Invited review

Are nanotheranostics and nanodiagnostics-guided drug delivery stepping stones towards precision medicine?

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The progress in medical research has led to the understanding that cancer is a large group of heterogeneous diseases, with high variability between and within individuals. This variability sprouted the ambitious goal to improve therapeutic outcomes, while minimizing drug adverse effects through stratification of patients by the differences in their disease markers, in a personalized manner, as opposed to the strategy of “one therapy fits all”. Nanotheranostics, composed of nanoparticles (NPs) carrying therapeutic and/or diagnostics probes, have the potential to revolutionize personalized medicine. There are different modalities to combine these two distinct fields into one system for a synergistic outcome. The addition of a nanocarrier to a theranostic system holds great promise. Nanocarriers possess high surface area, enabling sophisticated functionalization with imaging agents, thus gaining enhanced diagnostic ability in real-time. Yet, most of the FDA-approved theranostic approaches are based on small molecules. The theranostic approaches that are reviewed herein are paving the road towards personalized medicine through all stages of patient care: starting from screening and diagnostics, proceeding to treatment and ending with treatment follow-up. Our current review provides a broad background and highlights new insights for the rational design of theranostic nanosystems for desired therapeutic niches, while summoning the hurdles on their way to become first-line diagnostics and therapeutics for cancer patients.

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1. Introduction

For the last three decades, polymer therapeutics were labeled with imaging moieties, in order to investigate the pharmacokinetics, drug accumulation and biodistribution of drugs conjugated to, or entrapped in nanocarriers. Yet, only in the beginning of this millennium, it has emerged as a distinct field termed ‘Theranostics’ (Kelkar and Reineke, 2011). This youth-aggered field includes every material that combines therapy and diagnostics. Initially, this term referred to a two-step process, where the first step is diagnosis and the second step is therapy. In the first step of diagnosis, a predictive marker (e.g., tumor-specific genes and proteins that indicate sensitivity or resistance to a specific therapy) (Duffy, 2005) is found and, then, in the second step, the care-giving team can choose an appropriate treatment accordingly. The first diagnostic step is often termed as companion diagnostic, aimed to identify not only a gene or protein but also a specific target on the cell surface (such as an overexpressed receptor), facilitating patient verification for specific targeted delivery of a therapeutic system (second step). The folate receptor represents an example for a thoroughly investigated pivotal overexpressed receptor on various cancer cells used as a companion diagnostic (Assaraf et al., 2014). This companion diagnostic step differs from diagnostics alone: upon linking these two steps, the companion diagnostic test may influence the choice of therapy, with distinction from diagnostics alone, which defines a disease state only. This concept led to a predictive, personalized, and more precise medicine: a treatment that will be applied for selected patients with high probability to respond, in contrast to “one drug fits all” (Warenius, 2009). This rational, evolved to the development of advanced diagnostic probes that will enable real-time visualization and detection of certain enzymes and analytes that are present in the tumor and its supporting microenvironment on the molecular level (Lee and Li, 2011). Hence, a more precise therapy that fits patients on the basis of certain conditions (that detects enzyme or analyte activity in real-time) can be established. Apart from the precision of therapy for a certain medical condition, there are additional advantages in real-time imaging of the tumor or the therapeutic systems used to treat it. The tumor is a heterogeneous environment, composed of tumor cells, cancer stem cells (tumor initiating cells), fibroblasts, immune system cells (e.g. macrophages), and various functional proteins (Gerlinger et al., 2012). The heterogeneity and adaptive nature of cancer calls for diverse types of treatment options in accordance with disease progression and its molecular profile. Real-time diagnosis can be beneficial in comparison with conventional single-tumor biopsy sampling. The conventional biopsy is prone to sampling error, stemming from validating only a small tumor area within a larger heterogeneous tumor, and among its metastases, leading to misdiagnosis. Moreover, this heterogeneity may foster tumor adaptation and cause treatment failure upon Darwinian selection. These obstacles led to an unmet medical need in monitoring the therapy, which is enabled by combining the therapy and diagnostic modalities on a single platform. This platform may address different issues such as monitoring drug release, treatment efficacy, drug accumulation at the tumor site and biodistribution. Moreover, upon additional functionalities together in one system, one can address other obstacles towards curative therapy of malignant tumors, such as the drug resistance phenomena. An example for such system is multifunctional theranostic systems bearing four components—a drug, a targeting ligand, a chemosensitizer aimed at neutralizing a resistance mechanism and an imaging agent. This multifunctional, or “quadrodiagnostic” entity holds the potential to reach the tumor location, report upon it, treat the tumor and synergistically overcome drug resistance (Livney and Assaraf, 2013; Shapiro et al., 2011). The combination of therapy and diagnostics includes not only a drug and a diagnostic molecule that are physically loaded on one platform, but also a therapeutic and a diagnostic probe combined in a single session, usually referred to as “image-guided therapy”. This session can include photodynamic and photothermal therapy, radiosensitization and image-guided surgery.

An ideal theranostic probe should be endowed with the following features: (1) be able to detect the tumor location, (2) have a high signal-to-noise ratio (SNR), (3) have a high specificity to its target in order to achieve early detection which is crucial for better prognosis, and (4) be non-toxic or at least non-harmful at the concentration necessary to obtain the diagnostic signal. Possessing these properties, different theranostic approaches towards personalized medicine can be met. Such hybrid systems can be exploited for (1) early detection of cancer, (2) selection of the proper drug for the individual patient, (3) detection of a predictive biomarker (enzyme/analyte for specific therapy), (4) staging the disease to adapt the therapeutic strategy, (5) monitoring drug release in real-time, and (6) determining the therapeutic outcome during therapy and post-therapy in order to follow up efficacy and exclude remission. These diverse theranostic applications, meet varied clinical needs, starting from diagnosis and proceeding to clinical aspect of treatment and follow-up, together with drug discovery and development validation. The opportunities they bring to the unmet medical needs and the challenges they face, are reviewed in the current review and are presented in Fig. 1. Herein, we focus on optical imaging which holds great promise (as well as limitations) for clinical translation. Most of the described non-invasive applications demonstrated herein are meanwhile limited to preclinical studies on small animal models, and are presented as the proof of principle for these theranostic systems towards personalized medicine. Furthermore, in the present review, we describe the probes investigated in current clinical trials, and address the clinical aspects of
nanotheranostic systems and the obstacles they face on their way to obtain approval.

1.1. Imaging modalities

Nowadays, there are clinically available imaging modalities that can visualize anatomical changes (including X-ray, computed tomography (CT) ultrasound and magnetic resonance imaging (MRI)), and functional changes (including positron emission tomography (PET), single-photon emission computed tomography (SPECT), functional MRI (fMRI) and optical imaging). Years before the tumor demonstrates morphological alterations, there are molecular modifications within the tumor cells and the surrounding environment (Diaz-Cano, 2012). Those unique changes can evolve to detectable anatomical and morphological alterations that are demonstrated by benign or malignant tumors. Thus, in order to detect those abnormalities at an early stage, real-time visualization on the molecular level is required. Early detection is one of the major reasons for improved patient prognosis. Non-invasive molecular imaging aims to identify specific processes and molecular alterations way before the establishment of a tumor at a predisposed site within the whole organism. In addition, the aforementioned variations can indicate specific tumor characteristics, paving the way to treatment adjustment and monitoring its outcome. In order to gain information regarding molecular activity and its anatomical location, high sensitivity along with temporal and spatial resolutions are obligatory properties of the desired probe and modality. Such an ideal imaging modality does not exist yet as an independent tool, thus, the combination of different imaging modalities for improvement of each separate performance is common. For the purpose of intravital real-time imaging, each modality holds several advantages alongside limitations, and should be selected accordingly. For example, PET and SPECT are highly sensitive but suffer from low spatial resolution (thus are combined with CT for anatomic data) (Histed et al., 2012). Moreover, the labeling of a nanocarrier with a radionuclide is costly and special caution during synthesis and handling should be taken. Conversely, MRI imaging has a good spatial resolution (10–100 μm) and sensitivity (Weissleder and Pittet, 2008), however its use is expensive and does not fit all patients (such as patients bearing medical devices or metal implants or those suffering from renal diseases, disabling them from contrast agent administration). Optical imaging has great sensitivity (picomolar concentrations can be detected) (Weissleder et al., 1999a) and at the near-infrared (NIR) range, possesses good spatial resolution and greater depth penetration than other wavelengths (Pansare et al., 2012). Nevertheless, optical imaging holds several advantages over the other imaging modalities. Fluorescent molecular probes are relatively inexpensive, sensitive and enable simultaneous multicolor imaging and specificity. Furthermore, they do not hold long-term health risks like other commonly-used computed tomography methods (e.g., PET and SPECT), which expose the patient and caregivers to ionizing radiation (Kobayashi et al., 2010; Longmire et al., 2009). Consequently, optical imaging is suitable to regularly repeated examinations. The major drawback of optical imaging modality is the shallow tissue penetration, limiting its imaging ability to superficial tissue (if imaged non-invasively or to minimally invasive imaging in internal organs).

1.2. Exploiting nanotechnology for theranostic applications

In this review, we focus mainly on polymeric nanoparticles (NPs) (linear polymeric chains, dendrimers, polymeric micelles, polymersomes and polymeric NPs) in the context of optically-imagable theranostic systems and their different applications for personalized medicine. Due to their size above the glomerular filtration limit (around 6 nm) NPs tend to escape the renal clearance and circulate for prolonged periods of time. In addition, the leaky tumor vasculature leads to selective percolation at the tumor site. Together with the additional poor lymphatic drainage, NPs tend to display increased accumulation at the tumor site (Markovsky et al., 2012). This phenomenon is known as the enhanced permeability and retention (EPR) effect (Matsumura and Maeda, 1986). Most contrast agents (for various imaging modalities) are low molecular weight (MW), non-specific and poorly water-soluble, thus suffer from short imaging duration, low SNR and aggregation, respectively. Integration of nanotechnology with theranostics (i.e., nanotheranostics) holds several benefits; it may upgrade the diagnostic agent as well as it does for therapeutics, in the context of improved therapeutic index. As the drug, the diagnostic agent may benefit from
increased stability, altered biodistribution and a higher accumulation at the tumor site due to the EPR effect. Thus, conjugation to a nanocarrier results in enhanced specificity (Alexis et al., 2008), higher SNR, increased solubility, improved physicochemical properties and prolonged imaging duration. The prolonged time in the circulation increases the contact time of the probe, thus maximizing the chances to reach the desired site (i.e., the tumor) and the specificity is increased (Alexis et al., 2008). The macromolecular carrier can protect the drug or dye payload from degradation by endogenous environment, extending the plasma half-life.

