Chapter 6
Passive Targeting of Nanoparticles to Cancer

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Abstract Cancer is a leading cause of death globally. For the effectual treatment of cancer, it is crucial to advance our knowledge of the pathophysiology of cancer, discover novel anti-cancer agents, and expand new biomedical technology. A large number of possible barriers exist in the efficient delivery of small-sized drugs to solid tumors. After intravenous administration, many small-sized chemotherapeutic medicines have a larger volume of distribution, which is usually related to a narrow therapeutic index that is attributable to their elevated level of toxic effects in healthy tissues. A nanoparticle-based drug for targeting cancer is one of the auspicious advances to conquer the lack of tissue specificity associated with common chemotherapeutic drugs. Accordingly, the overall objectives are to lengthen a patient's lifespan, avoid recurrence of a cancer episode, and concurrently lessen the toxic effects of chemotherapeutic drugs. A range of approaches have been investigated for the nanoparticle-mediated targeting of drugs. Among them, a passive drug targeting approach has been the most commonly explored, and much preclinical learning has provided insight into its soundness. This approach is in accordance with the abnormality of tumor vasculatures, allowing nanoparticles the right of entry to tumors while avoiding distribution into healthy tissues. Thus, a passive drug targeting approach facilitates the advancement of a targeted nano-carrier structure loaded with chemotherapeutic agents for an improved effective profile with negligible toxic effects.

Keywords Cancer · Nanoparticles · Passive targeting · Tissue specificity

1 Introduction

Cancer is a leading cause of fatality worldwide, and the number of cancer-diagnosed patients is quickly rising [1]. According to estimates from the World Health Organization, approximately 84 million people died from cancer between 2005 and 2015 [2]; in 2012, approximately 14.1 million patients were diagnosed with cancer,

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of which 8.2 million cases were incurable. This figure is predicted to increase to 19.3 million new cases of cancer by 2025 [3]. At present, the management of cancer is a very important aim of research [4]. For the effectual treatment of cancer, it is crucial to advance our understanding of cancer pathophysiology. Traditional chemotherapeutic drugs are tremendously inadequate in their treatment profiles because of their very poor solubility, inauspicious pharmacokinetic profiles, and unfocused distribution within the body, which eventually results in serious toxic effects [5].

Currently, the treatment of cancer is a multidisciplinary effort that necessitates a close relationship between doctors, biological researchers, and biomedical engineers to form a delivery method that is strong enough to resist the fair number of challenges in a multifaceted microenvironment. There are many possible barriers to the effectual release of an active-form drug in solid tumors. After intravenous administration, most small-sized chemotherapeutic medicines have a larger volume of distribution, specifically related to a narrow therapeutic index attributable to their elevated level of toxic effects in healthy tissues [6, 7]. Accordingly, the overall objectives are to lengthen a patient's lifespan, improve a patient's quality of living, avoid recurrence of a cancer episode, and concurrently lessen the toxic effects of chemotherapeutic drugs. The vasculature of a tumor has to be altered to counterbalance the negative effects of chemotherapeutic drugs. A drug-loaded nanocarrier system has been used to conquer the lack of specificity that is generally associated with chemotherapeutic agents [8].

The tissue selectivity of current anticancer medicines is of an amplitude that allows effective and harmless cancer chemotherapy. Against this background, nanoparticle-based medicine that targets the tumor has been developed as an exciting advance to overcome the inadequacy of tissue specificity of traditional chemotherapeutic agents. Nanoparticles can be used for submicron-sized drug delivery systems that can predictably enhance the bio-distribution of systemically delivered chemotherapeutic agents. By transporting pharmacologically active drugs more specifically to tumor tissues and/or directing them far away from healthy tissues, nanoparticulate-based medicines can strike a balance between efficacy and the toxic effects of systemically administered chemotherapeutic interventions.

A variety of approaches have been investigated for nanoparticle-mediated targeting of drugs. Among them, a passive drug targeting approach has been the most commonly explored, with preclinical learning providing insight into the soundness of the approach [9–11]. A drug delivery system with passive targeting is burdened by several challenges as well as restrictions [12]. The major challenge is to accurately recognize and then guide the chemotherapeutic agent to a specific target. This is generally caused by the very limited solubility of the majority of these chemotherapeutic drugs, which have a deprived pharmacokinetic profile along with a high toxicity potential. These limits can be overcome by loading these drugs into nanocarriers and permitting passive targeting to occur as a result of the compromised vasculature. Although the nanosystem is intended for active targeting, mostly passive targeting occurs initially, with subsequent active targeting [13]. This approach is rooted in the irregularities of tumor vasculatures, which permit nanoparticles to access tumors while circumventing distribution in normal healthy

tissues. At present, targeted anti-cancer drugs alone have established successes, with well-known examples that include Gleevec® (imatinib mesylate), Herceptin® (trastuzumab), and Iressa® (gefitinib) [14]. Thus, this approach facilitates the advancement of a targeted nano-carrier structure loaded with chemotherapeutic agents for an improved effective profile with negligible toxic effects.

2 Passive Targeting

Passive targeting deposits a drug or drug-carrier structure at a specific site as a result of physico-chemical or pharmacological factors [15, 16]. Solid tumors present more favorable situations for the gathering of macromolecular drugs as well as colloidal-size drug delivery systems resembling micellar systems, liposomes, polymeric-drug conjugates, and polymeric nanoparticles. The enlarged vascular permeability together with the defective lymphatic drainage of fast-growing tumors provides for an enhanced permeability and retention (EPR) effect of the nano-systems in the tumor [17, 18].

There are a small number of commonly used techniques for targeting tumors and tumor cells due to the large phenotypic variety of cancerous cells and tumors. Many tumors and vascularized solid tumors, in addition to a few vascularized metastatic tumor lumps, show signs of an EPR effect that can be used for the passive targeting of anti-tumor drugs [19]. This effect happens because many solid tumors have a permeable vasculature along with missing or damaged lymphatic drainage, which induces the buildup of higher molecular-weight compounds in addition to smaller particles (~20–500 nm diameter) inside the tumor tissue.

3 Passive Targeting by Nanoparticles

Passive targeting by nanoparticles occurs because of the uniqueness of solid tumors (i.e., leaky vasculature and defective lymphatic drainage), which permits nanoparticles to build up in the tumor. This observable fact, which was first reported in 1986 by Matsumura and Maeda, is called the EPR effect [20, 21].

3.1 Enhanced Permeability and Retention Effect

A nanoparticle that fulfills the size and surface requirements for evading reticulo-endothelial system capture has a greater ability to travel in the bloodstream, as well as a higher possibility of arriving at the targeted tumor tissues. In particularly, macromolecules comprising nanoparticles build up in tumor tissues as a result of the distinctive pathophysiologic individuality of tumor vessels [22]. The rapidly

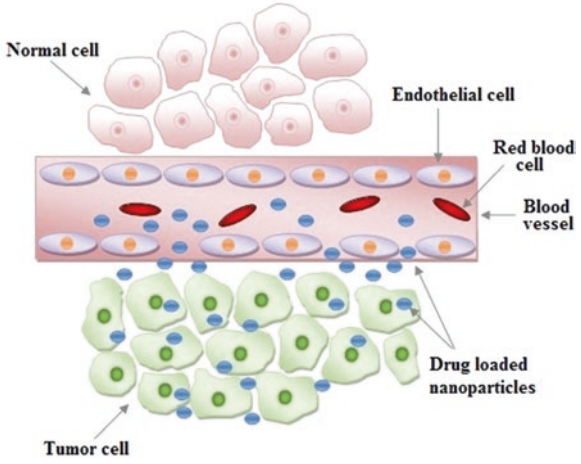

Fig. 6.1 Schematic drawing of the EPR effect

expanding cancerous cells require new vessels (neovascularization) or otherwise the diversion of existing vessels close to the tumor mass to provide them with oxygen and nutrients [23]. The resultant disparity of angiogenic regulators, such as growth factors, along with matrix metalloproteinases creates tumor vessels that are extremely disordered and enlarged, with abundant pores displaying expanded gap junctions among endothelial cells and compromised lymphatic drainage [23]. The EPR effect is an essential means through which macromolecules, together with nanoparticles, of a molecular weight greater than 50 kDa, are able to specifically build up in the tumor interstitium. A schematic drawing of the EPR effect is presented in Fig. 6.1, which illustrates the mechanism behind passive targeting [21].

