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Nanoparticle delivery systems, general approaches and their implementation in Multiple Myeloma

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Abstract

Multiple myeloma (MM) is a hematological malignancy that remains incurable, with relapse rates greater than 90%. The main limiting factor for the effective use of chemotherapies in MM is the serious side effects caused by these drugs. The emphasis in cancer treatment has shifted from cytotoxic, non-specific chemotherapies to molecularly targeted and rationally designed therapies showing greater efficacy and fewer side effects. Traditional chemotherapy has shown several disadvantages such as lack of targeting capabilities, systemic toxicity and side effects; low therapeutic index, as well as, most anticancer drugs have poor water solubility. Nanoparticle delivery systems (NPs) are capable of targeting large doses of chemotherapies into the target area while sparing healthy tissues, overcoming the limitations of traditional chemotherapy.

Here, we review the current state-of-the-art in nanoparticle-based strategies designed to treat multiple myeloma. Many nanoparticle delivery systems have been studied for myeloma using non-targeted NPs (liposomes, polymeric NPs, and inorganic NPs), triggered NPs, as well as targeted NPs (VLA-4, ABC drug transporters, bone microenvironment targeting). The results in preclinical and clinical studies are promising; however, there remains much to be learned in the emerging field of nanomedicine in myeloma.

Keywords

Multiple myeloma; Nanoparticles; Passive Targeting; Triggered Targeting; Targeted Targeting

1. INTRODUCTION

Multiple myeloma (MM) is a hematological malignancy of plasma cells localized in the bone marrow. Despite recent advances in therapy, MM remains incurable with relapse rates greater than 90% (1, 2). The main limiting factor for the effective use of chemotherapies in

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CONFLICTS OF INTEREST:

Dr. Azab receives research support from Verastem, Selexys, Karyopharm, Cell Works, Cleave Bioscience, Glycomimetics, and Vasculox; and is the founder and owner of Targeted Therapeutics LLC and Cellatrix LLC. Dr. de la Puente is co-founder of Cellatrix LLC. Moreover, Drs. Azab and de la Puente have a provisional patent application describing a novel nanoparticulate drug-delivery system for multiple myeloma.

MM is the serious side effects caused by these drugs. The development of specific and targeted therapies based on nanoparticle delivery systems in MM is under investigation.

Over the last decades, a large number of nanoparticle delivery systems have been designed for cancer therapy. The emphasis in cancer treatment has shifted from cytotoxic, non-specific chemotherapies to molecularly targeted and rationally designed therapies showing greater efficacy and fewer side effects (3). Many drugs are hydrophobic and poorly water-soluble; properties which help them to penetrate the cell membrane and reach intracellular targets, however, its therapeutic application is associated with poor absorption and low bioavailability (4-7). Therefore, NPs mainly aim to minimize drug degradation upon administration, prevent undesirable side effects, and increase drug bioavailability in the pathological area (8-10).

This review will provide an insight into the advances in nanoparticle delivery systems focused on oncology therapeutics and in particular in MM.

2. NANOPARTICLE DELIVERY SYSTEMS IN CANCER

Current treatment for cancer relies on chemotherapy as a major strategy. Traditional chemotherapy has shown several disadvantages such as lack of targeting capabilities, affecting normal healthy tissues; non-specific distribution, producing systemic toxicity and side effects; low therapeutic index, and most anticancer drugs have poor water solubility (11-13). Nanoparticle delivery systems are aimed to target higher doses of active agents into the tumor areas while sparing healthy tissues, overcoming the limitations of traditional chemotherapy (9, 10). The delivery of anticancer drugs through a nanoparticle delivery system offers multiple attractive opportunities (Table 1), including: 1) improved stability and delivery of poorly soluble in water drugs, permitting re-evaluation of drugs with poor pharmacokinetics, high cytotoxicity or poor cellular uptake (14); 2) controlled release in a specific location triggered by an specific stimuli, protection of the drug from harsh environment (acidic environment, proteases, or lysosomes of cells) extending half-life of the drug in the circulation, and controlled release over time to achieve a drug dose within a therapeutic window (10, 15-18); 3) targeting properties can be enhanced to deliver drugs specifically to a cell or tissue, reducing systemic side effects, increasing the concentration of the drug in the target area, and increasing therapeutic index for the chemotherapeutic agent (19); and 4) nanoparticle delivery systems offer a multifunctional approach to deliver combination therapy of drugs, as well as, imaging agents to improve detection, imaging, and treatment of cancer (20, 21).

Nanoparticle delivery systems are very diverse in function of shapes, sizes, and surface-properties (Figure 1): **Liposomes:** are vesicles made of a bilayer of amphiphilic lipids enclosing a hydrophilic core, which can carry hydrophilic drugs within the aqueous core area while hydrophobic drugs within the hydrophobic region of the bilayer(22) (Figure 1a). **Polymeric NPs:** are particles prepared from polymers, they can be biodegradable or non-biodegradable, synthetic or natural and the drug is dissolved, entrapped, encapsulated or attached to the matrix (23) (Figure 1b). **Micelles:** are artificial vesicles similar to liposomes but made of a monolayer of amphiphilic lipids enclosing a hydrophobic core, which can

carry hydrophobic anticancer agents (Figure 1c). **Dendrimers**: are repetitively branched molecules consisting of radially symmetric molecules of tree-like arms or branches (Figure 1d). **Polymersomes:** are artificial vesicles made of a bilayer of synthetic amphiphilic block copolymers enclosing a hydrophilic core, which can carry hydrophilic drugs within the aqueous core, therefore presenting a similar structure to liposomes (Figure 1e). **Inorganic NPs** are particles formed by the crystallization of inorganic salts, forming a three-dimensional arrangement with linked atoms (24) (Figure 1f).

The delivery of anticancer drugs through nanoparticle delivery systems can be achieved in three ways: take advantage of pathophysiological conditions of the tumors (passive targeting) (Figure 2), induction of drug release once in the tumor through triggered or inducible approaches (triggered targeting) (Figure 3), or addition of high-affinity ligands to target the tumor to the surface of nanoparticle delivery systems (active targeting) (Figure 4).

Passive targeting

Non-targeted NPs exploits passive targeting approaches taking advantage of pathophysiological conditions of the tumor microenvironment for specific delivery, such as enhanced permeation and retention (EPR) effect: most solid tumors have blood vessels with aberrant architecture and extensive production of vascular permeability factors stimulating extravasation within tumor tissues, in addition to lack of lymphatic drainage. Therefore, tumors exhibit enhanced vascular permeability, which will ensure a sufficient supply of nutrients and oxygen to tumor tissues for rapid growth (25-27). The tumor vasculature is formed by poorly aligned defective endothelial cells with wide fenestrations (up to 4 μm), leading to EPR effect, which facilitates transport of macromolecules (>40 kDa) and small-sized NPs (10 - 400 nm) into tumors (28-30). EPR is the gold-standard technique for drug delivery systems for cancer treatment (Figure 2). However, large tumors do not exhibit the EPR effect and show less accumulation of macromolecules in central areas (31).