We hereby discuss few aspects of personalized medicine derived from the theranostic approach and describe their role in different stages of patient care. We start from diagnosis, proceed to therapy adjustment and end with treatment follow-up. Through these stages, we address the challenges such systems composed of macromolecule carrier and its payload (therapeutics, and/or imaging moieties) face, and the strategies they provide in order to overcome them. To conclude, the clinical advances will be noted alongside to the challenges that limit these nanotheranostic systems from further clinical translation.

2. Diagnosis

2.1. Improving signal-to-noise ratio (SNR) using smart polymeric probes

The quest for methods to pin-point tumors continues to be a prominent challenge in cancer detection. Numerous examples of polymeric systems labeled with single, dual or even triple functionalities for MRI, PET/CT and fluorescence used for the detection of tumors were published in the past three decades (Kumar et al., 2009; Lee et al., 2012; Zhou and Lu, 2013). The polymeric backbone can carry a large amount of payload (i.e., drug and/or imaging agent) to the tumor site at a single administration. Increased region specificity and imaging sensitivity can be obtained due to higher accumulation in the tumor compared to healthy tissues and release of a large payload (Alexis et al., 2008), resulting in enhanced signal. The polymeric nanocarrier will be retained in the tumor due to the impaired lymphatic drainage, resulting in elongated duration of imaging procedure until cleared from the body by the ureter or bile systems. On the other hand, therapeutic polymer probes are designed for prolonged circulation. These polymeric probes bearing an imaging agent travel in the body and eventually accumulate in the tumor. The signal obtained from the tumor as well as from the whole body can result in a low SNR while circulating in the bloodstream; however, it may improve by the time it accumulates in the tumor. This low SNR will not be sufficient for the detection of small lesions or metastases (Kunjachan et al., 2014; Ogawa et al., 2009). This effect can be even intensified, since as mentioned earlier, NPs allow inclusion of several imaging agents on one platform, resulting in densely packed imaging agents that can increase the signal intensity compared to free dyes (excluding the case of narrow stocks-shifts resulting in self-quenching of fluorescent dyes) (Singh et al., 2012).

In order to improve the threshold of detection (i.e., increase the SNR), more sophisticated nanoprobe possessing an activatable switch were introduced. They were designed such that when they travel in the body, their fluorescent signal will be “OFF” and upon detection of a certain enzyme, analyte or change in pH, they will be switched to their “ON” state, resulting in a higher SNR (Ferber et al., 2014; Ogawa et al., 2009; Yuan et al., 2014c). Thanks to their polymer backbone, these sophisticated probes (discussed further in details), have the ability to amplify the signal upon an activation event by a strong quenching mechanism (Bremer et al., 2002), narrow range pH-changes sensing (Zhou et al., 2012) and a higher stability towards photobleaching (Ferber et al., 2014) compared to small molecules.

A noteworthy example of an outstanding enhancement in SNR (over 300-fold change) was demonstrated by Gao’s group (Wang et al., 2014b). The researchers synthesized two self-assembled polymeric micellar carriers bearing incredibly high Cy5.5 NIR dye payload, densely packed to allow self-quenching. These unique 24.5 nm carriers have shown ultra-sensitive pH transition detection ability of both tumor cells and their extracellular acidic surroundings. They differentiated sharp pH changes of 0.23 and 0.21 units around pH of 6.7 (in acidic tumor extracellular fluid) and 6.2 (intracellular endosomal and lysosomal pH), respectively (as compared with 2 pH units demonstrated for small molecular pH sensors), thus differentiating tumor over healthy tissue (characterized by physiological pH of ∼7.4).

Another approach to address the low SNR obtained by ~100–200 nm imaging agents-bearing NPs, is by reducing the NPs size to below 10 nm. The, <10 nm-sized NPs will be excreted rapidly from the kidneys and will result in reduced background signal in several hours from administration (Kunjachan et al., 2014; Mignot et al., 2013; Theek et al., 2014).

2.1.1. Evading off-target accumulation

Another aspect that diminishes the SNR (and also decreases the treatment’s efficacy) is the off-target effect, which can also be resolved by a macromolecular integration. NPs tend to escape renal clearance and to accumulate in the liver with correlation to size, surface charge and chemical composition (Longmire et al., 2008). Apart from causing liver toxicity in case the liver is not the treatment’s target, the imaging modality of theranostic particles will result in high signal obtained from the liver. Although not apparent in non-invasive imaging with low-tissue penetration wavelengths, organ resection will reveal the liver’s high signal intensity, as was demonstrated in many previous studies (Ferber et al., 2014; Kim et al., 2010; Mieszawska et al., 2013). A common method to reduce liver uptake and accumulation in the reticuloendothelial system (RES, a network of cells and tissues found throughout the body, including the spleen, liver, lungs, bone marrow and lymph nodes) for liposomes, thus increasing the specificity of the imaging, is by coating them with polyethylene glycol (PEG), a procedure named PEGylation (Gabizon, 2001). In general, polymers tend to reduce off-target accumulation, but many of them still suffer from enhanced liver uptake. Therefore, in some cases, PEG or other agents inducing “stealth-like” properties were conjugated to polymers as well. In fact, an example that highlights this property is a comparison of PEGylated poly(L-glutamic acid) containing meso-chlorin e6, a photosensitizer, and Cd(II)-D03A (P(Gd-D03A)-Mce6) to the non-PEGylated particles where the PEGylated system revealed reduced signal from the liver. This was accompanied by higher intensity signal from the tumor at 18 h after treatment by Vaidya et al., 2008). These aforementioned examples for the enhancement in SNR are presented in Fig. 2.

2.2. Nanotheranostics for early detection

2.2.1. Minimally invasive procedures

Early detection of malignant tissues is crucial for enhancement of prognosis. For several tumor types, including breast and colorectal cancers (CRC), prognosis is very favorable (exceeding 90%) when diagnosed at early stages, however at late stages, it drops below 20% (O’Connell et al., 2004; Sant et al., 2003). CRC is an excellent example for the ability to screen premalignant and malignant lesions by minimally invasive endoscopy procedures. Today, the gold standard for screening and early detection of CRC is colonoscopy. The addition of a targeting moiety can direct the theranostic-based system to a molecular target overexpressed on the surface of the tumor.
cells, also referred to as “tumor biomarker”. Such theranostic-based nanosystems, equipped with a targeting ligand towards an overexpressed target, were shown to detect CRC cells at higher extent than untargeted drug delivery systems. For example, Kogan-Zviagin et al., have examined the underglycosylated mucin-1 antigen (uMUC-1)-targeted N-(2-Hydroxypropyl)methacrylamide (HPMA) copolymer conjugated to the NIR fluorophore, IR783. They used EPPT1 synthetic peptide in order to target the uMUC-1 overexpressed in CRC cells, resulting in higher detection of tumors in the submucosal level after intra-colonic administration in comparison with non-targeted conjugate (Kogan-Zviagin et al., 2014). Another mucin-type tumor-associated carbohydrate antigen for topical imaging application, the Thomsen–Friedenreich (TF) antigen, was exploited. Peanut agglutinin (PNA), as the recognition molecule for TF was added to the nanobeacon surface to demonstrate real-time in vivo detection ability of CRC in pre-clinical animal model. In this study, Sakuma et al. (2015) combined colonoscopy with an optical imaging probe composed of rhodamine encapsulated in polystyrene polymer nanobeacon, further covered by PNA and PMAA polymers. It was indicated that the CRC tumors could be detected weeks before they were visualized by white light colonoscopy. In addition, the imaging probe was nontoxic due to its large size (above 300 nm), which prevented systemic absorption through the intestinal mucosa.

2.3. Disease staging – sentinel lymph node (SLN) mapping

Proper determination of the stage of a malignant disease has a great impact on the treatment strategy. Different cancer patients might undergo aggressive surgical resection, with or without presurgical chemotherapeutic or radiation therapy, according to their diagnosed disease state. The tumor-node-metastasis (TNM) staging is a common staging parameter for many solid cancers (including melanoma, cancers of the colon, breast and lung) (Patel and Shah, 2005). It considers the size of tumors, their invasiveness, their appearance in the proximal lymph nodes and their tendency to spread out to distant organs (metastases).

The sentinel lymph nodes (SLNs) are the primary location of metastases in several cancers (breast, melanoma, colon and gastric), carried by lymphatic drainage of the nearby tumor. Biopsy of the sentinel lymph nodes, utilized to determine the stage of disease and the need in subsequent lymphadenectomy, has a prognostic value (Scoggins et al., 2005). Standard of care for sentinel lymph nodes mapping is the use of isosulfan blue or 99Tc sulfur-colloid suspension each one separately or together (Giuliano et al., 1994; Krag et al., 1993). The use of isosulfan blue dye is limited by its rapid migration, while 99Tc-sulfur-colloid suspension suffers from poor migration from the injection site. Both materials accumulate preferentially in distal nodes (Hung et al., 1995; Vera et al., 2001). Thus, attempts were made to use the smaller particles population of filtered 99Tc-sulfur-colloid suspension. Reasonably, due to its shallow tissue penetration but high sensitivity, SLN mapping is one of the most investigated application for fluorescent macro-molecule imaging. Recent years have seen a flood of improved optical diagnostic systems aimed to locate the SLN without the need for radioactive tracer and/or blue dye. In order to map the SLN, the nanotheranostic system should be small enough to travel with the lymphatic flow but large enough to stay there in order to be detected. An appropriate hydrodynamic diameter for this end is considered to be within the range of 5–40 nm (Kim et al., 2004). In addition, an important demand from an SLN detecting probe is photostability during the surgical procedure. An example for such a theranostic system, a biodegradable pullulan–cholereter polymer nanogels labeled with fluorescent IRDye800, was presented by Noh et al. (2012), for the detection of SLN in both small and large animal models. The 30 nm-sized labeled-NIR dye probe (for rapid uptake and retention capabilities at SLNs) was administered into the front paw of a mouse. Two min post intradermal injection, the nanobeacon accumulated in the SLN with a detectable signal that kept rising for 30 min following administration. Next, the lymphatic flow from the injection site towards the SLN was examined.
in a large animal model. As designed, this polymeric theranostic system showed higher photostability and accumulation in the SLN in comparison with the low MW IRDye800.

