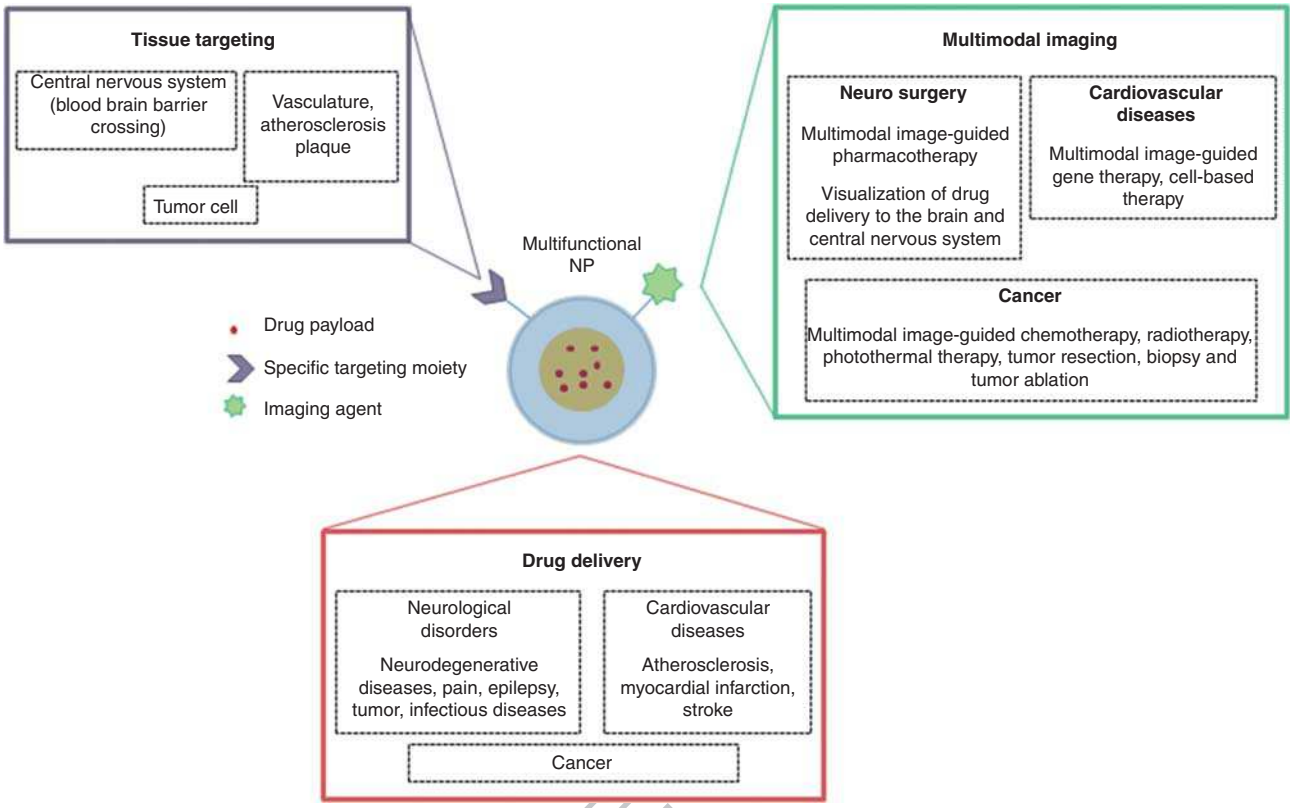

Figure 2. Multifunctional and multimodal NPs for image-guided therapy. Schematic representation of ideal NPs combining functional components for effective targeted imaging and drug-delivery. The potentialities and clinical application of the multifunctional and multimodal NPs are summarized.
NP: Nanoparticle.

in fact much slower. In this contest, a promising material is represented by mesoporous SiNPs and zeolite-L nanocrystals, that have been proved to be an interesting option for the delivery not only of molecular drugs, but also of nucleic acids (as DNA and PNA) [89,90].

5. Safety of NPs in medicine

A major open question dealing with NPs in medicine is the issue of safety. Although this is not a strong concern for pre-clinical applications in small animal models, it is fundamental for the translation at clinical level when nanoprobe toxicity needs to be carefully evaluated. The plethora of variables to be considered makes the toxicity evaluation of these systems hard to rationalize, with an evaluation process that frequently needs to follow a case-by-case basis.

Due to the large surface to area ratio, nanomaterials have frequently major toxicity effects than the corresponding bulk materials [91]. The toxic effect is mainly related to the release of components (i.e., ions and/or molecular fragments). In addition, the nanomaterial-cell membrane interaction can trigger cell surface effects, usually mediated by a 'protein corona' formed by the interaction with the macromolecules that a nanosystem encounters in the district of entry [92]. For these reasons, important parameters to be considered for NP safety are their size and aggregation behavior. Indeed, the same mass dose of a smaller system means a larger surface area that can result in an increased toxicity, since both components release and cell membrane interaction are increased. The NP aggregation behavior is strongly influenced by surface properties and also by the external environment conditions. Therefore, in the next future, the possibility to monitor with different instrumental techniques the colloidal behavior of nanosystems in real time in the biological environment, as well as the NP-cell membrane interactions, will probably increase the knowledge dealing with the *in vivo* biological response toward nanomaterials [93]. Beside NPs composition, the surface chemistry is the main variable to increase the circulation time, counteracting the NPs capture by the phagocytic system. Up to now NPs PEG is the main strategy to reduce opsonization and liver capture and to reduce unspecific binding in the biological environment [11].

A main issue for the safety of nanomaterials is also related to the need to avoid accumulation in specific tissues:

Table 4. Nanoparticles approved for clinical application and/or in clinical trials [106].

