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Recent progress in nanomedicine: therapeutic, diagnostic and theranostic applications

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In recent years, the use of nanomedicine formulations for therapeutic and diagnostic applications has increased exponentially. Many different systems and strategies have been developed for drug targeting to pathological sites, as well as for visualizing and quantifying important (patho-) physiological processes. In addition, ever more efforts have been undertaken to combine diagnostic and therapeutic properties within a single nanomedicine formulation. These so-called nanotheranostics are able to provide valuable information on drug delivery, drug release and drug efficacy, and they are considered to be highly useful for personalizing nanomedicine-based (chemo-) therapeutic interventions.

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Introduction

Nanomedicine is defined as the application of nanotechnology to medicine. It encompasses the use of submicrometer-sized tools for the diagnosis, prevention and treatment of diseases, for better understanding the complex underlying pathophysiology of diseases, and for improving the quality of life of patients [1,2,3••]. Significant efforts have been invested and significant progress has been made in the nanomedicine field over the last couple of years, and a number of nanomedicine formulations have been shown to hold considerable potential for enabling more effective and less toxic diagnostic and therapeutic interventions.

Interdisciplinary research on nanomedicine formulations, on disease diagnosis and on disease treatment has brought about a number of efforts to combine diagnosis and therapy within a single nanomedicine formulation. These so-called nanotheranostics are designed to facilitate several important aspects of drug delivery research, including, for example, the longitudinal assessment of the pharmacokinetics, the biodistribution and the target site accumulation of a given formulation, and the non-invasive visualization and quantification of drug release [4,5•,6–8]. In addition, by enabling patient preselection and treatment monitoring, nanotheranostics can be used for personalizing nano-chemotherapeutic interventions [9•,10,11].

Here, we briefly summarize the basic principles of nanomedicine-mediated drug targeting to pathological sites, we describe the progress made with nanotherapeutics, nanodiagnostics and nanotheranostics in recent years, and we discuss the potential of theranostic systems and strategies for enabling personalized nanomedicine treatments.

Therapeutic nanomedicine

Nanomedicines are advantageous over standard low-molecular-weight drugs in several different regards. They, for example, (1) reduce renal excretion and/or hepatic degradation, leading to prolonged circulation times; (2) reduce the volume of distribution, leading to less accumulation in healthy non-target tissues ('site-avoidance drug delivery'); (3) improve the ability of drugs to accumulate at pathological sites ('site-specific drug delivery'); and (4) improve the therapeutic index of drugs, by increasing their accumulation at the target site and/or reducing their localization in potentially endangered healthy organs [3••,12–14]. In addition, nanomedicine formulations assist low-molecular-weight (chemo-) therapeutic agents in overcoming several additional barriers to drug delivery to pathological sites. As exemplified by Table 1, this is particularly obvious in the case of anticancer drugs, for which a large number of chemical, anatomical, physiological and clinical barriers exist [15]. By assisting drug molecules in overcoming (some of) these barriers, and by better balancing their localization in diseased vs. healthy tissues, nanotherapeutics are able to improve the often far-from-optimal therapeutic index of i.v. administered (chemo-) therapeutic agents, they increase their accumulation at pathological

Table 1

Barriers limiting the delivery of i.v. administered (chemo-) therapeutic drugs to tumors. Note that several barriers are inter-related, and that not all barriers apply to all types of (chemo-) therapeutics.

Barriers to drug delivery to tumors			
Chemical barriers	Anatomical barriers	Physiological barriers	Clinical barriers
Low solubility	Vascular endothelium	Renal filtration	Low efficacy
Low stability	Perivascular space	Hepatic degradation	High toxicity
Low molecular weight	Cellular membrane	High tumor cell density	Need for hospitalization
Large volume of distribution	Nuclear membrane	High interstitial fluid pressure	Frequent administration
Charge interactions	Blood brain barrier	Drug efflux pumps	Low cost-effectiveness

Table adapted, with permission, from [15].

sites and their therapeutic efficacy, they reduce the incidence and intensity of side effects, and they thereby result in improved patient acceptance, compliance and prognosis.