Triggered targeting

A selective and controlled delivery of drugs in the tumors is still a challenge. The introduction of stimuli-responsive mechanisms to drug delivery systems help to provide better solutions for tumor drug targeting strategies. NPs can be induced to release the drugs once in the tumor through triggered or inducible approaches (32, 33). Several factors (such as pH (34-37), temperature (38-41), magnetic field (42, 43), ultrasound (44, 45) or light exposure (46, 47)) have been shown to increase the accumulation of NPs and/or enhance the release of the drugs from the NPs in the tumor (Figure 3).

Active targeting

The EPR effect and PEG-stealth have improved biodistribution and circulation of nanoparticle delivery systems. Stimuli-triggered NPs also have improved controlled release in the tumor tissue. However, better methods are required to increase the number of NPs that accumulate in the target tissue and reduce the amount of NPs that are concentrated in organs such as liver, spleen, and bone marrow due to clearance by mononuclear phagocytic system (MPS). The main approach in the active targeting consists of ligand-based targeting, these ligands facilitate binding to a marker or receptor overexpressed in the targeted tumor cells,

triggering receptor-mediated endocytosis, or targeting tumor microenvironment extracellular matrix or surface receptors on tumor blood vessel endothelial cells (48-54) (Figure 4).

Even though nanoparticles have shown very promising results in cancer, there are two main disadvantages of NPs, which need to be overcome for successful usage: 1) **Clearance:** while NPs smaller than 6 nm are rapidly cleared by the kidneys, larger NPs either accumulate in a lesion or are cleared by the mononuclear phagocytic system (MPS) accumulating in lymph nodes, spleen, and liver (55, 56). A strategy to improve circulation half-life consist on the conjugation of hydrophilic polymers such as polyethylene glycol (PEG) to the NPs surface, which confers them long-circulation due to stealth properties by reducing opsonization (57, 58). 2) **Toxicity:** Nanoparticles toxicity depends not only on their chemical composition, but also on the composition of any chemicals adsorbed onto their surfaces (59). As a solution, the surfaces of nanoparticles can be modified to make them less harmful.

3. NANOPARTICLE DELIVERY SYSTEMS FOR MYELOMA

Multiple myeloma (MM) is an incurable disease characterized by the proliferation of plasma cells in the bone marrow (60), and represents approximately 20% of deaths from hematological malignancies (61). Several chemotherapeutic regimens have been used in the management of MM, including anthracycline antibiotics (doxorubicin), glucocorticoids (dexamethasone and prednisone), and nitrogen mustard alkylating agents (melphalan) (62-66). The introduction of novel agents, such as immunomodulatory drugs (IMiDs) thalidomide, lenalidomide, and pomalidomide; and the proteasome inhibitors (PIs) bortezomib and carfilzomib has significantly improved survival in MM (66-69). Despite the introduction of the previous novel therapies, more than 90% of MM patients relapse or become refractory to those treatments (1, 2).

The main limiting factor for the effective use of chemotherapies in MM is the serious side effects caused by these drugs. The use of the PIs bortezomib and carfilzomib has led to significant improvement of the survival of MM patients (66). However, treatment with bortezomib is limited by its neurotoxicity, especially in the peripheral nerves, which leads to sensory axonal neuropathy (70). Carfilzomib is a second generation proteasome inhibitor, but the safety data from a meta-analysis reported thrombocytopenia, anemia, fatigue, nausea, and diarrhea as the most common adverse events, with dose-limiting neutropenia or peripheral neuropathy (71). Immunomodulatory drugs are emerging promising therapies in MM which showed synergistic effects when they are added to current treatments (69). Nevertheless, one-fourth of patients discontinued thalidomide because of toxicity, including peripheral neuropathy, constipation, somnolence, and fatigue as common side-effects (72). Moreover, cutaneous adverse neutropenia, deep vein thrombosis, infection, and hematologic cancer were observed in patients treated with lenalidomide (73, 74). Dose limiting neutropenia, thrombocytopenia, neuropathy, and deep vein thrombosis were common adverse effects observed in patients treated with pomalidomide (75).

Nanoparticle delivery systems offer a new strategy to increase the efficacy of the treatment and reduce side effects in normal tissues by delivering drugs to the target tissue, in this case the bone marrow niche in which myeloma cells developed. Table 2 and 3 provides an insight

into the preclinical and clinical advances in nanoparticle delivery systems used in multiple myeloma.

3. 1. Non-targeted therapies

Non-targeted approaches exploit passive targeting based on the pathophysiological conditions of the myeloma microenvironment for specific delivery of the drugs. Most of the nanoparticle delivery systems that have been studied for myeloma used non-targeted NPs.

a. **Liposomes** have been used to encapsulate bortezomib, a proteasome inhibitor with high neurotoxicity. Due to the poor water solubility of bortezomib, instead of loading the drug in the aqueous core (76), direct conjugation of bortezomib to the surface of liposomes via a reversible boronic ester linkage was used (77). Liposomal bortezomib NPs showed size ranges of 100 nm with high reproducibility and encapsulation efficiency of 80%. In vitro studies were performed to study proteasome inhibition, apoptosis, and cell viability. Liposomal bortezomib NPs inhibited proteasome activity, induced apoptosis and cytotoxicity on MM cells. In the in vivo studies SCID mice were injected subcutaneously with MM cells, treated with free drug or liposomal bortezomib NPs intravenously (i.v.) on days 1 and 4 at a dose of 1 mg/kg bortezomib equivalent, and analyzed for tumor progression and systemic toxicity. The results revealed that liposomal bortezomib NPs were efficacious in tumor growth inhibition, and reducing systemic side effects measured by body weight loss. Free drug group showed >20% weight loss and moribundity on day 7, which led to required sacrificed of the mice. In case of liposomal bortezomib NPs there was <10% loss in body mass during the 2 week study period (77) (Table 2). However, even though reduced side effects, distribution studies should have been done to verify the location of the NPs.

   Liposomes have been also used for incorporation of anti-estrogens to prevent oral administration of an anti-estrogen being widely distributed to the whole body and reaching unwanted tissues. Liposomes size was around 100 nm and encapsulation efficiency of >90%. Loaded liposomes were administered i.v. at a dose of 12 mg anti-estrogen/kg/week in a MM xenograft model. Loaded liposomes induced the arrest of tumor growth in contrary to free anti-estrogen or to empty liposomes (78) (Table 2). Further studies of the drug delivery of anti-estrogens in tumors which express estrogen receptors will be required, as well as, distribution of these NPs and systemic toxicities should be investigated.