Platform	Drug	Brand name	Indications	Status
Albumin-based particles	Paclitaxel	Abraxane	Breast cancer, non-small cell lung cancer, pancreatic cancer	Approved
Liposomes	Paclitaxel	DaunoXome	Kaposi's sarcoma associated with HIV	Approved
Liposomes	Doxorubicin	Doxil	Ovarian cancer, multiple myeloma (in combination with bortezomib), Kaposi's sarcoma associated with HIV	Approved
Liposomes	Doxorubicin	Myocet	Metastatic breast cancer	Approved
Liposomes	Amphotericin B	AmBisome	Systemic fungal infections	Approved
Liposomes	Cytarabine	Depocyt	Malignant lymphomatous meningitis	Approved
Liposomes	Morphine	DepoDur	Postsurgical analgesia	Approved
Liposomes	Verteporfin	Visudyne	Age-related macular degeneration, pathologic myopia, ocular histoplasmosis	Approved
Polymeric micelles	Paclitaxel	Genexol-PM	Breast cancer, lung cancer, ovarian cancer	Approved
		NK105	Stomach cancer, breast cancer	Phase II/III
Polymer–drug conjugate-based particles	Doxorubicin	Livatag	Hepatocellular carcinoma	Phase III
Polymer–drug conjugate-based particles	Paclitaxel	XYOTAX	Ovarian cancer, non-small cell lung cancer	Phase III
		PNU166148	Solid tumors	
Polymer–drug conjugate-based particles	Camptothecin	CT-2106	Colorectal, lung and ovarian cancers	Phase I/II
		MAG-CPT	Solid tumors	Phase II
		Pegamotecan	Gastric and gastroesophageal cancers	
Polymer–drug conjugate-based particles	Platinate	AP5346	Solid tumors	Phase II
Polymer–drug conjugate-based particles	Doxorubicin	FCE28068 (PK1)	Various cancers, particularly lung and breast cancers	Phase II
		FCE28069 (PK2)	Hepatocellular carcinoma	
Polymer–drug conjugate-based particles	Irinotecan	NKTR-102	Breast, ovarian, colorectal, and lung cancers, glioblastoma	Phase II/III
Polymeric	Docetaxel	BIND-014	Solid tumors	Phase I
Polymeric	small interfering RNA	CALAA-01	Solid tumors	Phase I

bio-elimination can be safely achieved mainly using biodegradable organic NPs (this is the reason why the majority of nanosystems used at clinical level exhibits this characteristic). In the case of inorganic 'hard' NPs, bioaccumulation can be avoided tailoring the NP dimension, since excretion from the body is a size-governed process. It is largely recognized that excretion with urines occurs when NPs are smaller than the renal excretion threshold, which is found to be around 6 nm [94]. However, such tiny dimensions involve the considerable drawback to carry a limited payload of active agents (i.e., drug, dyes or contrast agents). Considering that most of the nanoparticulate material suitable for medicine applications is above this threshold, this excretion pathway is a difficult task to reach and fecal excretion has to be quantitatively examined. In humans, NPs with diameter larger than ~ 6 nm undergo bioaccumulation with possible long-term toxicity effects, whose evaluation is problematic. An alternative strategy to avoid bio-accumulation of hard NPs contrast agents is to design a medium-term biodegradable matrix, able to develop smaller fragments that are finally excreted with urines. This is probably one of the needs for the development of medicine nanotools in the next future.

6. Expert opinion

The progressive breakthroughs in nanomedicine have supported the development of multifunctional and multimodal NPs. In particular, besides the loading with contrast agents and/or therapeutic compounds, the conjugation of targeting moieties on the surface of multifunctional nanomaterials can confer specific targeting to the imaging procedure and drug delivery, with the possibility to design strategies for concomitant diagnostic and therapeutic applications. Therefore, these systems are now being actively investigated and hopefully will form the next generation of NP-platforms, which will facilitate personalized and tailored treatment in particular, but nonexclusively, in the oncologic field. To be clinically acceptable, they should exhibit specific cell targeting, optimal clearance profiles and be nontoxic for normal tissues, with safety and overall performance fully evaluated in the long-term. Indeed, both FDA and SCENIHR recently published documents to guide the safe development of nanotechnology-based products for clinical practice [95,96].

At present, despite the intense advances in NP design, only few nanomedicines have been approved for human

Table 5. Advantages and limitations of different nanocarriers in drug delivery.

Nanoparticle	Advantages	Limitations
Inorganic nanoparticles		
Silica nanoparticles	Simple, cheap, mild conditions synthesis with size tunability. Well known surface chemistry for functionalization and targeting. Intrinsic non-toxic hydrophilic material. Optical properties are conferred by the doping materials. Development of systems with tunable emission properties based on FRET approach	Surface functionalization often needed to avoid aggregation and unspecific binding. Tiny systems have a moderate brightness. Self quenching phenomena whitin the NP at high doping regime
Gold nanoparticles	Simple synthesis. Ability to tailor the functionality of the surface. Intrinsic properties of the gold core ideal for photodynamic therapies, contrast imaging, and thermal ablation. High drug load	Biocompatibility, bioaccumulation. Uncoated gold nanoparticles are susceptible to aggregation in solution and can melt under laser irradiation. Possible catalytic effect in the case of very tiny gold clusters
Superparamagnetic iron oxide crystals	Possibility of using passive and active drug delivery strategies. High efficiency in drug targeting by locally applying an external magnetic field. Simple functionalization for the targeting. Visualization in MRI. Theranostic carriers	Tend to aggregate into larger clusters. Toxicity due to reactive surface
Colloidal semiconductor	Photostability. High fluorescent quantum yields, broad absorption and sharp emission peaks (multiplexing). Multiplex essay and targeting of the surface. Theranostic carriers	Synthetic procedure at high temperature with toxic or carcinogenic reagents, with batch to batch variability. Toxicity effect of metal core, bioaccumulation. Blinking behavior
Organic nanoparticles		
Polymer-based nanoformulations	Well established mild conditions synthetic approaches. Highly monodisperse systems, size tenability. Biodegradability. Functionalization for targeting	Mechanical stability. Long-term chemical stability. Prone to aggregation/deformation under mechanical stress
Liposomes	Low toxicity, biocompatibility, and biodegradability. Ability to modify size and surface. Encapsulate both hydrophobic and hydrophilic drugs	Low stability. Poor batch to batch reproducibility. Low drug entrapment

applications, suggesting that there is still a need for improving the understanding of their behavior in the human body (pharmacokinetics, biodistribution, extravasation, tumor accumulation, elimination). In particular, multifunctional/multimodal NPs are usually a combination of products whose synergy could enhance the effects of the single product, possibly changing both the biological as well as the imaging performance [97]. Multimodal NPs offers exciting opportunities of development and may have a huge impact on health care in several fields [98]. In particular, we believe that the design and characterization of innovative NP-based systems/devices containing more than one imaging modality and functionalized for specific 'molecular recognition' have great applicative potential into the medical field. For this purpose, more progresses will be dependent on the identification of specific ligand directed against selected cell targets (i.e., surface markers) that will amplify the specificity of the NPs to be used for both diagnostic and therapeutic applications.