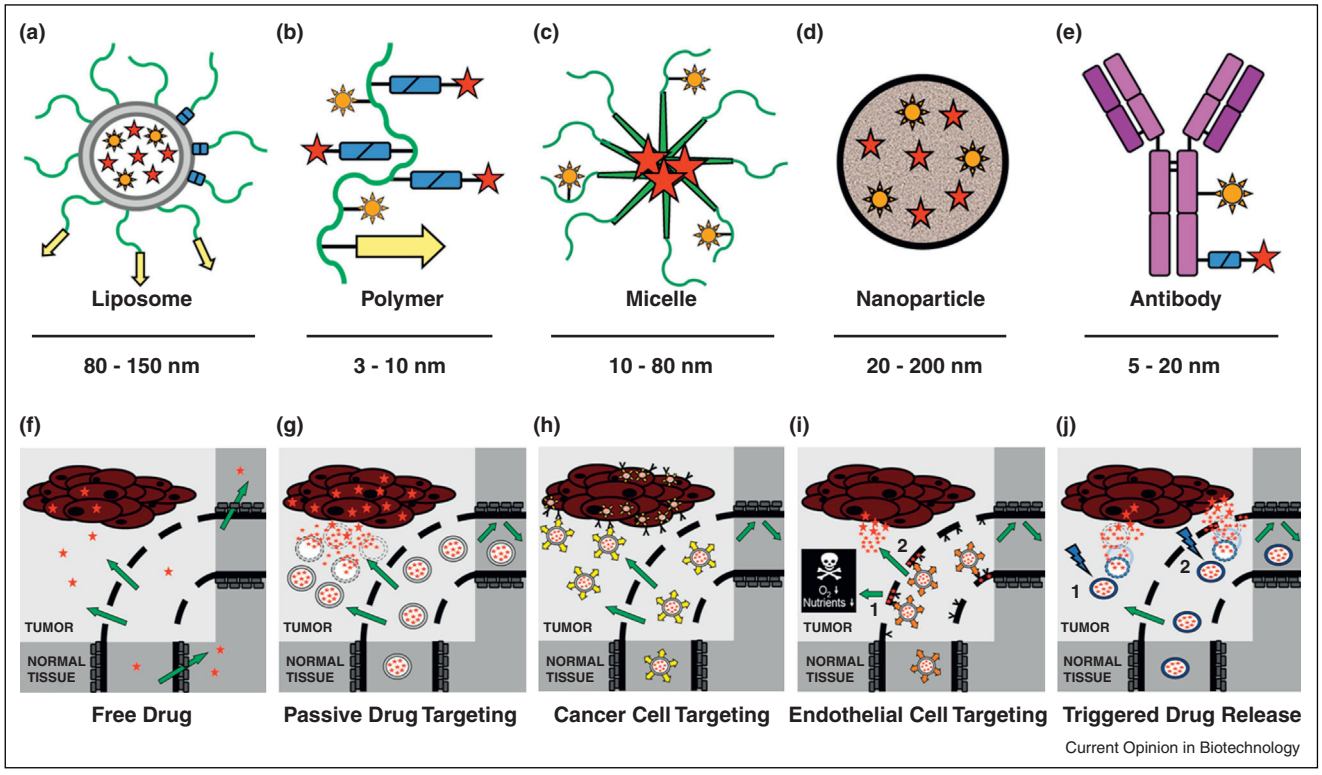
For obvious reasons, the vast majority of efforts in the nanomedicine field have thus far focused on cancer [4,14,16,17,18••]. Because solid tumors — as do inflamed tissues — generally present with leaky blood vessels, which as opposed to the vasculature in the majority of healthy tissues, allow for the extravasation of nanomaterials with sizes of up to 400 nm, nanomedicines are generally able to accumulate both effectively and selectively at such pathological sites, by means of a mechanism known as the Enhanced Permeability and Retention (EPR) effect [19,20]. Besides on the enhanced leakiness of tumorous and inflamed blood vessels, the EPR effect is also based on the fact that solid tumors tend to lack functional lymphatics, thereby limiting the removal of extravasated nanomaterials from the target site. Because EPR-mediated drug targeting exclusively relies on the pathological properties of the target tissue, that is, enhanced leakiness and poor lymphatic drainage, it is generally referred to as passive drug targeting (Figure 1g). Active drug targeting, on the other hand, relies on the use of antibodies, peptides or sugar moieties, which are physically or chemically incorporated into nanomedicine formulations to facilitate localization to and/or uptake by target cells (Figure 1h). Additional nanomedicine-based strategies to improve drug delivery to, drug release at and/or drug efficacy against target cells and tissues are based on active drug targeting to pathological blood vessels (e.g. to tumor endothelium; Figure 1i), and on the development of stimuli-responsive systems that can be triggered to release their contents, using, for example, hyperthermia or ultrasound (Figure 1j).