   The first nanoparticle delivery system approved by the FDA for clinical use in multiple myeloma was a PEGylated liposomal doxorubicin. It has been used use in combination with other anti-myeloma therapeutics (bortezomib, or vincristine and dexamethasone). Relapsed or refractory multiple myeloma patients received PEGylated liposomal doxorubicin (Tibotec Therapeutics) administered on day 4 at 30 mg/m^2 and bortezomib given on days 1, 4, 8, and 11 from 0.90 to 1.50 mg/m^2. Time to progression (TTP) was significantly prolonged in the combination arm (median TTP = 9.3 months) compared with bortezomib

monotherapy (median TTP = 6.5 months) (79-81) (Table 3). Newly diagnosed multiple myeloma patients received intravenous PEGylated liposomal doxorubicin (40 mg/m^2), vincristine (2.0 mg, Day 1), and oral or intravenous dexamethasone (40 mg per day for 4 days) every 4 weeks for six or more cycles and/or for two cycles after the best response. The overall response rate was 88% with 12% complete remission. TPP was 23.1 months, with 2-year and 3-year progression-free survival rates of 42% and 23%, respectively (82) (Table 3). These results are very encouraging for clinical efficacy of NPs on MM, although further follow-up results need to be supplemented.

b. **Polymeric NPs** such as PLGA-PEG NPs have been developed to encapsulate thymoquinone, an anti-inflammatory and anti-cancer natural product derived from the medicinal spice black cumin, which has problems of bioavailability (83). PLGA-PEG NPs encapsulating thymoquinone showed size around 200 nm with homogeneous distribution and encapsulation efficiency of 94%. Thymoquinone PLGA-PEG NPs had anti-proliferative effect on MM cells and these NPs were more potent than free drug sensitizing leukemic cells to TNF-α and paclitaxel-induced apoptosis (84) (Table 2). However, this study is very preliminary, only in vitro studies were realized. In vivo studies to prove efficacy and distribution will demonstrate the potential of thymoquinone NPs in myeloma.

   Other polymeric NPs are nanocolloids based on N,N,N-trimethyl chitosan have been developed to encapsulate camptothecin, a potent anticancer agent of plant origin, which is extremely water insoluble (85). In vitro cytotoxicity showed no statistical difference between loaded nanocolloids and free drug. However, loaded nanocolloids more effectively inhibited tumor growth and prolonged survival time than free drug in vivo. In this case the murine Balb/c myeloma model was treated with i.v. injections of loaded nanocolloids (2.5 mg/kg), free drug (2.5 mg/kg), or nanocolloids (25 mg/kg) every 3 days for 15 days (86) (Table 2). Further studies of distribution and systemic toxicities should be examined.

c. **Micelles** composed of biodegradable block copolymers of PEG and poly-(caprolactone) (PCL) were developed to improve the metabolic stability of the proteasome inhibitor carfilzomib against enzyme-mediated degradation and delivering of this poorly water soluble drug in a controlled manner (87). Drug loading efficiency was around 2 – 4% and encapsulation efficiency around 20 – 40%. Micelles showed improved stability profiles with at least 50% of the active carfilzomib remaining after 20 minutes of incubation in mouse liver homogenates. Micelles were tested in RPMI-8226 cell line and shown comparable anticancer effect compared with free carfilzomib (88) (Table 2). However, this study is very preliminary, only in vitro studies were realized. In vivo studies to confirm improved stability of carfilzomib, and efficacy and distribution of the micelles will demonstrate the potential of this approach in myeloma.

d. **Inorganic NPs** have been developed for the treatment of myeloma. Silica NPs have been combined with snake venom from *Walterinnesia aegyptia*, a natural toxin with antitumor potential (89). The particle size was 300 nm and the combination of the silica NPs with the snake venom was tested on cells from 5 myeloma patients and XG2 cell line. This combination decreased viability and induced apoptosis (90). In a follow-up publication enhanced anticancer efficacy was detected in a murine MM model with loaded NPs subcutaneously (s.c.) injected (1 μg/kg/day) in the tumor site compared to treatment with NPs or vehicle (91) (Table 2). Nevertheless, distribution of silica NPs combined with snake venom, systemic toxicities, and comparison to standard care of treatment in MM should be addressed in the future.

   Moreover, iron oxide NPs have been investigated in MM. While paclitaxel is an effective anticancer drug with poor solubility in water, Abraxane® (Celgene, Summit, NJ, USA) is a water-soluble commercially available nanoparticle albumin-bound paclitaxel-loaded Fe_3O_4 nanoparticle (92), approved by the FDA for the treatment of metastatic breast cancer. Paclitaxel Fe_3O_4 NPs were used to treat CD138- CD34- tumor stem-like cells in multiple myeloma-bearing mice. The NPs size was 7 nm, with good stability and sustained-drug release. Tumor growth was more inhibited when treated with placitaxel Fe_3O_4 NPs (0.6 - 2 mg/kg once a week for 2 weeks) compared to NPs alone or drug alone, as well as detection of induced apoptosis of tumor cells in treated mice (93) (Table 2). Nonetheless, distribution of these NPs, effect on CD138+ non-stem cell-like tumors, and systemic toxicities should be investigated.

The main disadvantages of non-targeted NPs are associated to problems in pharmacokinetic and pharmacodynamics, including their dependency on abnormal leakiness of blood vessels and lack of specificity. Therefore, new techniques are needed to improve the accumulation of NPs at the disease site.

3. 2. Triggered therapies

The combination of magnetic hyperthermia treatment through **magnetic NPs** and remote-control drug release from the same NPs using heat as the trigger to release the drugs have been used in MM. Doxorubicin magnetically responsive NPs were injected intratumorally (i.t.) 5 mg/kg (0.13 mg/kg doxorubicin equivalent) into mice bearing subcutaneous xenograft tumors and then exposed to the magnetic field. Combination of chemotherapy and magnetic hyperthermia NPs decreased tumor volume gradually, until reaching zero tumor volume at day 45 without further recurrence (94) (Table 2). In addition, no significant toxicity was found. These results with i.t injection showed promising results, i.v injection as more general approach and related-distribution should be tested in the future. One limitation of this approach is the ability to treat only the tumor site with well-defined localization in case of further clinical evaluation.

3. 3. Targeted therapies

In general, targeted NPs used the same nanoparticle delivery systems of non-targeted therapies, but in this case NPs contain a targeted-ligand to help NPs to be directed towards

tumor cells, tumor blood vessel endothelial cells, or the tumor microenvironment. The strategies used in MM to date are the following:

a. **Very Late Antigen-4 (VLA-4)** is an integrin receptor expressed on cancer of hematopoietic origin, such as MM, and is a key adhesion molecule in MM associated with cell adhesion mediated drug-resistance (CAM-DR). Therefore, VLA-4 is a relevant surface receptor to target MM cells (95, 96). The use of VLA-4 targeted NPs has been explored to enhance the targeting of VLA-4 expressing MM cells. VLA-4 antagonist peptide and pH-sensitive doxorubicin were incorporated in lipid NPs. These NPs had a size of 20 nm, induced cytotoxicity and apoptosis on MM cells in vitro, and overcame CAM-DR. In vivo, 6 mg/kg doxorubicin equivalent nanoparticles were injected on days 1, 3 and 5 on mice with palpable tumors. Free drug group showed >15% weight loss and moribundity on day 7, which led to required sacrifice of the mice. In case of targeted NPs there was around 10% loss in body mass. Targeted NPs demonstrated enhancement in tumor growth inhibition compared to non-targeted NPs, as well as, significant higher accumulation in the tumor (97) (Table 2).

