Chapter 4

Passive targeting in nanomedicine: fundamental concepts, body interactions, and clinical potential

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4.1 Introduction

Over the past few decades, researchers have been designing and applying nanoparticles for diagnosis and treatment of diseases inside the body. These *in vivo* biomedical applications of nanoparticles represent a major research area within the continuously growing field of nanomedicine.[1] While it is a fascinating concept to use systemically administered nanoparticles inside the body for medical applications, development and clinical translation of nanomedicines are challenging. One of these challenges is delivery.[2] Direct and efficient delivery of administered nanoparticles to diseased tissues and cells is required for most nanomedicines to ensure accurate diagnosis and effective treatment. However, biological barriers within the body, such as the mononuclear phagocyte system (MPS), limit nanoparticle delivery to diseased sites.[3]

To address this nanoparticle delivery challenge, researchers have been working on so-called "targeting" strategies.[4] The goal of these strategies is to deliver nanoparticles preferentially to diseased tissues while minimizing their accumulation in healthy organs and cells. Such targeted delivery approaches may have several clinical benefits, including (1) reduced treatment-related side effects; (2) improved imaging and diagnosis; and (3) enhanced therapeutic outcomes.

In this chapter, we focus on passive nanoparticle targeting strategies in the context of solid tumor management. This chapter begins with a brief introduction of nanomedicine (see Section 4.2), after which the journey of administered nanoparticles en route to malignant tissues and cells in the body is briefly explored (see Section 4.3). Section 4.4 provides a concise description of active and passive nanoparticle targeting strategies. In Section 4.5, we discuss fundamental concepts of passive nanoparticle targeting, including pathophysiological characteristics of solid tumors and nanoparticle design rules. Section 4.6 briefly explores limitations of passive nanoparticle targeting, and Section 4.7 explains nanoparticle–body interactions and biological barriers. We focus on clinical potential and relevance of passively targeted cancer nanomedicines in Section 4.8 and conclude our chapter with an outlook on how to further exploit the potential of this technology for biomedical applications (see Section 4.9).

4.2 What is "nanomedicine"?

Nanomedicine can be broadly defined as the biomedical and clinical applications of rationally engineered nanoscale materials with typical dimensions between 1 and 100 nm.[5] Materials in this nanoscale size regime are referred to as nanoparticles. Nanoparticles exhibit unique optical,[6] magnetic,[7] and biological properties[8] that are usually not observed in their corresponding bulk materials. Researchers are able to synthesize nanoparticles from inorganic (e.g., semiconductor quantum dots,[9] upconversion nanoparticles,[10] and iron oxide nanoparticles[11]) and organic materials (e.g., liposomes,[12] dendrimers,[13] and polymeric nanoparticles[14]) with defined physicochemical properties, including nanoparticle size, shape, and surface chemistry. Such high tunability of material properties allows

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researchers to engineer nanoparticles with unique capabilities for biomedical and clinical use. For example, nanoparticles can be synthesized to function as drug delivery vehicles for therapeutic applications or as imaging contrast agents for medical imaging and diagnosis. Application of these rationally engineered nanoparticles for cancer management is referred to as cancer nanomedicine.[15]

Medical applications of nanoparticles as carriers for therapeutic agents require encapsulation and/or surface modification strategies. Researchers can load nanoparticles with a variety of therapeutic agents, including small molecule drugs, chemotherapeutics, peptides, antibodies, and nucleic acid–based drugs. This can improve solubility and bioavailability of drugs *in vivo* and potentially lead to better therapeutic efficacy against diseased cells compared with administration of free drugs.[16,17] Combination of therapeutic and diagnostic capabilities into one nanoparticle is also possible, and such nanoparticles are referred to as "theranostic" nanoparticles in the literature.[18,19] To exert their intended biomedical function, diagnostic, therapeutic, and theranostic nanoparticles are typically administered via systemic administration.

4.3 Systemic nanomedicine and the journey of administered nanoparticles in the body

Systemic administration, i.e., administering nanoparticles directly into the body's circulatory system, is a frequently used approach in nanomedicine. The rationale for systemic nanomedicine is that intravenously (i.v.) administered nanoparticles transport directly with the bloodstream throughout the body and may eventually reach diseased tissues, such as a primary solid tumor or metastatic lesions. Upon accumulation, nanoparticles will exert their deliberate biomedical function in these diseased tissues (Fig. 4.1A).

Before i.v. administered nanoparticles reach malignant sites in the body, several key steps occur. First, exposure of nanoparticles to blood leads to formation of a so-called