Beside oncology, the technology based on multifunctional/multimodal NPs hold promises for improving the management of diabetes [99]. In particular, since one of its crucial pathogenic factors is the progressive loss of insulin producing beta-cells within the pancreatic Langerhans islets, measuring and/or monitoring *in vivo* the mass of beta-cells would be fundamental in providing more reliable and earlier diabetes diagnosis, more accurate clinical decisions, and significantly facilitate follow-up of therapy/transplantation.

Declaration of interest

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Review~~, US Spelling~~

Applications of nanoparticles in cancer medicine and beyond: optical and multimodal *in vivo* imaging, tissue targeting and drug delivery

Applications of nanoparticles in cancer medicine and beyond~~: optical and multimodal *in vivo* imaging, tissue targeting and drug delivery~~

S. Biffi *et al.*

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Introduction:

Nanotechnology has opened up the way to the engineering of new organized materials endowed with improved performances. In the past decade, engineered nanoparticles (NPs) have been progressively implemented by exploiting synthetic strategies that yield complex materials capable of performing functions with applications also in medicine. Indeed, in the field of 'nanomedicine' it has been explored the possibility to design multifunctional nanosystems, characterized by high analytical performances and stability, low toxicity and specificity towards a given cell target.

Area covered:

In this review article, we summarize the advances in the engineering of NPs for biomedical applications, from optical imaging (OI) to multimodal OI and targeted drug delivery. For this purpose, we will provide some examples of how investigations in nanomedicine can support preclinical and clinical research generating innovative diagnostic and therapeutic strategies in oncology.

Expert opinion:

The progressive breakthroughs in nanomedicine have supported the development of multifunctional and multimodal NPs. In particular, NPs are significantly impacting on the diagnostic and therapeutic strategies since they allow the development of: NP-based OI probes containing more than one modality-specific contrast agent; surface functionalized NPs for specific 'molecular recognition'. Therefore, the design and characterization of innovative NP-based systems/devices have great applicative potential into the medical field.

Keywords: ~~Đ~~diagnostic strategies, drug delivery, nanoparticles, optical imaging

1. Introduction

Nanotechnology is an emerging scientific area that has created a multiplicity of intriguing and versatile submicrometer-sized materials. Nanoparticles (NPs) in particular can be prepared from a variety of materials such as metals, metal oxides, semiconductors, proteins, polysaccharides and synthetic polymers [1]. Different types of NP have been developed with peculiar physicochemical properties (such as chemical reactivity, energy absorption and biological mobility) that distinguish them from bulk materials by virtue of their size and surface characteristics [1]. In recent years, these materials have emerged as important tools in medicine, with various applications ranging from contrast agents in molecular imaging to carriers for drug delivery [2,3].

Within the set of NPs with biomedical applications, two main families can be distinguished: inorganic NPs, such as silica nanoparticles (SiNPs), gold NPs (GNPs), superparamagnetic iron oxide crystals (SPIONs), colloidal semiconductor quantum dots (QDs), and organic NPs (polymer- and lipid-based NPs) (Table 1). In particular, among the inorganic nanoformulations, silica-based nanomaterials have been investigated mainly because of: i) the fine size-control, ; and ii) a chemistry suitable for surface modification [4,5,6]. Based on these properties, several investigators including our group have considered SiNPs for applications as sensors [7], drug delivery systems [8] and/or bioimaging probes [9,10,11,12]. Metallic (iron oxide, gold, silver) NPs have been used for a huge number of applications in various areas of medical treatment and are emerging as tool for imaging, diagnosis, and for the delivery of therapeutic agents to tumour cells [13,14,15]. Metallic NPs can be used in diagnosis and therapeutics due to their unique properties of small size, large surface area to volume ratio, tunable absorption, photostability and cellular uptake [15]. Among all metallic NPs the GNPs proved to be among the safest (non-toxic) agents for biomedical applications. GNPs, based on their unique physico-chemical and plasmonic properties, which can be tuned across the vis-NIR spectral band, have been exploited for chemical and biological sensing

16, genomics and immunoassays 16, multimodality imaging, tumor targeting, and as vehicle for various therapeutics 17. Additionally, GNPs have been used as photothermal therapeutics against cancer 17.

The intrinsic properties of SPIONs, such as inherent magnetism, broad safety margin and the availability of methods for fabrication and surface engineering, allow applications in bioimaging, drug delivery and thermal therapy 18. SPIONs have been reported as tracers for imaging for: tumours and metastases; CNS and magnetic resonance angiography; atherosclerotic plaque and thrombosis 19. Moreover, SPIONs can achieve a very high efficiency in drug targeting since, by locally applying an external magnetic field to the target organ, it is possible to drive the accumulation of the magnetic NPs into the site of drug action 18. QDs are of interest since the emission wavelength of these NPs can be continuously tuned by changing the particle size, and a single light source can be used for simultaneous excitation of different-sized QDs 20. Current and future applications of QDs include the *in vitro* and *in vivo* fluorescence imaging 20, 21, the use in microarrays 22, in drug delivery 23 or as sensitizers for photodynamic therapy (PDT) 23. Moreover, multifunctional integrated targeting, imaging and therapeutic functionalities based on QDs and fluorescence imaging system, have become effective materials for synchronous cancer diagnosis and treatment 24.