   Another study has developed liposomal carfilzomib NPs, such as the ones previously described for bortezomib (77), enriched with a VLA-4 antagonist peptide. NPs were stable and reproducible with size 70 nm, and loading efficiency of 98%. In vitro studies showed that VLA-4 targeted liposomal carfilzomib NPs were cytotoxic for MM cells and induced apoptosis. In vivo, SCID mice with subcutaneously injected tumors were treated with i.v injection at a dose of 5 mg/kg of carfilzomib free or in NPs on days 1, 2, 8, and 9, and analyzed for tumor progression and systemic toxicity. The results revealed that targeted liposomal carfilzomib NPs were efficacious in tumor growth inhibition, and reducing systemic side effects measured by body weight loss. Free drug group showed >15% weight loss and moribundity on day 4, which led to required sacrificed of the mice. In case of liposomal carfilzomib NPs (targeted and non-targeted) there was <10% loss in body mass. Non-targeted NPs demonstrated less enhancement in tumor growth inhibition compared to targeted liposomal carfilzomib NPs, which had preferential accumulation at the MM tumor (98) (Table 2).

   A recent publication in 2015 combined VLA-4 targeted NPs with an inhibitor of MYC-MAX dimerization (MYC transcription factor is an oncoprotein activated in MM), which had poor bioavailability and rapid metabolism (99). VLA-4 targeted perfluorocarbon NPs and polysorbate micelles were developed with size of 200 and 20 nm, respectively. In a KaLwRij metastatic mouse model NPs were injected i.v on days 3, 5, 7, 10, 12, and 14 at an equivalent dose of 0.145 mg/ml of inhibitor. The smaller targeted NPs loaded with the inhibitor conferred survival benefits compared to free drug, larger targeted NPs, or non-targeted NPs (100) (Table 2).

   The use of VLA-4 integrin targeting strategies has led to very promising results with several different drug combinations. However, VLA4-4 expression on

human myeloma cells is very heterogeneous, with even negligible expression in some MM cells, as well VLA-4 is expressed in many other non-myeloma cells (97, 100). Prescreening of myeloma cell surface biomarkers expression on a personalized medicine approach will be required in case of clinical approach.

b. **ATP-binding cassette (ABC) drug transporters,** such as ABCG2 (breast cancer resistance protein 1), enable cancer cells to not be affected by the cytotoxic effects of chemotherapy that kill most cells in a tumor (101). It has been shown that MM expresses ABC transporters (102-104). Previously described paclitaxel Fe_3O_4 NPs (93) were targeted to the ABCG2 transporter overexpressing MM cancer stem cells with monoclonal antibodies (mAbs). Multiple myeloma cancer stem cells (CSC) mouse model was locally treated once a week with equivalent of 10 μg mAb and 8 μg paclitaxel NPs for 4 weeks. Targeted NPs inhibited tumor growth, increased survival by inducing apoptotic pathways, and showed less toxic side effects in comparison with the single-agent treatments (105, 106) (Table 2). Targeted CSC therapy using mAb in combination with nanoparticle delivery systems is a promising strategy to target MM CSCs for refractory MM patients. This is because the therapeutic strategy has the specific action on the MM CSCs and the proven therapeutic efficacy at lower drug dosages as well as preventing the paclitaxel efflux from cells for generating the cytotoxic effects on the MM CSCs. Further investigation is required in this promising targeted approach.

c. **Bone microenvironment targeting,** aimed to target the bone mineral component of the bone marrow microenvironment instead myeloma cells directly. Seventy percent of the bone is made up of the inorganic mineral hydroxyapatite, which includes several types of calcium forms. Bisphosphonates-based drugs preferentially stick to calcium and bind to it upon systemic administration (107). Alendronate (a bisphosphonate) targeted PLGA-PEG NPs encapsulating bortezomib have been engineered to target selectively the bone material in the bone marrow. NPs were elaborated through nanoprecipitation and single emulsion with size 75 nm and 195 nm, and 5.4% and 24% encapsulation efficiency, respectively. In vitro, targeted NPs indicated good binding to hydroxyapatite and induction of apoptosis in same way that non-targeted NPs loaded with bortezomib. In vivo biodistribution after 24h of intraperitoneally (i.p) injection showed increase retention of targeted NPs in spleen, femurs and skull, with around 9-fold increase of targeted compared to non-targeted NPs in bone sections. SCID mice were injected with MM cells and after 21 days, treatment was injected i.p twice a week with 0.5 mg/kg bortezomib equivalent. Targeted and non-targeted NPs inhibited MM growth in vivo and increased survival in the same way that free drug. In addition, pre-treatment thrice a week for 21 days with 0.3 mg/kg bortezomib equivalent before injection of MM cells inhibited myeloma growth and increased survival compared to free drug (108) (Table 2). Nonetheless, non-significant differences were found between targeted and non-targeted NPs. The study showed that bortezomib, as a pretreatment regimen, modified the bone microenvironment, and loaded NPs enhanced

survival and decreased tumor burden. However, biodistribution studies in organs such as lung, liver and kidney should be tested, and the reduction of off-side effects provided.

4. FUTURE DIRECTIONS

The application of NPs to improve delivery of drugs is a revolutionary approach to advance the treatment of cancer and many other diseases. The emphasis of NPs focus on the delivery of drugs more efficiently to reduce side effects. Several liposomes and polymeric NPs have been approved by the FDA for clinical use in cancer, and many others are under investigation in several clinical trials, including indications such as solid tumors, and even hematological malignancies (acute myeloid leukemia) (12, 109). NPs offer the option to deliver therapeutics with improved pharmacokinetics, safety profiles, and biodistribution, allowing us to reconsider chemotherapies previously discarded due to lack of targeting capabilities, non-specific distribution, and side effects.

Contributions of NPs to Multiple Myeloma treatment

Nanoparticle delivery systems can improve MM treatment in two main ways: 1) increase the specificity of treatments based on improve potency and efficacy by hitting the right target, the bone marrow niche in which myeloma cells developed. 2) At the same time, NPs offer the advantage to reduce side effects in normal tissues by delivering drugs to the target tissue. This will be allowed through delivery of higher doses to the MM cells and lower the doses to other tissues. Therefore, the ability to improve potency or efficacy of drug treatments combined with the reduction of side effects may improve disease outcomes besides suppressing drug adverse effects.

Challenges of NPs for Multiple Myeloma treatment

The EPR effect is an important factor for delivery of nanoparticle systems in general, which has been confirmed mainly for solid tumors. Although encouraging results have been shown with liposomes, polymeric NPs, micelles, and inorganic NPs using non-targeted approaches (82, 86, 88, 93), MM is a hematological malignancy and it is still not clear that the EPR effect will have a role in how non-targeted NPs exploit passive targeting based on the pathophysiological conditions of the myeloma microenvironment.