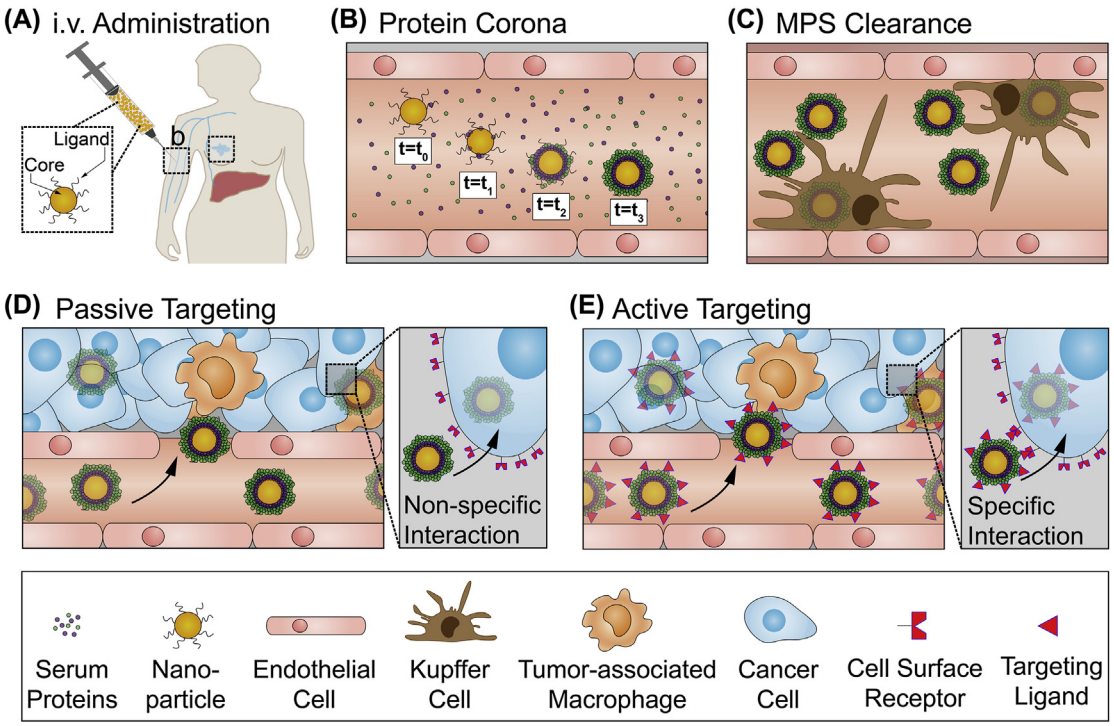

FIGURE 4.1 Schematic overview representing biological barriers and transport mechanisms of systemically administered nanoparticles in biomedical applications. (A) Systemic (intravenous, i.v.) administration of engineered nanoparticles into the circulatory system is a commonly used administration route in biomedicine. Engineered nanoparticles typically comprise organic and/or inorganic nanoscale core materials. The nanoparticle core is often surface modified with organic polymers and ligand molecules. (B) Protein corona formation is a dynamic process, which starts immediately upon i.v. administration into the circulatory system. The nanoparticle protein corona changes dynamically over time. (C) Cells of the mononuclear phagocyte system (MPS), such as liver macrophages (Kupffer cells), may line the luminal surface of liver sinusoid blood vessels. Kupffer cells have been reported to remove opsonized nanoparticles quantitatively from the bloodstream. (D) The passive targeting mechanism suggests nanoparticle transport through interendothelial gaps (paracellular transport) of compromised blood vessels. In cancer nanomedicine, nonspecific interaction with tumor cells may occur upon paracellular nanoparticle transport. (E) Ligand-coated nanoparticles follow the same transport pathway as passively targeted nanoparticles. In contrast to passive targeting nanoparticles, ligand-coated nanoparticles may then interact specifically with tumor cells, potentially leading to increased nanoparticle retention and improved cell uptake.

protein corona that covers the nanoparticle surface (Fig. 4.1B). Certain proteins within the corona, called opsonins, may then direct nanoparticles to phagocytic cells in organs of the MPS. Major MPS organs include the liver and spleen. Liver macrophages, called Kupffer cells, are one type of phagocytic MPS cells. They can remove nanoparticles efficiently and in large quantity from the bloodstream (Fig. 4.1C).[20,21] Consequently, nanoparticles will not be able to accumulate within solid tumors once they have been engulfed by MPS cells and removed from the blood. Therefore, organs and cells of the MPS represent major biological barriers that significantly limit nanoparticle blood circulation times and delivery. Nanoparticles need to overcome these biological barriers for efficient delivery to diseased sites.

Crossing the tumor endothelium is another key step in the journey of nanoparticles from the administration site to diseased tissues *in vivo*. It has been reported in the literature that the vascular wall of tumor blood vessels is compromised.[21a] In contrast to healthy blood vessels, tumor blood vessels may exhibit specific pathological characteristics that nanoparticles exploit during accumulation within cancerous tissues.[22] Briefly, systemically administered nanoparticles may transport from the tumor blood vessel lumen through interendothelial gaps into the tumor interstitial space.[23] This is a fundamental mechanism for both passive and active nanoparticle targeting (Fig. 4.1D and E).[24,25]

4.4 Active versus passive nanoparticle targeting strategies

The literature divides targeted nanoparticle delivery strategies into two major categories: (1) passive and (2) active targeting.[24] The goal of these targeting strategies is to deliver nanoparticles and their therapeutic/diagnostic payloads preferentially to diseased tissues while minimizing nanoparticle accumulation in healthy organs and cells. Although we focus on passive targeting in this chapter, the fundamental mechanisms of nanoparticle accumulation within malignant tissues are similar for both strategies. In cancer nanomedicine, both targeting strategies exploit a tumor's pathophysiological characteristics for nanoparticle accumulation. In addition, researchers use empirically derived nanoparticle design rules with the intention to improve tumor delivery (Fig. 4.1D and E).

In contrast to passive targeting nanoparticles, active targeting nanoparticles are engineered with specific nanoparticle surface ligands (Fig. 4.1E).[26] These surface ligands are referred to as targeting ligands. Typical examples of targeting ligands are biomolecules, including nucleic acids, antibodies, and peptides. Such biomolecules can recognize and bind to specific cell surface receptors on cancerous cells with high affinity.[24] Hence, active targeting approaches in nanomedicine are rationally designed strategies to exploit specific biomolecular interactions that may occur between nanoparticle surface ligands and cell surface receptors. In comparison with passive targeting, the underlying rationale for the use of active targeting is twofold: (1) improved retention of passively accumulated nanoparticles at diseased sites as a result of specific interaction between surface ligands and cell surface receptors, and (2) increased specific interaction of nanoparticles with targeted diseased cells while minimizing nontargeted nanoparticle−cell interactions (Fig. 4.1E).[27]

4.5 Fundamental concepts of passive targeting strategies

The term "passive targeting" has been widely used in nanomedicine to describe findings related to accumulation of nanoparticles in solid tumors. Upon i.v. administration of nanoscale materials, Matsumura and Maeda reported in 1986 two key observations that represent the foundation of passive nanoparticle targeting.[28] The first observation was spontaneous accumulation of administered macromolecular drug carriers in areas of solid tumors with leaky vasculature. The second observation was retention of intratumoral nanoparticles due to compromised lymphatic drainage. Together, these observations form the basis of a concept termed "enhanced permeability and retention (EPR) effect."[29]

Passive targeting and EPR effect are closely related. To better understand this relationship, we will briefly discuss the pathophysiology of tumor vasculature that enables enhanced vascular permeability.

One of the hallmarks of metabolically active cancers is sustained angiogenesis, i.e., formation of new blood vessels and neovasculature.[30,31] This provides a nutrient and oxygen supply to tumors and helps with removing metabolic waste products. Chronically activated angiogenesis, however, may lead to formation of highly abnormal tumor vessels.[22] Such blood vessels often exhibit a chaotic and disorganized course, branch irregularly, and are structurally different from healthy vessels (Fig. 4.2A).[32−35]

Dvorak and coworkers identified six distinctly different types of tumor blood vessels with characteristic architectures (Fig. 4.2A).[35] These different vessels are referred to as (1) feeding artery; (2) mother vessel; (3) glomeruloid microvascular proliferation (GMP); (4) vascular malformation; (5) capillary; and (6) draining vein. The first angiogenic tumor blood vessel type is called "mother vessel," which forms from existing normal venules and capillaries. Mother vessels are characterized by (1) thin layer of flattened endothelial cells; (2) disrupted basement membrane; and (3) little or no pericyte cell coverage. These