On the other hand, the organic nanoformulations currently investigated in the field of nanomedicine include: PEG, poly(glutamic acid), poly(lactic-co-glycolic acid) (PLGA), as well as N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers and polysaccharides (i.e., chitosan, cyclodextrin). In particular, PLGA has generated tremendous interest due to its excellent biocompatibility, biodegradability, and mechanical strength 25. For these reasons, the US FDA approved the use of PLGA and polylactide polymers in microspheres (to be used via parenteral administration route), implants and periodontal drug-delivery systems 26. PLGA NPs have been

used as carriers for different compounds including drugs, peptides, proteins, nucleotides and vaccines [27]. Upon surface engineering, PLGA have been assessed for targeted delivery to malignant cells with high affinity [28,29,30,31]. Finally, among the lipid-based NPs, liposomes have received a lot of attention during the past 30 years as convenient delivery vehicles for biologically active compounds [32,33]. The aqueous core can be used to encapsulate hydrophilic drugs, whereas lipophilic drugs can be incorporated into the external NP membrane. Moreover, the liposome surface can be easily functionalized to enhance their *in vivo* stability or to enable their preferential delivery. Lipidic NPs are the first nanomedicine delivery system to make the transition from concept to clinical application, and they are now an established technology platform with considerable clinical acceptance [32,33].

In the present review, we attempt to provide a general overview of the main properties of the NP-systems, either in use or under investigation, for optical bioimaging and drug delivery applications, focusing our attention on same examples of pre-clinical investigations in nanomedicine aimed to the generation of innovative diagnostic and/or therapeutic NP-based strategies in oncology.

2. NPs for optical imaging

Optical imaging (OI) is an emerging imaging modality with high potential for improving diseases diagnosis and treatment. The commonly used OI methods for biomedical applications include fluorescence imaging, bioluminescence imaging, optical coherence tomography, photoacustic imaging and Raman spectroscopy [34,35]. OI relies on non-ionizing radiation and uses light as the primary imaging modality to characterize local anatomy down to cellular and molecular levels. Thus, OI technologies are becoming critical tools to image structural, functional, and molecular information using their unique photon absorption or scattering profiles. The most important limitation for *in vivo* OI is represented by a limited tissue penetration [36]. However, this issue can

be at least partially addressed by the use of near-infrared (NIR) OI, due to reduction in scattering and minimal absorption within the NIR spectral window. Thus, NIR modalities are the OI approaches of choice to *in vivo* human studies and have dominated the field of intraoperative image-guided surgery recently [37]. In this contest, the technical advancements enabled the development of important clinical applications such as: intraoperative sentinel lymph node mapping for cancer staging [38,39], optical image guided surgery for detection of tumo~~u~~r margins and malignant masses [40,41,42], and video-angiography during surgery [43]. Several OI probes have been developed and have been used in clinical trials until now: indocyanine green (ICG), 5-aminolevulinic acid (5-ALA) and methylene blue (MB) [44]. ICG has FDA approval for clinical applications and is currently utilized in NIR fluorescence for sentinel lymph node mapping, intraoperative identification of solid tumo~~u~~rs and angiography during reconstructive surgery [40,45]. 5-ALA has been described as a non-fluorescent pro-drug that leads to intracellular accumulation of fluorescent porphyrins in malignant gliomas, thus enabling more complete resections of contrast-enhancing tumo~~u~~r, leading to improved progression-free survival in patients with malignant glioma [46]. More recently, the use of MB has been reported for intraoperative NIR fluorescence imaging of a paraganglioma, allowing the identification of an otherwise undetectable lesion [47]. Anyhow, the small organic fluorescent dyes present drawbacks such as low fluorescence quantum yield, which results in reduced brightness, photobleaching, a very fast body clearance, and the lack of precise targeting properties. All these critical aspects can be overcome by the use of nanostructured probes. Indeed, the performances of NP-contrast agents, when compared with conventional OI methods, are characterized by: i) increased contrast ~~efficiency~~ efficiency [44]; ii) increased circulation (blood residence) ~~time~~ time [3]; iii) possibility of combining different functions (diagnosis and therapy~~)~~) [48]; iv) improved tumo~~u~~r ~~penetration~~ penetration [2,49]; v) multi-spectral ~~capabilities~~ capabilities [21] and; vi) multi-modal detection, as NPs provide a versatile carrier system to simultaneously load modality-specific contrast agents [48]. So far, a wide range of fluorescent NPs suitable for OI have

been designed and tested in preclinical studies [50] and the characteristics of the most relevant NP types are summarized in (Table 2). Among the different types of fluorescent NPs, the main distinctive property of QDs in comparison with traditionally used organic dyes, is their outstanding resistance to photobleaching. QDs have also a high photoluminescence quantum yield and high molar extinction coefficient, especially in the UV-VIS wavelength range. They can exhibit a broad absorption with narrow and symmetric emission spectra spanning from VIS to NIR depending on QDs composition and size [51]. A possible drawback of QDs is related to their composition; since they typically contain highly poisonous heavy metal, there is some concern about their possible toxicity. In this context, biodegradable organic NPs together with silica (that is recognized as safe by FDA) NPs are now receiving increasing attention as OI probes [10,12,52]. In this respect, we have recently reported a synthetic strategy for the development of silica-PEG core-shell nanostructures doped with a donor–acceptor couple, able to display a tunable intensity profile across the NIR spectrum [12,53,54]. It is worth mentioning that recently tiny dye-doped SiNPs, called Cornell dots (C dots), have entered clinical investigation in melanoma patients [9,55]. These NPs consist of NIR fluorescent molecules encapsulated in a silica core and surrounded by a silica shell, which is functionalized with both cyclic RGD peptide and radioactive iodine. Its applications in melanoma include real-time, intraoperative detection and imaging of nodal metastases, differential tumour burden and lymphatic drainage patterns. It is of interest the evidence that this multimodal nanoplatform has advanced to the clinical translation by combining key benefits of NIR fluorescence imaging (enhanced sensitivity/contrast) with those of positron emission tomography (PET), (depth penetration, quantitation). By exploiting NIR fluorescence imaging the authors were able to elucidate sub-millimetre structures of metastatic disease in small local/regional nodes that PET could not, though PET enabled deeper penetration and quantitation [9,55].