Targeted approaches containing targeted-ligands against a marker or receptor overexpressed in the targeted tumor cells is challenging in MM. The characterization of MM by a single surface marker is being questioned over the last decade. CD138, the gold-standard surface marker to detect MM cells, is shown to be affected by drug exposure and hypoxia (110). Other secondary antibodies used in combinations such as CD38, CS1 and CD20 are highly expressed in many other cell types (111). Other markers such as VLA4-4 is expressed in several cell types and the expression in MM cells is very heterogeneous (97, 100). The lack of a constitutively expressed unique surface marker in MM limits the specific active targeting of NP systems.

In addition, myeloma is a complex disease involving multiple pathways and mutations. Inhibition of a pathway by a single drug may be insufficient to achieve therapeutic efficacy.

As a result, combination therapy will let to use drugs at lower doses, reducing cytotoxic effects but increasing efficacy due to the synergistic effect of several agents targeting different pathways (112). The effectivity of combination therapy by NPs will depend on the ability of NPs to carry multiple therapeutic agents with different physicochemical properties and pharmacological behaviors, which adds more challenges to the technical development of NPs in MM.

Strategies to improve NPs for MM treatment

NPs offer multifunctionality, combining both diagnostics and therapeutics, combination known as theranosis. For successful theranosis, the efficient delivery of imaging agents and drugs is critical to provide sufficient imaging signal or drug concentration in the targeted disease site (113, 114). This will allow for monitoring drug delivery and image-guided therapy of the target site. In addition, the investigation of new molecular targets will help the ability to improve delivery at the tumor level. These benefits could include advances in detection, imaging, and treatment of myeloma. On the other hand, NPs can be designed to target multiple antitumor moieties at the same time to maximize the accuracy of tumor targeting. In this case, further investigation of myeloma cell surface markers is required to identify alternative targets with adequate expression levels.

Another consideration will be to contemplate the disadvantages of current in vitro testing techniques to effectively evaluate the behavior of nanoparticle delivery systems. The discrepancy between preclinical and clinical outcomes can be attributed to the failure of classic two dimensional culture models to accurately recapitulate the complex biology of MM and drug responses observed in patients. Three-dimensional (3D) culture systems are gaining strength as in vitro systems to assess and predict drug sensitivity in myeloma (115-117). 3D in vitro systems could help to evaluate NPs as drug delivery systems and predict better the in vivo performance of NPs.

In summary, the promising results in myeloma preclinical studies will lead to further investigation, and in a short period of time nanoparticle-based therapies will undergo more clinical investigation. Safety of these devices will be an important consideration before moving forward. Therefore, there remains much to be learned in the emerging field of nanomedicine in myeloma.

5. CONCLUSIONS

The main limiting factor for the effective use of chemotherapies in MM is the serious side effects caused by these drugs. The development of specific and targeted therapies based on nanoparticle delivery systems in MM is under investigation offering a new strategy to increase the efficacy of the treatment and reduce side effects in normal tissues by delivering drugs to the target tissue. Many nanoparticle delivery systems have been studied for myeloma using non-targeted NPs (liposomes, polymeric NPs, and inorganic NPs), triggered NPs, as well as targeted NPs (VLA-4, ABC drug transporters, bone microenvironment targeting). The promising results in myeloma preclinical and clinical studies will lead to further investigation. Therefore, there remains much to be learned in the emerging field of nanomedicine in myeloma.

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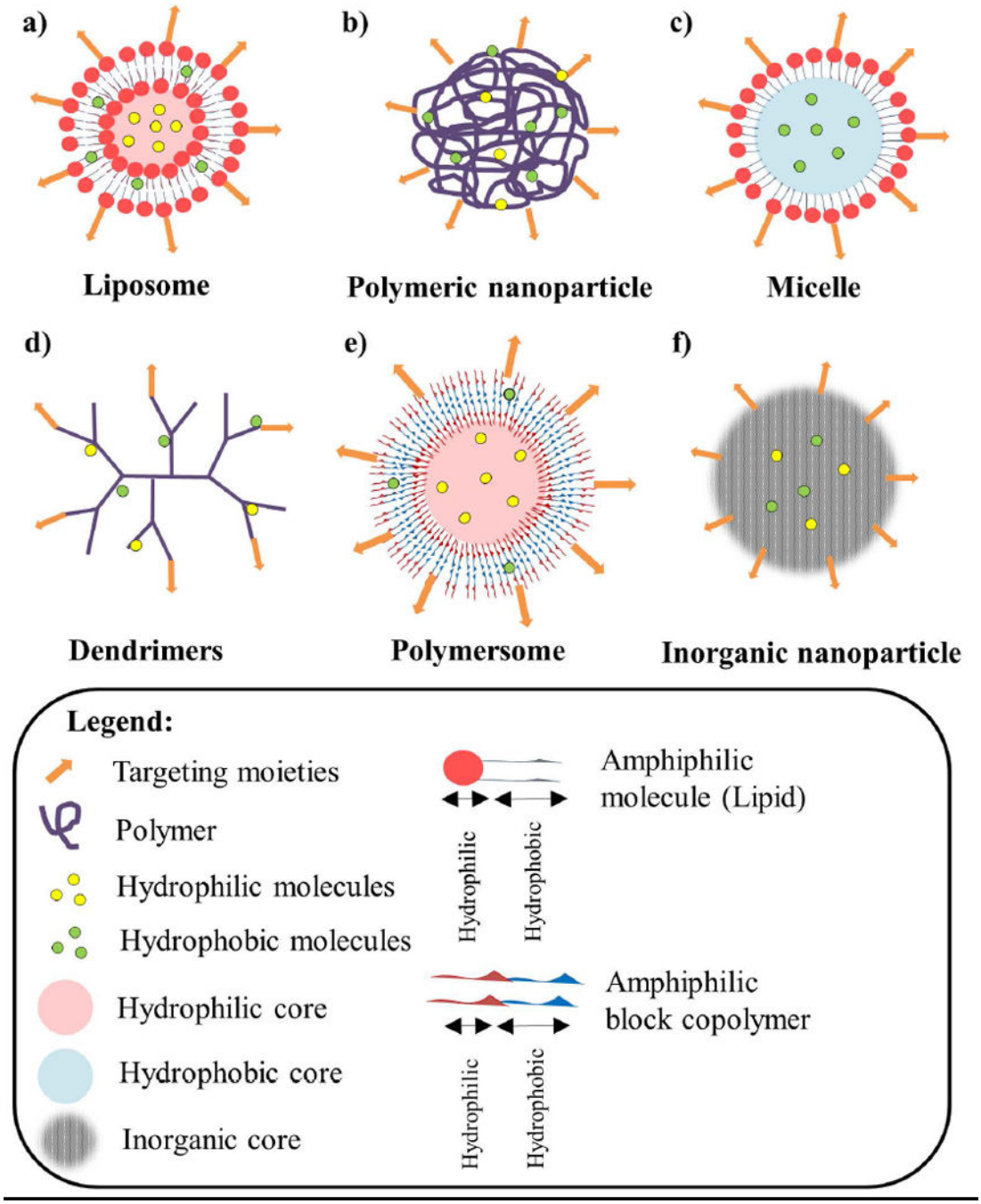