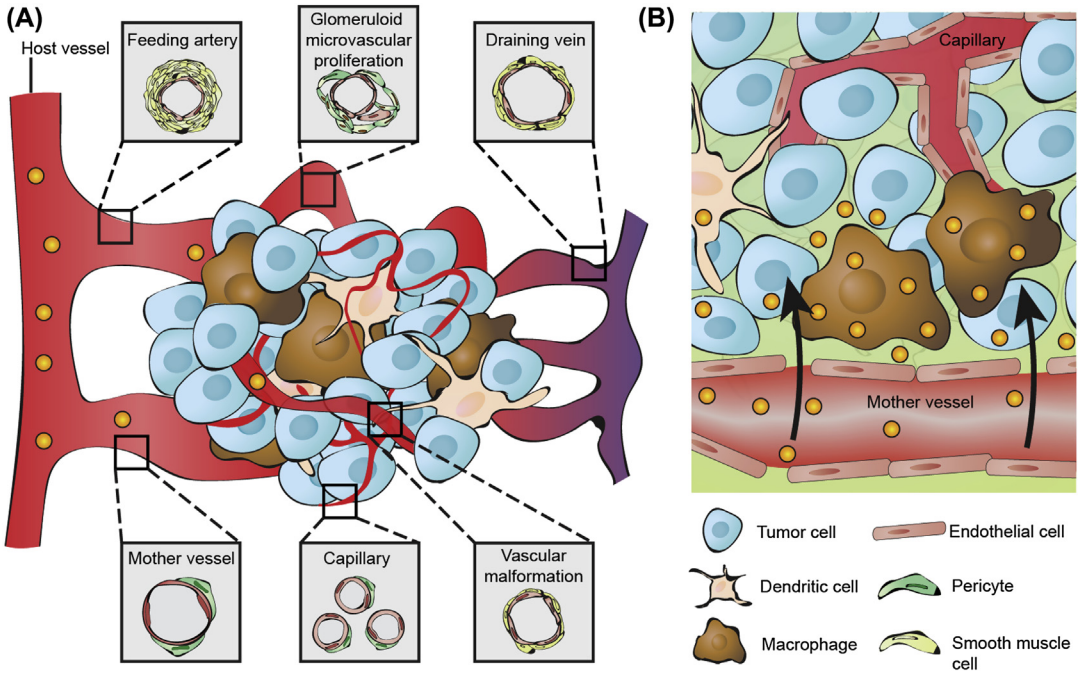

FIGURE 4.2 Heterogeneity of tumor blood vessels, nanoparticle extravasation mechanism, and pathological features of tumor endothelium. (A) Schematic representation of six different types of tumor blood vessels identified by Dvorak and coworkers: (i) feeding arteries; (ii) mother vessels; (iii) glomeruloid microvascular proliferations (GMPs); (iv) vascular malformations; (v) capillaries; and (vi) draining veins. (B) According to the EPR effect, nanoparticles extravasate from tumor blood vessels via passive diffusion through interendothelial gaps. This transport route is referred to as paracellular transport pathway. *Modified with permission from Wilhelm S. et al. Analysis of nanoparticle delivery to tumours.* Nature Reviews Materials *2016;1, 2016:14.*

features contribute to the abnormal hyperpermeability of mother vessels to plasma proteins and likely to nanoparticles (Fig. 4.2B). In addition, mother vessels and GMPs are typical examples of tumor blood vessels that may exhibit interendothelial gaps.[32,36,37] Such gaps between endothelial cells increase the leakiness of tumor vasculature. The size of these gaps can range from a few nanometers to several hundred nanometers, as reported from the analysis of mouse tumor models.[23] In addition to discontinuous endothelium with gaps and pores, mother vessels may also exhibit fenestrated endothelium, which may allow nanoparticle extravasation.[38]

Extravasation is a term used to describe the transport of i.v. administered nanoparticles from the lumen of tumor blood vessels into the tumor interstitium. If extravasation of nanoparticles occurs through interendothelial gaps, the underlying transport pathway is referred to as paracellular route, i.e., transport through gaps between adjacent endothelial cells (Fig. 4.2B).[39] The paracellular route is a passive transport mechanism and requires a nanoparticle concentration gradient between blood and tumor interstitium. This concentration difference then drives nanoparticles passively (mostly by diffusion) across the endothelium and facilitates nanoparticle tumor accumulation (Fig. 4.2B).[40] Since a large enough concentration gradient is required to enable nanoparticle diffusion across the endothelium, high nanoparticle bolus doses and long blood circulation times of administered nanoparticles may increase the efficiency of this passive tumor targeting mechanism.[41]

The EPR effect suggests that administered nanoparticles can accumulate in solid tumors with discontinuous endothelium if the following nanoparticle design criteria are met: (1) smaller nanoparticle size than the cutoff size of tumor interendothelial gaps, and (2) long blood circulation times of nanoparticles. The rationale for long nanoparticle blood circulation times is to increase the chance for paracellular nanoparticle extravasation. The longer the i.v. administered nanoparticles are able to remain in the bloodstream at high concentrations, the higher the chance for paracellular nanoparticle transport into the tumor. Both active and passive nanoparticle targeting strategies are designed to exploit the EPR effect for tumor delivery, i.e., both targeting strategies use concentration-dependent passive paracellular transport across discontinuous endothelium for nanoparticle tumor accumulation (Figs. 4.1D,E, and 4.2B).

Another key principle of nanoparticle targeting is nanoparticle retention within the tumor space. Nanoparticle retention occurs due to poor lymphatic drainage of solid tumors.[42] In normal tissues, the lymphatic system drains excess fluid from the tissue to maintain an interstitial fluid

balance.[43] In solid tumors, however, lymphatic vessels are compressed by the high density of continuously proliferating cancer cells, which may cause the collapse of these vessels and can lead to the poor infiltration of lymphocytes.[44,44a] Collapsed lymphatic vessels are no longer able to efficiently drain fluid from the tumor tissue, which may result in elevated interstitial fluid pressures inside tumor tissues.[43] Nanoparticle tumor retention due to impaired lymphatic drainage is a key concept of the EPR effect and may be observed to a similar extent for both passive and active targeting nanoparticles.