The EPR effect can be used to overcome a dilemma affecting nearly every kind of cancer therapy currently in use: deficiency in tumor selectivity. However, the use of "selectivity" could be deceptive. Although nanoparticles are administered into the circulation, there is no "selectivity" with reference to the EPR effect. The nanoparticles are distributed all over the body, with the aim of "passive targeting" through the EPR effect to attain an inconsistent distribution of the nanoparticles by concentrating on the tumors.

The EPR effect is an effect of tumor vasculature irregularity, similar to the increased vascular permeability and hypervascularization [24]. Along with other growth factors, vascular endothelial growth factor (VEGF) causes the development of neo-vasculature and angiogenesis. This recently created tumor vasculature is irregular in structure and structural design, imparting fenestrations in the vessels that are induced by inadequately aligned endothelial cells, a deficit in smooth muscle, and raised levels of vascular permeability [25, 26]. In addition, the hyperproduction of vascular mediators plus VEGF, similar to nitric oxide, peroxynitrite, prostaglandins, and matrix metalloproteinases, result in better vascular permeability of the vasculature in tumor tissue [27, 28].

It is also feasible that a reduction in the boundary of the tumor-affected vasculature, which is a result of the tumor naturally pushing otherwise confining vessels, may possibly produce high hydrostatic pressure nearby. Researchers also revealed that an increase in fluid pressure in the vasculature increases the particle deposition on the endothelial cells ex vivo [29]. With this pressure-deposition system, it is possible that nanoparticles would comprise a high level of linkage to the tumor vasculature. As a result, nanoparticles may stick more regularly to endothelial cells in tumor-affected regions, which makes co-localization of the nanoparticle with tumors possible.

The therapeutic effectiveness of passively targeted nanoparticles is influenced by the heterogeneity of the EPR effect inside as well as among dissimilar tumors. An inconsistent endothelial gap (ranging from 1 to 100 nm) give rise to non-uniform extravasations of nanoparticles into the tumor [30]. The outside edge of the tumor is not as leaky as the hypoxic core, which suggests that nanoparticles extravasate more regularly at the core than the margin. However, numerous investigations have pointed out the opposite—that nanoparticles administered intravenously extravasate more often in the tumor margin [31, 32]. In addition, apart from the permeability, nanoparticle extravasation is also directed by perfusion, which has both spatial and temporal heterogeneity inside a tumor; this adds another point of complication to the scheme for nanoparticle extravasation [33]. Nanoparticle characteristics such as particle size, shape, and surface charge have an influence on the EPR effect, which in turn affects circulation time, penetration speed, and intracellular internalization [34, 35]. Furthermore, physiochemical properties such as size and shape also affect nanoparticle extravasation and accumulation [36, 37].

Nanoparticles that cross the vasculature and then extravasate into the tumor are hindered by the interstitial tumor milieu, which serves as an obstacle to their profound infiltration into tumor tissue. The diffusional obstruction can be considerably decreased by reducing the particle size, thus enhancing its diffusion into the interstitial milieu. Wong and co-workers suggested a multistage technique in which a 100-nm gelatin particle is condensed to 10 nm in size after its extravasation into the tumor tissue through degradation by tumor-associated matrix metalloproteinases (MMPs) [38]. Other groups have also reported comparable advances by diverse nanocarriers [39–41]. Passive targeting is mostly achievable during diffusion-mediated transport, in which size is the main factor. A size range of 40–200 nm is considered to be most favorable for an extended circulation time, augmented buildup inside the tumor mass, and decreased renal clearance [42].

Other physicochemical characteristics of nanoparticles, including elasticity, shape, and surface charge, can also influence the contact of nanoparticles with physiological barriers and the microenvironment of a tumor; as a result, they can be critical factors in the design of nanoparticles and the maximization of their biological function. Such as, a nanoparticle's shape is a vital feature in determining their blood circulation, ability to marginate in blood vessels, and their ability to intake through tumor cells and macrophages [43–46]. For microparticles with elliptical shapes, the macrophage may first contact these particles beside the major axis; the particles are then quickly internalized in less than 6 min. However, if the initial contact is beside

the minor axis, then the particles are not internalized for an extended time, up to 10 h. It is simply a result of their symmetry that these spherical particles are speedily internalized. In such cases, this effect of shape was separate from the size of particle. The only difference in response to the size of the particles was the degree of uptake, which was experiences only in particles having volumes that were considerably more than the volume of the cell [47]. In another investigation, it was reported that sphere-shaped particles were taken up at a rate that was approximately 5 times greater than that of rod-shaped particles, therefore indicating the importance of a nanoparticle's shape on the mechanism of uptake [48].

The elasticity of nanoparticles can also influence their biological effects, comprising blood circulation as well as tumor uptake. Softer nanoparticles (10 kPa) were associated with extended blood circulation in comparison with harder nanoparticles (3000 kPa) in an in vivo demonstration by Anselmo and co-workers [49].

In measurements of the magnitude of internalization of nanoparticles into cells, surface properties also have played an incredibly significant role. The surface characteristics can be somewhat customized by the polymer composition, which results in an additional degree of hydrophobicity or hydrophilicity for these particles. The surface modification of these polymers with the addition of polyethylene glycol (PEG) has been identified to defend the nanosystems from opsonization along with clearance by the reticulo-endothelial (RES) system [50]. Moreover, the circulation time of nanoparticles was extended by increasing the molecular weight of PEG chains. This PEG protection can provide greater defense against negatively charged nanoparticles and also put off instantaneous clearance of these particles. By modifying the nanoparticles' size, shape, or (in a few cases) surface dimensions, one can regulate the passive targeting.

Nanoparticles can remain in circulation for a long period of time, while not being able to infiltrate the tight endothelial junctions that exist in healthy vasculature and avoiding clearance via the mononuclear phagocyte system (MPS). This allows the EPR effect to bring similar passive targeting to solid tumors for anticancer drug-loaded nanoparticles. The tumor vasculature is therefore an important goal in cancer management by means of nanoparticles. Through tumor vasculature targeting, the tumor itself is targeted circuitously; otherwise, the tumors supply a line of nutrients and routes for metastasis that are able to be affected. In general, the physiochemical characteristics of nanoparticles can to a large extent affect their accretion, preservation, and permeation in tumors. Conversely, the optimization of physiochemical characteristics of nanoparticles is explicit to the target tumor's pathophysiology, as demonstrated by Sykes et al.; as a result, they should be customized to every type of tumor to exploit therapeutic efficacy [51].

