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# Tumor-targeted nanomedicines for cancer theranostics

**Alexandra G. Arranja**[#1], **Vertika Pathak**[#2], **Twan Lammers**[2,3,4,*], and **Yang Shi**[2,*]

[1]Department of Chemical Engineering, Delft University of Technology, 2628BL Delft, The Netherlands [2]Department of Nanomedicines and Theranostics, Institute for Experimental Molecular Imaging (ExMI), RWTH Aachen University Clinic, 52074 Aachen, Germany [3]Department of Targeted Therapeutics, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, 7500 AE Enschede, The Netherlands [4]Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, 3584 CG Utrecht, The Netherlands

[#] These authors contributed equally to this work.

## Abstract

Chemotherapeutic drugs have multiple drawbacks, including severe side effects and suboptimal therapeutic efficacy. Nanomedicines assist in improving the biodistribution and the target accumulation of chemotherapeutic drugs, and are therefore able to enhance the balance between efficacy and toxicity. Multiple different types of nanomedicines have been evaluated over the years, including liposomes, polymer-drug conjugates and polymeric micelles, which rely on strategies such as passive targeting, active targeting and triggered release for improved tumor-directed drug delivery. Based on the notion that tumors and metastases are highly heterogeneous, it is important to integrate imaging properties in nanomedicine formulations in order to enable non-invasive and quantitative assessment of targeting efficiency. By allowing for patient pre-selection, such next generation nanotheranostics are useful for facilitating clinical translation and personalizing nanomedicine treatments.

### Keywords

nanomedicines; tumor-targeting; in vivo imaging; theranostics; clinical translation

## Introduction

Cancer is not one, but many heterogeneous diseases characterized by rapid and uncontrolled cellular expansion as a result of genetic and epigenetic alterations, and it annually affects millions of people worldwide (1). Current therapies for cancer include surgery, radiotherapy, chemotherapy and immunotherapy. Early diagnosis of tumors facilitates the treatment of patients with surgery and/or with radiotherapy, however, in patients with tumors that cannot be resected or irradiated, or that have already metastasized, the only available treatment options are chemotherapy and immunotherapy (2). The clinical usefulness of chemotherapy

[*]Corresponding authors: tlammers@ukaachen.de (TL), yshi@ukaachen.de (YS).


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is limited by the low ability of drug molecules to reach tumors (3), by the fact that tumors tend to become resistant during the course of therapy (4–6) and by the development of immediate and long-term severe side effects (7–9), together compromising the efficiency of chemotherapy treatment.

Drug targeting strategies that enable tumor-targeted drug delivery and alter the balance between efficacy and toxicity of chemotherapeutic drugs are highly needed to overcome such limitations of chemotherapy (2, 10). In recent years, nanotechnology-based drug delivery systems have been extensively investigated to realize tumor-targeted chemotherapy. These so-called ‘nanomedicines’ aim at targeted delivery of chemotherapeutic drugs to the tumor site utilizing strategies such as passive targeting, active targeting and triggered drug release, while at the same time decreasing accumulation in off-target tissues, together leading to an improved therapeutic index (11, 12). Clinically relevant nanomedicines are liposomes, polymer-drug conjugates and polymeric micelles (Figure 1A) (13–16). Unlike conventional small molecule drugs, which are rapidly cleared from the blood circulation, nanomedicines have prolonged circulation half-lives, increased bioavailability and enhanced tumor disposition (Table 1, Figure 1B). However, to achieve tumor-targeted drug delivery, nanoparticulate drug delivery systems have to overcome several biological barriers as presented in Figure 1C (17).

The first nanomedicine formulation that was approved by the US Food and Drug Administration (FDA) is Doxil (PEGylated liposomal doxorubicin) (18). The most pronounced improvement of Doxil compared to free doxorubicin is the substantially reduced cardiotoxicity, which compromises the clinical use of free doxorubicin (19). The clinical success of Doxil has led to the development of many other nanomedicine formulations (20). Besides lipid-based formulations, these also include polymer-drug conjugates and polymeric micelles (21, 22) (Figure 1). The latter are especially attractive for the delivery of chemotherapeutic drugs with low water-solubility, and approximately a dozen of nanomedicines based on polymeric micelles are currently in clinical trials for different types of cancers.

To better understand the in vivo fate of nanomedicines, and to obtain information on pharmacokinetics, target site accumulation and therapeutic efficacy, it is of great value to integrate therapy with non-invasive imaging (23, 24). Such information can be used to assess the suitability of nanomedicine-based therapeutic interventions, via the pre-selection of patients most likely to respond to nanotherapy. This review summarizes the basic principles of nanoparticle-based tumor targeting, and it discusses the benefit of integrating imaging to pre-select patients and personalize nanomedicine treatments.

## 2 Mechanisms of nanomedicine-based tumor targeting

### 2.1 General considerations

When designing nanocarriers for tumor targeting, it is essential to consider their physicochemical characteristics including size and surface properties to attain their accumulation at the pathological site. For example, intense interactions between nanoparticles and serum proteins may cause rapid clearance from the circulation. The

surface properties of nanocarriers, such as the charge and hydrophobicity, affect protein opsonisation resulting in activation of the complement system and rapid uptake by phagocytes. Compared to hydrophobic and positively charged particles, neutral and hydrophilic particles are generally less prone to opsonisation (25, 26). Apart from surface properties, particle size also critically affects the in vivo fate of nanomedicines. Hydrophilic nanoparticles smaller than ~5 nm are efficiently eliminated via renal filtration whereas larger nanoparticles (> ~200 nm) tend to rapidly accumulate in healthy organs such as liver, spleen and lungs (17, 27). Interestingly, nanocarriers with sizes between ~5 and ~250 nm can extravasate from leaky tumor vessels allowing for efficient accumulation over time, in part also because tumors tend to lack functional lymphatic drainage (28, 29).