Besides solid tumors, the EPR effect is also found in atherosclerosis, which is associated with chronic inflammation of arterial blood vessels. This opens the possibility to target inflamed tissue sites of atherosclerosis with nanoparticles.[45,46] Researchers have used nanoparticles to image and treat atherosclerotic plaques.[42–44] Similar to nanoparticle extravasation mechanisms in solid tumors, atherosclerotic plaques may exhibit mirconeovasculature that is permeable to systemically administered nanoparticles.[46] Atherosclerotic plaques form by deposition of lipids, cholesterol, calcium, cellular waste products, and other compounds on the inner walls of arterial blood vessels.[46] Nanoparticles may be transported through leaky vasculature of atherosclerotic plaques into the tissue interstitium, where they accumulate over time as a result of underdeveloped lymphatics.[46] To probe and model translocation of poly(D,L-lactide-*co*-glycolide) PLGA-based nanoparticles in atherosclerotic endothelium, Langer *et al.* reported in 2014 an *in vitro* model based on an endothelialized microfluidic chip.[47] In the future, such *in vitro* models may provide key insights in nanoparticle transport mechanisms with clinical relevance to help overcome endothelial barriers. Results from these studies may lead to new nanoparticle design principles for *in vivo* applications in diagnosis and treatment of atherosclerotic plaques and solid tumors.

4.6 Limitations of passive nanoparticle targeting

According to the EPR effect, solid tumors exhibit pathophysiological features, including leaky vasculature and impaired lymphatic drainage that may facilitate nanoparticle targeting. These features may theoretically result in enhanced vascular permeability and retention of nanoparticles within tumors. However, it is important to mention that the extent of the EPR effect can vary substantially between and within tumors.[48,49] Solid tumors are highly heterogeneous with large variability of vascular permeability, lymphatic drainage, blood perfusion rates, interstitial tissue pressures, extracellular matrix (ECM) density, and ECM composition.[50–52] All of these factors may individually and/or collectively affect EPR. While the EPR effect has been derived based on observations in mouse tumor models, the extent to which EPR occurs in humans is controversial and subject to debate.[53–55] Current data suggest that the EPR effect is highly variable across and within individual tumors.[56]

Even for the same tumor, the extent of vascular permeability, lymphatic drainage, and even blood perfusion rates of existing tumor vessels may vary significantly for different tumor areas. This intratumoral heterogeneity suggests that EPR may be highly variable within the same tumor, which impedes uniform distribution of nanoparticles throughout the tumor tissue.[57] The resulting heterogeneous intratumoral nanoparticle and drug distribution may lessen therapeutic effects and may lead to resistance of cancer cells to chemotherapeutics.

The idea of passive nanoparticle targeting suggests that rationally designed nanomedicines may exploit EPR features for tumor accumulation. Since the proposal of the EPR effect in the mid-1980s, passive targeting has evolved into a key rationale for developing nanoscale therapeutic and diagnostic agents for cancer management. However, it should be emphasized that passive targeting does not necessarily result in quantitative and efficient tumor delivery of administered nanoparticles. A recent meta-analysis of preclinical studies published between 2005 and 2015 reported that only about 1% of the injected nanoparticle dose (median value) accumulates within solid tumors of mouse models.[38] The majority of administered nanoparticles interact with nonmalignant cells and accumulate in healthy organs such as the liver and spleen rather than in "targeted" cancerous tissues.[21] The potential reasons for nanoparticle accumulation in healthy organs will be discussed in the next section, where we focus on nanoparticle body interactions and biological barriers that impede efficient nanoparticle delivery.

4.7 Nanoparticle–body interactions and biological barriers

Nanomedicines may provide improved clinical benefits if nanoparticles are able to reach diseased sites in the body efficiently and effectively.[24] However, achieving efficient and targeted nanoparticle *in vivo* delivery requires a better fundamental understanding of how administered nanoparticles and biological systems interact. These interactions are referred to as "nano–bio" interactions in the literature and are highly complex and multiparametric.[58] Nano–bio interactions occur on multiple different scales that range from biomolecular and cellular to tissue and system levels (Fig. 4.1B and C).[59] The fate of administered nanoparticles in the body is eventually determined by these multilevel nano–bio interactions.[60] In this section, we discuss

nanoparticle−body interactions and corresponding biological barriers categorized into three major types: (1) nanoparticle−blood interactions; (2) nanoparticle−MPS interactions; and (3) nanoparticle−kidney interactions. We conclude the section with a brief discussion of nanoparticle design strategies to control nano−bio interactions and to overcome biological barriers.

4.7.1 Nanoparticle−blood interactions

Upon i.v. administration, nanoparticles interact with cellular and acellular components of the blood.[61] As nanoparticles get transported with the bloodstream, they may be engulfed by circulating blood cells, such as circulating monocytes and other phagocytes.[62,63] In addition, serum proteins and other biomolecules within the bloodstream adsorb onto the nanoparticle surface (Figs. 4.1B and 4.3A).[61] This protein/biomolecule adsorption leads to the formation of a so-called protein corona (or biomolecular corona), which is an energetically favorable biochemical process.[64−70] The literature differentiates the nanoparticle protein corona into two compartments: (1) hard protein corona and (2) soft protein corona (Fig. 4.3A).[71,72] Whereas the hard corona typically comprises tightly bound surface proteins, the soft corona is more dynamic and allows exchange of surface proteins with lower binding affinity over time (Fig. 4.3A).[73,74] Recent studies have investigated the dynamic changes of the protein corona *in vivo* and how they affect the biological fate of nanoparticles.[75,76]

Nanoparticle protein corona formation is one of the most important and most complex nano−bio interactions. Its importance stems from the fact that formation and presence of a protein corona change the synthetic identity of nanoparticles into a biological identity[66,77,78] (Fig. 4.3B). The synthetic identity refers to intentionally engineered physicochemical properties of nanoparticles that include nanoparticle size, shape, surface chemistry, and surface functional groups.[79] The new biological identity determines the physiological response and biological fate of nanoparticles *in vivo*. For example, certain proteins called opsonins within the protein corona may be recognized by phagocytic cells in the liver,[80] which may then lead to liver accumulation of opsonized nanoparticles as a result of phagocytic cell uptake.[20,21] The nanoparticle protein corona can also change nanoparticle aggregation state and surface charge, which may further affect the biological fate of nanoparticles in the body.[64,78]

As shown schematically in Fig. 4.3C−F, nanoparticle protein corona formation may affect their intratumoral targeting capabilities. Once intentionally engineered active targeting nanoparticles accumulate in the tumor space, they may lose their binding specificity toward targeted cells as a result of nanoparticle protein corona formation (Fig. 4.3E and F). The reason for this is that the nanoparticle protein corona may sterically hinder specific ligand−receptor interactions. Targeting surface ligands may get buried within the protein corona.[81] For passive and active targeting nanoparticles, protein corona formation may direct nanoparticles to off-target cells, such as tumor-associated macrophages (TAMs) and other immune cells within the tumor microenvironment (Fig. 4.3C−F).[82,83,83a]