Upconversion NPs are emerging as a new class of OI contrast agents. They are extremely interesting particularly for their ability to emit anti-Stokes shifted light (upconversion) with

relatively high brightness, thus providing low imaging background and deep tissue penetration 56. Also, polymer–encapsulated organic NPs have attracted increasing attention for optical properties and outstanding performance as imaging agents 57. In this context, we have shown that incorporation of the fluorescent probe Cy5.5 into PLGA-NPs engineered with an anti-CD20 antibody (Rituximab) allowed to visualize the CD20-positive tumour mass by OI 31. As newly emerging optical nanoprobes, surface-enhanced Raman scattering (SERS) tags have been gaining great interest in the application of biomedical imaging and phototherapies 58,59. In particular, SERS tags combine metallic (Au or Ag) NPs and specific organic Raman reporter molecules, such as organic dyes. A protective shell together with antibodies or ligands is also employed for targeting, which endows the SERS tag with specific targeting ability and biocompatible properties. Fluorescent-SERS dual mode tags showed great potential for bioimaging due to the combined advantages of intuitive, fast imaging of fluorescence and multiplex capability of SERS technique 60.

Besides NPs made by an intrinsically fluorescent material, the entrapment of NIR-emitting organic dyes into NPs is versatile approach to optimize the OI performances of a fluorescent nanoprobe. With this approach the nanoparticle matrix can shield the doping dyes against chemical and/or biological degradation and frequently improves the resistance to photobleaching and the emission properties 50. A similar behaviour was shown for the encapsulation of the NIR fluorescent dye IR-783 within the human serum albumin that reduced the dye photobleaching significantly 61.

3. Nanoplatforms for multimodal OI

The current imaging modalities vary in sensitivity, resolution and quantitative capabilities, with each modality offering its own unique benefits and intrinsic limitations. Obviously, multi-modality imaging – combining different imaging modalities – has the potential to overcome the limitations

of a single imaging modality, and therefore, is a central task for current and future clinical imaging research [62]. As already discussed, the main limitation of OI is the relatively small penetration, due to the scattering and absorption of light in tissues [36]. Thus, the combination of OI with other imaging modalities, such as MRI, PET, CT and/or photoacustic, results in deeper tissue imaging providing accurate anatomical information. In this regard, multimodal imaging may greatly take advantage from the development of multifunctional NP-based contrast agents able to combine different physical imaging properties on the same nanoplatform (Table 3). Moreover, the conjugation of recognition moieties on the surface of multifunctional nanomaterials confers specific targeting properties to the imaging procedure (Figure 1) and may allow concomitant therapeutic applications [48] (Figure 2).

For instance, as outlined in (Figure 1), NP-based multimodal contrast agents offer several applications for multimodality imaging in oncology surgery. While OI allows for the intra-surgery discrimination of tumour margins, pre-surgery analyses with multimodal NPs that combine OI and MRI or PET modalities offer high spatial resolution and assessment of the depth of tumour penetration, lymph node involvement and presence of distant metastatic diseases [63, 64, 65]. Further increment in imaging performance has been obtained by the design of trimodality imaging probes combining contrast agents for OI, MRI and PET [66, 67, 68]. In recent years, the emerging development of multimodal NP carrying therapeutic agents has received considerable attention for imaging-guided therapy (Figure 2) that combine the possibility to improve the targeting specifically to the diseased tissue and also to monitor the efficacy of the treatments [18, 19, 69]. The clinical area that certainly benefits the most from the combination of imaging and treatment is cancer research. Several studies have reported multifunctional and multimodal NPs applied in pre-clinical and clinical settings for: i) delivery of therapeutic agents to tumour cells [70, 71] and ii) assessment of the cancer therapeutic response [72]. Increasing evidences have also demonstrated the feasibility and

potency of utilizing multifunctional and multimodal NPs for CNS diseases by using specific targeting moieties able to cross the blood brain barrier and, thus, allowing the delivery of therapeutic agents to the brain [73,74] beside the guide surgical resection/ablation of brain tumours [67]. Finally, in the context of cardiovascular diseases, image-guide therapy has been applied for: i) intramyocardial injection of therapeutics guided by multimodal imaging [75]; and ii) for early and accurate evaluation of the response to pro-angiogenic therapy in preclinical diabetic stroke models [76].

4. NPs for drug delivery

The development of drug delivery systems able to improve the bioavailability, tissue uptake and pharmacokinetics of therapeutic agents is central to biomedical research and pharmaceutical industry. In this respect, clinical results suggest that NPs carrying therapeutic agents can exhibit enhanced efficacy, while simultaneously reduce side effects, owing to properties such as cell/tissue targeted delivery and active cellular uptake [3,77]. By itself, injected NPs preferentially accumulate in tumour tissues due to the enhanced permeability and retention effects and therefore are ideally suited for the delivery of chemotherapeutics in cancer therapy [78]. Among the examples of NP carrying chemotherapeutics listed in (Table 4) approved by FDA, we can mention: Genexol-PM, a polymeric micellar formulation of paclitaxel [79] and Doxil, a liposomal formulation of doxorubicin [80], both of which are used as first-line treatment of different tumours. Genexol-PM has significantly improved the maximum tolerated dose of paclitaxel, allowing delivery of higher doses without additional toxicity [79]. Doxil has dramatically prolonged doxorubicine circulation time and enhanced drug release at the tumour site [80]. Nanotherapeutics are rapidly progressing and are being implemented to solve several limitations of conventional drug delivery systems. While some nanomedicine-based drug delivery systems have already been marked and others are in clinical

trials (Table 4), most are in the stage of preclinical development. Advantages and disadvantages of the different types of nanocarriers are summarized in (Table 5).