3.2 Tumor Microenvironment

Another passive targeting approach uses the distinctive tumor environment in a system referred to as the tumor-activated therapy of prodrugs. Rapidly growing, hyperproliferative cancerous cells exhibit a higher metabolic rate; however, the delivery

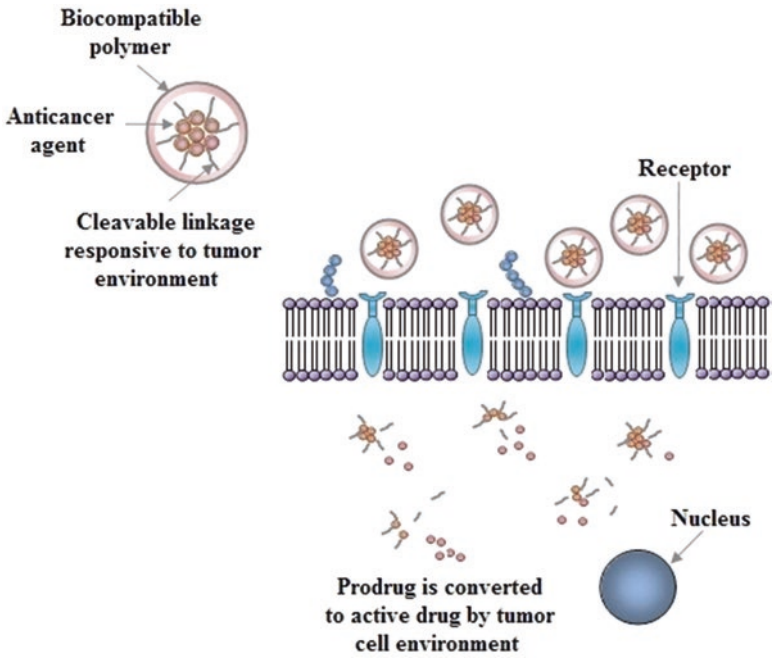

Fig. 6.2 Tumor-targeted delivery of a prodrug

of oxygen plus nutrients is generally not enough to support this. Consequently, tumor cells make use of glycolysis to obtain additional energy, resulting in an acidic milieu [52]. Furthermore, cancerous cells articulate and discharge distinctive enzymes, such as matrix metalloproteinases, which are involved in their motion as well as endurance mechanisms [53]. The drug is coupled to a tumor-specific particle and stays inert, waiting to arrive at the target (Fig. 6.2). The anticancer drug is coupled with a biocompatible polymer through an ester link. The association is hydrolyzed by a cancer-specific enzyme or through higher or lower pH at the site of tumor, at which point the nanoparticle delivers the drug [54].

For example, an albumin-bound structure of doxorubicin that integrated a matrix metalloproteinase-2-specific octapeptide series between the anticancer drug and the carrier was reported to be proficiently and specially cleaved by matrix metalloproteinase-2 in an in vitro examination [55].

3.3 Direct Local Delivery

Another passive targeting technique is the straight local release of anticancer agents to the tumor cells. This technique has the understandable benefit of excluding the drug from systemic circulation. However, administration can be extremely intrusive

because it entails injections or otherwise surgical procedures. For a few tumors that are not easy to access, such as lung cancers, this approach is almost impossible to employ [56].

4 Nanoparticle Delivery System for Cancer Through Passive Targeting

At present, numerous passively targeted nanoparticles are in clinical use, such as Genexol-PM™ in Korea and ProLindac™ and Opaxio™ in United States [57, 58]. Furthermore, a number of additional nanocarriers, including AZD2811, CPX-1, and NK911, have confirmed safety and/or therapeutic effectiveness in clinical investigations [59–61]. From a great number of nanosystems, only a small number of nanomedicines are accepted for use in cancer treatment, as depicted in Table 6.1 [58].

Even if most of these nanosystems change the pharmacokinetics, toxicological profile, or drug solubility, some have also demonstrated noteworthy endurance advantages and improved therapeutic effectiveness compared with the parent medicine in clinical investigations. Abraxane™ (nanoparticle albumin-bound paclitaxel) is one example that established considerably high response rates in comparison with standard paclitaxel in a phase III clinical trial of patients with metastatic breast cancer [62]. Likewise, the U.S. Food and Drug Administration (FDA)-approved CPX-351 (Vyxeos™) a liposomal preparation of cytarabine-daunorubicin combination, has demonstrated better endurance of 9.56 months compared with 5.95 months for cytarabine and daunorubicin delivered in their free forms in patients with recently identified vulnerable acute myeloid leukemia [63].

Our knowledge of EPR efficiency is restricted by the limited information gained from pre-clinical tumor models to precisely classify solid tumors in individuals. In reality, the most frequently used subcutaneous tumor xenografts are rapidly expanding, resulting in extremely high-EPR tumors that might provide an inaccu-

Table 6.1 Examples of passively-targeted nanosystems approved for anticancer therapy

Name	Type of formulation	Bioactive compound	Indication
DaunoXome®	Non-PEGylated liposomes	Daunorubicin	Kaposi sarcoma
Myocet®	Non-PEGylated liposomes	Doxorubicin	Breast cancer
Onco TCS®	Non-PEGylated liposomes	Vincristine	Non-Hodgkin lymphoma
Doxil®/ Caelyx®	PEGylated liposomes	Doxorubicin	Breast cancer, ovarian cancer, multiple myeloma, Kaposi sarcoma
Abraxane®	Albumin-based	Paclitaxel	Breast cancer
Oncaspar®	PEG-L-asparaginase	Asparagine specific enzyme	Acute lymphoblastic leukemia

rate assessment of the therapeutic advantages of nanocarriers in treatments that depend on EPR-based passive targeting [64]. Moreover, there is restricted patient-based investigational information on the EPR phenomenon itself in addition to its effects on the buildup of a drug in the tumor site, which can be interpreted as clinical effectiveness [64]. Additional research on the EPR effect in different human tumors as well as the progress of advanced preclinical models are therefore necessary for the design of nanoparticles with improved tumor penetration and therapeutic effects [8, 50].

The link between tumor vascularization and EPR-based passive targeting has been explored by Theek and co-workers in a subcutaneous tumor model [65]. Through the use of both contrast-enhanced ultrasound and computed tomography-fluorescence molecular systems, the authors established heterogeneous buildup of 10-nm near infrared-labeled polymeric nanocarriers (pHPMA-Dy750) inside and among the tumors (5–12%). In the same way, copper-64-loaded PEGylated liposomes were investigated by Hansen and his group. Moreover, the EPR effect of PEGylated liposomes was evaluated with a micro-positron emission tomography/computed tomography (PET/CT) imaging technique [66]. Evaluation of eleven dogs bearing spontaneous solid tumors revealed that the EPR effect is a predominant feature in a few solid tumors (e.g., carcinoma), resulting in a higher accumulation of liposomes; however, this might not be widespread to every solid tumor.

An FDA-approved 30-nm carboxymethyl dextran-coated magnetic nanoparticle (ferumoxytol) could be used as a substitute or companion element for intratumoral transfer, pharmacokinetics, and distribution of a therapeutic nanocarrier rooted in poly(D,L-lactic-co-glycolic acid)-*b*-poly(ethylene glycol) (PLGA-PEG), as demonstrated by Miller et al. [67]. Lee et al. developed 64Cu-labeled human epidermal growth factor receptor-2 (HER2) targeted liposomes along with PET/CT to measure the accumulation of s drug in 19 patients with HER2-positive metastatic breast cancer [68]. The maximum accumulation of liposomes was found at 24–48 h; patients were categorized in accordance with 64Cu-liposomal abrasion deposition by a cut-point that was similar to a response threshold as determined in preclinical investigations. Patients with elevated 64Cu-liposomal abrasion deposition were associated with additional positive therapy results. These investigations reveal that the use of imaging systems for the assessment and characterization of EPR may ultimately allow clinicians to preselect patients with higher-EPR tumors who are expected to respond to passively targeted nanosystems with enhanced therapeutic effects.

A meta-analysis of pre-clinical data based on a nano-carrier delivery system for tumors reported during the past 10 years found that a mean of approximately 0.7% of the injected dose of nanocarriers arrives at the target tumors [69]. This figure appears to be very small on the surface, elevating concerns about the competence of the EPR effect and the management of low-EPR tumors. However, a delivery effectiveness of approximately 0.7% for nanocarriers is considerably better than the delivery effectiveness of most chemotherapeutic preparations that are commonly used in hospitals, including docetaxel, doxorubicin, and paclitaxel [70–73]. In a preclinical investigation, van Vlerken et al. established the delivery effectiveness of 0.6% of the injected dose for paclitaxel-loaded nanocarriers in comparison with

0.2% of the injected dose for free paclitaxel [70]. This outcome is hopeful and indicates the benefits of nanocarrier systems for the tumor-targeted delivery of drugs.