4.7.2 Nanoparticle−MPS interactions

Off-target nanoparticle accumulation due to protein corona formation does not just occur inside a tumor. The body itself has a network of MPS organs and immune cells in place that effectively remove foreign nanomaterials from the bloodstream (Fig. 4.4).[84] These organs include the liver, spleen, lymph nodes, skin, bone marrow, and other organs with resident phagocytic macrophages.[85] These resident macrophages are typically derived from circulating monocytes and may exhibit a range of phenotypical diversity.[86]

Biodistribution analyses have shown that accumulation of nanoparticles in MPS organs and cells is a universal phenomenon, which has been observed for different types of materials, such as micelles and polymeric nanoparticles, liposomes, carbon nanotubes, quantum dots, and gold nanoparticles.[87−90] This off-target nanoparticle accumulation in MPS organs is a significant challenge for the development and clinical translation of nanomedicines, as it impedes efficient delivery of nanoparticles to diseased sites. In addition, MPS accumulation of nanoparticles may cause severe toxicity-related side effects, particularly in organs such as the liver and spleen.[91]

Fig. 4.4 summarizes nanoparticle interactions with the liver. Tsoi et al. reported that when blood enters liver sinusoids, its fluid velocity decreases by approximately 1000-fold compared withblood velocities in arteries and veins in systemic circulation.[85] Reduced blood velocity within the liver sinusoid is in part responsible for increased nanoparticle uptake by phagocytic cells near the vascular inlet. Besides the relative location of cells within the liver sinusoidal microarchitecture, a cell's phenotype, nanoparticle internalization, and dissociation kinetics are also important factors that determine nanoparticle−cell interactions in the liver. In addition, immune cells, such as hepatic B cells, have been shown to interact with hard nanoparticles as efficiently as Kupffer cells and liver sinusoidal endothelial cells.[85] It has been suggested that Kupffer cells recognize opsonins within the nanoparticle protein corona via scavenger receptors.[92] This molecular recognition may then trigger nanoparticle uptake into macrophages. While organic nanoparticles can be more readily degraded and eliminated upon accumulation in MPS cells, inorganic nanoparticles can reside in these cells