Even if sufficient tumor accumulation is reached, the therapeutic efficacy of nanomedicines greatly depends on the penetration depth of the formulations into the tumor interstitium (30, 31). Both tumor microenvironment and the physicochemical characteristics of the nanocarriers are key factors affecting penetration. The tumor microenvironment is generally characterized by extensive stromal components such as collagen, hyaluronan, proteoglycans networks, as well as by a high interstitial fluid pressure, which altogether present a formidable biological barrier for efficient tumor penetration (32). Among the different strategies used to enable tumor penetration, the most straightforward method is minimizing the size of the nanocarriers (33). In the following sections, we discuss targeting strategies used to improve the accumulation and retention of nanomedicines at the tumor site, and to increase the therapeutic efficacy of the compounds through modulation of their drug release properties.

## 2.2 Passive targeting

Solid tumors are characterized by leaky blood vessels and lack of functional lymphatics. Consequently, nanoparticles with sizes up to ~250 nm are prone to extravasation across the abnormal endothelial lining and are efficiently retained in tumors over time. This phenomenon is termed the ‘Enhanced Permeability and Retention (EPR) effect’ and forms the basis for passive tumor targeting with nanomedicines (Figure 2) (28, 29).

A classic example of this phenomenon is the abovementioned formulation Doxil with a particle size of ~100 nm, which is highly suitable for EPR-mediated passive targeting. Clinical studies have shown that Doxil presents a prolonged circulation time, leading to a 60-fold higher area under the plasma concentration-time curve (AUC) of doxorubicin than the free drug. Simultaneously, the volume of distribution of Doxil is significantly decreased and the clearance is drastically reduced by 250-fold comparing to free doxorubicin. The capacity of Doxil to accumulate and extravasate in tumors via the EPR effect has been confirmed in malignant effusions, AIDS-related Kaposi’s sarcoma (ARKS) skin lesions and a variety of solid tumors presenting on average 15-fold higher tumor accumulation than the free drug (19). Furthermore, liposomal encapsulation of doxorubicin significantly reduced the cardiotoxicity while keeping the therapeutic effect of the drug in a phase III clinical trial (34). In another randomized phase III clinical trial in patients suffering from recurrent epithelial ovarian carcinoma, Doxil resulted in superior overall survival rates comparing to topotecan (median survival 108 weeks for Doxil vs. 71 weeks for topotecan; $P = 0.008$) (35).

Prolonged circulation kinetics is important to exploit the EPR effect. There are several prerequisites which have to be met in order to ensure a long blood circulation time of nanoparticles. First of all, as already alluded to before, an appropriate particle size is required: formulations with a size ≤5 nm are quickly eliminated via the kidneys and those with a size ≥200 nm tend to be rapidly taken up by macrophages (27). Secondly, efficient surface coating of nanoparticles with neutral hydrophilic polymers, such as poly (ethylene glycol) (PEG) (36), is necessary to decrease protein opsonization and recognition by the mononuclear phagocyte system (MPS), thereby increasing the circulation half-life times of nanoparticles (37). To date, there are a number of PEGylated nanomedicines used in the clinic, including Doxil, Daunoxome (PEGylated liposomal daunorubicin), and PEGylated poly (amino acid) based polymeric micelles (e.g., NK911, NK105, NK102, NC-6004, NC-4016 and NC-6300) (22, 34, 38).

Stability is another decisive factor for the longevity of nanoparticles in the blood circulation. This is particularly an issue for nanoparticles self-assembled from amphiphilic polymers, e.g. polymeric micelles, and generally less critical for liposomes. Due to the massive dilution effect of nanoparticles upon intravenous (i.v.) injection as well as to interactions of polymeric building blocks with serum proteins and other blood components, rapid disintegration of polymeric micelles and other self-assembled polymeric nanoformulations are frequently observed (39). For example, polymeric micelles based on poly (ethylene glycol)-*b*-p(*N*-(2-Hydroxypropyl) methacrylamide) (PEG-*b*-p(HPMAm)) copolymers and poly(ethylene glycol)-polycaprolactone (PEG-PCL) were rapidly dissociated upon i.v. injection (40, 41). To enhance the stability of nanoparticles in the blood circulation, various physicochemical strategies have been applied, with various degrees of success (41, 42).

In this context, it has to be pointed out that even for nanocarriers with sufficient intrinsic particle stability; the retention of drug payloads is of great importance for efficient tumor targeting. Various reports have shown that drugs loaded in stable nanoparticles leaked out before the carriers reached the target site (43–45). Therefore, premature drug release from nanocarriers severely hampers the tumor targeting efficiency of nanomedicines. Physical interactions and chemical conjugation of drugs to carriers are effective to enhance drug retention in nanoparticles, which has been exemplified by several nanomedicine formulations based on polymeric micelles in clinical trials (22).