For anatomically deep-tissue located tumors, where optical imaging is limited, both the tumor and the SLN detection are usually addressed by the combination with other imaging modality on a single hybrid probe. There are several examples for the combination of MRI with optical imaging, to provide pre- and intra-operative information, respectively, during the SLN mapping and resection procedure. Such dual-labeled imaging nanosystem was exemplified by Koyama et al. (2007), who synthesized a dendrimer-based contrast agent combining MR and optical imaging by conjugation with chelated Gd and Cy5.5 fluorophore, respectively. The MR was used pre-operative to detect the tumor location, while the Cy5.5 fluorescent signal directed the real-time intraoperative SLN detection once the skin was incised. In addition, this probe provided both high sensitivity and anatomical data by a single administration. Melancon et al. (2007) introduced a 46±6 nm macromolecule consisting of biodegradable poly(l-glutamic acid) conjugated to Gd and NIR813 fluorescent dye. The researchers found their system to co-localize with isosulfan blue when injected together subcutaneously to healthy mice while the surrounding tissue was left signal-free. The importance of the combined imaging agent was shown by the pre-operative detection of the SLN location by MRI only 3 min post injection and by the enhanced sensitivity of the fluorescent signal which detected axillary LN that were missed by MR imaging when lower dose was consumed. Next, SLN containing metastases were visualized by the dual probe after intralinguinal injection to both healthy mice and to orthotopic head and neck tumor-bearing mice. Effective removal of NIR fluorescence-detected SLN was performed and histopathologic examination of identified fluorescent SLN confirmed micrometastases infiltration.

An important study dealing with the choice of a suitable tracer and an imaging system was provided by Tanaka et al. (2006). The researchers demonstrated optimized protocols for SLN mapping using a large animal model system of spontaneous melanoma. For this purpose, three different lymphatic tracers were developed, possessing different sizes and fluorescent dyes. Two organic dyes and one inorganic core structures with a hydrodynamic diameter of ~7 and 15 nm (which alters to ~20 nm in the presence of serum), respectively, were used. Indocyanine green (ICG) was adsorbed into human serum albumin (HSA) (an FDA approved formulation), and NIR fluorophore CW800 was covalently attached to HSA. The inorganic core tracer was type II NIR QD with an organic surface, negatively charged. All of the tracers successfully identified the SLN 15 s post-peritumoral injection. The smaller particles may have an advantage in identifying all of the various sized-SLNs, while the larger can be retained in the SLN for longer times in order to be detected. Although the presented reports exemplified herein for SLN mapping and diagnosis suggest different particles’ sizes for optimal performance, recent clinical trials are conducted using 7 nm particles (will be detailed in the clinical trials section) (Phillips et al., 2014). Consequently, the desired size will have to be optimized by the results of the clinical trials.

2.4. Precision medicine – preselection of therapy-responding patients

Individualizing the therapy to responding patients holds a great potential to enhance the treatment’s efficacy and success. It is therefore highly important to identify biological variability between patients (i.e., tumor vascularization, receptor overexpression on tumor cells, and analytes or enzymes overexpressed in the tumor and its microenvironment) by real-time imaging. We hereby delineate the differences contributing to patient variability (represented in Fig. 3), elaborating the need to develop an appropriate treatment for the individual tumor pathological properties.
2.4.1. Characterizing EPR-mediated passive drug targeting to tumors

Theranostic particles that consist of an imaging modality conjugated to a NP (either polymeric, micellar, dendrimers-shaped, liposomal or metal cored), with or without a therapeutic modality (such as a chemotherapeutic drug), are relying on the extravasation-dependent (passive) accumulation of nanoparticles in tumors due to the EPR effect (Maeda, 2001, 2012; Matsumura and Maeda, 1986). The importance of this passive accumulation characteristics manifests in the various FDA-approved NP-based drugs for cancer therapy (Pillai, 2014). In terms of therapeutic effect, drug-bearing NPs of 20–100 nm were shown to better accumulate, longer circulate and overall have better therapeutic outcomes and decreased toxicity (due to lower dose required) than small-molecule free drugs (Markovsky et al., 2012). In addition, the larger the MW of the polymeric backbone, the larger is its tumor-selective accumulation (Seymour et al., 1995). Such MW-dependent improved pharmacokinetics of NPs over free imaging agent was demonstrated by Lammers et al., using Iodine-131-conjugated N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer. Conjugates of 23, 31 and 65 kDa were injected intravenously (IV) to rats bearing subcutaneous AT1 tumors. Higher MW conjugates have circulated longer and demonstrated prolonged tumor signal/accumulation compared with lower MW particles and with free imaging agent (Lammers et al., 2010).

Although very effective in some cancer xenograft mouse models, another consideration regarding passive accumulation by the EPR effect is the high variability of vascularization and vascular permeability in human tumors (Prabhakar et al., 2013). For instance, gastric and pancreatic cancers as well as metastatic cancers are known to have low vascularization, therefore exhibit low tumor accumulation of NPs compared to highly vascularized cancers (Cheng et al., 2012; Maeda, 2012). Moreover, several xenogeneic and syngeneic tumor mouse models were shown to exhibit hypovascular areas and in some cases, angiogenic tumors were not as leaky as required for nano-delivery systems. Therefore, there is an unmet need to better understand the variability of the EPR effect in different tumor types, gaining the ability to select the patients who will benefit from anti-angiogenic and/or EPR-mediated passive targeted treatments. Schmiedler et al. (2008) have used paramagnetic fluorocarbon NPs targeted towards the α5β1 and αvβ3 integrins overexpressed on neovascularure of tumors to demonstrate such low-angiogenic MDA-MB-435 tumors by MR imaging. Their imaging contrast has shown that 90% of the enhanced signal originated in the tumor’s periphery. Accordingly, anti-angiogenic therapy did not decrease tumor volume and did not prolong the survival of mice. Recently, Theek et al. (2014), presented a very good correlation between the tumor vasculature to the drug accumulation. The EPR-mediated passive targeting of NIR fluorescently-labeled imaging agent, HPMA copolymer-Dy750, was evaluated in CT26 murine colon carcinomas-bearing mice. The accumulation of this ~10 nm-sized conjugate was evaluated by fluorescence molecular tomography (FMT) while tumor vascularization was validated by contrast-enhanced functional ultrasound (ceUS) imaging. Visualizing the EPR effect in different tumor types and by different imaging modalities can facilitate patient stratification for treatment, in order to increase the efficacy while minimizing adverse effects, and the annual expense of useless therapy.

2.4.2. Characterizing ligand-mediated targeted drug delivery

To address the issues raised above regarding passive tumor targeting, to increase specificity, reduce off-target toxicity and overcome biological barriers, ligand targeting was introduced to the world of NPs. Ligand targeting has undoubted advantages when conjugated to small molecules such as chemotherapeutic drugs (Beeram et al., 2012; Burris et al., 2011; Krop et al., 2010; Krop et al., 2012; Verma et al., 2012) or dyes (van Dam et al., 2011). Furthermore, the addition of targeting ligands to NPs bearing imaging capabilities can prompt signal enhancement due to improved accumulation in the desired site. Such targeting towards the IGF1 receptor overexpressed in pancreatic primary human xenografts in mice (representing the clinical settings of poor vascularization as biological barrier for the treatment) resulted in 3-fold increased signal intensity in mice injected with NIR-830 dye conjugated to IGFIR targeted particles compared with NIR-830 dye conjugated non-targeted particles (Zhou et al., 2015). In addition, targeting of receptors that are overexpressed in brain tumor cells can help penetrate through the blood brain barrier (Gabathuler, 2010). Dixit et al. (2015), designed a PEGylated gold NP modified with a transferrin peptide to target towards glioblastoma U-87 MG cells. A photodynamic prodruk labeled-targeted particles, Pc 4, exhibited 6-fold tumor accumulation compared with Pc 4 labeled untargeted particles. Nevertheless, whether there is an advantage for active targeting in case of NPs on top of their passive tumor-accumulation is still debated (Bertrand et al., 2014; Sykes et al., 2014).

2.4.3. Characterizing tumor proteolytic enzymes/analyte activity

The diagnostic guided drug delivery approach, composed of two-steps theranostic system, begins with molecular imaging which is followed by the tailored therapy (Lee and Li, 2011). Using real-time diagnostic test, we can detect not only the presence but also the activity of special biomarkers (e.g., enzyme or analyte) in the tumor (Kisin-Finert et al., 2014; Redy-Keisar et al., 2014; Weinstand et al., 2014). Visualization of such activity requires the design of sophisticated probes capable of sensing and reporting the activation event, usually termed “Turn-ON” probes. These probes are designed to be none to minimally-detectable when traveling in the circulation and become highly fluorescent upon activation. Several proteolytic enzymes are known to be overexpressed in tumors cells (among them matrix metalloproteinases (MMPs) and cathepsins), presumably to adapt to rapid cell cycling, growth demands, degradation of regulatory proteins and other acquired aggressive characteristics such as intravasation, extravasation and metastasis (Garcia et al., 1996; Keppler et al., 1996; Kim et al., 1998). These enzymes have specific substrate recognition sites. Thus, a smart probe can be composed of a polymeric carrier and a dye, connected by a known enzyme-cleavable peptide, in a manner that will allow activation of the probe following the proteolytic cleavage, i.e. acting as an optical sensor reporting on enzyme activity in the tumor. To date, a variety of polymeric backbone carriers have been developed for bioimaging, including poly(amino acids), polysaccharides, dendrimers, graft-, and block- or random-co-polymers (Kim et al., 2007).