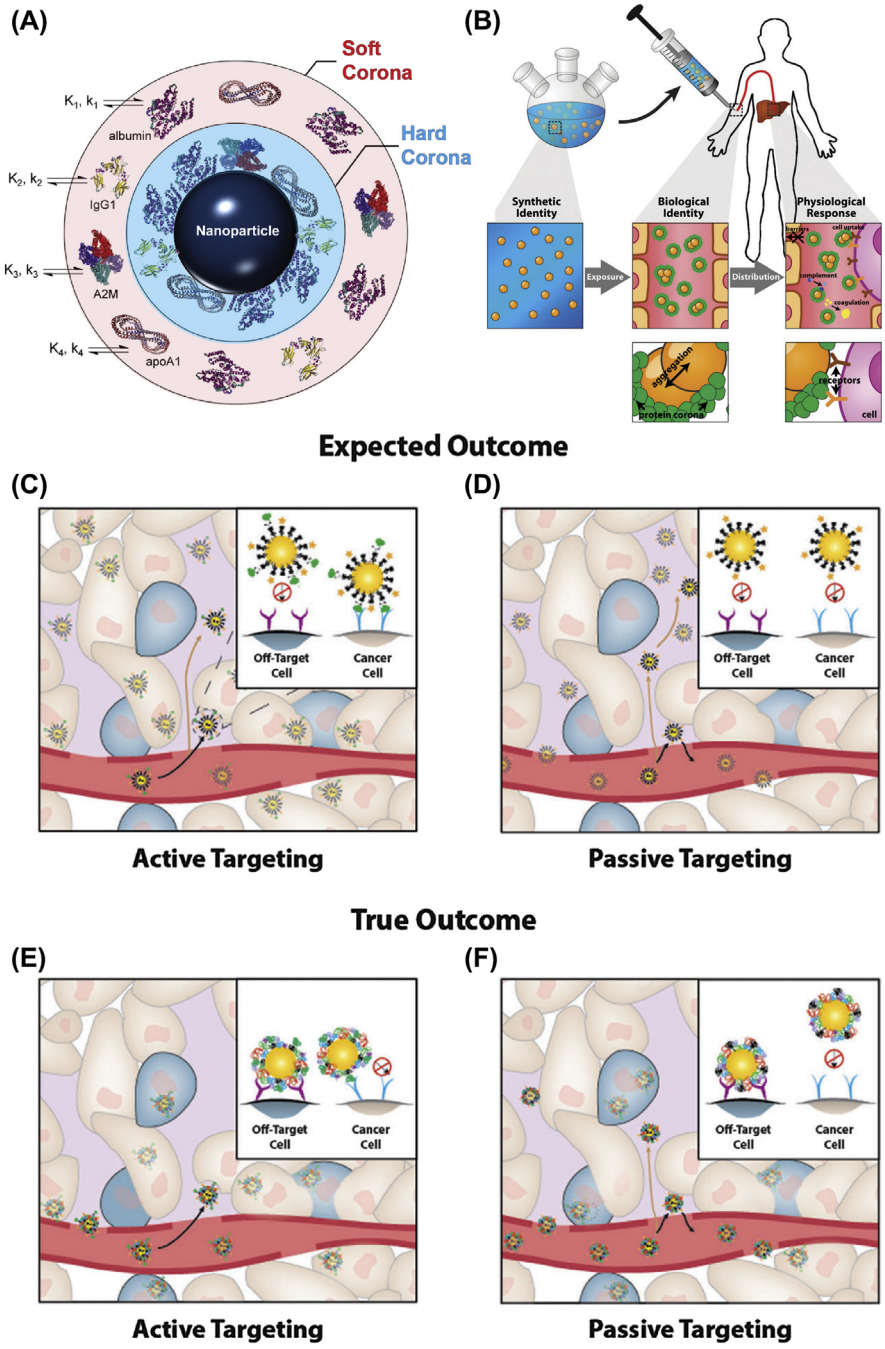

FIGURE 4.3 Nanoparticle–blood interactions and protein corona formation. (A) Protein corona formation is a dynamic process. The adsorption of proteins on the nanoparticle surface is a kinetic (k) and thermodynamic (K) function of individual protein properties and nanoparticle physicochemical characteristics. The protein corona can be classified into (i) hard corona (high-affinity proteins with strong binding) and (ii) soft corona (low-affinity proteins with weak binding). (B) Upon intravenous administration, blood serum proteins adsorb on the nanoparticle surface. This changes the rationally engineered synthetic identity of nanoparticles into a biological identity. This new biological identity is presented to the body and determines nanoparticle interactions with cells, tissues, and organs. (C–F) Protein corona formation may affect cancer cell targeting capabilities of nanoparticles. Active and passive targeting nanoparticles may enter the tumor interstitium through interendothelial gaps according to the EPR effect. (C,D) In a hypothetical situation without nanoparticle protein corona formation, ligand-functionalized active targeting nanoparticles may interact with specific cell surface receptors on targeted cancer cells to facilitate receptor-mediated endocytosis for selective nanoparticle cell uptake. Passive targeting nanoparticles do not exhibit specific cell interactions. (E,F) Upon formation of a protein corona, active targeting nanoparticles may lose their specific targeting capabilities, as protein corona formation masks surface-bound ligands. Both active and passive targeting nanoparticles may show nonspecific cellular uptake that is enabled by the protein corona. *(A) Modified with permission from Fleischer, CC, Payne CK. Nanoparticle–cell interactions: molecular structure of the protein corona and cellular outcomes. Accounts Chem Res 2014;47:2651–2659. (E &F) Panels b–f modified with permission from Lazarovits J, Chen Y, Sykes EA, Chan WC. Nanoparticle-blood interactions: the implications on solid tumour targeting.* Chem Commun Camb Engl *2015;**51**:2756–2767.*

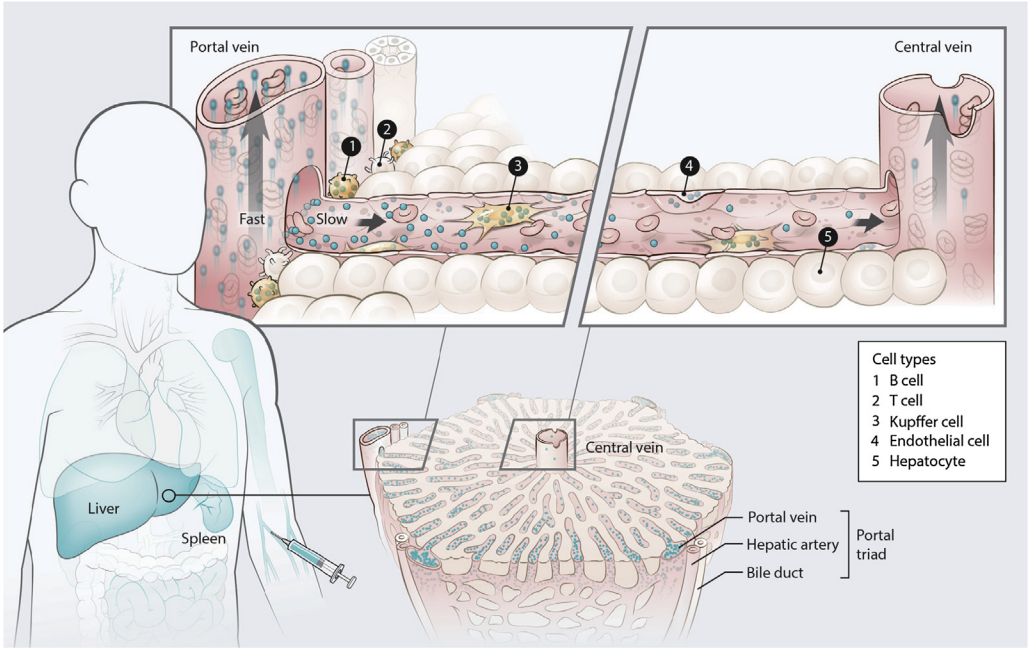

FIGURE 4.4 Nanoparticle liver interactions. Intravenously administered nanoparticles interact with the mononuclear phagocyte system (MPS). The MPS consists of organs, such as liver, spleen, bone marrow, and phagocytic cells. The intensity of the blue color in the figure indicates the extent of nanoparticle uptake within MPS organs. As nanoparticles transport from the peripheral circulation into the liver, their velocity reduces 1000-fold. In consequence, nanoparticles could interact with a variety of hepatic cell types, resulting in gradual nanoparticle clearance from the bloodstream. A concentration gradient of nanoparticles along the length of the liver sinusoid has been observed. The number of nanoparticles leaving the liver through the central vein is lower than the amount that enters via the portal triad. B and T cells border the portal triad and are exposed to a high concentration of incoming nanoparticles. In general, B cells have a higher phagocytic potential than T cells. Nanoparticles that escape the first set of cellular interactions transport along the liver sinusoid and may interact with endothelial and Kupffer cells. Hepatocytes are separated from the blood vessel by a layer of fenestrated endothelium and do not seem to take up nanoparticles efficiently. Nanoparticles that escape blood clearance during a pass through the liver return to the systemic circulation via the central vein and may ultimately return back into the liver (or another MPS organ). This process may repeat itself until nanoparticle clearance from the bloodstream is complete. *Reproduced with permission from Chan WC. Nanomedicine 2.0.* Accounts Chem Res *2017;50:627–632.*

for long periods of time, which may affect long-term toxicity and safety of nanomedicines.[93]

The spleen is another important MPS organ that may clear systemically administered nanoparticles efficiently from the blood. Anatomically, a spleen's red pulp and white pulp are separated by a marginal zone and sinuses with pores of ~3 μm (Fig. 4.5).[94] The red pulp accounts for ~75% of the spleen. It contains macrophages and is involved in degradation of erythrocytes.[86] Macrophages in the marginal zone, splenic B cells, and dendritic cells provide biological barriers against pathogens.[95] Macrophages within the white pulp are involved in innate immunity and clearing of apoptotic cells and may remove circulating nanoparticles.[96] Overall, splenic macrophages have been shown to exhibit lower phagocytic potency toward circulating nanoparticles compared with liver macrophages, such as Kupffer cells.[84] Nanoparticles with sizes larger than 200 nm in diameter are typically more likely to be cleared by splenic macrophages, whereas liver macrophages interact more strongly with smaller nanoparticles (100 nm or less).[97]

To reduce MPS sequestration of systemically administered nanoparticles, researchers have explored strategies to modulate and inhibit MPS functions and phagocytic response.[21] For example, it has been demonstrated that transient depletion of MPS macrophages, such as hepatic Kupffer cells, with toxic compounds (e.g., gadolinium chloride and clodronate-containing liposomes) can substantially prolong nanoparticle blood circulation times.[20,98–100] Tavares et al. reported 18–150 times greater nanoparticle delivery efficiency to solid tumors upon depletion of liver Kupffer cells via clodronate–liposome treatment.[20] Wolfram *et al.* used a chloroquine-based liver preconditioning strategy to reduce nanoparticle accumulation in the liver by temporarily inhibiting phagocytosis of Kupffer cells.[101] Overwhelming MPS cells by administering large bolus doses of organic and inorganic nanoparticles is another approach to saturate the phagocytic response of Kupffer cells.[102–104] This approach does not require depletion and elimination of macrophages to reduce nanoparticle uptake by the MPS. While most of these examples focus on modulation and inhibition of the liver, it will be important to systematically assess how other MPS organs (e.g., spleen, lymph nodes, skin, bone marrow, and other organs and tissues) individually and collectively