### 2.3 Active targeting

Actively targeted nanomedicines are surface-functionalized with recognition motifs which specifically bind to receptors (over-) expressed at the target site, e.g. by tumor or endothelial cells (Figure 2). Frequently used targeting ligands include small molecules (e.g. folic acid (46)), peptides (e.g. RGD (47)), proteins (e.g. transferrin (48–50)), nanobodies (51) and aptamers (52). Besides nanocarriers, also radioisotopes and drugs have been modified with targeting ligands, to enable molecular imaging and radio-immunotherapy. Although a large number of studies have exclusively shown significantly higher cell uptake of actively targeted nanoparticles in vitro, different results on tumor targeting efficiency using nanoparticles with or without active targeting ligands have been reported. For example, actively targeted magnetic nanoparticles (MNP) modified with anti-HER2 monoclonal

antibody showed 10-30 fold higher concentrations in tumor tissues than the non-targeted counterpart (53). Similar results have been observed in studies using layered double hydroxides (LDHs) modified with folic acid (46) and paclitaxel loaded nanocarriers modified with RGD peptide (47). Nevertheless, several in vivo head-to-head comparisons of passively vs. actively targeted formulations revealed no significant difference in tumor accumulation (54, 55). As exemplified by a study by Kirpotin and colleagues, HER2 antibody-modified PEGylated liposomes were much more efficiently internalized in vitro than antibody-free liposomes, while the extent and the pattern of in vivo tumor accumulation of both actively and passively targeted liposomes were identical (Figure 3C) (54). This phenomenon has also been reported by another study employing RGD- and NGR modified polymeric nanomedicines (55).

The concept of active targeting has been tested in clinical trials. An interesting example of an actively targeted nanomedicine formulation is BIND-014, which has recently completed a phase II trial in patients suffering from NSCLC and metastatic castration resistant prostate cancer. BIND-014 refers to docetaxel-loaded poly (lactic-co-glycolic acid)-poly (ethylene glycol) (PLGA-PEG) nanoparticles decorated with the anti-PSMA targeting moiety ACUPA, which recognizes receptors overexpressed on the surface of prostate cancer cells and angiogenic blood vessels. As shown in Figure 3B, in an initial phase I trial, it was found that BIND-014 induced therapeutic responses, in spite of lower dosing as compared to the free drug (52).

Taken together the advantage of using active targeting in nanomedicines is still questionable and more factors should be carefully considered when analyzing the potential of EPR-based versus receptor-mediated targeting. Nevertheless, actively targeted nanomedicines are particularly useful for the delivery of biotherapeutics, such as proteins and nucleic acids, because they have to enter cells to exert their effects, but cannot cross the cellular membrane by themselves. Currently, there are several early-stage clinical trials ongoing with actively targeted nanoformulations for the delivery of biotherapeutics, such as CALAA 01, SGT-53, SGT-94 and DCR-MYC (56–59). Conversely, intracellular delivery into cancer cells is much less critical for nanoparticles loaded with standard small molecule drugs, as the vast majority of classical low-molecular-weight chemotherapeutics are capable of crossing cell membranes via passive diffusion, e.g. upon release from the nanocarrier in the tumor interstitium or in tumor-associated macrophages.

It has to be kept in mind in this regard that depending on circumstances, active targeting may also be beneficial for nanocarriers containing standard chemotherapeutic drugs, e.g. if the targeting ligand possesses intrinsic pharmacological activity. A nice example in this context is based on the single domain antibody (also known as nanobody) EGa1, which selectively binds to EGFR, which is frequently overexpressed on and overly active in cancer cells. The EGa1 nanobody blocks EGFR signaling and is currently under clinical investigations for the treatment of EGFR-expressing tumors (60). When using EGa1 as a targeting ligand attached to the surface of core-crosslinked polymeric micelles, tumor growth inhibition was achieved even in the absence of entrapped drug molecules. When doxorubicin, which was employed as a model drug, was incorporated in the EGa1-targeted polymeric micelles, antitumor

responses and animal survival were substantially enhanced, as a result of the combined therapeutic effects of both the drug and the pharmacologically active targeting ligand (51).

It is important to note here, that in case of active targeting to extravascular receptors, nanoformulations first have to accumulate in tumors via passive targeting. Therefore, actively targeted nanoparticles have to meet the requirements for passive targeting discussed above (e.g., proper particle size, stealth property, sufficient stability, prolonged circulation and efficient drug retention). It is long debated which type of targeting (active/passive) is better, the above examples demonstrates that in some specific cases, active targeting to cancer cells is absolutely necessary to make formulations effective, however in most other cases it will not have a greater impact.

### 2.4 Triggered Drug Release

Passive and active targeting are prominently based on the EPR effect to achieve efficient tumor accumulation. EPR, however, is a highly variable biological phenomenon, with large inter- and intra-individual differences in different types of tumors and metastases, and therefore can fail in some cases. A recently developed approach to overcome this problem is intravascularly triggered drug release, which relies on the spatial-temporal control of drug release from nanoparticles at the tumor site by internal or external stimuli (Figure 2). Thereby, even in non-leaky tumors, targeted delivery can be achieved by triggering drug liberation within tumor blood vessels, and by then allowing the small molecule drugs to extravasate out of the blood vessels and into the tumor interstitium (63–65). For example, ThermoDox, a formulation based on thermosensitive liposomes loaded with doxorubicin, contains single chain lysolipids with a phase transition temperature (Tc) of 41.5°C. Upon local heating, the lipid bilayer deforms and doxorubicin is released. ThermoDox has been tested in a phase III clinical trial for the treatment of hepato-cellular carcinoma in connection with radiofrequency ablation-based heating. To monitor the accumulation of temperature-responsive (and standard) nanomedicines at the target site, and to verify and quantify drug release, imaging is increasingly integrated in such setups.