Pioneering work was provided by the Weissleder group in 1999 (Weissleder et al., 1999a), which synthesized and characterized a “Turn-ON” probe with “stealth-like” properties. Their primary research goal was not only early detection of small tumors by enhancing the SNR of NPs imaging probes, but also sensing different enzymes activity by multiple fluorescently-activatable nanocarriers. Their reported nanosystem, composed of poly-l-lysine (PL) partially protected by a methoxypolyethylene glycol (MPEG) side chains, and partially bare lysine-lysine proteases-cleavable recognition sites, was conjugated to multiple NIR dyes, Cy5.5. Due to multiple dyes conjugated to the polymeric delivery vehicle in close proximity, the phenomenon of homo-fluorescence resonance energy transfer (FRET) or “self-quenching”) occurred. This probe was tested in vivo, presenting detection ability of >300 μm sized tumors with depth detection threshold of approximately 7–10 mm 24 h post administration. Furthermore, this group presented additional macromolecule-based NIR fluorescent probes, capable of sensing both different proteolytic enzymes (Tung et al., 1999; Tung...
et al., 2000) and their activity level (Bremer et al., 2002), thus enabling “molecular profiling” of tumors for therapy adjustment.

The ability to sense a certain biomarker in vivo requires high specificity for the target and high SNR. The former can be achieved by a macromolecule carrier that exploits the EPR effect and recognizes a certain enzyme, while the latter demands strong quenching technique. This kind of specific and enhanced SNR was exemplified by Lee et al. (2009), for the identification of MMP activity. A “Turn-ON” system of self-assembled chitosan nanoparticle (CNP) equipped with a dark quencher and a NIR dye was generated. Due to both FRET and self-quenching, this system demonstrated strong quenching and higher SNR properties. IV administration to MMP-2/9-positive mouse squamous cell carcinoma SCC7 tumor-bearing mice showed an approximately 15.4-fold difference between tumor and healthy tissue. Later on, Man Yoon et al., presented similar NPs for the detection of MMP7 in an azoxymethane (AOM)-induced mouse colon cancer model. The CNP system was able to distinguish between normal, adenoma, and adenocarcinoma tissues (Yoon et al., 2011).

Apart from the aforementioned proteolytic enzymes, other important players in the biological system are biological thiol. They play a significant role in maintaining cell survival, serving as antioxidants, contributing to the tertiary structure of proteins and are involved in the inhibition of apoptosis. Comprehensive research on biological thiol was done on glutathione (GSH), a key intracellular reducing agent. Major alterations in its concentration may indicate a disease state including cancer (Townsend et al., 2003). Recently, Ang et al. (2014), reported the fabrication of NP-based system with an entrapped Turn-ON fluorescent probe for sensing biological thiol compounds. These polyacrylate-based NPs were generated by self-assembly of β-cyclodextrin and conjugation to different polyacrylate and PEG polymers in the presence or absence of adamantane. The fluorescent probe was quenched in the absence of thiol compounds due to a photoinduced electron transfer (PET) mechanism between the pyridine (as a quencher) and the disulfide bond-connected coumarin fluorophore. Upon thiol compound interaction, the disulfide bond was reduced and the fluorescence signal of the coumarin was restored. These studies pointed out that although overexpressed in cancer cells, thiol groups are also present at low levels in normal cells, which may result in false positive detection. Thus, they added a folic acid ligand-targeting moiety in addition to the passive EPR-mediated targeting and demonstrated the difference in specificity in vitro. This example is a proof of concept for the thiol compounds’ sensing ability in cancer cells. These systems demonstrated higher specificity and signal amplification compared to small molecules for the detection of biomarkers that can be utilized as targets for prodrug activation in the individual patient.

2.5. Tailoring drug treatment to the individual patients

Using an optical sensor can elucidate a specific mechanism by which certain tumor cells proliferate and thrive in a given cancer patient. Thus, a drug delivery system that utilizes the same mechanism in order to release an anti-cancer drug can be tailored. This drug delivery system can reach the tumor by the EPR effect, while bearing the drug attached by a cleavable spacer specifically adapted to the enzyme recognized by the diagnostic test. In other words, a selective and specific mechanism of drug release may be achieved, hence paving the way to precision medicine. As a proof of concept to this approach, our group recently demonstrated a two-step “Turn-ON” theranostic system, composed of HPMA-copolymer conjugated to multiple and self-quenched Cy5 NIR dyes through cathepsin B-cleavable linker, Gly-Phe-Leu-Gly (GFLG) (Ferber et al., 2014). We first detected the in vivo enzyme activity in mammary adenocarcinoma tumor bearing mice, and further applied the complement therapeutic step by using anticancer drug paclitaxel (PTX) conjugated to the same polymeric backbone by the same peptide recognition site. Both the fluorescent signal activation rate of the companion diagnostic and the drug release kinetics were first examined in vitro, which led to the assumption that the drug activation and the PK profile can be further deduced from the imaging of probe’s activation and biodistribution.

Alternatively, commercial drugs that possess a specific molecular pathway for drug activation or release, thus potentially suitable only for a subset of patients, can use such pre-screening mechanism of patients, in order to be administered only to the patients who will benefit from the treatment. This may lead to a reduction in both the drugs coverage costs by insurance companies and the side effects for unsuitable patients. Although it should be taken into consideration that changing the payload on the polymeric carrier from imaging agent to drug, may alter the physicochemical characteristics, resulting in different PK properties of a pre-examined molecular imaging probe.

The aforementioned macromolecule-based theranostic systems used through the diagnostics stage of patient care are summarized in Table 1.

3. Therapy

One of the major benefits of a theranostic system and a milestone towards precision medicine is the ability to monitor treatment efficacy in real-time, which enables educating decisions regarding treatment continuation, timeline, dose, etc. Three aspects of treatment monitoring are being practiced in research laboratories: The first aspect follows drug release, indicating the treatment has reached its destination, accumulated there and released the therapeutic cargo. The second aspect includes theranostic systems in which the therapeutic modality is inherent to the diagnostic system. The third aspect monitors the therapeutic outcome.

3.1. Monitoring drug release

Integrating imaging and therapeutic capabilities on a single platform can be a useful tool in preclinical study and clinical trials (Lu, 2010). During preclinical study, the combined theranostic system can non-invasively facilitate the PK and PD profiling of the drug delivery system in real-time, including biodistribution, accumulation in the tumor site or in healthy tissues (off-target). This enables the optimization of a drug delivery system while maximizing the study efficacy by performing continuous examination of tumors progression in a single animal. In addition, using Turn-ON theranostic probes which are activated upon drug release event by a certain enzyme or analyte (Redy-Keisar et al., 2015; Weinstein et al., 2010) can contribute to the investigation of the drug itself rather than the nanocarrier. The drug release mechanism can be visualized in real-time and in a non-invasive manner, hence, gaining a strong research tool which can shorten the lag between bench to bedside. Monitoring drug release is based upon a “switchable” imaging agent that is switched off when delivered via the vascular system and activated upon its release in the target tumor tissue. Several mechanisms allow activatable fluorescence. A FRET-based activatable probe for in vitro monitoring of doxorubicin (DOX) release was recently shown by the Calderón group (Kruger et al., 2014). Their theranostic nanoprobe was composed of a pH-sensitive hydrazone bond linking between a donor-acceptor FRET pair (fluorescent native molecule, DOX, attached to the indocarbocyanine (IDCC) dye, respectively, in close proximity) to dendritic polyglycerol (PG) (Fig. 4). The carrier was rationally designed and synthesized to bear a MW of 200 kDa, previously reported to be
Table 1

<table>
<thead>
<tr>
<th>Application</th>
<th>Aspects</th>
<th>Macromolecular carrier</th>
<th>Targeting ligand</th>
<th>Activation mechanism</th>
<th>Turn-ON mechanism</th>
<th>References</th>
</tr>
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<td>Tumor imaging</td>
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<td>Phospholipid based micelles</td>
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<td>X</td>
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<td>X</td>
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<td></td>
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<td>X</td>
<td>X</td>
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<td>Evading off-target</td>
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<td>X</td>
<td>X</td>
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<td>accumulation</td>
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<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Early detection</td>
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<td></td>
<td>X</td>
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<td>Transferrin</td>
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<td></td>
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<td>X</td>
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<td>X</td>
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<td>Bremer et al. (2002)</td>
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<td>X</td>
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<tr>
<td></td>
<td>nanoparticles</td>
<td>Polyacrylate-based nanoparticles</td>
<td>Folic acid</td>
<td>X</td>
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<td>Yoon et al. (2011)</td>
</tr>
<tr>
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<td>HPMA copolymer</td>
<td>X</td>
<td>X</td>
<td></td>
<td>Ang et al. (2014)</td>
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<tr>
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<td>enzymatic/analytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ferber et al. (2014)</td>
</tr>
</tbody>
</table>


within the size range exploited better accumulation and cellular uptake (Reichert et al., 2011). The FRET quenching efficacy was 94% of the intact theranostic probe, and terminated upon cell internalization and the subsequent cleavage, mostly inside the low-pH acidic, endolysosomal system.