A large number of nanotherapeutics have been designed and evaluated over the years, relying, for example, on liposomes, polymers, micelles, nanoparticles and antibodies as carrier materials (Figure 1a–e). The vast majority of these formulations have been used for drug targeting to tumors, and rely on EPR-mediated passive drug targeting. Regarding the former, it is important to note that in recent years, increasing numbers of efforts have been initiated in which therapeutic nanomedicines are used for drug targeting to non-cancerous disorders, including, for example, rheumatoid arthritis and atherosclerosis [12,14,21,22•,23•]. As mentioned above, also inflammatory diseases are characterized by leaky blood vessels, and the accumulation of long-circulating nanotherapeutics within such lesions (via 'site-specific drug delivery'), together with their ability to attenuate localization in healthy non-target tissues ('site-avoidance drug delivery'), enables the use of potent anti-inflammatory agents such as corticosteroids, at much higher i.v. doses, thereby providing a clear rationale for novel inflammation-targeted nanotherapeutic treatments.

Regarding the latter, that is, passive vs. active targeting, it should be realized that incorporating targeting ligands in nanomedicine formulations is much less useful than generally assumed, making sense only in a limited number of specific cases, whereas in other cases, it only complicates formulation design. As an example of this, it has been shown that active targeting to tumor cells, using, for example, HER2-antibody targeted liposomes or transferrin-targeted gold nanoparticles, does not increase tumor concentrations as compared to passively targeted formulations [24,25]. In these set-ups, active targeting did change the local distribution of the agents within tumors, with much higher levels eventually ending up in tumor cells (vs. in macrophages), but it did not enhance their overall accumulation at the target site.

In our opinion, active targeting does make sense in case of agents which are poorly — or not at all — internalized, as is the case for negatively charged macromolecules such as DNA and siRNA. In such cases, active targeting is absolutely necessary to confer proper cellular uptake and therapeutic efficacy [26,27]. For nanomedicines containing standard (chemo-) therapeutic drugs, on the other hand, active targeting only seems to be useful if the targeting ligand adds something 'special', that is, a feature not conveyed by the passively targeted formulation such as intrinsic antitumor efficacy (thereby enabling targeted combination therapy) [28,29]. On other occasions, the added advantage of active drug targeting appears to be — at best — modest. This can be best exemplified