## 3 Nanoparticles as imaging and theranostic agents

### 3.1 General considerations

The barriers presented in Figure 1C have to be overcome before nanomedicines can efficiently accumulate at pathological sites, and this process can be visualized and quantified using appropriate imaging setups and strategies in order to better understand tumor targeting using nanomedicines. Apart from therapeutic applications, nanoparticles have also been employed for diagnostic purposes. However, the use of nanoparticles for diagnosis presents several limitations (66–68). To explain, comparing to standard imaging probes that generally have low molecular weight, short half-lives and large volumes of distribution, nano-sized diagnostic agents, on the other hand, have prolonged circulation kinetics and small volumes of distribution, limiting their ability to produce high signal-to-background images. Therefore, using nanoparticles for diagnostic purposes only makes sense in a relatively small number of situations (66).

The complex journey of nanocarrier materials from the site of injection to tumors reveals the need for developing methodologies that enable in vivo tracking of the administered drug delivery systems. Non-invasive imaging modalities are able to provide in vivo monitoring of nanoparticles regarding their pharmacokinetics, biodistribution, tumor disposition, drug release and therapeutic efficacy (23, 24) (Figure 4). The labelling of nanomedicine formulations with imaging agents results in theranostic platforms that have several advantages and applications. Most importantly, alluding to the abovementioned notion that the EPR effect is highly variable, nanomedicine-based chemotherapeutics are only effective in certain subgroups of patients, and therefore theranostic nanomedicines that can be used to non-invasively and quantitatively assess tumor targeting efficiency are highly needed. The obtained imaging information can then be employed to pre-select suitable patients with acceptable/high tumor accumulation of nanomedicine for further treatment, thereby contributing to (more) personalized medicines (23, 68, 69).

### 3.2 Imaging technologies

Multiple non-invasive imaging modalities are available, e.g., computed tomography (CT), magnetic resonance imaging (MRI), optical imaging (OI), ultrasound (US), gamma camera scintigraphy, positron emission tomography (PET) and single photon emission computed tomography (SPECT). Each modality has its own advantages and disadvantages relating to differences in terms of spatial and temporal resolution, sensitivity in probe detection and signal penetration depth (67, 70), and the choice of the appropriate imaging system depends on the type of information required (24). For instance, OI is frequently used for in vivo imaging of small animals because of its safety, and time- and cost-effectiveness. However, it is impractical to image deep tissues or large living objectives. This can be overcome by using radionuclide-based imaging technologies such as PET and SPECT, which provide deep tissue penetration and high sensitivity. However, PET and SPECT have relatively poor spatial resolution and lack anatomical information. To obtain anatomical information, they are therefore often combined with MRI and CT, which enable sensitive and high-resolution soft- and hard-tissue visualization (24). Similarly, also OI profits from such hybrid imaging protocols. In most preclinical situations, OI is unable to accurately assign the probe signal to animal organs and consequently only gives moderately informative feedback on nanomedicine biodistribution. When combined with CT, organs of interest can be segmented and the anatomic information obtained from CT can be fused with the fluorescent signal obtained from OI, thereby allowing for more accurate biodistribution assessment (71).

### 3.3 Theranostic nanomedicine

An example showing the importance of in vivo imaging of nanomedicines is presented in Figure 3D-E. Gamma camera images obtained 72 hours after i.v. injection of radiolabeled PEGylated liposomes revealed significant differences in accumulation in different types of tumors. As can be seen, liposomes strongly accumulated in sarcomas, (Figure 3D), likely due to higher EPR, whereas breast carcinomas were associated with poor liposome accumulation (Figure 3E) (61, 62). This observation exemplifies that the EPR effect may highly vary depending on the tumor type (and likely also between tumors of the same type, and between different tumors and metastases within one patient). It thereby implies that visualizing and quantifying the tumor accumulation of nanocarriers, and integrating

imaging-based patient preselection in clinical trials, is necessary to stratify potential responders from non-responders.

Apart from the use of in vivo imaging for monitoring target site accumulation, imaging – and in particular MRI – can also be used to monitor payload release. As the MRI signal is highly dependent on the availability of freely diffusing water molecules surrounding the probes, distinct MRI signals are generated when paramagnetic MR contrast agents are present at different locations, i.e. inside or outside of liposomes, correlating to encapsulated vs. released payloads (72–74). This notion has been exploited to monitor triggered drug release from temperature-sensitive liposomes (TSL) (75–79). As an example, De Smet and colleagues intravenously administered temperature-sensitive liposomes co-loaded with the $T_1$ MR contrast agent Gd–HPDO3A and with doxorubicin to rats bearing 9L gliosarcoma tumors. Two groups of animals were employed, controls and animals in which tumors were heated with high intensity focused ultrasound (HIFU), and the release of the MR probe in tumors triggered by HIFU-mediated heating was monitored by MRI (76). The group that received hyperthermia showed significant changes in the $T_1$ in tumor tissue as a result of the heat-induced payload release. The group that received TSL without hyperthermia showed minimal payload release at the target site. These studies demonstrate the potential of using imaging to non-invasively monitor drug release from nanomedicines.