Another theranostic Turn-ON mechanism is the aggregation-induced emission (AIE) proposed by Yuan et al. (2014c), to monitor the release of DOX and Pt compound from a drug delivery system. This unique prodrug formed nanoaggregates with an average size of 120 ± 14.2 nm in an aqueous medium. When conjugated through Pt(IV), FRET occurred between the DOX and the tetrphenylenetetrazole (TPE) molecule. Upon cellular uptake and intracellular reduction of the active Pt(II) drug, TPE and DOX were released, ending the FRET process and enabling strong fluorescence of TPE molecules by AIE. This study has demonstrated both the synergistic therapeutic effect of DOX and Pt and the ability to report upon drug release event. In both the above given examples, the native fluorescence of the chemotherapeutic drug was utilized for the Turn-ON mechanism. Considering not all drugs are natively fluorescent, a broader application of monitoring drug release from a polymeric carrier can be obtained by an independent activation process, thus the requisite fluorescent drug is optional. Many reports regarding low MW drug-release-reporting systems (without the use of fluorescent drugs) were published in the past 5 years, most of which are based upon FRET mechanism and intramolecular charge transfer (ICT). The latter is an elegant modality to achieve a selective signal upon drug release. Redy-Keisar et al. (2015), in collaboration with our group recently demonstrated such platform based on the fluorogenic dye QCy7. Upon the removal of a triggering substrate, a distinct change in the τ-electron system resulted in restoration of the NIR dye fluorescence, which was followed by the drug release event. Attaching such activatable molecule to a polymeric backbone can provide a robust research tool for real-time monitoring of drug release from its carrier.
3.2. Image-guided therapy

Several systems employ the inherent imaging properties of the drug or the carrier themselves in order to treat the malignant disease and simultaneously report on the treatment (Fig. 4). These include the use of microbubbles for ultrasound therapy and imaging, iron oxide NPs for MR imaging, photodynamic therapy or the use of high atomic number elements for radiosensitization and CT imaging.

3.2.1. Microbubbles

Microbubbles are FDA-approved ultrasound contrast agents that operate as “gas filled” cavities. They resonate with application of ultrasound waves, thus increase sensitivity of ultrasound imaging (Blomley et al., 2001). Microbubbles have recently gained therapeutic status in a series of clinical trials testing sonothrombolysis of blood clots (Alexandrov et al., 2008; Molina et al., 2009; Unger et al., 2002). They can be further conjugated to a ligand to perform as specific markers, mostly to tumor’s angiogenesis or inflammation (Kiessling et al., 2012). Moreover, microbubbles can act as drug-carrying cavities when drugs are either incorporated to the lipid layer or to the outer shell, releasing their content in the target tissue with application of low intensity ultrasound waves (Chen and Hwang, 2013). Microbubbles are known to assist delivery of drugs across biological barriers such as the blood brain barrier (BBB) in a process known as “sonoporation” (Kiessling et al., 2012). Lammers et al., have used poly(n-butyl-cyanoacrylate) based microbubbles to deliver model macromolecular drug represented by FITC to the brain of mice. Ultrasound super paramagnetic iron oxide NPs were incorporated to allow MRI imaging. Following IV administration, BBB permeation was imaged by power Doppler ultrasound, while release kinetics induced by high intensity ultrasound waves was followed by MR and fluorescence imaging (Lammers et al., 2015). The therapeutic applications of microbubbles face several obstacles including limited amount of loadable drugs and safety concerns when carrying chemotherapeutics (Chen and Hwang, 2013).

3.2.2. Photodynamic therapy (PDT)

PDT is a clinically FDA-approved process that employs light-activated molecules for the treatment of several diseases, including cancer. The components of a PDT system are: (1) a light sensitive molecule, i.e., a photosensitizer (PS), (2) intracellular molecular oxygen, O2 and (3) a light source (lamp, laser, light emitting diode (LED)). Having these three components, photosensitizers (PSs) can produce cytotoxic singlet-oxygen when irradiated with an appropriate wavelength of light (Dolmans et al., 2003). PDT has several benefits alongside limitations. It is relatively non-expensive, non-invasive, can be locally administered and do not present severe side effects. However, the PS agents usually have short half-life in the tissue and can accumulate in healthy tissues. From a manufacturing point of view, their synthesis is not simple and they are relatively unstable. These limitations can be overcome by the use of nanotechnology. Conjugating PS to a macromolecule carrier contributes to a favorable tumor-accumulation (improves the selectivity towards the tumor, passively, by extravasation-dependent targeted strategy via the EPR effect or actively by ligand-receptor mediated targeting). Thus, the local concentration at the therapeutic site is increased. Combining an imaging ability with the PDT system possesses great potential for improved treatment. In such systems, only after detection of the location and concentration of the therapeutic system, a light-triggered phototoxic treatment can be “activated”, minimizing undesired side effects resulting from wrongly timed- or placed-treatment (pre- or post-PS tumor accumulation).

Since one of the most important factors for PDT activation is the efficient production of singlet oxygen from its PS component and intracellular O2, its photostability and high radiation-induced activity is greatly important. Fluorescence quenching and hence dramatic reduction in reactive oxygen species (ROS) generation
is observed often due to π–π stacking aggregate formation of hydrophobic and rigid planar structures of PSs. To address this problem, Yuan et al. (2014a), recently fabricated an AIE-polymeric matrix encapsulating the PS TTD (2-(2,6-bis(E)-4-(phenyl)-4′-(1,2,2-triphenylvinyl)1,1′-biphenyl-4-yl)aminostyrlyl)-4H-pyran-4-ylidene)malononitrile), containing an enhanced fluorescent signal (and thus ROS generation activity). In addition, this ∼30 nm-sized nanoprobe was equipped with a targeting moiety, cationic RGD (cRGD) peptide towards αvß3, an overexpressed receptor on endothelial cells, to increase specificity. Under appropriate light irradiation, the AIE nanoprobe exhibited an ability to effectively generate ROS in vitro and bright fluorescence for imaging. In addition, many photosensitizers are generally excited by visible (UV/vis) light, which is readily absorbed by tissues, thus restricting PDT applicability. To overcome this obstacle, a new generation of bio-probes for PDT were developed. These include mainly lanthanide ion (Ln3+, such as Er3+, Tm3+, Ho3+)-doped upconversion nanoparticles (UCNPs), that are capable of converting NIR light to UV/vis light via energy transfer from UCNPs to the PS upon NIR excitation. Recently several groups described novel smart systems for imaging-guided PDT that are based upon UCNPs to enhance the theranostic system performances by signal efficiency and cell internalization. Wang et al. (2014a), showed a UCNP-based nanoplatform containing a two-color imaging abilities together with improved PDT efficiency by dual targeting with aminophenylboron acid (APBA) and hyaluronic acid (HA) ligands. Liu et al., introduced another UCNP-based system that converts NIR light to shorter NIR light in order to improve the imaging performance. This research group utilized an effective ligand-exchange strategy to directly conjugate the PS, C60MA, to the surface of the UCNP, which resulted in higher energy transfer in the NIR range (using 980 nm continuous wave excitation, and imaging in 808 nm wavelength) (Liu et al., 2015). Owning to this newly applied method, the energy transfer distance between the donor (UCNPs) and the acceptor (C60MA) were dramatically shortened, enhancing \(^{1}O_2\) production and improving both the therapeutic efficiency and the imaging capabilities. The short treatment duration (15 min) and low irradiation power density applied in 980 nm confirmed that the NIR image-guided PDT performed by sufficient irradiation power density was far below the exposure limit of human skin. In order to improve cellular uptake and accumulation of NIR-guided PDT, Zhuang Liu’s group have developed pH-sensitive UCNPs (Wang et al., 2013). This pH-sensitive system is negatively charged at pH 7.4, and switch to positive charge at pH 6.8 due to the removal of a charge-reversive polymer containing dimethylacrylate acid (DMMMA)-PEG coat. Hence, the negatively-charged-shell is removed and positively charged NPs uptake is enhanced via interactions with negatively charged membrane, preferably in the tumor extracellular acidic environment. The researchers utilized the Mn-doped optical and paramagnetic properties and demonstrated in vivo dual imaging of significantly enhanced cell internalization in comparison with a control system lacking pH sensitivity.

3.2.3. Photothermal therapy (PTT)

Photothermal therapy (PTT) is another cancer therapeutic strategy that mostly utilizes NIR light absorbing agents to eradicate cancer cells by heating. Compared with traditionally used therapeutic methods, PTT displays a series of advantages such as high specificity, minimal invasiveness and high efficiency. Similar to PDT, the development of imageable photothermal agents to enable educated therapeutic planning before and during the operation of PTT is on demand. Recently, a novel albumin-based theranostic nanocomplex for multimodal imaging guided photothermal therapy was described (Chen et al., 2014). To enable multichannel optical imaging together with efficient photothermal cytotoxicity, Gd\(^{3+}\) ions were chelated and a NIR fluorophore, IR825, was absorbed onto chemically-modified human serum albumin (HSA). The researchers have presented a new strategy, relying on photothermal therapy to assist the inhibition of metastases, by burning SLN after surgery. It was shown by in vivo fluorescence and MR imaging that about 30 min after an intratumoral injection, HSA-Gd-IR825 (about 10 nm in size) could successfully reach nearby metastatic lesions, i.e., the SLNs (sentinel lymph nodes). Finally, the photothermal ablation of these SLNs induced by 808 nm NIR laser irradiation for 10 min in combination with primary tumor resection significantly prolonged the animals’ survival compared to thermally untreated mice. This study demonstrates the ability of monitoring and affecting the therapy outcome through proper image-guided techniques.

3.2.4. Combination of photodynamic and photothermal therapy

Although PDT is considered a promising therapy method, there are limitations restricting its clinical application, such as photobleaching, self-destruction upon prolonged light exposure and most importantly, limited penetration depth of the excitation light. To overcome these limitations, several groups have been recently developing multimodal synergistic modalities combining PTT followed by PDT. We selected two interesting examples of newly developed nanosystems for dual-imaging guided combined, sequential PTT/PDT treatment. Guo et al. (2014) presented a micelle-based system loaded with the photosensitizer Ce6 and the cyanine dye Cyptate with an average size of circa 60 nm. In vitro analysis showed the lysosomal disruption by the radiation-induced PDT as proposed to enhance lysosomal escape and synergistic effect with the next PDT step. In a pertaining study, lysosomal photodestruction was introduced as a novel modality to eradicate multidrug resistant (MDR) cancer cells which highly sequestered in their abundant lysosomes, hydrophobic weak base anticancer drugs. Adar et al., utilized the photosensitizer and cytotoxic fluorescent, Imidazolacridinone (IA), for lysosomal photodestruction in MDR cancer cells that present higher amount of lysosomes sequestering hydrophobic weak base materials, upon blue light illumination (\(\lambda_{ex}=470\) nm). The decrease in IC\(_50\) values, indicated the restoration of the MDR cells drug sensitivity (Adar et al., 2012). In a subsequent study, this group showed the correlation between treating with hydrophobic weak basic chemotherapeutic drugs and elevation in lysosomes biogenesis and drug entrapment, resulted in increasing the MDR (Zhitomirsky and Assaraf, 2015). These novel studies emphasize the importance of lysosomes destruction in order to evade MDR and improve the treatment outcome as demonstrated in the micelle-based system combining PTT and PDT synergistic effect following lysosomal disruption. The Second system, introduced by Yan et al. (2015), contained sinoporphyrin sodium (DVdms) as a photosensitizer loaded in Pegylated graphene oxide (GO-Peg-DVdms) and was ∼20 nm in size (Yan et al., 2015). The nanocarrier improved the fluorescent signal of the free PS via an intramolecular charge transfer mechanism (Geng et al., 2013). Both nanocomplexes have exhibited strong dual-imaging abilities (optical and photoacoustic), accompanied by precise anatomical tumor localization and high contrast, while demonstrating significantly higher therapeutic efficacy in vivo, compared to single PDT or PTT treatment.