Nevertheless, the delivery effectiveness of nanocarrier systems can be additionally enhanced to make the best use of their therapeutic advantages. Enhancing EPR effects with angiotensin II-induced hypertension or heat-based vasodilation might be an answer; however, such a system could make the clinical transformation of nanocarriers difficult. One more possible and comparatively adaptable solution, particularly for low-EPR tumors, is are the gracefully engineered delivery methods that make use of non-EPR advances for tumor targets. For example, injectable nanoparticle generators (iNPGs) that tackle the many physiological barriers were developed by Xu and co-workers [74]. An iNPG is a discoidal micrometer-sized nanoporous silicon particle that can be laden with drug polymer conjugates, controlling tumor growth because of normal tropism along with improved vascular dynamics. The iNPG delivers the drug polymer conjugate by self-assembling to make nanoparticles that are transferred to the perinuclear area, thus bypassing the drug efflux pump. Superior efficiency was observed with iNPG in MDA-MB-231 and 4 T1 mouse models of metastatic breast cancer compared with its individual components as well as other existing therapeutic dosage forms. The delivery effectiveness of nanocarriers can be appreciably enhanced by such realistically engineered systems.

The cell-mediated delivery of nanocarriers may be an additional EPR-free advance to improve tumor targeting in low-EPR tumors or certain metastatic tumor sites that are inaccessible to passive targeting. This method uses the aptitude of definite cell types to house or travel to such tumors [75]. Huang et al. bridled the innate capability of T-cells to travel throughout the lymphatic system via conjugating nanocapsules by encapsulating the topoisomerase I drug SN-38 to the surface of the cell [76]. The authors reported an approximately 90-fold increase in the concentration of SN-38 in lymph nodes found by cell-mediated delivery compared with the free drug when injected systemically at 10-fold high doses, as well as an extended median endurance by 35 days without toxic effects. In addition to targeting low-EPR tumors, the immune cell-mediated delivery of nanocarriers can also result in better tumor accumulation in dispersed tumors as well as metastases. This may possibly unlock novel opportunities for more secure targeted delivery of immune-modulating compounds, such as IFN-γ, which can encourage the segregation of tumor-promoting M2 macrophages to antitumor M1 macrophages. In addition, the use of tumor-infiltrating lymphocytes or chimeric antigen receptor T-cells for targeted delivery of immune-modulating agent-loaded nanocarriers may facilitate a synergetic dual-arm therapy, thus boosting anti-tumor immune responses by tumor targeting and/or intonation of immunosuppressive cells. However, this advance is restricted to medications with few toxic effects to common carrier cells.

The coating of nanoparticles can help to manage the interaction of nanoparticles with proteins in the bloodstream [77, 78]. Targeting approaches are being used to ensure that an adequate quantity of nanoparticles reach tumor cells. In a passive targeting approach, the individual receives the benefit of the elevated endocytic

uptake of cancer cells as well as the permeable vasculature in the region of tumors, which allows for high uptake of nanoparticles compared with healthy tissues [79].

As soon as the organism detects a foreign body in the bloodstream, specific serum proteins (opsonins) will adsorb on the surface of the body, tagging it for discharge from the body [80]. By attaching suitable molecules, such as PEG, on the surface of the nanoparticles, this response has been avoided [50, 81]. The PEG-coating of nanoparticles repulses the opsonins, un-labeling them to coat the surface as a result [82]. Although nanoparticles have a propensity to focus on tumor tissue as a result of the irregular blood vessel wall configuration around tumor tissues, in addition to a weakly urbanized lymphatic system that restricts discharge of macromolecules from tumor tissue [83], the EPR effect is useful in such cases. Coating a nanoparticle with PEG increases its blood circulation time, therefore resulting in high passive uptake because of the EPR effect. The ability of the coating layer to offer passive targeting circumstances is influenced by a number of factors, such as nanoparticle core size, length, and surface density of capping molecules, which has been previously studied both computationally and experimentally [84, 85]. Non-targeted strategies take advantage of passive targeting on the basis of the pathophysiological circumstances of the myeloma microenvironment for precise release of the medicine. Most nanoparticle delivery systems explored for myeloma have used non-targeted nanoparticles.

Liposomal bortezomib nanoparticles have 100-nm size ranges with great reproducibility and 80% encapsulation efficiency. For investigation of proteasome inhibition, apoptosis, and cell viability, in vitro studies have been performed. It was observed that liposomal bortezomib nanoparticle systems restrained proteasome action, encouraged apoptosis, and enhanced cytotoxicity on manifold myeloma cells. In the in vivo investigations, multiple myeloma cells were injected subcutaneously in the severe combined immune-deficient mice, processed by free drug or liposomal bortezomib nanoparticles intravenously on the first and fourth days at 1 mg/kg bortezomib equivalent dose, and examined for the succession of the tumor and systemic toxicities. The outcomes showed that liposomal bortezomib nanoparticles were effective in the suppression of tumor growth, in addition to lessening the systemic side effects, including body weight loss. The free drug group exhibited >20% weight loss and moribundity on the seventh day, which required sacrifice of the mice, whereas the liposomal bortezomib nanoparticle group exhibited <10% weight loss for the duration of the 2 weeks [86].

PEGylated liposomal doxorubicin was the initial nanoparticle delivery method approved by the FDA for medical use in multiple myeloma. It has been used with additional anti-myeloma agents, such as bortezomib or vincristine and dexamethasone. Patients with regression or refractory multiple myeloma received PEGylated liposomal doxorubicin (Tibotec Therapeutics) delivered on the fourth day at 30 mg/m^2 as well as bortezomib administered on days 1, 4, 8, and 11 at 0.90–1.50 mg/m^2. The time to progression (TTP) was considerably extended in the combining arm (median TTP = 9.3 months) in relation to bortezomib monotherapy (median TTP = 6.5 months) [87, 88].

Thymoquinone-encapsulated PLGA-PEG nanoparticles exhibited a size of approximately 200 nm with uniform distribution and 94% encapsulation efficiency. PLGA-PEG based thymoquinone nanoparticles had anti-proliferative effects on multiple myeloma cells; these nanoparticles were more effective than free drug-sensitizing leukemic cells to TNF-α as well as paclitaxel-induced apoptosis [89]. However, this investigation is very preliminary, with only in vitro studies reported. In vivo examinations are required to confirm the effectiveness and delivery for thymoquinone nanoparticles in myeloma. Other polymeric nanoparticles that are nanocolloids derived from *N*,*N*,*N*-trimethyl chitosan have been formulated to encapsulate camptothecin, a powerful anticancer drug with a plant source. There was no statistical dissimilarity observed between loaded nanocolloids and the free drug in the in vitro cytotoxicity study. Conversely, loaded nanocolloids more efficiently restrained growth of the tumor and extended survival time compared with the free drug in vivo [90].

Silica nanoparticles have been conjugated with snake venom derived from *Walterinnesia aegyptia*, a natural toxin that exhibits antitumor activity [91]. The obtained nanoparticles had 300-nm particle size. Silica nanoparticles containing snake venom were examined in the cells of five patients with myeloma, as well as a XG2 cell line. It was observed that this combination had the ability to decrease viability and encourage apoptosis [92]. Furthermore, iron oxide-based nanoparticles have been studied for multiple myeloma. Paclitaxel is an effectual anticancer medicine with poor aqueous solubility. However, Abraxane® (Celgene, Summit, NJ, USA), an albumin-bound paclitaxel-loaded iron oxide nanoparticle, is a water-soluble commercially available nanoparticle approved by the FDA for the management of metastatic breast cancer [93]. In myeloma-bearing mice, 7-nm paclitaxel iron oxide nanoparticles were used to treat CD138-CD34-tumor stem-like cells. The inhibition of tumor growth was greater with paclitaxel iron oxide nanoparticles (0.6–2 mg/kg once a week for 2 weeks) in comparison with nanoparticles only or paclitaxel only; in addition, they were found to encourage the apoptosis of cancer cells in treated mice [94].