To improve the therapeutic index (the ratio of the toxic to the therapeutic dose) of drug formulations, reducing their toxicity to normal tissues, a second generation of NPs for drug delivery includes surface functionalities that enable the specific 'molecular recognition' of the target tissue or active/triggered release of the payload at the site of the disease 81. The engineering of a targeting ligand onto the NP surface result in localized drug delivery, which should translate into greater efficacy and reduced drug side effects 82,83. Cancer treatment stands to benefit from targeted drug delivery, as tumour cells express many molecules on their surface that distinguish them from normal cells 84. In this context, targeted polymeric NPs are emerging as an important class of therapeutic, among these, BIND-014 has been the first targeted polymeric NP for cancer chemotherapy to reach clinical development 85. BIND-014 is a polymeric NP containing the chemotherapeutic docetaxel and is targeted to recognize the prostate-specific membrane antigen, a tumour antigen expressed on prostate cancer cells and on the neo-vasculature of most non-prostate solid tumours. Initial clinical data in patients with advanced solid tumours indicate that BIND-014 displays an improved pharmacokinetic and pharmacological profile in comparison with docetaxel 86,87. CALAA-01 is another notable example of targeted NP for cancer therapy, which has been evaluated in a Phase I clinical trial for the treatment of patients with solid tumours 88. It is a polymeric NP that encapsulates small interfering RNA and is functionalized with human transferrin protein (Tf), to target the Tf receptor overexpressed on the surface of most cancer cells. It is to note that all the NPs mentioned so far are made by soft materials; the advancement of hard carriers is in fact much slower. In this contest, a promising material is represented by mesoporous SiNPs and zeolite-L nanocrystals, that have been proved to be an interesting option for the delivery not only of molecular drugs, but also of nucleic acids (as DNA and PNA) 89,90.

5. Safety of NPs in medicine

A major open question dealing with NPs in medicine is the issue of safety. Although this is not a strong concern for preclinical applications in small animal models, it is fundamental for the translation at clinical level when nanoprobe toxicity needs to be carefully evaluated. The plethora of variables to be considered makes the toxicity evaluation of these systems hard to rationalize, with an evaluation process that frequently needs to follow a case-by-case basis.

Due to the large surface to area ratio, nanomaterials have frequently major toxicity effects than the corresponding bulk materials [91]. The toxic effect is mainly related to the release of components (i.e., ions and/or molecular fragments). In addition, the nanomaterial-cell membrane interaction can trigger cell surface effects, usually mediated by a 'protein corona' formed by the interaction with the macromolecules that a nanosystem encounters in the district of entry [92]. For these reasons, important parameters to be considered for NP safety are their size and aggregation behaviour. Indeed, the same mass dose of a smaller system means a larger surface area that can result in an increased toxicity, since both components release and cell membrane interaction are increased. The NP aggregation behaviour is strongly influenced by surface properties and also by the external environment conditions. Therefore, in the next future, the possibility to monitor with different instrumental techniques the colloidal behaviour of nanosystems in real time in the biological environment, as well as the NP-cell membrane interactions, will probably increase the knowledge dealing with the *in vivo* biological response toward nanomaterials [93]. Beside NPs composition, the surface chemistry is the main variable to increase the circulation time, counteracting the NPs capture by the phagocytic system. Up to now NPs PEG is the main strategy to reduce opsonization and liver capture and to reduce unspecific binding in the biological environment [11].

A main issue for the safety of nanomaterials is also related to the need to avoid accumulation in specific tissues: bio-elimination can be safely achieved mainly using biodegradable organic NPs

(this is the reason why the majority of nanosystems used at clinical level exhibits this characteristic). In the case of inorganic 'hard' NPs, bioaccumulation can be avoided tailoring the NP dimension, since excretion from the body is a size-governed process. It is largely recognized that excretion with urines occurs when NPs are smaller than the renal excretion threshold, which is found to be around 6 nm 94. However, such tiny dimensions involve the considerable drawback to carry a limited payload of active agents (i.e., drug, dyes or contrast agents). Considering that most of the ~~N~~nano-particulate material suitable for medicine applications is above this threshold, this excretion pathway is a difficult task to reach and f~~a~~ecal excretion has to be quantitatively examined. In humans, NPs with diameter larger than ~ 6 nm undergo bio-accumulation with possible long-term toxicity effects, whose evaluation is problematic. An alternative strategy to avoid bio-accumulation of hard NPs contrast agents is to design a medium-term bio-degradable matrix, able to develop smaller fragments that are finally excreted with urines. This is probably one of the needs for the development of medicine nanotools in the next future.

6. Expert opinion

The progressive breakthroughs in nanomedicine have supported the development of multifunctional and multimodal NPs. In particular, beside~~s~~ the loading with contrast agents and/or therapeutic compounds, the conjugation of targeting moieties on the surface of multifunctional nanomaterials can confer specific targeting to the imaging procedure and drug delivery, with the possibility to design strategies for concomitant diagnostic and therapeutic applications. Therefore, these systems are now being actively investigated and hopefully will form the next generation of NP-platforms, which will facilitate personalized and tailored treatment in particular, but non-exclusively, in the oncologic field. To be clinically acceptable, they should exhibit specific cell targeting, optimal clearance profiles and be nontoxic for normal tissues, with safety and overall performance fully

evaluated in the long-term. Indeed, both FDA and SCENIHR recently published documents to guide the safe development of nanotechnology-based products for clinical practice [95,96].

At present, despite the intense advances in NP design, only few nanomedicines have been approved for human applications, suggesting that there is still a need for improving the understanding of their behaviour in the human body (pharmacokinetics, biodistribution, extravasation, tumour accumulation, elimination). In particular, multifunctional/multimodal NPs are usually a combination of products whose synergy could enhance the effects of the single product, possibly changing both the biological as well as the imaging performance [97]. Multimodal NPs offers exciting opportunities of development and may have a huge impact on health care in several fields [98]. In particular, we believe that the design and characterization of innovative NP-based systems/devices containing more than one imaging modality and functionalized for specific 'molecular recognition' have great applicative potential into the medical field. For this purpose, more progresses will be dependent on the identification of specific ligand directed against selected cell targets (i.e., surface markers) that will amplify the specificity of the NPs to be used for both diagnostic and therapeutic applications.