Imaging can also be used to assist in evaluating whether active targeting really benefits to achieve better tumor targeting efficiency. In a recent study from Schleich et al., superparamagnetic iron oxides (SPIOs) were co-loaded with paclitaxel in poly(lactic-co-glycolic acid) (PLGA) nanoparticles functionalized with RGD to enable quantification and visualization of the accumulation of nanoparticles into the tumors using Electron Spin Resonance spectroscopy and MRI. They observed an 8-fold increase of accumulation in the tumor of double targeted nanoparticles (active via the RGD peptide and magnetic targeting via the SPIOs) when compared to the passively targeted nanoparticles (80). Xiao et al. prepared micelles functionalized with NOTA chelator and labeled with $^{64}$Cu enabling in vivo PET imaging. A higher tumor accumulation of cRGD peptide modified micelles loaded with doxorubicin was observed by PET imaging. The actively targeted micelles showed a high tumor accumulation at 4 h post-injection (~5.7 %ID/g) when comparing to the non-targeted micelles (~2.5 %ID/g). Interestingly, when a blocker of cRGD peptide receptor was injected along with the actively targeted micelles, the values of tumor accumulation of both micelles were similar. These experiments suggest that imagable nanoparticles can be a promising candidate for a theranostic platform (81).

Similarly, our group conducted a head-to-head comparison between actively and passively targeted fluorophore-labeled polymer-drug conjugates of ˜10 nm using hybrid imaging technique, combining 3D CT and fluorescence molecular tomography (CT-FMT), in which the quantitative 3D fluorescence obtained using FMT (detection limit in the order of nano- to picomoles) is combined with the anatomical information provided by the high spatial resolution of μCT images (micrometers resolution). We showed that upon modification with the peptide RGD, which binds to angiogenic endothelium, actively targeted nanocarriers rapidly accumulate in tumors. Over time, however, passively targeted polymeric counterpart achieved high tumor concentrations (55).

## 4 Conclusions

Significant progress has been made in the development of nanomedicines, which has resulted in improved pharmacokinetics, in higher tumor accumulation, in enhanced antitumor efficacy and/or in decreased side effects. Tumor-targeting efficiency of nanomedicines depends on the physicochemical properties of nanocarriers (e.g. size and surface chemistry), as well as on the pathophysiological characteristics of the target tissue. There are still many challenges to achieve a general consensus on how tumor accumulation and therapeutic efficacy of nanomedicines are affected by their physicochemical properties. Currently, the majority of nanomedicines employed in the clinic rely on passive targeting, i.e. the EPR effect. More efforts should be undertaken to better understand the in vivo behavior of passively and actively targeted nanomedicines, as well as of nanoformulations that can be externally triggered to release their contents at the target site.

Although functionalization with targeting ligands enhances cellular uptake of nanomedicines in vitro, the in vivo tumor accumulation often does not increase. Despite great number of research studies conducted on actively targeted nanoparticles, majority of them did not make it to the clinics. Therefore, the added value of including targeting moieties is still a controversial topic and should be further investigated. Nevertheless, actively targeted nanomedicines are particularly useful for the delivery of biotherapeutics and for combination therapy (with pharmacologically active ligands).

In addition, imaging has to be integrated more intensively in nanomedicine treatments to particularly facilitate patient preselection in order to improve clinical translation. Since tumors and metastases are highly heterogeneous, therapeutic efficacy of nanomedicines has significant intra-individual difference. Developing imaging strategies to monitor tumor accumulation and therapeutic efficacy of nanomedicines will realize preselection of patients who have reasonably high tumor accumulation and efficacy of injected nanomedicines, which will assist in treating the suitable patients with nanomedicines, and exclude non-responders who can take other treatment modalities. By integrating imaging properties in nanomedicine formulations, theranostic nanomedicines have higher potential to achieve more robust clinical benefit.