In most passive targeting nanosystems, surface coating with PEG is performed for biocompatibility and "stealth" purposes [50, 95, 96]. Significantly, improved hydrophilicity on the surface of the nanoparticle can obstruct its uptake by cancerous cells, thus hindering the competent delivery of a drug to tumors with passive targeting nanoparticles [50, 97, 98]. Nevertheless, PEG-based block copolymers have been used in many passive targeting polymeric nanoparticles, including Genexol-PM, SP1049C and NK911. Among them, SP1049C is a pluronic-based polymeric micelle nanoparticle of doxorubicin. At present, it is being investigated in Phase II clinical trials for metastatic cancer of the esophagus versus the usual chemotherapeutic protocols [99]. Another polymeric micellar nanoparticle that acts through a passive targeting mechanism is NK911 containing PEG, doxorubicin, and poly(aspartic acid), which is currently being investigated in Phase II clinical trials for a variety of cancers [100]. Likewise, Opaxio™ and passively targeted paclitaxel/poly(L-glutamic acid) nano-construct are established as effectual in ovarian cancers [101, 102]. CRLX101 (previously IT-101), a camptothecin-cyclodextrin polymeric

conjugate, has shown better pharmacokinetic effectiveness in preclinical and clinical investigations [103]. NC-6004 is a cisplatin-incorporated PEG-poly(glutamic acid) block copolymer micellar nanotherapeutics, whereas ProLindac™ is a diaminocyclohexane-platinum hydroxypropylmethacrylamide prodrug, which are both in the final stage of clinical investigation [104].

A passive targeting lipid nanoparticle system is also moving to the progressive stages of clinical trials, and profound attempts are being made to put these methods into medical practice. Promising liposomal nanoparticles in clinical trials include Thermodox®, a thermosensitive liposomal doxorubicin nanoparticle that delivers the drug at approximately 39 °C; it is presently being examined in Phase III clinical investigations in addition to radiofrequency excision in hepatocellular carcinoma patients [105]. SPI-77 is a PEGylated liposomal cisplatin nanoparticle, which is currently in Phase II clinical investigations for patients with recurring epithelial ovarian tumors [106]. CPT-11, a nanosized liposomal irinotecan formulation, is in Phase I clinical investigations for patients with glial cell tumors [107]. A number of liposomal nanoformulations containing two dissimilar categories of anticancer drugs, such as cytarabine and daunorubicin, are also being investigated [108].

5 Conclusion

The recent advancements in novel nanoparticulate strategies have necessitated greater accuracy in delivering medicine to cancerous cells, while also sparing nearby healthy tissues. The tumor microvasculature is the focus of theories on the passive targeting of nanoparticles. Despite the widespread investigations and progress in nanotechnology, only a small number of nanoparticle-based drug delivery approaches have been approved and are in practice for cancer management. This is because the pathophysiological characteristics of the tumor microenvironment and the molecular mechanisms inherent in tumor angiogenesis are fairly diverse and are reliant on the nature of cancers. Hence, further investigations and greater knowledge on these features of tumor tissues are essential to ensure a successful future for EPR effect-based chemotherapy of cancer with a passive drug-targeting strategy.

References

1. BWKP, S., & Wild, C. P. (2015). *World cancer report 2014*. Lyon: International Agency for Research on Cancer.
2. Ogawara, K., Yoshizawa, Y., Un, K., Araki, T., Kimura, T., & Higaki, K. (2013). Nanoparticle-based passive drug targeting to tumors: Considerations and implications for optimization. *Biological & Pharmaceutical Bulletin, 36*(5), 698–702.
3. Center for Disease Control and Prevention. (2015, February 2). *Global cancer statistics*. Retrieved from http://www.cdc.gov/cancer/international/statistics.htm

4. Bazak, R., Houri, M., El Achy, S., Hussein, W., & Refaat, T. (2014). Passive targeting of nanoparticles to cancer: A comprehensive review of the literature. *Molecular and Clinical Oncology, 2*(6), 904–908.
5. Danhier, F., Feron, O., & Préat, V. (2010). To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. *Journal of Controlled Release, 148*(2), 135–146.
6. Singla, A. K., Garg, A., & Aggarwal, D. (2002). Paclitaxel and its formulations. *International Journal of Pharmaceutics, 235*(1–2), 179–192.
7. Zhang, S., Liu, X., Bawa-Khalfe, T., Lu, L. S., Lyu, Y. L., Liu, L. F., et al. (2012). Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nature Medicine, 18*(11), 1639–1642.
8. Byrne, J. D., Betancourt, T., & Brannon-Peppas, L. (2008). Active targeting schemes for nanoparticle systems in cancer therapeutics. *Advanced Drug Delivery Reviews, 60*(15), 1615–1626.
9. Araki, T., Kono, Y., Ogawara, K., Watanabe, T., Ono, T., Kimura, T., et al. (2012). Formulation and evaluation of paclitaxel-loaded polymeric nanoparticles composed of polyethylene glycol and polylactic acid block copolymer. *Biological & Pharmaceutical Bulletin, 35*(8), 1306–1313.
10. Thigpen, J. T., Aghajanian, C. A., Alberts, D. S., Campos, S. M., Gordon, A. N., Markman, M., et al. (2005). Role of pegylated liposomal doxorubicin in ovarian cancer. *Gynecologic Oncology, 96*(1), 10–18.
11. Wakaskar, R. R. (2017). Challenges pertaining to adverse effects of drugs. *International Journal of Drug Development and Research, 9*(3), 1–2.
12. Sparreboom, A., Scripture, C. D., Trieu, V., Williams, P. J., De, T., Yang, A., et al. (2005). Comparative preclinical and clinical pharmacokinetics of a cremophor-free, nanoparticle albumin-bound paclitaxel (ABI-007) and paclitaxel formulated in Cremophor (Taxol). *Clinical Cancer Research, 11*(11), 4136–4143.
13. Bae, Y. H. (2009). Drug targeting and tumor heterogeneity. *Journal of Controlled Release, 133*(1), 2–3.
14. Wakaskar, R. R. (2017). Passive and active targeting in tumor microenvironment. *International Journal of Drug Development and Research, 9*(2), 37–41.
15. Garnette, M. C. (2001). Targeted drug conjugates: Principles and progress. *Advanced Drug Delivery Reviews, 53*(2), 171–216.
16. Kim, J. H., Kim, Y. S., Park, K., Lee, S., Nam, H. Y., Min, K. H., et al. (2008). Antitumor efficacy of cisplatin-loaded glycol chitosan nanoparticles in tumor-bearing mice. *Journal of Controlled Release, 127*(1), 41–49.
17. Lamprecht, A., Ubrich, N., Yamamoto, H., Schäfer, U., Takeuchi, H., Maincent, P., et al. (2001). Biodegradable nanoparticles for targeted drug delivery in treatment of inflammatory bowel disease. *The Journal of Pharmacology and Experimental Therapeutics, 299*(2), 775–781.
18. Chytil, P., Etrych, T., Konák, C., Sírová, M., Mrkvan, T., Boucek, J., et al. (2008). New HPMA copolymer-based drug carriers with covalently bound hydrophobic substituents for solid tumour targeting. *Journal of Controlled Release, 127*(2), 121–130.
19. Maeda, H., Wu, J., Sawa, T., Matsumura, Y., & Hori, K. (2000). Tumor vascular permeability and the EPR effect in macromolecular therapeutics: A review. *Journal of Controlled Release, 65*(1–2), 271–284.
20. Rosenblum, D., & Peer, D. (2014). Omics-based nanomedicine: The future of personalized oncology. *Cancer Letters, 352*(1), 126–136.
21. Matsumura, Y., & Maeda, H. (1986). A new concept for macromolecular therapeutics in cancer chemotherapy: Mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Research, 46*(12), 6387–6392.
22. Maeda, H. (2001). The enhanced permeability and retention (EPR) effect in tumor vasculature: The key role of tumor-selective macromolecular drug targeting. *Advances in Enzyme Regulation, 41*(1), 189–207.