Beside oncology, the technology based on multifunctional/multimodal NPs hold promises for improving the management of diabetes [99]. In particular, since one of its crucial pathogenic factors is the progressive loss of insulin producing beta-cells within the pancreatic Langerhans islets, measuring and/or monitoring *in vivo* the mass of beta-cells would be fundamental in providing more reliable and earlier diabetes diagnosis, more accurate clinical decisions, and significantly facilitate follow-up of therapy/transplantation.

Article highlights.

Engineered nanoparticles (NPs) have been progressively implemented to obtain high analytical performances and stability, low toxicity and specificity towards a given cell target.

NPs are important tools in medicine, with various applications ranging from imaging, diagnosis and delivery of therapeutic active agents.

Recent clinical advances in ~~O~~o_o_ptical imaging (OI) point out its potential for medical applications. Anyhow, the small organic fluorescent dyes present drawbacks such as: low fluorescence quantum yield, photobleaching, a very fast body clearance, and the lack of precise targeting properties. All these critical aspects can be overcome by the use of NPs.

The combination of OI with other imaging systems results in deeper tissue imaging that allows accurate anatomical information. In this regard, multimodal imaging may greatly take advantage from the development of NP-based contrast agents able to combine different physical imaging properties on the same nanoplatform.

The conjugation of targeting moieties on the surface of NPs can confer specific targeting to the imaging procedure and drug delivery.

It is concluded that the design and characterization of innovative NP-based systems/devices containing more than one modality-specific imaging contrast agent and functionalized for specific ‘molecular recognition’ have great applicative potential into the medical field.

This box summarizes key points contained in the article.

Declaration of interest

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Table 1. Relevant nanoparticles for biomedical applications.

Nanoplatform	Physico-chemical properties	Biomedical applications	~~References~~Ref.
Inorganic nanoparticles~~:~~			
~~–~~Silica nanoparticles ~~(SiNPs)~~	Diameter from 5 to 300 nm; uniform and tunable pore size; high chemical and mechanical stability; surface functionalizatio n and bioconjugation	Drug delivery; bioimaging; lymph node mapping; sensors;	4, 5, 6, 7, 8, 9, 10, 11, 12
~~–~~Gold nanoparticles ~~(GNPs)~~	Diameter from 5 to 300 nm; tunable localized surface plasmon resonance; large absorption and scattering cross-	Genomics and biosensorics; immunoassay s and clinical chemistry; Fluorescence Resonance	13, 14, 15, 16, 17

	sections; localized enhanced electromagnetic field; surface functionalization and bioconjugation	Energy Transfer (FRET) technologies (e.g., for measuring protein interactions, protein conformational changes); photothermal therapy; drug delivery; optical imaging (OI); X-ray CT; MRI	
–Superparamagnetic iron oxide crystals ~~(SPIONs)~~	Iron core of 4 – 5 nm in diameter coated by either inorganic materials (silica, gold) or organic	MRI; lymph node mapping, drug delivery; gene delivery;	18, 19

	materials (phospholipids, fatty acids, polysaccharides, peptides, polymers); superparamagnetic; surface functionalization and bioconjugation	photothermal therapy	
–Colloidal semiconductor ~~(QDs)~~	Diameters of 2-20 nm; nanocrystals and core-shell nanocrystals based on different semiconductor materials; the band-gap energy that determines the energy of the fluorescent light is inversely	Oligonucleotide array; Fluorescence Resonance Energy Transfer (FRET) technologies (i.e., for measuring protein interactions, protein conformation	20, 21, 22, 23

	proportional to the size of the quantum dot; surface functionalization and bioconjugation	al changes); OI of cells and tissues; lymph node mapping; photodynamic therapy; drug delivery; gene delivery	
Organic nanoparticles:			
–Polymer-based nanoformulations	Diameter from 5 to 300 nm; controlled release property; surface functionalization and bioconjugation	Drug delivery; bioimaging	25,26,27,28,29,30,31
–Liposomes	Diameters from 15 nm up to several μm; vesicular structures with an aqueous core	Gene delivery; drug delivery	32,33

	surrounded by a hydrophobic membrane bilayer composed by phospholipids structure; surface functionalizatio n and bioconjugation	

Table 2. Relevant nanoparticle-based contrast agents for optical imaging.

Nanoplatform	Optical properties	~~References~~Ref.
Quantum dots ~~(QDs)~~	High photoluminescence quantum yield; high molar extinction coefficients; broad absorption and narrow emission spectra; large distinction	24

	between the excitation and emission spectra	
Silica nanoparticles ~~(SiNPs)~~	Tunable intensity profile across the NIR spectrum; large pseudo Stokes-shifts	54
Upconversion nanoparticles	Large anti-Stokes shift; NIR excitation; high signal-to-noise ratio; high photostability	56
Polymer-based NPs and lipid-based NPs embedding organic NIR dyes	Protection of the NIR dyes against chemical and/or biological degradation, improvement of their photophysical properties	57

Table 3. Example of nanoparticle-based multimodal contrast agents for multimodal optical imaging.

Nanoparticle	Imaging modalities	Applications	Ref.~~References~~
Iron oxide	Magnetic resonance/positron emission tomography (PET)/optical	Imaging of sentinel lymph nodes	66
Graphene	PET/optical	Image-guided photodynamic cancer therapy	70
Upconversion nanoparticle	CT/magnetic resonance/optical	Image-guided cancer therapy	71
Dendrimer-based nanoparticle	Magnetic resonance/optical	Image-guided therapy of stroke	76
Polymeric nanoparticle	Magnetic resonance/optical	Imaging for tumor detection	100
Chitosan nanoparticle	PET/optical	Imaging for tumor	101

		detection	
Upconversion nanoparticle	Single-photon emission CT/CT/magnetic resonance/optical	Tumor angiogenesis imaging	102
Reduced graphene oxide-iron oxide nanoparticle	Photoacustic/magnetic resonance/optical	Image-guided photothermal cancer therapy	103
Dendrimer-based nanoparticle	Magnetic resonance/optical	Image-guided surgery of brain tumors	104
Plant viral nanoparticle platform tobacco mosaic virus	Magnetic resonance/optical	Imaging of atherosclerotic plaques *in vivo*	105

Table 4. Nanoparticles approved for clinical application and/or in clinical trials 106.