Figure 1

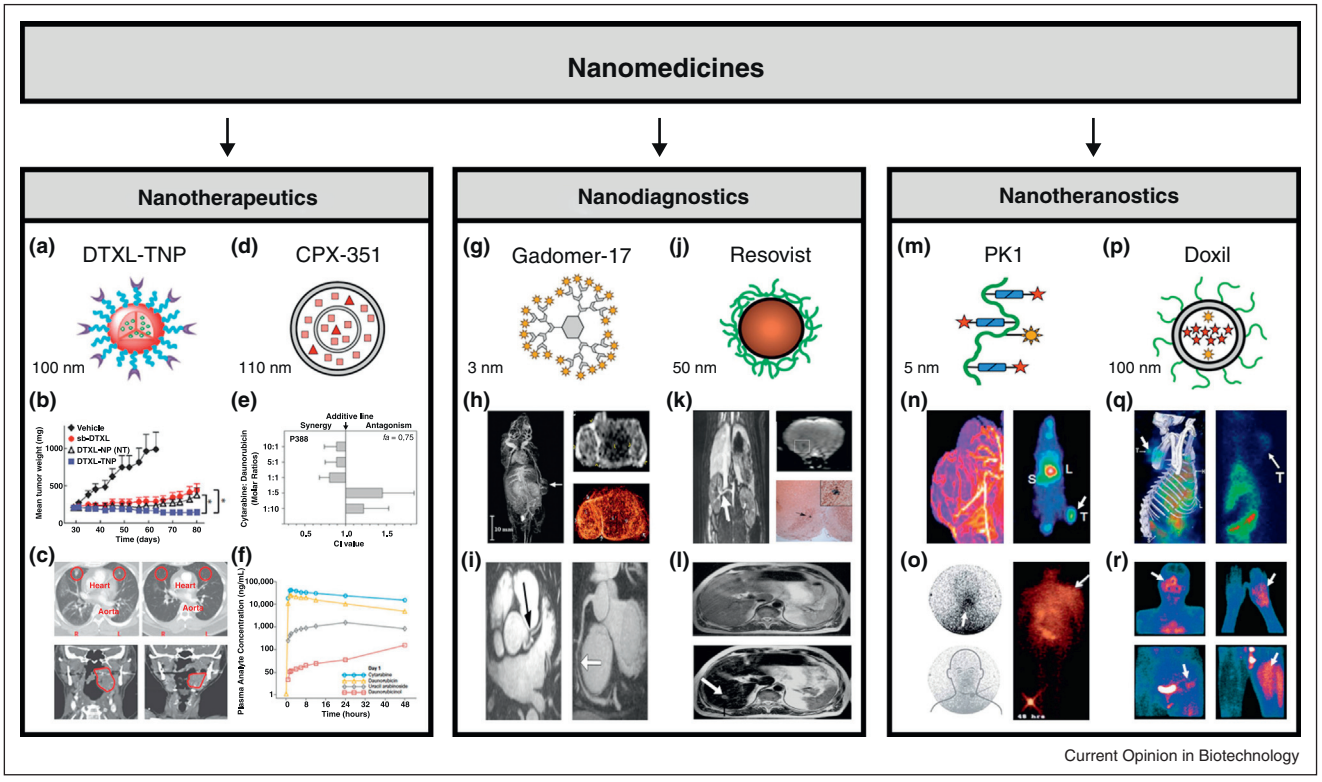


Nanomedicine systems and strategies. **(a–e)** Examples of clinically relevant nanotherapeutics and nanotheranostics. Liposomes and liposomal bilayers are depicted in gray, polymers and polymer-coatings in green, linkers allowing for drug release and for sheddable stealth coatings in blue, targeting ligands in yellow, antibodies in purple, imaging agents in orange, and conjugated or entrapped (chemo-) therapeutic agents in red. **(f–j)** Drug targeting strategies. (f) Upon the i.v. injection of a standard low-molecular-weight (chemo-) therapeutic drug, which is often rapidly cleared from the blood, only low levels of the agent accumulate at the target site, while localization to healthy non-target tissues tends to be high. (g) Upon using a passively targeted nanomedicine formulation, by means of the EPR effect, the accumulation of drugs in tumors and in tumor cells can be substantially increased, while their localization in healthy organs and tissues can be attenuated. (h) Active targeting to internalization-prone cell surface receptors (over) expressed by cancer cells aims to improve the cellular uptake of nanomedicine formulations. This is particularly useful for the intracellular delivery of agents which are poorly internalized by cells such as DNA and siRNA. (i) Active targeting to receptors (over) expressed by angiogenic endothelial cells can on the one hand aim to increase drug delivery to tumor endothelium, thereby eradicating tumor blood vessels, and depriving tumor cells of oxygen and nutrients (i-1). On the other hand, reasoning that tumor blood vessels are continuously exposed to long-circulating nanomedicines, endothelial cell targeting might also be useful for improving the overall tumor accumulation of chemotherapeutic drugs (i-2). (j) Stimuli-responsive nanomedicines can be triggered to release their contents by externally applied stimuli such as hyperthermia and ultrasound. This can be done either upon (EPR-mediated) accumulation at the target site (j-1), or while the formulations are still present in the circulation (j-2).
Image reproduced, with permission, from [8,31••].

by an excellent recent study by Hrkach and colleagues, in which PSMA-targeted PLA/PLGA-nanoparticles containing docetaxel (DTXL-TNP) were elaborately optimized from a formulation point-of-view, but in which the added value of active PSMA-targeting was not very large (Figure 2a–c) [30]. These efforts underline the importance of carefully overthinking in which situations active targeting of nanomedicines might be beneficial, in which cases it might be better — from a formulation, production and upscaling point of view — to refrain from active targeting.