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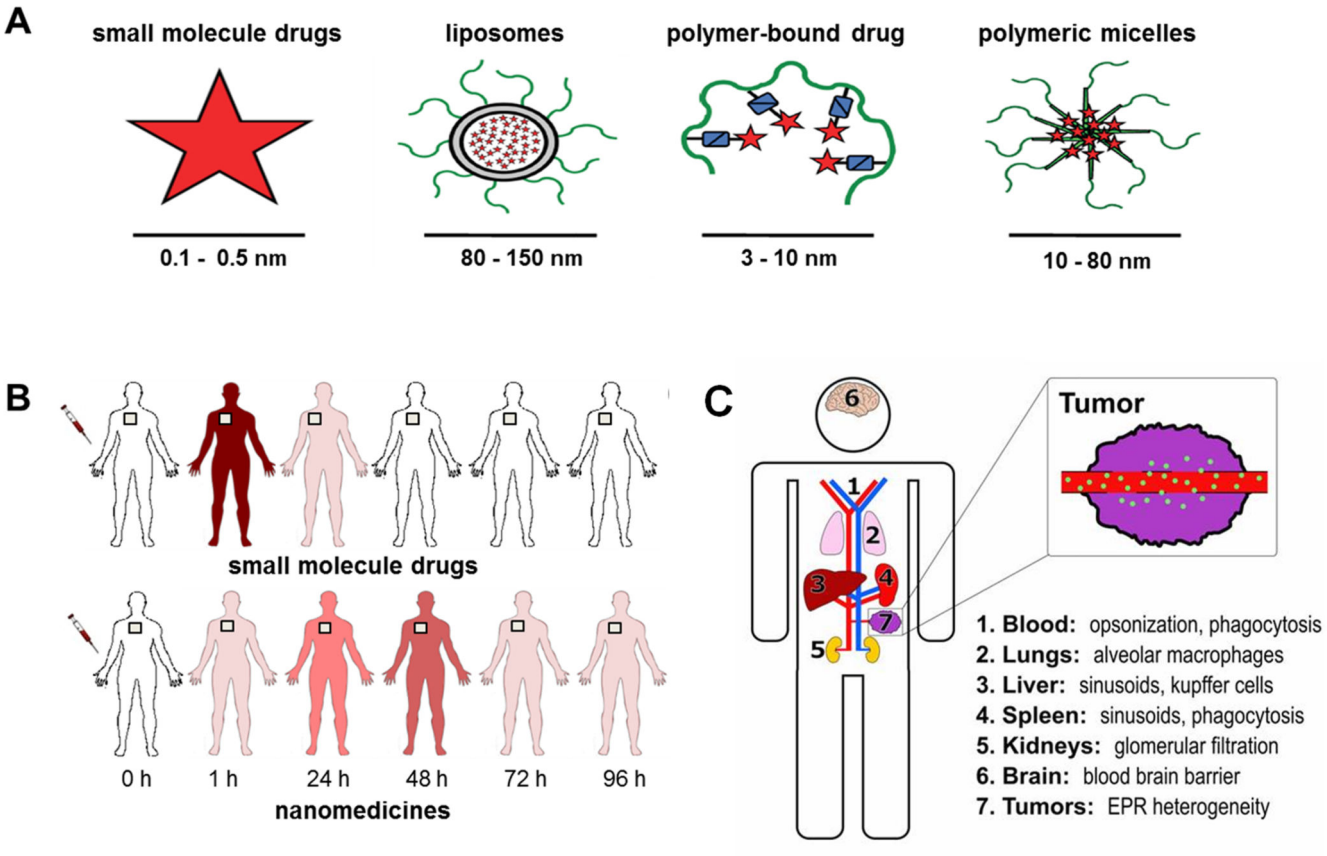


**Figure 1. Nanomedicines and tumor targeting.**

**A.** Representative examples of nanomedicines. Drugs are depicted as red stars, polymers in green, drug linkers in blue, and liposomal bilayers in grey (adapted with permission from (13)). **B.** Schematic representation of the biodistribution of conventional small molecule drugs vs. nanomedicine formulations upon intravenous administration. Compared to small molecule drugs, nanomedicines circulate for prolonged periods of time and achieve higher concentrations at the tumor site (tumors are depicted as squares). **C.** Various barriers that nanomedicines have to overcome to achieve efficient tumor-targeted drug delivery.




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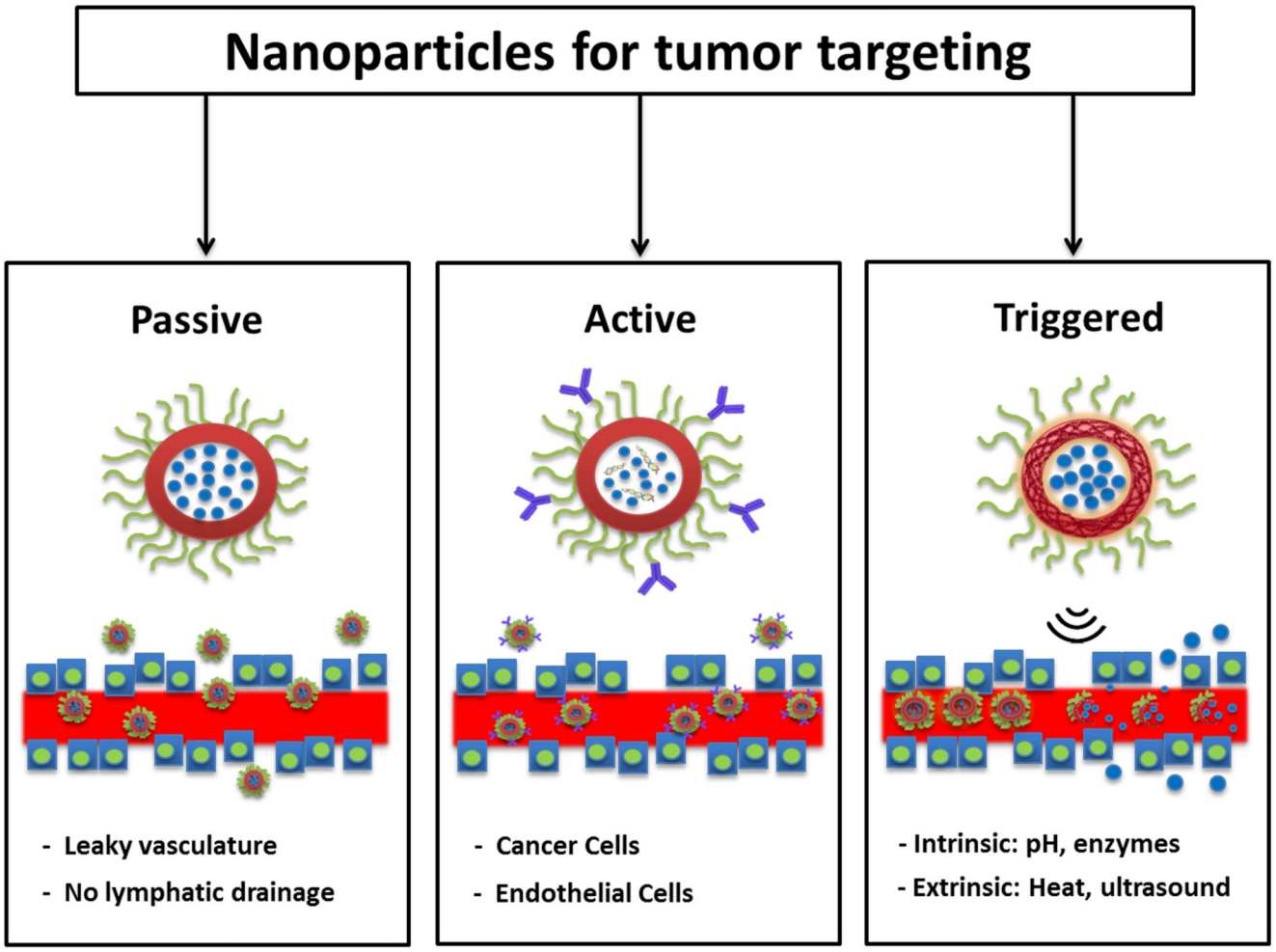