23. Carmeliet, P., & Jain, R. K. (2000). Angiogenesis in cancer and other diseases. *Nature, 407*(6801), 249–257.
24. Greish, K. (2010). Enhanced permeability and retention (EPR) effect for anticancer nanomedicine drug targeting. In S. R. Grobmyer & B. M. Moudgil (Eds.), *Cancer nanotechnology. Methods in Molecular Biology (Methods and Protocols)* (Vol. 624, pp. 25–37). Totowa, NJ: Humana Press/Springer.
25. Gazit, Y., Baish, J. W., Safabakhsh, N., Leuniq, M., Baxter, L. T., & Jain, R. K. (1997). Fractal characteristics of tumor vascular architecture during tumor growth and regression. *Microcirculation, 4*(4), 395–402.
26. Narang, A. S., & Varia, S. (2011). Role of tumor vascular architecture in drug delivery. *Advanced Drug Delivery Reviews, 63*(8), 640–658.
27. Maeda, H., Fanq, J., Inutsuka, T., & Kitamoto, Y. (2003). Vascular permeability enhancement in solid tumor: Various factors, mechanisms involved and its implications. *International Immunopharmacology, 3*(3), 319–328.
28. Wu, J., Akaike, T., Hayashida, K., Okamoto, T., Okuyama, A., & Maeda, H. (2001). Enhanced vascular permeability in solid tumor involving peroxynitrite and matrix metalloproteinases. *Japanese Journal of Cancer Research, 92*(4), 439–451.
29. Mann, M. J., Gibbons, G. H., Hutchinson, H., Poston, R. S., Hoyt, E. G., Robbins, R. C., et al. (1999). Pressure-mediated oligonucleotide transfection of rat and human cardiovascular tissues. *Proceedings of the National Academy of Sciences of the United States of America, 96*(11), 6411–6416.
30. Chauhan, V. P., & Jain, R. K. (2013). Strategies for advancing cancer nanomedicine. *Nature Materials, 12*(11), 958–962.
31. Yuan, F., Dellian, M., Fukumura, D., Leunig, M., Berk, D. A., Torchilin, V. P., et al. (1995). Vascular permeability in a human tumor xenograft: Molecular size dependence and cutoff size. *Cancer Research, 55*(17), 3752–3756.
32. Lee, H., Hoang, B., Fonge, H., Reilly, R. M., & Allen, C. (2010). In vivo distribution of polymeric nanoparticles at the whole-body, tumor, and cellular levels. *Pharmaceutical Research, 27*(11), 2343–2355.
33. Ernsting, M. J., Murakami, M., Roy, A., & Li, S.-D. (2013). Factors controlling the pharmacokinetics, biodistribution and intratumoral penetration of nanoparticles. *Journal of Controlled Release, 172*(3), 782–794.
34. Chou, L. Y. T., Ming, K., & Chan, W. C. W. (2011). Strategies for the intracellular delivery of nanoparticles. *Chemical Society Reviews, 40*(1), 233–245.
35. Albanese, A., Tang, P. S., & Chan, W. C. W. (2012). The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annual Review of Biomedical Engineering, 14*(1), 1–16.
36. Cabral, H., Matsumoto, Y., Mizuno, K., Chen, Q., Murakami, M., Kimura, M., et al. (2011). Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size. *Nature Nanotechnology, 6*(12), 815–823.
37. Kolhar, P., Anselmo, A. C., Gupta, V., Pant, K., Prabhakarpandian, B., Ruoslahti, E., et al. (2013). Using shape effects to target antibody-coated nanoparticles to lung and brain endothelium. *Proceedings of the National Academy of Sciences of the United States of America, 110*(26), 10753–10758.
38. Wong, C., Stylianopoulos, T., Cui, J., Martin, J., Chauhan, V. P., Jiang, W., et al. (2011). Multistage nanoparticle delivery system for deep penetration into tumor tissue. *Proceedings of the National Academy of Sciences of the United States of America, 108*(6), 2426–2431.
39. Tong, R., Hemmati, H. D., Langer, R., & Kohane, D. S. (2012). Photoswitchable nanoparticles for triggered tissue penetration and drug delivery. *Journal of the American Chemical Society, 134*(21), 8848–8855.
40. Tong, R., Chiang, H. H., & Kohane, D. S. (2013). Photoswitchable nanoparticles for in vivo cancer chemotherapy. *Proceedings of the National Academy of Sciences of the United States of America, 110*(47), 19048–19053.

41. Li, H. J., Du, J. Z., Du, X. J., Xu, C. F., Sun, C. Y., Wang, H. X., et al. (2016). Stimuli-responsive clustered nanoparticles for improved tumor penetration and therapeutic efficacy. *Proceedings of the National Academy of Sciences of the United States of America, 113*(15), 41640–44169.
42. Liechty, W. B., & Peppas, N. A. (2012). Expert opinion: Responsive polymer nanoparticles in cancer therapy. *European Journal of Pharmaceutics and Biopharmaceutics, 80*(2), 241–246.
43. Toy, R., Peiris, P. M., Ghaghada, K. B., & Karathanasis, E. (2014). Shaping cancer nanomedicine: The effect of particle shape on the in vivo journey of nanoparticles. *Nanomedicine (London, England), 9*(1), 121–134.
44. Smith, B. R., Kempen, P., Bouley, D., Xu, A., Liu, Z., Melosh, N., et al. (2012). Shape matters: Intravital microscopy reveals surprising geometrical dependence for nanoparticles in tumor models of extravasation. *Nano Letters, 12*(7), 3369–3377.
45. Barua, S., Yoo, J. W., Kolhar, P., Wakankar, A., Gokam, Y. R., & Mitragotri, S. (2013). Particle shape enhances specificity of antibody-displaying nanoparticles. *Proceedings of the National Academy of Sciences of the United States of America, 110*(9), 3270–3275.
46. Ananta, J. S., Godin, B., Sethi, R., Moriggi, L., Liu, X., Serda, R. E., et al. (2010). Geometrical confinement of gadolinium-based contrast agents in nanoporous particles enhances T1 contrast. *Nature Nanotechnology, 5*(11), 815–821.
47. Champion, J. A., & Mitragotri, S. (2006). Role of target geometry in phagocytosis. *Proceedings of the National Academy of Sciences of the United States of America, 103*(13), 4930–4934.
48. Chithrani, B. D., & Chan, W. C. (2007). Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. *Nano Letters, 7*(6), 1542–1550.
49. Anselmo, A. C., Zhanq, M., Kumar, S., Vogus, D. R., Menegatti, S., Helgeson, M. E., et al. (2015). Elasticity of nanoparticles influences their blood circulation, phagocytosis, endocytosis, and targeting. *ACS Nano, 9*(3), 3169–3177.
50. Alexis, F., Pridgen, E., Molnar, L. K., & Farokhzad, O. C. (2008). Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Molecular Pharmaceutics, 5*(4), 505–515.
51. Sykes, E. A., Dai, Q., Sarsons, C. D., Chen, J., Rocheleau, J. V., Hwang, D. M., et al. (2016). Tailoring nanoparticle designs to target cancer based on tumor pathophysiology. *Proceedings of the National Academy of Sciences of the United States of America, 113*(9), E1142–E1151.
52. Pelicano, H., Martin, D. S., Xu, R. H., & Huang, P. (2006). Glycolysis inhibition for anticancer treatment. *Oncogene, 25*(34), 4633–4646.
53. Deryugina, E. I., & Quigley, J. P. (2006). Matrix metalloproteinases and tumor metastasis. *Cancer Metastasis Reviews, 25*(1), 9–34.
54. Chari, R. V. (1998). Targeted delivery of chemotherapeutics: Tumor-activated prodrug therapy. *Advanced Drug Delivery Reviews, 31*(1–2), 89–104.
55. Mansour, A. M., Drevs, J., Esser, N., Hamada, F. M., Badary, O. A., Unger, C., et al. (2003). A new approach for the treatment of malignant melanoma: Enhanced antitumor efficacy of an albumin-binding doxorubicin prodrug that is cleaved by matrix metalloproteinase 2. *Cancer Research, 63*(14), 4062–4066.
56. Kim, G. J., & Nie, S. (2005). Targeted cancer nanotherapy. *Materials Today, 8*(8), 28–33.
57. Shi, J., Kantoff, P. W., Wooster, R., & Farokhzad, O. C. (2017). Cancer nanomedicine: Progress, challenges and opportunities. *Nature Reviews. Cancer, 17*(1), 20–37.
58. Sanna, V., Pala, N., & Sechi, M. (2014). Targeted therapy using nanotechnology: Focus on cancer. *International Journal of Nanomedicine, 9*(1), 467–483.
59. Awada, A., Bondarenko, I. N., Bonneterre, J., Nowara, E., Ferrero, J. M., Bakshi, A. V., et al. (2014). A randomized controlled phase II trial of a novel composition of paclitaxel embedded into neutral and cationic lipids targeting tumor endothelial cells in advanced triple-negative breast cancer (TNBC). *Annals of Oncology, 25*(4), 824–831.