A final important issue to keep in mind when evaluating the prospects of nanotherapeutics and drug targeting to pathological sites (in particular to tumors), relates to potential pitfalls associated with nanomedicine formulations [31••]. This is because, as standard low-molecular-weight drugs, nanomedicines also suffer from several shortcomings: (1) how prominent and how general is the EPR effect in patients? And what can we do to enhance EPR?; (2) what about the penetration of 'large' nanomedicines? Do 100 nm-sized formulations really penetrate tumors?; (3) how general and how useful is active targeting (see above)?; (4) aren't many of the advanced and chemically highly elegant nanotherapeutics we are developing far too complex to be scaled up by the pharmaceutical industry?; (5) do nanotherapeutics really improve efficacy, or do they only lower toxicity?

Figure 2



Therapeutic, diagnostic and theranostic applications of nanomedicines. **(a–c)** PSMA-targeted PLA/PLGA-nanoparticles containing docetaxel (DTXL-TNP) were extensively optimized from a formulation point of view, their efficacy was evaluated in three different mouse tumor models (b), and their pharmacokinetics were assessed in mice, rats, monkeys and humans. In addition, initial responses in patients with lung cancer metastases and tonsillar tumors were monitored (c). **(d–f)** Multilamellar liposomes containing cytarabine and daunorubicin (CPX-351) were extensively evaluated *in vitro* and *in vivo*, to identify the optimal ratio for synergistic drug efficacy (e). Liposomes containing an optimal 'ratiometric' mixture of 5:1 cytarabine vs. daunorubicin were subsequently evaluated in patients suffering from relapsed and refractorary acute myeloid leukemia, showing prolonged presence of drugs and metabolites in blood (f), and a significant number of complete responses in pretreated patients. **(g–i)** Gadomer-17 is a polylysine dendrimer containing 24 gadolinium complexes. It is significantly larger and it is highly useful for DCE-MRI, MR lymphography and MR angiography, enabling the visualization of tumor blood vessels and coronary arteries in animal models and in patients (h, i). **(j–l)** Carboxydextran-coated iron oxide nanoparticles (Resovist) have been employed for MR angiography and stem cell tracking in preclinical models (k), as well as for visualizing metastatic liver lesions in patients (l). **(m–p)** HPMA-based polymeric nanomedicines can be functionalized both with drugs and with imaging agents, enabling the *in vivo* visualization of their circulating properties and tumor accumulation in tumor-bearing mice and rats (n), as well as their ability to target solid tumors and metastases in patients (o). **(p–r)** Liposomes such as Doxil, can also be easily co-loaded with drugs and imaging agents. In HNSCC-bearing nude rats, Doxil co-functionalized with the beta-emitter and gamma-emitter rhenium-186 not only enabled the monitoring of tumor accumulation using 3D SPECT-CT and 2D gamma-scintigraphy (q), but also the combination of radionuclide therapy with chemotherapy. Technetium-99m-labeled Doxil can be used to visualize and quantify tumor accumulation in patients, suffering, for example, from different types of sarcomas (r), thereby enabling patient preselection and (more) personalized nano-chemotherapeutic treatments.
Images are adapted, with permission, from [30••,32,33••,34,38–42,50–52].

Shouldn't we consider integrating nanotherapeutics in combined modality regimens (Figure 2d–f; [32,33••, 34–37])?; (6) how important is solid tumor treatment from a clinical point of view? Shouldn't we be focusing on metastatic disease?; (7) how good (or bad) are the animal models we are routinely using?; and (8) why not use imaging to predict which patients are likely to respond to targeted therapeutic interventions, and thereby personalize nanomedicine treatments (see below)? Arguably, working on these general (physio-) logical aspects of nanomedicine-mediated drug targeting is at least equally important as working on the formulations themselves.