3.2.5. Radiosensitization

Radiosensitization by high atomic number elements is mostly attributed to their increased ability to absorb X-ray radiation and emit secondary electrons further causing local DNA damage. Such elements are also known as good CT-imaging contrast agents (Dorsey et al., 2013). Gold NPs were vastly utilized for radiosensitization (Bogdanov et al., 2015; Hainfeld et al., 2008) and for CT imaging (Curry et al., 2014; Hainfeld et al., 2006; Popovtzer et al., 2008). Al Zaki et al. used gold-loaded polymeric micelles (GPMs)
for selective radiosensitization of mice harboring human fibrosarcoma HT1080 tumors. Gold NPs accumulation in these tumors was evaluated by CT imaging and revealed a significant tumor accumulation when compared with muscle tissue at 24 and 48 h following administration. Mice irradiated with 6 Gy in 24 h after administration of GPNs demonstrated increased long-term survival compared to mice treated solely with radiation (Al Zaki et al., 2014). Although showing impressive results in mouse models (Hainfeld et al., 2013; Joh et al., 2013), theranostic use of high atomic number elements is limited due to their possible toxicity (Dorsey et al., 2013), high toxicity of X-ray radiation (Siala et al., 2011; Siala et al., 2014; Siala et al., 2009; Zagar and Marks, 2012) and lack of machinery able to generate high voltage X-ray radiation that might be needed for clinical applications (Dorsey et al., 2013). Even though such drug-release reporting systems are very informative regarding the treatment’s target location, they do not report on the treatment’s efficacy or therapeutic outcome. The latter is mainly achieved by measuring the reduction in tumor’s volume in theranostic systems targeted towards solid cancers.

3.2.6. Iron oxide NPs

Due to their superparamagnetic core, iron oxide NPs act as contrast agents in MRI imaging. Surface coating allows functionalization and conjugation of various drugs and targeting moieties to the iron oxide NPs. These properties make iron oxide NPs great candidates for theranostic applications. You et al., developed a polyaspartic acid, dextran-coated, iron oxide NPs carrying O(6)-methylguanine methyltransferase (MGMT) siRNA and decorated by chlorotoxin (CTX) targeting peptide towards MMP-2 and a glioblastoma-specific chloride ion channel. Tumor uptake of these particles was confirmed by MRI imaging. In addition, follow up of tumor volume by MRI imaging pointed out the therapeutic effectiveness of the MGMT-siRNA carrying NPs when administered in combination with temozolomide (Yoo et al., 2014).

3.3. Monitoring the therapeutic activity and outcome

The validity of the reduction in tumor volume in response to cytotoxic agents as treatment’s efficiency criteria has been the issue of a major debate during the past decades, with the main concern being the long-term response (Therasse et al., 2000, 2006; Prabhatkar et al., 2013), pointed out the FDA’s withdrawal from breast cancer indication of the targeted drug bevacizumab even though it exhibited tumor shrinkage; tumor response to therapy was not further reflected in patients’ overall survival. In another clinical trial evaluating gastrointestinal stromal tumor (GIST) response to imatinib (Gleevec) treatment, it was found that tumor size solely could not reflect positive responses to the treatment as measured by tumor density CT scans or 18F-FDG metabolism in PET (Choi et al., 2004). In light of the criticism, theranostics monitoring short-term response have emerged with systems combining a reporter which reacts to immediate anticancer alterations in tumor tissues (Hu et al., 2015; Yuan et al., 2014b; Zhang et al., 2013; Zhang et al., 2011). Zhang et al., developed PEG-coated core-crosslinked polymeric micelles (CCPM) conjugated to annexin A5, a ligand of phosphatidylserine, a phospholipid normally present at the inner leaflet of the plasma membrane; phosphatidylserine is flip-flopped to the outer leaflet during apoptosis (Fig. 4). CCPM were dual-labeled with the NIR fluorophore Cy7 and with 111In radioisotope. The annexin A5 CCPM-labeled NPs have shown 1.9-fold tumor to blood ratio and 2.6-fold tumor to muscle ratio in chemotherapy-treated EL4 lymphoma-bearing mice compared with non-treated mice, indicating higher apoptosis in response to treatment. Imaging by SPECT/CT was conducted at 72 h after chemotherapy treatment and 48 h after administration of CCPM NPs (Zhang et al., 2011).

The aforementioned macromolecule-based theranostic systems for treatment monitoring are summarized in Table 2.

4. Image-guided surgery

Image-guided surgery is the use of pre-surgery images of the area to be resected during an invasive procedure in order to: 1. Detect and completely remove tumor margins and metastatic lesions, and 2. Avoid damage to healthy tissues. Positive tumor margins following resection are associated with poor prognosis, higher rates of recurrence and lower survival rates in various cancers (Catena et al., 2012; Eldeeb et al., 2012; Jones et al., 1996; Kimbrough et al., 2013; McCann et al., 2013; Scheepers et al., 2009; Snijder et al., 1998; Tummalala et al., 2013). On the other hand, damage to healthy tissues, can also affect life span as was demonstrated by a clinical trial performed on glioblastoma multiforme patients who underwent surgical resection. In addition to the obvious effect on quality of life, the trial found a positive correlation between cortical damage expressed by motor or language deficits to overall survival (McGirt et al., 2009). Maneuvering between the two edges of damaging healthy tissues to leaving residual disease is the main challenge in tumor resection that could certainly benefit from relying on more than the surgeon’s eye. The area of image-guided surgery has evolved rapidly during the last two decades, as reflected by the various imaging techniques and agents already in clinical use or in advanced stages of clinical evaluation (Manglore et al., 2013; Peck et al., 2009; Parker et al., 2015; Carlson et al., 2001; Sondak et al., 2013; Wallace et al., 2013). In the current review, we chose to focus on recent developments in theranostic delivery systems aimed to assist imaging during resection.

Major efforts were invested to develop optical imaging technologies for image-guided surgery in order to meet the challenges of intra-operative cancer diagnosis and precise tumor margin resection. Intraoperative procedures can utilize the advantages of sensitivity, while the lack of deep tissue penetration may not be a concern. Poly(ethylene glycol)–block-poly(e-caprolactone) (PEG-b-PCL) micelles were presented by Cho et al. (2014a), as the carrier of both neoadjuvant chemotherapy (NACT) and an apoptosis targeted-NIR fluorescent probe, for the detection of tumor during surgical resection (followed by the NACT IP administration). The tandem administration of these two polymeric systems (in 24 h interval) to an ES-2-luc-bearing xenograft model for ovarian cancer -bearing mice enabled the visualization of therapy-induced apoptosis during surgical procedure. In addition, the accumulation and bright signal obtained from the imaging probe, resulted in detection of 1 mm diameter tumors 48 h post IV administration. The apoptosis targeting peptide, GFNRLKA-GAKIRFGS, demonstrated higher accumulation in tumor versus the non-targeted NIR-micellar imaging probe. The optical guidance during the surgical procedure led to ~90% tumor resection in comparison with 30% tumor removal of control group, using only white light.

In order to enhance the detection ability, a combination of imaging modalities is often used. For example, a dual modality of photoacoustic- and fluorescent-based nanoprobes was successfully used by Xi et al. (2014b), on a highly invasive breast carcinoma in a murine model. The researchers used a multifunctional NIR 830-labeled amphiphilic polymer-coated, 10 nm magnetic IONPs particles targeted with AFT recombinant peptide, towards urokinase plasminogen activator receptor (uPAR), overexpressed in breast cancer tissues. They first located the primary tumor by photoacoustic imaging (PAI) and removed it. Secondly, they inspected the surgical wound for complete resection or residual disease by the fluorescence guidance. The PAI, based upon US waves that return post light radiation, enabled a higher depth penetration of.
Table 2
Macromolecular thaneranostic systems used for treatment monitoring in real-time.

<table>
<thead>
<tr>
<th>Application</th>
<th>Aspects</th>
<th>Macromolecular carrier</th>
<th>Targeting ligand</th>
<th>Activation mechanism</th>
<th>Turn-ON mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monitoring drug release</td>
<td></td>
<td>Dendritic polyglycerol</td>
<td>X</td>
<td>pH-induced</td>
<td>FRET disruption</td>
<td>Kruger et al. (2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nanoaggregates</td>
<td>cRGD</td>
<td>Intracellular reduction</td>
<td>AIE</td>
<td>Yuan et al. (2014c)</td>
</tr>
<tr>
<td>Image guided therapy</td>
<td>Microbubbles</td>
<td>Cyanoacrylate based</td>
<td>X</td>
<td>High intensity</td>
<td>X</td>
<td>Lammers et al. (2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>microbubbles</td>
<td>cRGD</td>
<td>ultrasound waves</td>
<td></td>
<td>Yuan et al. (2014a)</td>
</tr>
<tr>
<td></td>
<td>Polymeric encapsulation matrix</td>
<td>UCNP-CsHA</td>
<td>Dual targeting</td>
<td>980 nm light</td>
<td>X</td>
<td>Wang et al. (2014a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PEG-coated UCNP</td>
<td>Folic acid</td>
<td>980 nm light</td>
<td></td>
<td>Liu et al. (2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UCNP nanoparticles</td>
<td>X</td>
<td>980 nm light</td>
<td></td>
<td>Wang et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>HSA-based nanocomplex</td>
<td>X</td>
<td>808 nm light</td>
<td></td>
<td></td>
<td>Chen et al. (2014)</td>
</tr>
<tr>
<td>PDT</td>
<td></td>
<td>Polymeric micelles</td>
<td>X</td>
<td>PDT–785 nm and</td>
<td>X</td>
<td>Guo et al. (2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PDT–660 nm light</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PEGylated graphene</td>
<td>X</td>
<td>PDT–630 nm and</td>
<td>X</td>
<td>Yan et al. (2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>oxide</td>
<td></td>
<td>PDT–808 nm light</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTT</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDT and PTT combination</td>
<td></td>
<td>Gold-loaded polymeric</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>micelles (CPMs)</td>
<td></td>
<td></td>
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<tr>
<td>Radiosensitization</td>
<td></td>
<td>Dextran-coated IONP</td>
<td>CTX targeting peptide</td>
<td></td>
<td></td>
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<tr>
<td>Iron oxide</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>nanoparticles</td>
<td></td>
<td>PEG-coated</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>core-crosslinked</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>micelles (CCPM)</td>
<td></td>
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</table>


Table 3
Macromolecular thaneranostic systems used for image-guided surgery.