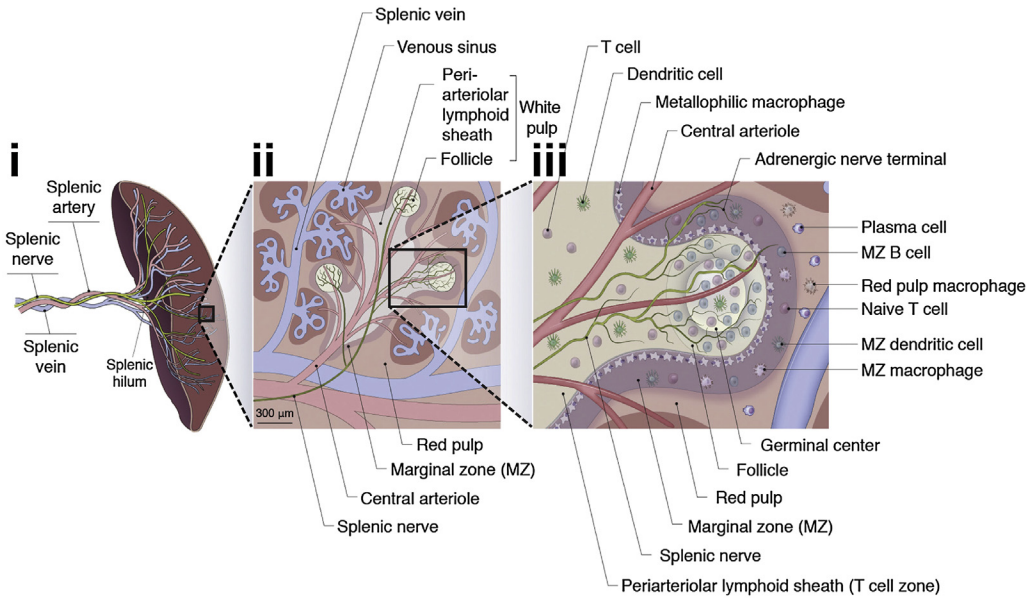

FIGURE 4.5 Spleen anatomy and microarchitecture. (i) Gross schematic illustration of the spleen. The splenic vein directs filtered blood from the spleen back into recirculation. (ii) Schematic of major zones within the spleen, including nonlymphoid red pulp, which filters blood, and the lymphoid white pulp, comprised of the periarteriolar lymphoid sheath and follicles. (iii) Microarchitecture of spleen with specific cell types. *Modified with permission from Noble B, Brennan FH, Popovich PG. The spleen as a neuroimmune interface after spinal cord injury.* J Neuroimmunol *2018.*

contribute to nanoparticle sequestration and elimination.[20,20a] A better fundamental understanding of nanoparticle−MPS interactions from a whole-organ perspective to cellular and molecular levels may allow researchers to design nanoparticles that are potentially able to overcome MPS biological barriers for more efficient nanoparticle delivery to diseased sites.

4.7.3 Nanoparticle−kidney interactions

Kidneys are major organs for clearance and elimination of nanoparticles through blood filtration.[105] Blood enters the kidney through paired renal arteries and exits through paired renal veins. Kidneys comprise three main anatomical regions: (1) renal cortex; (2) medulla; and (3) pelvis. In cortex and medulla, nephrons are basic structural units that contain the renal corpuscle, through which blood is transported into glomeruli. Increased blood pressure within the glomerular cavity causes filtration of fluid, solutes, and waste into the Bowman's space. Unfiltered fluid is transported back into the main bloodstream via efferent arterioles. Filtered fluid that may contain waste products, solutes, and nanoparticles flows from the Bowman's space into proximal tubules. The luminal surface of these tubules is covered with densely packed microvilli that balance secretion into urine and reabsorption.[105]

Nanoparticle filtration within the kidney occurs along the glomerular filtration membrane (GFM, Fig. 4.6). This membrane consists of four major structural units: (1) endothelial glycocalyx; (2) endothelial cells; (3) glomerular basement membrane (GBM); and (4) podocytes. The endothelial glycocalyx has been reported to help in the retention of proteins.[106] The GFM endothelium exhibits fenestrations 70−90 nm in size, where as the GBM exhibits pores with sizes of 2−8 nm. Podocytes that cover the GBM face toward the Bowman's space. The podocyte layer has a pore size of 4−11 nm. This four-layer anatomical architecture controls renal filtration and clearance of materials not only by size but also by charge (Fig. 4.6).[107−109]

The kidney filtration threshold for inorganic nanoparticles has been reported to be 5.5 nm.[110] For proteins and soft macromolecules, studies have shown that those materials with hydrodynamic diameter (HD) slightly larger than 6 nm can still be renally cleared and eliminated.[105] This has been attributed to the mechanical deformation capabilities of these soft organic nanomaterials in comparison with more rigid inorganic nanoparticles.[109] Nanoparticles with sizes larger than the renal filtration cutoff are more likely to interact with MPS organs and cells than kidneys.[21] However, non−renal-clearable nanoparticles with sizes smaller than 100 nm can still interact with the glomerulus but will not be able to cross the GBM for elimination into urine (Fig. 4.6A and B).[105]

A recent study by Zheng *et al.* reported that the GFM acts as a nanoparticle-size "bandpass" filter (Fig. 4.6C−E).[111] For the GFM, the following trends for size-dependent renal clearance of nanoparticles have been reported: (1) HD > 100 nm: minimal nanoparticle transport across endothelium; (2) HD = 6−100 nm: able to transport through endothelium but blocked by GBM; (3)