60. Burris, H. A., Wang, J. S., Johnson, M. L., Falchook, G. S., Jones, S. F., Strickland, D. K., et al. (2017). A phase I, open-label, first-time-in-patient dose escalation and expansion study to assess the safety, tolerability, and pharmacokinetics of nanoparticle encapsulated Aurora B kinase inhibitor AZD2811 in patients with advanced solid tumours. *Journal of Clinical Oncology, 35*(15), TPS2608–TPS2608.
61. Batist, G., Sawyer, M., Gabrail, N., Christiansen, N., Marshall, J. L., Spigel, D. R., et al. (2008). A multicenter, phase II study of CPX-1 liposome injection in patients (pts) with advanced colorectal cancer (CRC). *Journal of Clinical Oncology, 26*(15), 4108–4108.
62. Gradishar, W. J., Tjulandin, S., Davidson, N., Shaw, H., Desai, N., Bhar, P., et al. (2005). Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. *Journal of Clinical Oncology, 23*(31), 7794–7803.
63. Lancet, J. E., Uy, G. L., Cortes, J. E., Newell, L. F., Lin, T. L., Ritchie, E. K., et al. (2016). Final results of a phase III randomized trial of CPX-351 versus 7 + 3 in older patients with newly diagnosed high risk (secondary) AML. *Journal of Clinical Oncology, 34*(15), 7000.
64. Bogart, L. K., Pourroy, G., Murphy, C. J., Puntes, V., Pellegrino, T., Rosenblum, D., et al. (2014). Nanoparticles for imaging, sensing, and therapeutic intervention. *ACS Nano, 8*(4), 3107–3122.
65. Theek, B., Gremse, F., Kunjachan, S., Fokong, S., Pola, R., Pechar, M., et al. (2014). Characterizing EPR-mediated passive drug targeting using contrast-enhanced functional ultrasound imaging. *Journal of Controlled Release, 182*(1), 83–89.
66. Hansen, A. E., Petersen, A. L., Henriksen, J. R., Boerresen, B., Rasmussen, P., Elema, D. R., et al. (2015). Positron emission tomography based elucidation of the enhanced permeability and retention effect in dogs with cancer using copper-64 liposomes. *ACS Nano, 9*(7), 6985–6995.
67. Miller, M. A., Gadde, S., Pfirschke, C., Engblom, C., Sprachman, M. M., Kohler, R. H., et al. (2015). Predicting therapeutic nanomedicine efficacy using a companion magnetic resonance imaging nanoparticle. *Science Translational Medicine, 7*(314), 314ra183.
68. Lee, H., Shields, A. F., Siegel, B. A., Miller, K. D., Krop, I., Ma, C. X., et al. (2017). 64Cu-MM-302 positron emission tomography quantifies variability of enhanced permeability and retention of nanoparticles in relation to treatment response in patients with metastatic breast cancer. *Clinical Cancer Research, 23*(15), 4190–4202.
69. Wilhelm, S., Tavares, A. J., Dai, Q., Ohta, S., Audet, J., Dvorak, H. F., et al. (2016). Analysis of nanoparticle delivery to tumours. *Nature Reviews Materials, 1*(1), 16014.
70. van Vlerken, L. E., Duan, Z., Little, S. R., Seiden, M. V., & Amiji, M. M. (2008). Biodistribution and pharmacokinetic analysis of paclitaxel and ceramide administered in multifunctional polymer-blend nanoparticles in drug resistant breast cancer model. *Molecular Pharmaceutics, 5*(4), 516–526.
71. Cui, Y., Zhang, M., Zeng, F., Jin, H., Xu, Q., & Huang, Y. (2016). Dual-targeting magnetic PLGA nanoparticles for codelivery of paclitaxel and curcumin for brain tumor therapy. *ACS Applied Materials & Interfaces, 8*(47), 32159–32169.
72. Peer, D., & Margalit, R. (2004). Tumor-targeted hyaluronan nanoliposomes increase the antitumor activity of liposomal doxorubicin in syngeneic and human xenograft mouse tumor models. *Neoplasia, 6*(4), 343–353.
73. Shi, J., Xiao, Z., Kamaly, N., & Farokhzad, O. C. (2011). Self-assembled targeted nanoparticles: Evolution of technologies and bench to bedside translation. *Accounts of Chemical Research, 44*(10), 1123–1134.
74. Xu, R., Zhang, G., Mai, J., Deng, X., Segura-Ibarra, V., Wu, S., et al. (2016). An injectable nanoparticle generator enhances delivery of cancer therapeutics. *Nature Biotechnology, 34*(4), 414–418.
75. Levy, O., Brennen, W. N., Han, E., Rosen, D. M., Musabeyezu, J., Safaee, H., et al. (2016). A prodrug-doped cellular Trojan Horse for the potential treatment of prostate cancer. *Biomaterials, 91*(1), 140–150.