Figure 1. A summary of nanoparticles for drug delivery in cancer

a) Liposomes are vesicles made of a hydrophobic bilayer of amphiphilic lipids (composed of a hydrophilic head group and a hydrophobic tail) enclosing a hydrophilic core, which can carry hydrophilic drugs within the aqueous core area while hydrophobic drugs within the hydrophobic region of the bilayer; **b)** Polymeric NPs are made of polymers and can encapsulate hydrophilic and hydrophobic molecules. **c)** Micelles are made of a hydrophobic monolayer of amphiphilic lipids enclosing a hydrophobic core, which can carry hydrophobic anticancer agents. **d)** Dendrimers are repetitively branched molecules consisting of radially

symmetric molecules of tree-like arms or branches, which can encapsulate hydrophilic and hydrophobic molecules. **e)** Polymersomes are artificial vesicles made of a bilayer of synthetic amphiphilic block copolymers enclosing a hydrophilic core, which can carry hydrophilic drugs within the aqueous core and hydrophobic drugs within the hydrophobic region of the bilayer. **f)** Inorganic NPs are particles formed by the crystallization of inorganic salts, forming a three-dimensional arrangement with linked atoms, which can encapsulate hydrophilic and hydrophobic molecules. All the previous nanoparticles can be modified to contain specific targeting moieties for active targeting strategies.

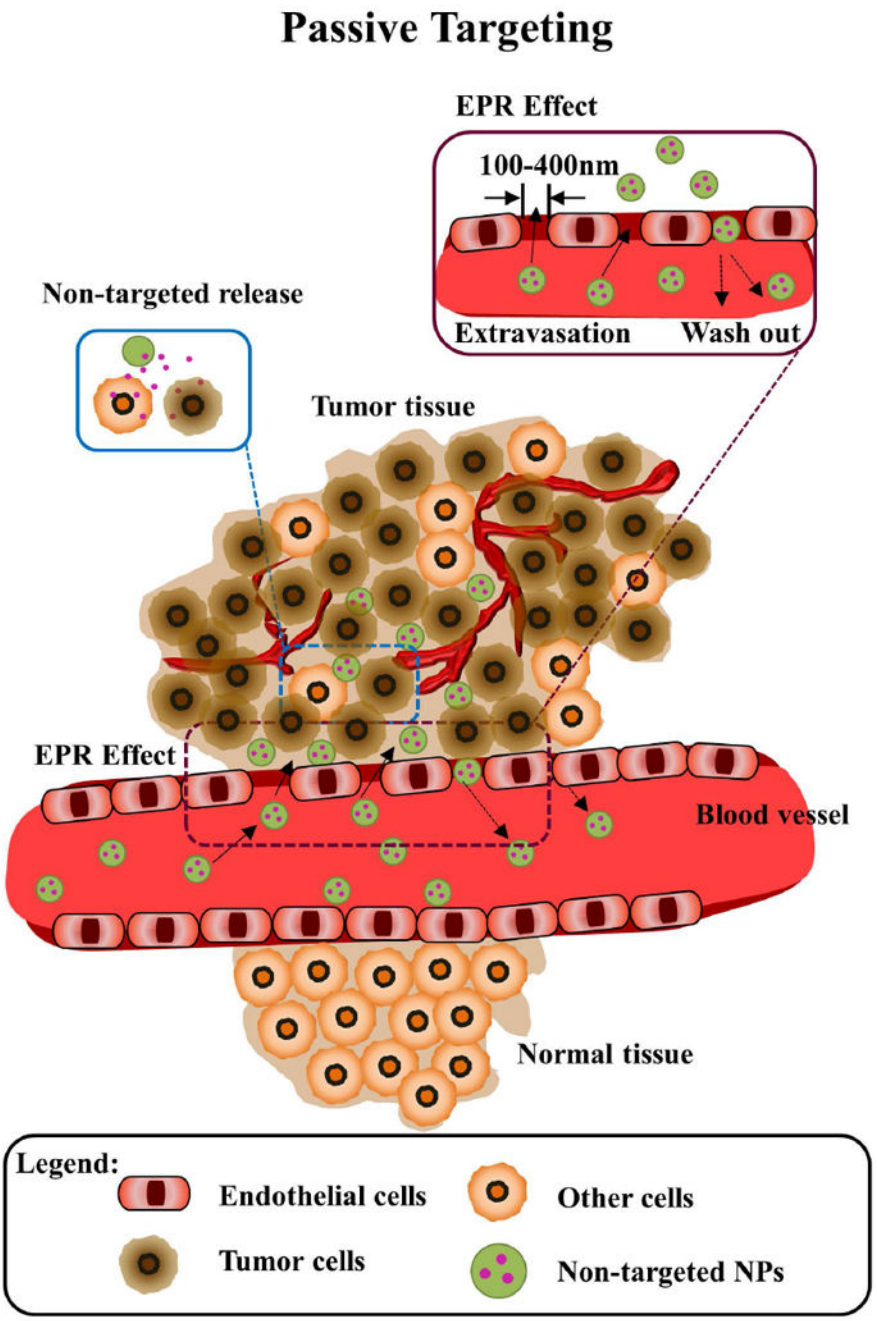

Figure 2. Schematic of passive (non-targeted) nanoparticles in cancer

Normal tissues lack of gaps between adjacent endothelial cells discarding nanoparticles to extravasate from the vasculature. Tumor tissues present large gaps (100-400 nm) between the endothelial cells on the tumor vasculature. The enhanced permeability and retention (EPR) effect allows untargeted nanoparticles of appropriate size to bind, internalize and release drugs into cancer (brown) and non-cancer cells (orange) cells due to unspecific surface adsorption inside tumor tissues. A significant part of nanoparticles can be washed out back to the blood circulation, resulting in low tumor accumulation.