Diagnostic nanomedicine

The impact of non-invasive imaging on assessing and improving the potential of nanomedicine-based therapeutic interventions has greatly expanded in the last couple of years. In addition, a number of nanomedicine formulations have been designed solely for diagnostic purposes. It should be realized in this regard, however, that as compared to standard low-molecular-weight diagnostics, there are only a limited number of imaging applications which really profit from the use of (i.v. administered) nanomedicine formulations, especially in the clinical situation.

Preclinically, labeling nanomedicines with contrast agents undoubtedly is highly useful, for example, for better understanding the properties and the potential of the formulations developed. In this context, non-invasive imaging can be used to provide feedback on the circulation properties, the target site accumulation and the off-target localization of therapeutic/theranostic nanomedicines, to report on (triggered) drug release at the target site, and to longitudinally monitor therapeutic efficacy [3••,4,5•,6–8,9•,10,11]. In addition, in line with the ESF-definition of nanomedicine [1], nanodiagnostics are highly useful for visualizing and better understanding several important (patho-) physiological principles of diseases and disease treatments, enabling, for example, magnetic resonance (MR) angiography and MR lymphography, and the labeling and tracking of (stem) cells.

Clinically, however, nanodiagnostics are only useful in a limited number of situations. This is because there are very stringent pharmacokinetic and elimination criteria for i.v. administered diagnostic agents, ruling out the vast majority of applications for which nanodiagnostics are routinely employed in animal models. Among the applications for which nanodiagnostics such as Gadomer-17 and Resovist, have been used in patients are the MR monitoring of (the perfusion of) tumor blood vessels and coronary arteries [38–40], the imaging of labeled stem cells [41] and the visualization of primary and/or metastatic liver lesions [42] (Figure 2g–l). A small number of additional clinical studies have focused on the use of Resovist-like iron oxide nanoparticles such as Endorem and Sinerem, for monitoring neural stem cells [43], lymph node metastases [44], intranodally injected cancer vaccines [45], and macrophage activity in atherosclerosis [46]. Several additional studies in these and other pathological settings are currently ongoing, and the results of these trials are eagerly awaited.

Given the fact that many different studies have been performed in recent years aiming to use nanodiagnostics for visualizing tumors and receptor structures overexpressed by tumor blood vessels, it is also important to mention here that in principle, the physicochemical and/or pharmacokinetic properties of nanomedicines are suboptimal for such purposes. For diagnostic agents targeting extravascular structures such as receptors overexpressed by cancer cells, it is mandatory that they rapidly extravasate out of blood vessels, and efficiently penetrate and distribute within the extravascular/interstitial space. Subsequently, the unbound/non-internalized fraction should rapidly re-enter the blood stream, and should be rapidly eliminated from the body. Given their size of 5–150 nm and their prolonged circulation times, passively and actively targeted nanodiagnostics do accumulate to a reasonable extent in tumors, but as compared to low-molecular-weight diagnostics such as ^{18}F-FDG and ^{68}Ga-DOTA-TOC, their penetration and their intratumoral distribution are much lower, and their re-entering into the blood stream and their elimination are much slower. Consequently, the signal-to-background ratios obtained upon using 5–150 nm-sized imaging agents are much lower than those obtained upon using standard low-molecular-weight imaging agents, making them much less useful for tumor cell-specific diagnostic purposes.

Conversely, when intending to monitor tumor angiogenesis and image intravascular targets, for example, via the RGD-mediated active targeting of integrin-receptors overexpressed by tumor endothelial cells, the background component becomes too dominant, because of non-specific EPR-mediated accumulation. As a result of this, without complex pharmacokinetic modeling, it is impossible to differentiate between the active binding of nanodiagnostics to receptors overexpressed by tumor blood vessels and the passive accumulation of extravasated nanodiagnostics within the tumor interstitium. Arguably, for such tumor vasculature-targeted diagnostic approaches, very large imaging agents, which are unable to extravasate (i.e. low background in tumor interstitium) and which possess very short circulation times (i.e. low background in circulation), are optimal. VEGF-R2-targeted microbubbles (MB) are a clinically relevant example of this, and are currently in early-stage trials for prostate cancer staging and treatment monitoring using ultrasound imaging [47,48]. Because of their size of 1–5 μm and their half-life time of ~1 min, MB are able to render very high signal-to-background ratios when specifically targeting receptor structures overexpressed by tumor blood vessels. These insights exemplify the importance of carefully and critically overthinking the use of i.v. administered nanodiagnostics for imaging tumors, tumor cells and tumor blood vessels.