Platform	Drug	Brand name	Indications	Status
Albumin-based particles	Paclitaxel	Abraxane	Breast cancer, non-small cell lung cancer, pancreatic cancer	Approved

Liposomes	Paclitaxel	DaunoXome	Kaposi's sarcoma associated with HIV	Approved
Liposomes	Doxorubicin	Doxil	Ovarian cancer, multiple myeloma (in combination with bortezomib), Kaposi's sarcoma associated with HIV	Approved
Liposomes	Doxorubicin	Myocet	Metastatic breast cancer	Approved
Liposomes	Amphotericin B	AmBisome	Systemic fungal infections	Approved
Liposomes	Cytarabine	Depocyt	Malignant lymphomatous meningitis	Approved
Liposomes	Morphine	DepoDur	Postsurgical analgesia	Approved
Liposomes	Verteporfin	Visudyne	Age-related macular	Approved

			degeneration, pathologic myopia, ocular histoplasmosis	
Polymeric micelles	Paclitaxel	Genexol-PM	Breast cancer, lung cancer, ovarian cancer	Approved
		NK105	Stomach cancer, breast cancer	Phase II/III
Polymer–drug conjugate-based particles	Doxorubicin	Livatag	Hepatocellular carcinoma	Phase III
Polymer–drug conjugate-based particles	Paclitaxel	XYOTAX PNU166148	Ovarian cancer, non-small cell lung cancer Solid tumors	Phase III
Polymer–drug conjugate-based particles	Camptothecin	CT-2106 MAG-CPT Pegamotecan	Colorectal, lung and ovarian cancers Solid tumors Gastric and gastroesophageal cancers	Phase I/II Phase II
Polymer–drug conjugate-based particles	Platinate	AP5346	Solid tumors	Phase II

Polymer–drug conjugate-based particles	Doxorubicin	FCE28068 (PK1) FCE28069 (PK2)	Various cancers, particularly lung and breast cancers Hepatocellular carcinoma	Phase II
Polymer–drug conjugate-based particles	Irinotecan	NKTR-102	Breast, ovarian, colorectal, and lung cancers, glioblastoma	Phase II/III
Polymeric Polymeric	Docetaxel small interfering RNA	BIND-014 CALAA-01	Solid tumors Solid tumors	Phase I Phase I

Table 5. Advantages and limitations of different nanocarriers in drug delivery.

Nanoparticle	Advantages	Limitations
Inorganic nanoparticles~~:~~		
Silica nanoparticles ~~(SiNPs)~~	Simple, cheap, mild conditions synthesis with size tunability. Well known surface chemistry for functionalization and targeting.	Surface functionalization often needed to avoid aggregation and unspecific binding. Tiny systems have a moderate brightness. Self quenching phenomena

	Intrinsic non-toxic hydrophilic material. Optical properties are conferred by the doping materials. Development of systems with tunable emission properties based on FRET approach.	whitin the NP at high doping regime.
Gold nanoparticles ~~(GNPs)~~	Simple synthesis. Ability to tailor the functionality of the surface. Intrinsic properties of the gold core ideal for photodynamic therapies, contrast imaging, and thermal ablation. High drug load.	Biocompatibility, bioaccumulation. Uncoated gold nanoparticles are susceptible to aggregation in solution and can melt under laser irradiation. Possible catalytic effect in the case of very tiny gold clusters
Superparamagnetic iron oxide crystals ~~(SPIONs)~~	Possibility of using passive and active drug delivery	Tend to aggregate into larger clusters. Toxicity due to reactive surface.

	strategies. High efficiency in drug targeting by locally applying an external magnetic field. Simple functionalization for the targeting. Visualization in MRI. Theranostic carriers~~.~~	
Colloidal semiconductor ~~(QDs)~~	Photostability. High fluorescent quantum yields, broad absorption and sharp emission peaks (multiplexing). Multiplex essay and targeting of the surface. Theranostic carriers~~.~~	Synthetic procedure at high temperature with toxic or carcinogenic reagents, with batch to batch variability. Toxicity effect of metal core, bioaccumulation. Blinking behavio~~u~~r~~.~~
Organic nanoparticles~~:~~		
Polymer-based nanoformulations ~~(PLGA)~~	Well established	Mechanical stability.

	mild conditions synthetic approaches. Highly monodisperse systems, size tenability. Biodegradability. Functionalization for targeting.	Long-term chemical stability. Prone to aggregation/deformation under mechanical stress
Liposomes	Low toxicity, biocompatibility, and biodegradability. Ability to modify size and surface. Encapsulate both hydrophobic and hydrophilic drugs.	Low stability. Poor batch to batch reproducibility. Low drug entrapment.

Figure 1. Multifunctional and multimodal NPs for tumor molecular imaging. Schematic representation of the structure of multimodal NPs and their applications in imaging. **(A)** Multiple functionalities such as magnetic, X-ray absorber, radionuclide, optical dye and targeting moiety can be integrated into a single NP system, which acts as a multimodal molecular imaging platform. **(B)** MRI and ~~Computed Tomography (~~CT~~)~~ provide valuable preoperative information for surgical planning (i.e., assessment of anatomical extent of the primary tumor and of the regional lymph node metastasis). **(C)** ~~Positron Emission Tomography (~~PET~~)~~ has the advantage of detecting deep-seeded

cancer metastasis. **(D)** Fluorescence-guided surgery aids surgeons in the intraoperative identifications and removal of malignant lesions.

CT: Computed tomography; NP: Nanoparticle; PET: Positron emission tomography.

Figure 2. Multifunctional and multimodal NPs for image-guided therapy. Schematic representation of ideal NPs combining functional components for effective targeted imaging and drug-delivery. The potentialities and clinical application of the multifunctional and multimodal NPs are summarized.

NP: Nanoparticle.