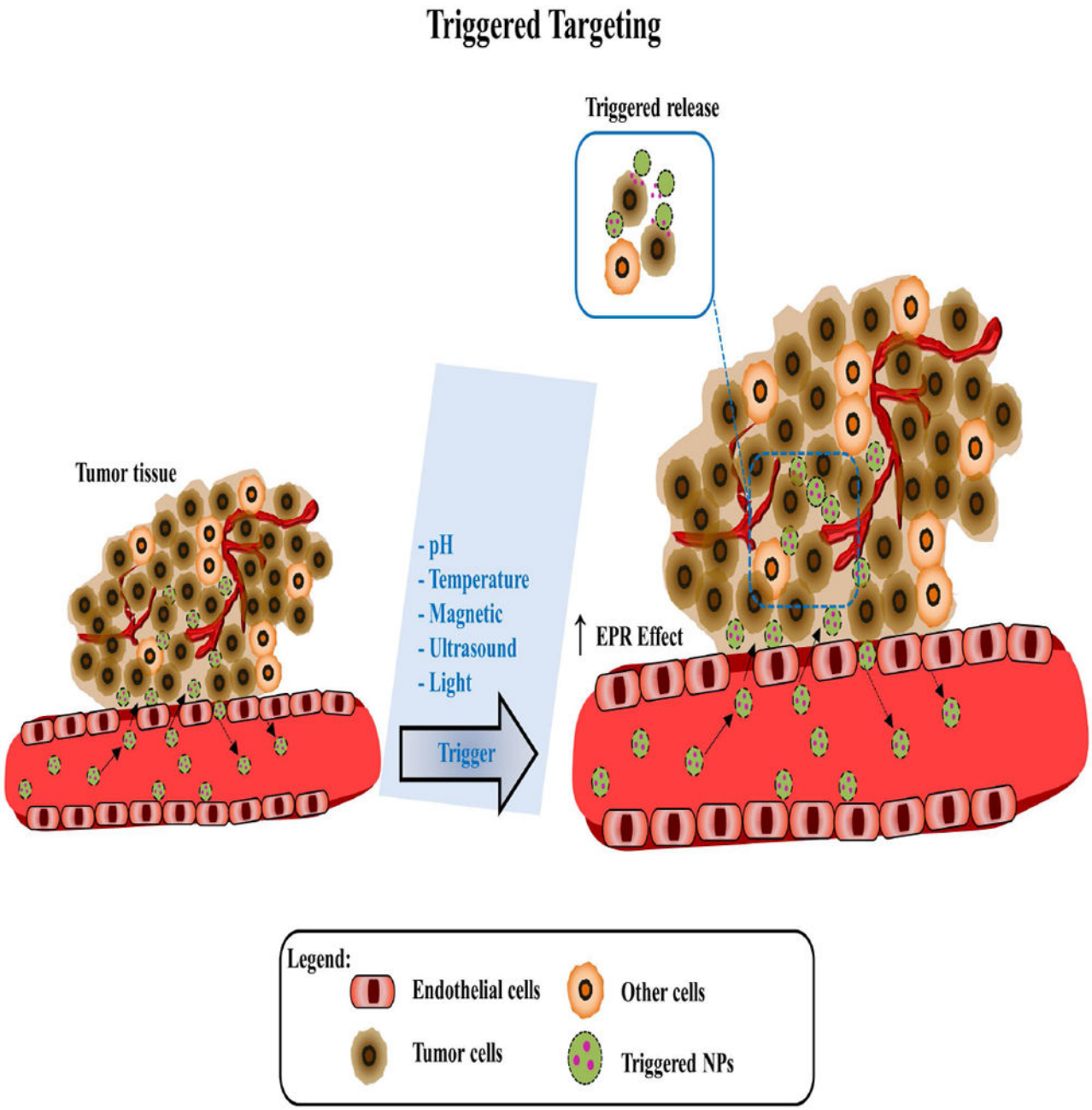

Figure 3. Schematic of triggered (inducible) nanoparticles in cancer

Triggered nanoparticles extravasate from the vasculature accumulate in the tumors due to increase EPR effect by biological factors of the tumor microenvironment or external factors (pH, temperature, magnetic field, ultrasound, and light exposure). The release is triggered by those factors into cancer cells (brown).

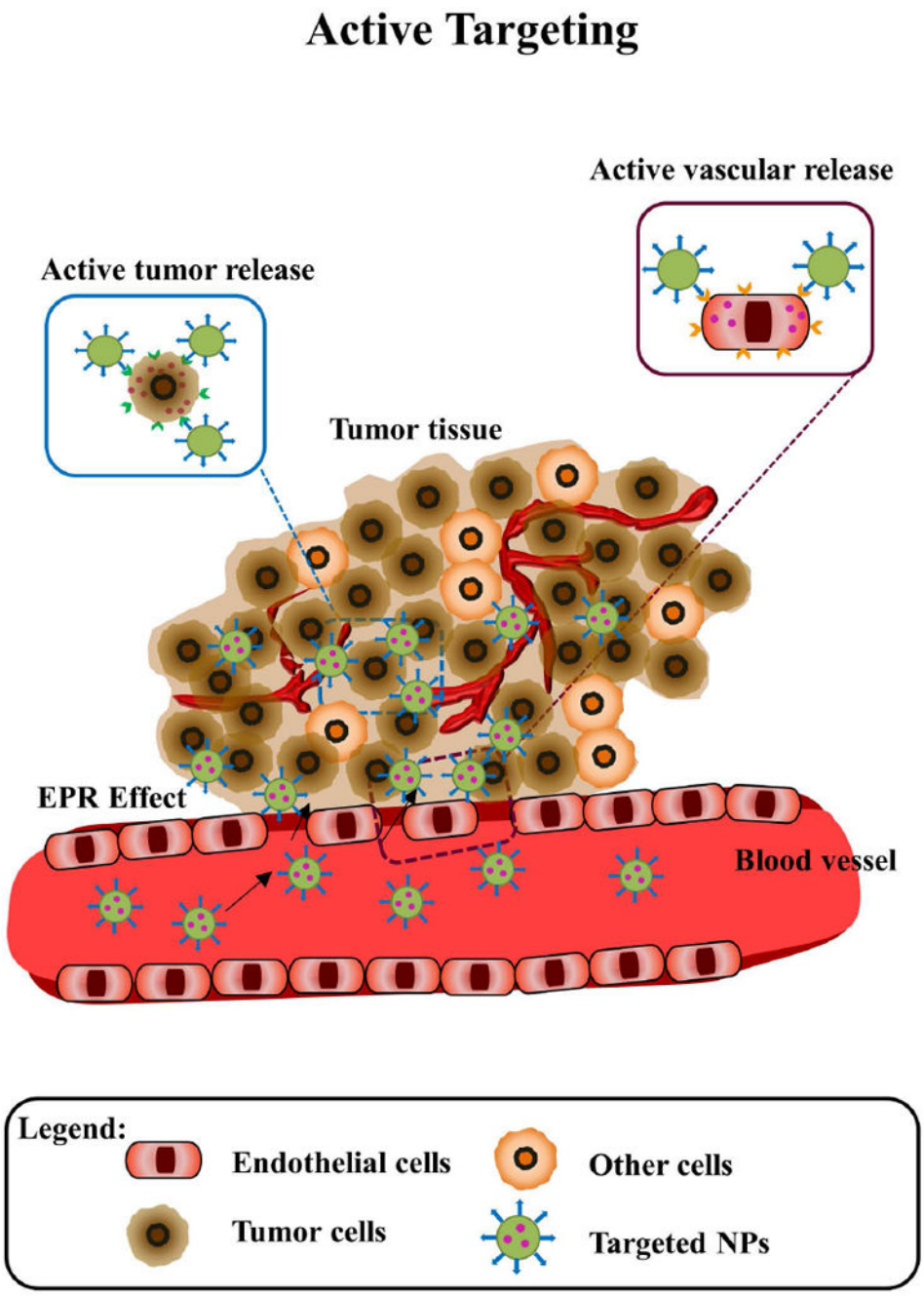

Figure 4. Schematic of active (targeted) nanoparticles in cancer

Nanoparticles extravasate from the vasculature due to EPR effect, then targeted nanoparticles bind and internalize into tumor tissues, the retention and uptake of these nanoparticles in cancer cells (brown) is augmented due to specific antigen-antibody/ ligand-receptor interactions, and wash out of nanoparticles is reduced.