Theranostic nanomedicine

Recent advances in nanomedicine research have resulted in a number of formulations containing both drugs and imaging agents within a single formulation. As already alluded to above, these so-called nanotheranostics can be used for various different purposes, including, for example, for monitoring the biodistribution and the target site accumulation of nanomedicines, for visualizing and quantifying (triggered) drug release, and for longitudinally assessing therapeutic efficacy [3••,4,5•,6–8,9•,10,11]. In addition, as will be detailed below, via their ability to be used for preselecting patients, they also seem to hold significant potential for personalizing nanomedicine-based (chemo-) therapeutic interventions.

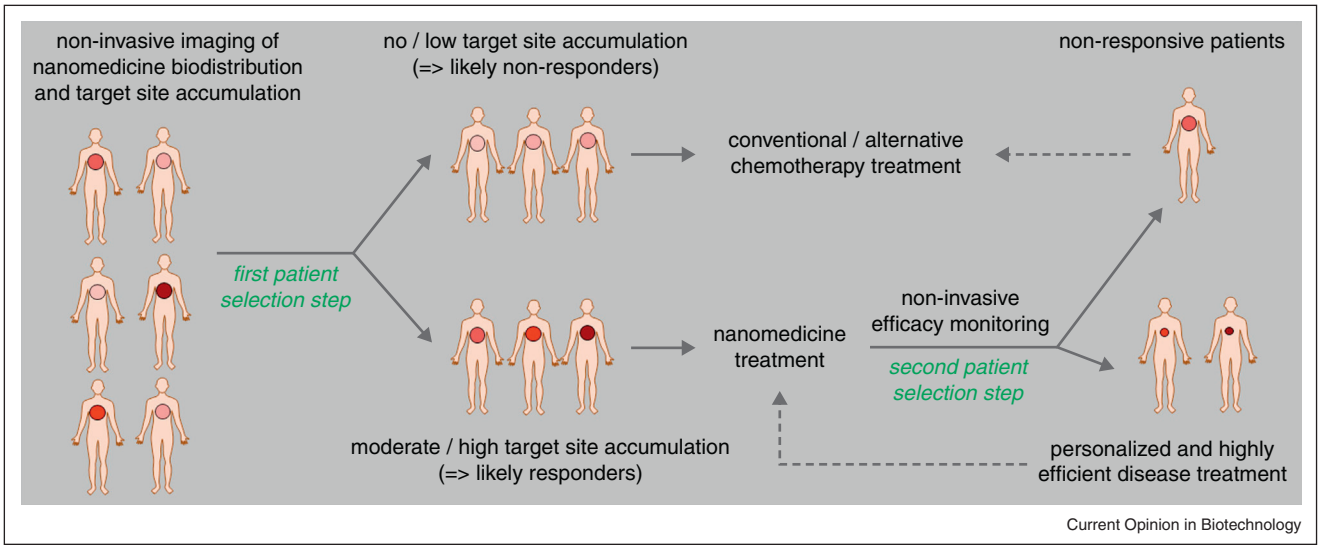
In general, the term 'theranostics' refers to the combination of disease diagnosis and therapy. Theranostic strategies range from set-ups in which patients are preselected on the basis of initial target site accumulation studies using a given diagnostic radionuclide-labeled antibody, followed by subsequent radio-immunotherapy

with the same antibody coupled to a therapeutic radionuclide [49], to set-ups in which imaging agents and (chemo-) therapeutic drugs are co-incorporated within a single nanomedicine formulation. It is important to take into account in this regard that as opposed to many popular claims in the literature stating, for example, that nanotheranostics can be used for the simultaneous diagnosis and treatment of diseases, their actual suitability for real disease diagnosis is questionable, especially when the term disease diagnosis is taken in its strictest sense. On the basis of what, for instance, should the choice for a given (chemo-) therapeutic agent be based, when no proper disease diagnosis or staging has been performed yet? In the case of nanotheranostics, combining diagnosis and therapy should therefore be interpreted in its broadest sense, with diagnosis not referring to the identification, the localization and/or the staging of a given pathology, but to the preselection of patients, to the prediction of potential therapeutic responses, and/or to the longitudinal monitoring of treatment efficacy. In addition, information with regard to the balance between the target site accumulation and the off-target localization of nanomedicines in potentially endangered healthy tissues can be useful from a 'diagnostic' point of view, especially in the initial phases of clinical evaluation, as this might enable the identification of patients likely to develop side effects.