76. Huang, B., Abraham, W. D., Zheng, Y., Bustamante Lopez, S. C., Luo, S. S., & Irvine, D. J. (2015). Active targeting of chemotherapy to disseminated tumors using nanoparticle-carrying T cells. *Science Translational Medicine, 7*(291), 291ra294.
77. Monopoli, M. P., Åberg, C., Salvati, A., & Dawson, K. A. (2012). Biomolecular coronas provide the biological identity of nanosized materials. *Nature Nanotechnology, 7*(12), 779–786.
78. Krpetić, Ž., Anguissola, S., Garry, D., Kelly, P. M., & Dawson, K. A. (2014). Nanomaterials: Impact on cells and cell organelles. In G. D. Capco & Y. Chen (Eds.), *Nanomaterial. Impact on cell biology and medicine* (pp. 135–156). Dordrecht: Springer.
79. Barreto, J. A., O'Malley, W., Kubeil, M., Graham, B., Stephan, H., & Spiccia, L. (2011). Nanomaterials: Applications in cancer imaging and therapy. *Advanced Materials, 23*(12), 18–40.
80. Malam, Y., Loizidou, M., & Seifalian, A. M. (2009). Liposomes and nanoparticles: Nanosized vehicles for drug delivery in cancer. *Trends in Pharmacological Sciences, 30*(11), 592–599.
81. Illés, E., Szekeres, M., Kupcsik, E., Tóth, I. Y., Farkas, K., Jedlovszky-Hajdú, A., et al. (2014). PEGylation of surfacted magnetite core-shell nanoparticles for biomedical application. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 460*(1), 429–440.
82. Thierry, B., & Griesser, H. J. (2012). Dense PEG layers for efficient immunotargeting of nanoparticles to cancer cells. *Journal of Materials Chemistry, 22*(18), 8810–8819.
83. Ranganathan, R., Madanmohan, S., Kesavan, A., Baskar, G., Krishnamoorthy, Y. R., Santosham, R., et al. (2012). Nanomedicine: Towards development of patient-friendly drug-delivery systems for oncological applications. *International Journal of Nanomedicine, 7*(1), 1043–1060.
84. Walkey, C. D., Olsen, J. B., Guo, H., Emili, A., & Chan, W. C. W. (2012). Nanoparticle size and surface chemistry determine serum protein adsorption and macrophage uptake. *Journal of the American Chemical Society, 134*(4), 2139–2147.
85. Haume, K., Mason, N. J., & Solov'yov, A. (2016). Modeling of nanoparticle coatings for medical applications. *European Physical Journal D: Atomic, Molecular, Optical and Plasma Physics, 70*(9), 181.
86. Ashley, J. D., Stefanick, J. F., Schroeder, V. A., Suckow, M. A., Kiziltepe, T., & Bilgicer, B. (2014). Liposomal bortezomib nanoparticles via boronic ester prodrug formulation for improved therapeutic efficacy *in vivo*. *Journal of Medicinal Chemistry, 57*(12), 5282–5292.
87. Orlowski, R. Z., Nagler, A., Sonneveld, P., Bladé, J., Hajek, R., Spencer, A., et al. (2007). Randomized phase III study of pegylated liposomal doxorubicin plus bortezomib compared with bortezomib alone in relapsed or refractory multiple myeloma: Combination therapy improves time to progression. *Journal of Clinical Oncology, 25*(25), 3892–3901.
88. Voorhees, P. M., Orlowski, R. Z., Mulkey, F., Watson, P., Geyer, S., Sanford, B. L., et al. (2015). Long-term outcomes for newly-diagnosed multiple myeloma patients treated with pegylated liposomal doxorubicin and bortezomib: Final results of CALGB (Alliance) 10301, a multicentre phase II study. *British Journal of Haematology, 171*(3), 373–377.
89. Ravindran, J., Nair, H. B., Sung, B., Prasad, S., Tekmal, R. R., & Aggarwal, B. B. (2010). Thymoquinone poly (lactide-co-glycolide) nanoparticles exhibit enhanced anti-proliferative, anti-inflammatory, and chemosensitization potential. *Biochemical Pharmacology, 79*(11), 1640–1647.
90. Li, Z., Li, X., Cao, Z., Xu, Y., Lin, H., Zhao, Y., et al. (2012). Camptothecin nanocolloids based on N, N, N-trimethyl chitosan: Efficient suppression of growth of multiple myeloma in a murine model. *Oncology Reports, 27*(4), 1035–1040.
91. Badr, G., Al-Sadoon, M. K., El-Toni, A. M., & Daghestani, M. (2012). *Walterinnesia aegyptia* venom combined with silica nanoparticles enhances the functioning of normal lymphocytes through PI3K/AKT, NFkappaB and ERK signaling. *Lipids in Health and Disease, 11*(1), 27.
92. Sayed, D., Al-Sadoon, M. K., & Badr, G. (2012). Silica nanoparticles sensitize human multiple myeloma cells to snake (*Walterinnesia aegyptia*) venom-induced apoptosis and growth arrest. *Oxidative Medicine and Cellular Longevity, 2012*, 386286.

93. Elsadek, B., & Kratz, F. (2012). Impact of albumin on drug delivery–new applications on the horizon. *Journal of Controlled Release, 157*(1), 4–28.
94. Yang, C., Wang, J., Chen, D., Chen, J., Xiong, F., Zhang, H., et al. (2013). Paclitaxel-Fe_3O_4 nanoparticles inhibit growth of CD138(−) CD34(−) tumor stem-like cells in multiple myeloma-bearing mice. *International Journal of Nanomedicine, 8*(1), 1439–1449.
95. Otsuka, H., Nagasaki, Y., & Kataoka, K. (2003). PEGylated nanoparticles for biological and pharmaceutical applications. *Advanced Drug Delivery Reviews, 55*(3), 403–419.
96. Avgoustakis, K. (2004). Pegylated poly(lactide) and poly(lactide-co-glycolide) nanoparticles: Preparation, properties and possible applications in drug delivery. *Current Drug Delivery, 1*(4), 321–333.
97. Knop, K., Hoogenboom, R., Fischer, D., & Schubert, U. S. (2010). Poly(ethylene glycol) in drug delivery: Pros and cons as well as potential alternatives. *Angewandte Chemie (International Ed. in English), 49*(36), 6288–6308.
98. Hatakeyama, H., Akita, H., & Harashima, H. (2011). A multifunctional envelope type nanodevice (MEND) for gene delivery to tumours based on the EPR effect: A strategy for overcoming the PEG dilemma. *Advanced Drug Delivery Reviews, 63*(3), 152–160.
99. Valle, J. W., Armstrong, A., Newman, C., Alakhov, V., Pietrzynski, G., Brewer, J., et al. (2011). A phase 2 study of SP1049C, doxorubicin in P-glycoprotein-targeting pluronics, in patients with advanced adenocarcinoma of the esophagus and gastroesophageal junction. *Investigational New Drugs, 29*(5), 1029–1037.
100. Matsumura, Y., Hamaguchi, T., Ura, T., Muro, K., Yamada, Y., Shimada, Y., et al. (2004). Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin. *British Journal of Cancer, 91*(10), 1775–1781.
101. Tong, R., & Cheng, J. (2007). Anticancer polymeric nanomedicines. *Polymer Reviews, 47*(3), 345–381.
102. Singer, J. W. (2005). Paclitaxel poliglumex (XYOTAX, CT-2103): A macromolecular taxane. *Journal of Controlled Release, 109*(1–3), 120–126.
103. Young, C., Schluep, T., Hwang, J., & Eliasof, S. (2011). CRLX101 (formerly IT-101)-a novel nanopharmaceutical of camptothecin in clinical development. *Current Bioactive Compounds, 7*(1), 8–14.
104. Lammers, T., Kiessling, F., Hennink, W. E., & Storm, G. (2012). Drug targeting to tumors: Principles, pitfalls and (pre-) clinical progress. *Journal of Controlled Release, 161*(2), 175–187.
105. Tagami, T., Ernsting, M. J., & Li, S. D. (2011). Efficient tumor regression by a single and low dose treatment with a novel and enhanced formulation of thermosensitive liposomal doxorubicin. *Journal of Controlled Release, 152*(2), 303–309.
106. Seetharamu, N., Kim, E., Hochster, H., Martin, F., & Muggia, F. (2010). Phase II study of liposomal cisplatin (SPI-77) in platinum-sensitive recurrences of ovarian cancer. *Anticancer Research, 30*(2), 541–545.
107. Clinical Trials.gov. *A phase I trial of nanoliposomal CPT-11 (NL CPT-11) in patients with recurrent high-grade gliomas*. Retrieved from http://clinicaltrials.gov/show/NCT00734682
108. Tardi, P., Johnstone, S., Harasym, N., Xie, S., Harasym, T., Zisman, N., et al. (2009). *In vivo* maintenance of synergistic cytarabine: Daunorubicin ratios greatly enhances therapeutic efficacy. *Leukemia Research, 33*(1), 129–139.