Table 1

Comparison of traditional chemotherapy and nanoparticle delivery systems properties.

Property	Traditional Chemotherapy	Nanoparticle delivery systems
Drug solubility	Poor; affecting pharmacokinetics	Improved; increased stability
Controlled release	None	Controlled release time and location, and protection from environment
Targeting abilities	Non-specific targeting; non-specific distribution; systemic side effects; low drug concentration in target area; low therapeutic index	Specific targeting; lower systemic side effects; improved drug concentration in target area; improved therapeutic index
Multi-functionality	None	Combination therapy for detection, imaging and treatment

Table 2

Preclinical nanoparticle delivery systems in MM.

Type	Formulation	Drug	In vitro efficacy	In vivo efficacy	Biodistribution/ Toxicity	Ref.
Non- targeted	**Liposome**	Bortezomib	Proteasome inhibition, induction of apoptosis, and cytotoxicity	Dose: 1 mg/kg twice i.v. Tumor growth inhibition	Reduced body weight loss No biodistribution	Ashley, J.D., et al., J Med Chem, 2014. 57(12): p. 5282-92.
		Anti-estrogens	N/A	Dose: 12 mg/kg/week i.v. Tumor growth inhibition	N/A	Maillard, S., et al., J Steroid Biochem Mol Biol, 2005. 94(1-3): p. 111-21.
	Polymeric NPs (PLGA-PEG)	Thymoquinone	Anti-proliferative effect	N/A	N/A	Ravindran, J., et al., Biochem Pharmacol, 2010. 79(11): p. 1640-7
	Polymeric NPs (Chitosan)	Camptothecin	Cytotoxicity	Dose: 2.5 mg/kg thrice i.v. Tumor growth inhibition and PST	N/A	Li, Z., et al., Oncol Rep, 2012. 27(4): p. 1035-40
	Micelles	Carfilzomib	Improved stability and cytotoxicity	N/A	N/A	Ao, L., et al., J Pharmacol Exp Ther, 2015. 355(2): p. 168- 73.
	Inorganic NPs (Silica)	Snake venom from *Walterinnesia aegyptia*	Induction of apoptosis, and cytotoxicity	Dose: 1 μg/kg/day s.c. in the tumor site Enhanced anticancer efficacy	N/A	Sayed, D., et al., Oxid Med Cell Longev, 2012. p. 386286. Al-Sadoon, M.K., et al., Cell Immunol, 2013. 284(1-2): p. 129-38.
	Inorganic NPs (Iron oxide)	Placitaxel	Identification of CD138- CD34- tumor stem cells	Dose: 0.6 - 2 mg/kg once a week for 2 weeks Tumor growth inhibition of CD138- CD34- tumor stem-like cells	N/A	Yang, C., et al., Int J Nanomedicine, 2013. 8: p. 1439-49.
Triggered	**Magnetic NPs**	Doxorubicin	N/A	Dose: 0.13 mg/kg + magnetic field Tumor growth inhibition	No significance toxicity No biodistribution	Hayashi, K., et al., Theranostics, 2014. 4(8): p. 834-44.
Targeted	**VLA-4**	Doxorubicin	Induction of cytotoxicity and apoptosis, overcame CAM-DR	Dose: 6 mg/kg thrice Tumor growth inhibition and higher accumulation in the tumor	Reduced body weight loss	Kiziltepe, T., et al., Blood Cancer J, 2012. 2(4): p. e64
		Carfilzomib	Induction of apoptosis and cytotoxicity	Dose: 5 mg/kg twice a week i.v. Tumor growth inhibition and higher accumulation at the tumor	Reduced body weight loss	Ashley, J.D., et al., J Control Release, 2014. 196: p. 113-21
		MYC inhibitor	Induction of apoptosis and cytotoxicity	Dose: 0.145 mg/ml thrice a week i.v. Survival benefits	N/A	Soodgupta, D., et al., Mol Cancer Ther, 2015. 14(6): p. 1286- 94

Type	Formulation	Drug	In vitro efficacy	In vivo efficacy	Biodistribution/ Toxicity	Ref.
	ABC drug transporters	Placitaxel	Inhibition of CD138- CD34- tumor stem-like cells growth	Dose: 10 μg mAb and 8 μg placitaxel for 4 weeks Tumor growth inhibition and PST in CSC models	Lower toxic side effects	Yang, C., et al.,Nanomedicine, 2014. 9(1): p. 45-60. & J Biomed Nanotechnol, 2014. 10(2): p. 336-44.
	Bone micro- environment	Bortezomib	Targeted NPs presented good binding, and induction of apoptosis	Dose: 0.5 mg/kg twice a week i.p. Tumor growth inhibition and PST	Biodistribution by imaging No side effects studies	Swami, A., et al., Proc Natl Acad Sci U S A, 2014. 111 (28): p. 10287-92

Legend: NPs, Nanoparticles; N/A, Not available; i.v., intravenous; s.c., subcutaneous; i.t., intratumoral; i.p., intraperitoneal; PST, prolonged survival time; CAM-DR, cell adhesion mediated drug-resistance; ABC, ATP-binding cassette; CSC, cancer stem cells.

Table 3

Clinical nanoparticle delivery systems in MM

Type	Phase	Study design	Results	Ref.
Non-targeted. PEGylated liposomal doxorubicin	Phase I	Advanced hematologic malignancies patients received PegLD (day 4 at 30 mg/m^2) in combination with bortezomib (days 1, 4, 8, and 11 from 0.90 to 1.50 mg/m^2).	Common toxicities were Grade 3 or 4. The MTD was 1.50 and 30 mg/m^2 of bortezomib and PegLD, respectively. Antitumor activity was seen against multiple myeloma, 36% CR or near-CR, and another 36% PR.	Orlowski, R.Z., et al., Blood, 2005. 105(8): p. 3058-65.
	Phase III	Relapsed or refractory multiple myeloma patients received either intravenous bortezomib 1.3 mg/m^2 on days 1, 4, 8, and 11 of an every-21-day cycle with PegLD 30 mg/m^2 on day 4 or bortezomib alone.	TTP was significantly prolonged in the combination arm (median TTP = 9.3 months) compared with bortezomib monotherapy (median TTP = 6.5 months)	Orlowski, R.Z., et al., J Clin Oncol, 2007. 25(25): p. 3892-901.
	Phase II	Newly diagnosed multiple myeloma patients received intravenous PegLD (40 mg/m^2), vincristine (2.0 mg, Day 1), and oral or intravenous dexamethasone (40 mg per day for 4 days) every 4 weeks for six or more cycles and/or for two cycles after the best response.	The most common toxicities were Grade 3. The overall response rate was 88%: 12% CR. TPP was 23.1 months, with 2-year and 3-year progression-free survival rates of 42% and 23%, respectively. The patient survival rate at 3 years was 67%.	Hussein, M.A., et al., Cancer, 2002. 95(10): p. 2160-8.

Legend: PegLD, PEGylated liposomal doxorubicin; MTD, maximum tolerated dose; CR, complete response; PR, partial response; TTP, time to progression