An important advantage of nanotheranostics over nanotherapeutics is that they can be used to longitudinally monitor the biodistribution and the target site accumulation of the formulations in question. Both in animal models and in patients, for instance, radiolabeled polymers and liposomes have on numerous occasions been shown to enable non-invasive (by drawing regions-of-interest over the heart) pharmacokinetic analyses. In addition, by non-invasively visualizing and quantifying the target site accumulation of nanomedicines, their ability to selectively deliver drugs to pathological sites (e.g. to tumors; 'site-specific drug delivery') can be analyzed, as well as their ability to attenuate accumulation in potentially endangered healthy tissues (e.g. in the heart or the brain; 'site-avoidance drug delivery').

Examples of this are provided in Figure 2m–r, depicting the effective and selective accumulation of polymeric and liposomal nanotheranostics in tumors. Figure 2n shows a ~5 nm-sized *N*-(2-hydroxypropyl)methacrylamide (HPMA)-based polymeric drug carrier circulating within tumor blood vessels in a syngeneic rat prostate tumor at 0.5 h post i.v. injection, as well as the strong EPR-mediated tumor accumulation of the same carrier material in the same tumor at 168 h post i.v. injection [34]. Figure 2o extends these findings to the clinical situation, showing that also in patients with head-and-neck tumors and with large subclavicular breast cancer metastases, the EPR-mediated accumulation of radiolabeled HPMA copolymer-based doxorubicin (PK1) can be clearly visualized and quantified [50]. Similarly, Figure 2q, r show that also for radiolabeled Doxil (i.e. PEGylated liposomal

Figure 3



Rationale for image-guided and personalized nanomedicine. By combining non-invasive imaging information on the target site accumulation (1st selection step) and therapeutic efficacy (2nd patient selection step) of theranostics nanomedicines, patients can be preselected. They can then either be assigned to nanomedicine treatment (in case of moderate to high tumor accumulation and proper antitumor efficacy), or to conventional/alternative chemotherapeutic interventions (in case of low tumor accumulation and/or improper efficacy). In addition, during the first patient selection step, patients presenting with high levels of nanomedicine accumulation in potentially endangered healthy organs can be excluded from nanomedicine treatment, to attenuate the incidence and/or intensity of side effects.Image reproduced, with permission, from [9•].

doxorubicin), EPR-mediated passive drug targeting to tumors can be visualized and quantified, both in nude rats bearing head-and-neck cancer xenografts (Figure 2q), and in patients suffering from different types of sarcomas (Figure 2r) [51,52].

Such imaging insights are considered to be highly useful for personalizing nanomedicine-based chemotherapeutic interventions [9•]. As depicted schematically in Figure 3, by using nanotheranostics, and by rationally combining non-invasive imaging and tumor-targeted drug delivery, patients can be preselected. In the first selection step, only individuals showing medium to high levels of tumor accumulation are selected for treatment with the nanomedicine formulation in question, while patients showing low or no target site localization are allocated to alternative treatments. In the second selection step, non-invasive imaging is then used to monitor therapeutic efficacy, and to identify those patients responding well to tumor-targeted nanochemotherapeutic interventions. Though somewhat more labor-intensive and cost-intensive, such theranostic approaches are highly useful for facilitating and fostering early phase clinical trials involving nanomedicines, not only from an industrial point of view, but also from a clinical and patient point of view, ensuring that only patients showing sufficient (EPR-mediated) tumor targeting are included in the trial and in the eventual efficacy analyses. Concomitant use of imaging information to exclude patients showing very high levels of off-target localization (e.g. because of co-morbidities and/or reduced renal or hepatic elimination), can also help reduce the incidence and intensity of 'unexpected' side-effects. Consequently, nanotheranostics, and the combination of drug targeting and imaging, hold significant potential for realizing the promise of personalized medicine.

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