<table>
<thead>
<tr>
<th>Application</th>
<th>Macromolecular carrier</th>
<th>Targeting ligand</th>
<th>Activation mechanism</th>
<th>Turn-ON mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Image guided surgery</td>
<td>Polymeric micelles</td>
<td>GFNFKMAGAKIRFGS targeted towards apoptotic cells</td>
<td>X</td>
<td>X</td>
<td>Cho et al. (2014b)</td>
</tr>
<tr>
<td></td>
<td>Polymer coated-IONP</td>
<td>X</td>
<td>X</td>
<td></td>
<td>Xi et al. (2014a)</td>
</tr>
<tr>
<td></td>
<td>Polymeric micelles</td>
<td>Sialic acid</td>
<td>X</td>
<td></td>
<td>Wu et al. (2015)</td>
</tr>
</tbody>
</table>


31 mm and improved the visualization of the primary tumor. The re-resected fluorescently-marked suspected tumor tissues were evaluated and found by pathological analysis to contain residual disease.

In an attempt to further increase the signal obtained from the tumor over the background (i.e., SNR) in addition to the deep penetration of PAI, Wu et al. (2015), have presented a multifunctional, targeted, pH-sensitive activatable nanovesicles. NIR fluorescent dye prone to switch to its optical and optoacoustic active state, was encapsulated in a biocompatible carrier of poly(styrene-alter-(maleic acid)]) decorated with surface-anchored sialic acid (SA) for tumor targeting. The resulting pNIR@P@SA system (~86.6 nm) generated a bright NIR fluorescent and photoacoustic signal in tumors about 24 h post IV injection to nude mice bearing subcutaneous H-22 hepatocellular carcinoma cell xenografts. A high tumor to healthy tissue signal contrast was achieved. Furthermore, pNIR@P@SA was able to discern liver tumor foci as small as 4 mm in size which is significantly below the defined minimal residual disease (1 cm). The aforementioned macromolecule-based thaneranostics for image-guided surgery are summarized in Table 3.

5. Clinical trials

The rapid evolution of the imaging area is expressed in the sharp growth curve of imaging-based clinical trials as listed by the US National Institute of Health (NIH), starting from only 123 studies initiated in the years 1990 to 2000 and growing to already more than 6000 trials that were launched between 2010 until today. Only a small portion, however, are NP-based thaneranostics, while the vast majority utilize small-molecules based molecular-imaging. Nanotheranostics have the potential to revolutionize personalized medicine and are currently far from full utility. Some of the unique features of nanotheranostics listed in this review have found parallel small-molecule based technologies that are currently clinically tested: 1. Nanotheranostics are “switchable” agents that can actively mark selected tissues such as tumors or inflammation (Ang et al., 2014; Ferber et al., 2014; Lee et al., 2009; Weissleder et al., 1999b), 5-Aminolevulinic acid (5-ALA) is an endogenous precursor of hemoglobin that produces porphyrins mainly in some types of malignant brain tissues. These porphyrins fluoresce under illumination with violet blue light. Orally administered 5-ALA is clinically tested for assisting complete resection of various brain tumors.
A separated nanotheranostic system can subsequently utilize the same delivery vehicle for imaging and therapy, in order to preselect patients on the basis of predicted response and receptor expression patterns. $^{90}$Y-edotreotide, or 111I-edotreotide, is a similar conjugate which contains the $\gamma$-emitter radioisotope instead of yttrium-90. This compound was evaluated clinically for treatment of neuroendocrine tumors, expressing the somatostatin receptor (Bushnell et al., 2010; Menda et al., 2010). These 111I-edotreotide, a similar conjugate which contains the $\gamma$-emitter radioisotope instead of yttrium-90, is utilized for PET/CT imaging. The latter conjugate is preclinically tested as a predictor of body-distribution and for the determination of the preferred dosage pretreatment with $^{90}$Y-edotreotide (Barrett et al., 2008; Yang et al., 2014).

3. Nanotheranostics can introduce an imaging agent and a targeting moiety in a single platform, in order to target specific cell population or tissue, while utilizing enlarged complex size for EPR-based accumulation. A folate-fluorescein isothiocyanate FITC conjugate is clinically tested for fluorescent imaging during exploratory laparotomy in ovarian cancer (van Dam et al., 2011). Many gas filled lipid-based microbubbles and paramagnetic iron oxide NPs are clinically examined as contrast agents for ultrasound imaging (Bokor et al., 2001; Gandon et al., 1991; Leen et al., 2002; Moriyasu and Itoh, 2009; Omoto et al., 2009; Stark et al., 1988; Uemura et al., 2013) and are beyond the scope of this review. Paramagnetic iron oxide NPs undergo clinical evaluation as MRI contrast enhancers since the late 1980s (Stark et al., 1988; Weissleder et al., 1989); this vast research has resulted in various FDA-approved commercialized products (Mahmoudi et al., 2011). These particles are composed of an iron oxide core and coated with a biocompatible polymer (Harisinghani et al., 2003; Mahmoudi et al., 2011). Due to their uptake by RES cells, they have been mainly studied for the imaging of liver cancer (Gandon et al., 1991; Nakamura et al., 2000) and for the non-invasive imaging of lymph node metastases (Harisinghani et al., 2003; Motoyama et al., 2012), although various other applications have been proposed as well (Alam et al., 2012; Dousset et al., 2006; Elizondo et al., 1990). From a historical perspective, theranostics had a developmental role in the area of nanotherapeutics since its infancy. HPMA doxorubicin conjugate (PK1), the first polymer therapeutics to enter clinical trials, was labeled with $^{131}$I radiosotope as a part of a phase I clinical trial. Biodistribution follow-up by $\gamma$-camera revealed tumor-accumulation in 6 out of 21 patients. This low fraction of accumulation was considered an artifact stemming from low SNR attributed to the high radiation of $^{131}$I radioisotope and the low sensitivity of the $\gamma$-reader (Vasey et al., 1999; Julyan et al., 1999). A subsequent clinical trial followed the biodistribution of PK2–HPMA doxorubicin conjugate targeted to liver hepatocytes by galactosamine. As a lesson from the former trial, the lower emitter $^{131}$I radiosotope was covalently conjugated to PK2 and SPECT images were added to $\gamma$-readings and compared with parallel CT scans. This imaging technique has revealed the higher uptake of normal liver compared with the malignant liver tissue, although tumor signal was still significantly higher than the background (Seymour et al., 2002; Julyan et al., 1999).

Recent years have provided a tailwind to the theranostic polymers area, with the FDA approval of Technetium (99mTc) tilmanocept (99mTc–diethylenetriamine penta-acid–mannosyl–dextran, marketed as Lymphoseek), 99mTc chelated to diethylenetriamine penta acid (DTPA) moieties conjugated to 10 kDa dextran backbone, targeted towards the CD206 receptor expressed on reticuloendothelial cells by multiple mannose moieties (Marcinow et al., 2013). This ~7 nm lymph node specific marker is injected near the tumor, rapidly cleared from the injection site and allows the identification of the sentinel lymph nodes using hand-held $\gamma$-detector within few hours. In a phase II clinical trial performed on 31 breast cancer patients and 49 melanoma patients, out of 55 patients subjected to whole body scan, 52 patients demonstrated radioactive hot-spots. An average of 2.22 lymph nodes per patient were marked by (99mTc) tilmanocept, 13.7% of which contained metastatic disease as was further analyzed by histopathology. Only one breast cancer patient had a positive lymph node that was not tracked by (99mTc) tilmanocept $\gamma$-counts (Leong et al., 2011). Another ligand-targeted theranostics delivery system which was designed for sentinel lymph nodes diagnosis is currently recruiting patients for phase 0 clinical trial. cRGDy-PEG-Cy5-C dots comprise of a Silicone-based shell encapsulating the NIR fluorescent dye Cy5, coated with PEG polymer conjugated to cyclic RGDy (cRGDy) peptide targeted towards the $\alpha v \beta 3$ integrin overexpressed on angiogenic endothelial cells and on various cancer cells to yield ~7 nm NPs. Pre-clinical examination has included the conjugation of $^{124}$I radioisotope to the cRGDy ligand in order to follow the biodistribution, clearance, tumor accumulation and signal intensity in mice and miniswine melanoma models (Benezra et al., 2011; Bradbury et al., 2013). These $^{124}$I-radiolabeled particles were further tested in a pilot clinical trial for their pharmakoetics, biodistribution and clearance following a single IV injection in 5 metastatic melanoma patients. Results obtained from whole body PET-CT scans at 2,4, 24 and 72 h post administration and plasma samples demonstrated $t_{1/2}$ of 13–21 h in renal and hepatobiliary clearance, favorable PK, and no RES uptake. Altogethe $^{124}$I-cRGDy-PEG-Cy5-C dots exhibited a good safety profile. Interestingly, PET-CT scans demonstrated low intensity lesion accumulation in 2 out of 5 patients, since settings were not optimized for this cause. Despite the low accumulation, based on previous animal studies and the established expression patterns of $\alpha v \beta 3$, $^{124}$I-cRGDy-PEG-Cy5-C dots were proposed for future applications as diagnostics for pre-selection of patients destined to integrin-targeted therapy, non-invasive imaging agents for tumors and neovascularature and for monitoring therapeutic efficiency (Phillips et al., 2014).