**Figure 2. Mechanisms for nanomedicine-mediated tumor targeting.**

Nanomedicines are depicted as a round red sphere, surface coating with polymers is depicted in green, and entrapped therapeutic agents in blue. In case of passive targeting, extravasation of nanoparticles occurs via leaky blood vessels and the EPR effect. In case of active targeting, recognition motifs such as antibodies are conjugated to the outer surface of the nanoformulations to induce binding to receptors (over-) expressed by cancer cells and endothelial cells. In case of triggered drug release, external or internal stimuli are employed to induce content release specifically at the target site.

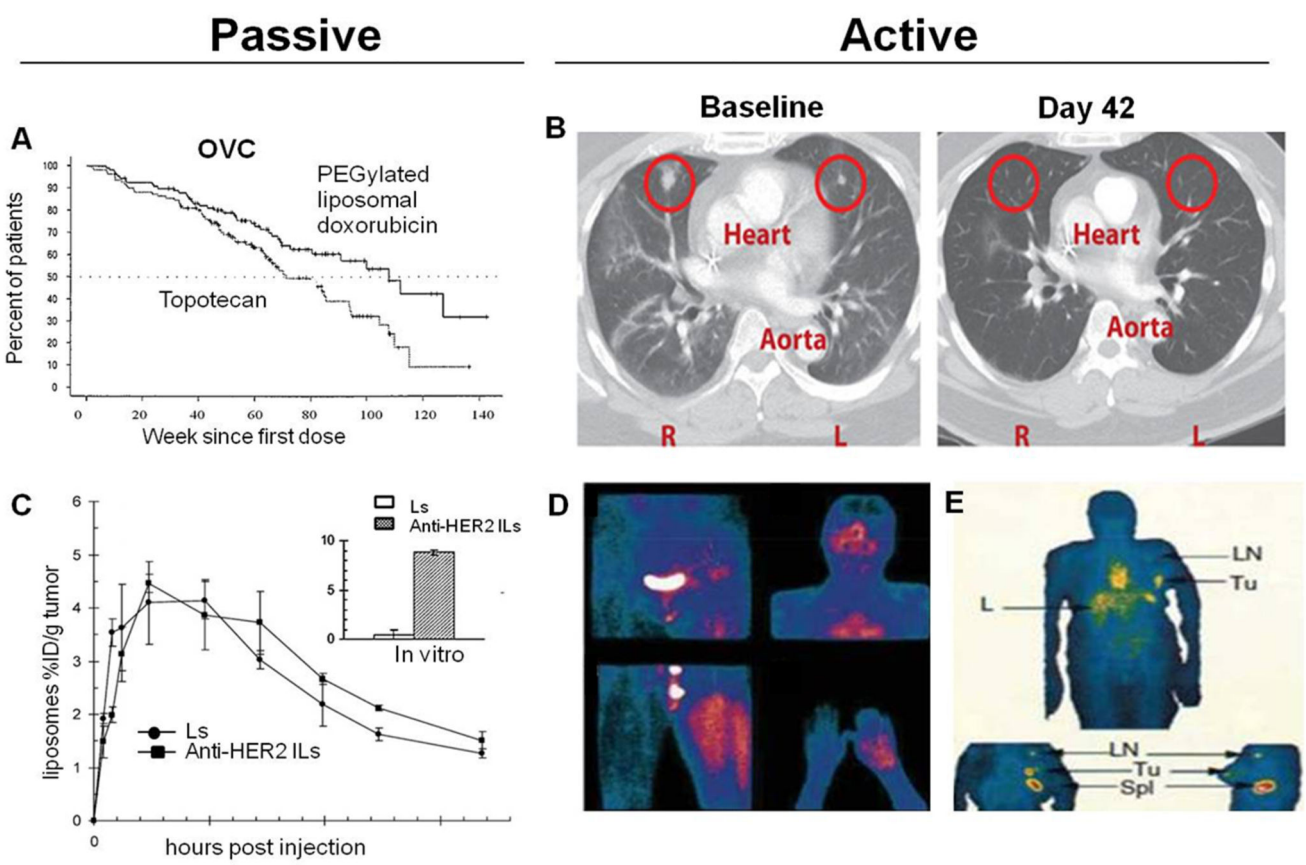


**Figure 3. Performance of passively and actively targeted nanomedicines.**

**A.** Survival benefit obtained using PEGylated liposomal doxorubicin (Doxil) as compared to topotecan in patients suffering from ovarian cancer (35). **B.** Phase I results obtained using BIND-014, a docetaxel-loaded polymeric nanoparticle actively targeted to the prostate-specific membrane antigen (PSMA; which is overexpressed on prostate cancer cells and also on activated endothelium), showing the regression of lung metastases via CT scans (52). **C.