6. Clinical translation

In recent years, there has been a great progress in the development of cameras and lasers for optical fluorescence imaging in the IR range (Mieog et al., 2011; Troyan et al., 2009). In parallel, there is a vast clinical use of low MW organic dyes such as ICG and methylene blue for determining cardiac output, hepatic function and liver blood flow, and for ophthalmic angiography. In 2015, the fluorescence imaging system, Xiralite®, gained FDA approval for visualization of microcirculation in the hands (for inflammation and perfusion-related disorders). As already mentioned, the integration of nanotechnology and the imaging world is lagging behind, despite the great potential of the former to improve the area of imaging by using different polymer based nanosystems, as demonstrated throughout the current review. These improvements include biodistribution alteration in agreement with the clinical need by means of size, shape, lipophilic or hydrophobic nature, surface charge and structure of the nanosystem. Major consideration for optical imaging is the limited depth of penetration, thus it can be directed to superficial tissues, minimally invasive procedures such as colonoscopy, or diagnostic-/treatment-based surgical procedures (such as sentinel lymph node detection and tumor resection, respectively). For in vivo whole body imaging applications, a dual imaging modality combining optical and MRI/CT/PET should be superior over a single one, exploiting the benefits that each modality brings. Several examples for dual-imaging were demonstrated herein in preclinical studies for SLN detection, image-guided surgery, and PDT/PTT treatments. The next clinical translated application of nanotheranostics will probably...
rise for SLN mapping (as similar system is already included in phase 0 clinical trial patient recruitment (Benezra et al., 2011; Bradbury et al., 2013)).

In general, the road towards clinical translation is long and comprised of developmental hurdles, caregivers’ compliance, expenses and profitability (i.e., cost effectiveness). From a developmental point of view, the theranostic systems mentioned in this review are composed of three main components (and the different combinations between them): (1) the polymeric nanocarrier, (2) the imaging and or the therapeutic agent, and (3) a targeting ligand. Selecting a thoroughly explored and characterized polymeric system (from the great experience of polymer therapeutics) may overcome the developmental difficulties when compared with the use of newly characterized and unfamiliar polymers for imaging purposes. This is the reason that stands behind the vast amount of publications utilizing the FDA approved PEG as a carrier or as a coating agent. Nevertheless, it should be stated that the research of macromolecular-carrier systems still has a long journey to explore the physico-chemical characteristics in order to achieve optimized duration of circulation, biodistribution and tissue uptake. Furthermore, the production of uniform particles and the batch-to-batch reproducibility is another milestone to overcome. Such reproducibility issues are attributed to the recent discontinuation of the various formerly approved super paramagnetic iron oxide (SPIONPs) used to serve as contrast enhancers for MR imaging (Corot and Warlin, 2013).

As for the imaging agent, its first and foremost important characteristic is non-toxicity or reasonable safety profile. The second is the ability to shine brightly over a dark background in order to facilitate the detection of small malignant lesions, and its third desired characteristic is the ability to report on molecular changes for early and differentiated diagnosis. Organic fluorescent dyes comply with these criteria but lack the deep-tissue non-invasive diagnosis. Thus, in order to be clinically approved, the right medical application should be embraced in accordance with its imaging modality performances. In addition, a superior specificity and positive effect on patient health in comparison with an existing gold standard should be proved. For the third component, the development of ligand-targeted NPs is a complexed-multi-stage process which includes additional stages of synthesis, characterization and purification. In addition, targeting NPs can increase immunogenicity, leading to shorter circulation time (Cheng et al., 2012). In general, the conjugation of a ligand/drug/imaging agent adds to the heterogeneity of the system, which manifests in difficult characterization and low reproducibility resulting in lower chances to meet the demands of clinical trials and market use (Mullen et al., 2010). As an example for the inability to scale-up and produce sufficient amount for clinical trials demands due to chemical and biological inconsistency was the case of the Baker group. Their multifunctional dendrimer-based system was composed of a drug, targeting moiety and an imaging agent. They found that only 5% (or less) of the nanostructure contained the designed amount of drug or targeting agent, which probably caused the biological inconsistency (Thomas et al., 2012). Furthermore, another problem standing in the way of ligand targeted systems for clinical embracing is their sensitivity-dependence and false-positive or negative validation. Various receptors for targeting ligands aimed for cancer detection and therapeutics are also overexpressed in inflammatory conditions, thus detection should be treated with skepticism as cases of false-positive may occur. Alternatively, in the case of low targeting affinity, there would not be a sufficient signal from the tumor, leading to false-negative evaluation. Basic research should be conducted for the quest of highly specific predictive biomarkers that would be recognized in the tumor tissue and not in other healthy tissues. Finally, the biggest hurdle for clinical translation of theranostic NPs is the cost effectiveness, which is bundled within all three components mentioned. The different applications in accordance with precision medicine would fit only a certain condition and/or patient. The use of macromolecular based theranostics would suit longer procedures due to the EPR effect, in comparison with low MW theranostics. The macromolecular different physico-chemical characteristics can be fine-tuned and optimized for different retention and clearance time. As a result, for each nanotheranostic system, there will be a very narrow market, a common problem of personalized medicine profitability. Moreover, the ligand targeting nanosystems, generally demonstrate enhanced sensitivity versus untargeted particles. Although for such a complexed system, the addition of ligand can further shrink the patient's population. This very expensive development of agents which will only fit a limited fraction of patients would probably improve patient care but repel investors. Taken together, the nanotheranostic field holds major limitations along medical benefits and the answer to the title question is controversial. The incorporation of the nanotheranostics within the applications for personalized medicine discussed herein (during screening, diagnosis, treatment and follow-up) is far from clinical translation and requires improvements in analytical characterization methods, enhanced signal emitting optical imaging agents, and batch-to-batch reproducible polymeric scaffolds.

7. Future perspectives

Nanotheranostics have the potential to revolutionize personalized medicine and are currently far from being fully exploited. Theranostic NPs hold great potential leading to the improvement of various treatment strategies, among them, therapeutic decision making. The proper treatment strategy adjustment, is crucial for the patient treatment success. Within the broad umbrella of theranostic NP systems presented in our current review, several examples are capable of dealing with this challenging demand. We demonstrated here the utilization of companion diagnostic for the selection of the appropriate treatment used for patient stratification according to vascular hyper-permeability and leakiness, certain enzymes, analytes or receptors overexpressed in the cancer cells or their surrounding environment. In addition, biomarker identification is another major challenge for personalized medicine. The improvement of the above ambitious goals is critical for decreasing cancer death rates and increasing the number of cancer survivors. Although not a simple task, we believe that overcoming the obstacles mentioned herein for the development of theranostic NPs might change the way we diagnose, monitor and treat cancer.

8. Conclusions

Theranostic systems, combining imaging and therapeutic modalities, can synergistically act to improve all stages of patient care, starting form screening and diagnostics, proceeding to treatment, and ending with treatment follow-up. Nanotechnology possesses great opportunities to the theranostic field, along with various challenges that limit the clinical translation of current available nanotechnology-based theranostic systems. Combining small molecule-based imaging modalities with polymeric nanoparticles can increase their solubility, prolong their stability and passively target them to tumors based on their vascular hyper-permeability and lymphatic drainage characteristics, resulting in enhanced signal. These systems can be used to characterize hypervascularization and to stratify patients that will benefit from NPs-based therapy.

Great progress was achieved in the theranostic NPs field, considering the abundance of applications studied in the past decade. The imaging modalities evolved from “simple” tagged probes (with
MR, radiologic and fluorescence imaging capability) to “smart” probes, are capable of sensing their surrounding enzymes, analytes, pH changes and overexpressed receptors while reporting on them in real-time. This shift to “smart” fluorescent Turn-On probes improved the low SNR resulting from long circulating fluorescently tagged NPs. In addition, their use does not expose the caregiving teams to ionizing radiation. Yet, these reported Turn-On probes are not flawless and face several hurdles. The complicated manufacturing procedures reduce batch-to-batch reproducibility and homogeneity, and overall increase expenses over profit and hamper the transition from bench to bedside. In addition, the limitation of low depth of penetration (even for NIR probes) to and from the tissue is a major drawback, narrowing the applicability of the use of these probes for non-invasive diagnostic applications. Evidently, in recent years, a vast amount of studies has focused on minimally to fully-invasive procedures guided by real-time optical imaging. These procedures include endoscopy screening for cancer, SLN identification for staging the disease and image-guided surgery for complete resection of the tumor in order to minimize residual disease and damage to healthy tissues around it. An important reinforcement for this statement is the Phase I and 0 clinical trials of polymeric systems for SNL mapping validating the diagnosis by radiotracer and fluorescence-tagged polymeric and polymeric system-coated systems. Yet, no theranostic NPs system bearing an independent fluorescence reporting system has advanced to the stage of clinical evaluation. It may be contributed to the low SNR in comparison with radiotracers, and the compliance of caregivers to change the known procedures with new materials, demanding different new imaging instruments and training. In addition, the photostability of the fluorophore is another issue to consider for prolong procedures under white light and NIR exposure.

From an economic perspective, although very complexed systems (and thus potentially possess high development costs), as mentioned in the clinical translation section, they can save expenses of useless treatment protocols provided to non-responding patients, leading to adverse effects which result in additional demand of treatment costs.

In light of all the pros and cons of the theranostic systems, we expect that the greatest impact of the described theranostic precision nanomedicines will be within (i) image-guided surgery, (ii) detection and preselection of patients with superficial disease, and (iii) clinical drug development in Phase I PK clinical trials. The current review will hopefully help to rationally design theranostic systems, taking into consideration the different hurdles each tumor model and/or application of therapy strategy brings along.

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