** HER2-antibody-targeted liposomes are taken up much more strongly by cancer cells in vitro than antibody-free liposomes (inset), but this does not result in improved tumor accumulation in vivo (54). **D-E.** Accumulation of radiolabeled liposomes in tumors in patients, showing a relatively high degree of EPR-mediated accumulation in sarcomas (**D**), and a more moderate accumulation in breast cancer (**E**). Tu: tumor, LN: lymph node, L: liver, Spl: spleen (61, 62).




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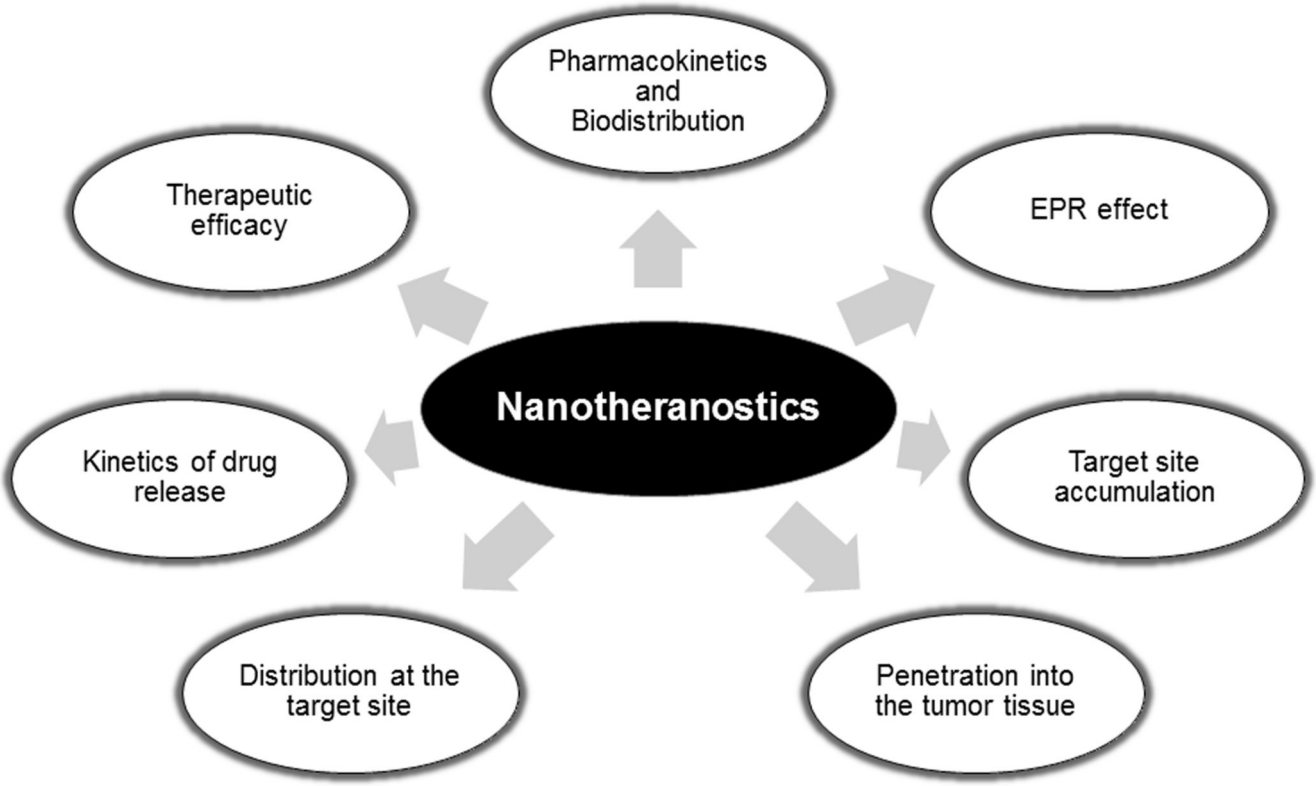


**Figure 4. Applications of nanotheranostics.**

By combining diagnostic and therapeutic properties within a single nanomedicine formulation, several important aspects of tumor-targeted drug delivery can be visualized and quantified. Adapted with permission from (68).

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**Table 1**

**Pharmacokinetics of small molecule drugs versus nanomedicines**

| | Small molecule drugs | Nanomedicines |
|---|---|---|
| Blood elimination | rapid | slow |
| Circulation half-life ($t_{1/2}$) | short | long |
| Volume of distribution ($V_d$) | high | low |
| Area under the curve (AUC) | low | high |
| Tumor accumulation | poor | good |