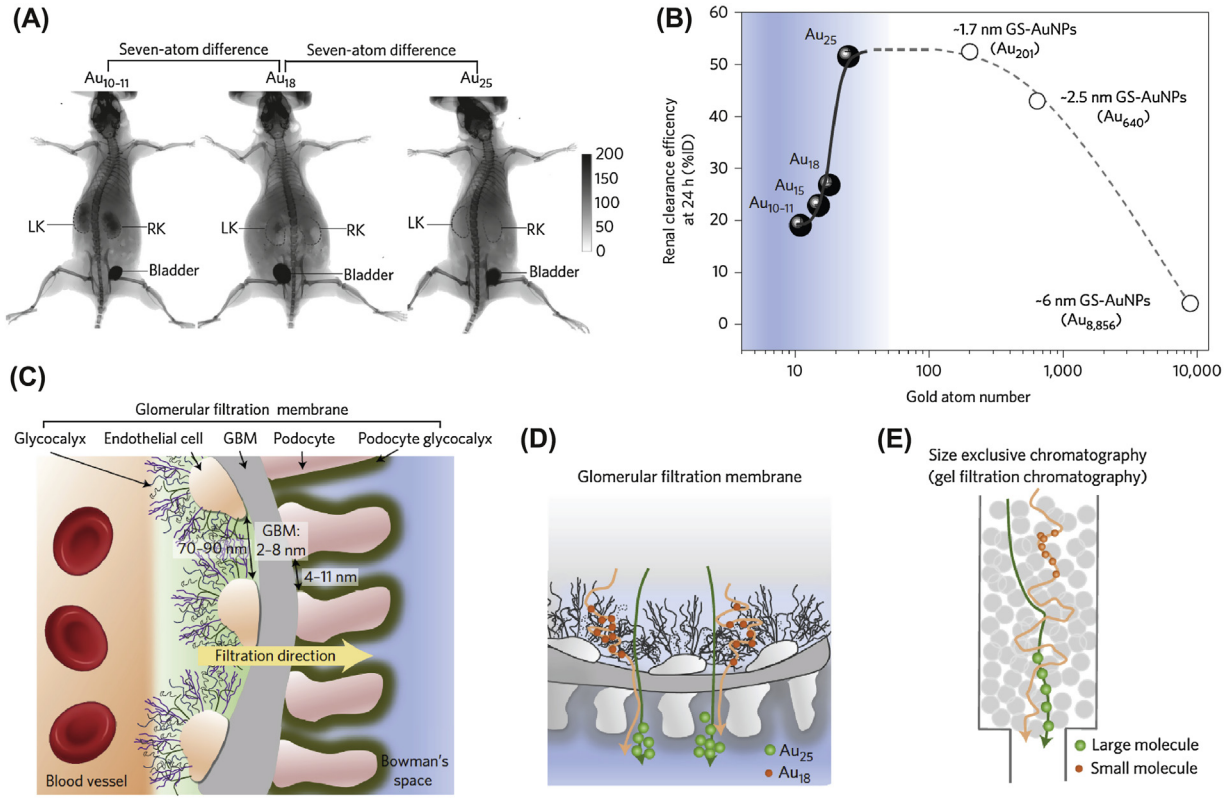

FIGURE 4.6 Nanoparticle−kidney interactions and glomerular filtration of nanoparticles. (A) Whole-body X-ray-based biodistribution images of mice 40 min post intravenous administration of gold nanoclusters (AuNCs) Au_{10-11}, Au_{18}, or Au_{25}. Kidney retention time increased in the following order: $Au_{10-11} > Au_{18} > Au_{25}$. LK, left kidney; RK, right kidney. (B) Renal clearance efficiencies in percentage of the injected dose (%ID) unit of Au_{10-11}, Au_{15}, Au_{18}, and Au_{25}, 1.7 nm (Au_{201}), 2.5 nm (Au_{640}), and 6 nm (Au_{8856}) glutathione-coated AuNCs/AuNPs at 24 h p.i. versus number of gold atoms. Importantly, for AuNCs < Au_{25}, the renal clearance efficiency decreased exponentially with decreasing number of gold atoms per AuNCs. (C) The glomerular filtration membrane (GFM) consists of an endothelial glycocalyx, endothelial cells, the glomerular basement membrane (GBM), and podocytes. (C)−(E). The GFM exhibits capabilities similar to a size "bandpass" filter for nanoparticles. Nanoparticles with a hydrodynamic diameter (HD) > 100 nm cannot transport through the endothelium. Nanoparticles with HD = 6−100 nm cannot cross the GBM. Nanoparticles with HD between 2 and 6 nm can traverse the GFM. In general, smaller nanoparticles will cross the GFM faster than larger ones. Nanoparticles with HD between 1 and 2 nm cross the membrane with similar velocity and efficiency. Nanoparticles with HD < 1 nm physically interact with the endothelial glycocalyx, resulting in inverse size-dependent glomerular filtration. In general, smaller nanoparticles clear more slowly than larger ones. *Modified with permission from Du B, et al. Glomerular barrier behaves as an atomically precise bandpass filter in a sub-nanometre regime.* Nat Nanotechnol. *2017;12.*

HD = 2−6 nm: size-dependent nanoparticle interactions with GBM and podocytes—smaller nanoparticles are cleared faster than larger nanoparticles; (4) HD = 1−2 nm: these materials exhibit comparable renal clearance characteristics with those nanoparticles with HD 2−6 nm; and (5) HD < 1 nm: these materials interact substantially with glycocalyx, which decreases renal clearance efficiency of nanoparticles with decreasing size (Fig. 4.6B).[105] In addition to size-dependent renal clearance, there is also nanoparticle surface charge dependence.[107,108] The GFM glycocalyx is overall negatively charged. In consequence, renal nanoparticle clearance rates decrease in the following order: positively charged nanoparticles > neutral nanoparticles > negatively charged nanoparticles.[105]

In summary, the renal system represents a nanoparticle size and charge-dependent biological barrier for administered nanomedicines. This barrier may be able to significantly reduce blood circulation times of small nanoparticles. Ultimately, this may impede efficient delivery of nanoparticles to diseased tissues.

4.7.4 Controlling nano−bio interactions through nanoparticle design

Efficient and targeted delivery of nanoparticles to diseased sites is a major quest in nanomedicine research. Researchers have been able to develop nanomaterial design strategies that allow, to a certain extent, the control of *in vivo* fate of nanomedicines by modulating nano−bio interactions.[3] One of these strategies is nanoparticle surface modification with antifouling polymers, such as poly(ethylene glycol), i.e., PEG.[112,113] Nanoparticle surface modification with PEG is referred to as PEGylation. PEGylation reduces nonspecific serum protein adsorption onto the nanoparticle surface and

therefore reduces the formation of a protein corona.[64,65] This in turn may increase nanoparticle blood circulation times and theoretically increase passive and active targeting capabilities of PEGylated nanoparticles and ligand-conjugated nanoparticles.[114]

To mitigate the negative effect of protein corona formation on active targeting, Dai *et al.* developed a PEG surface modification strategy for antibody-decorated gold nanoparticles.[81] Antibodies, such as the FDA-approved HER2 targeting monoclonal antibody Trastuzumab (Herceptin), are conjugated with 5-kDa PEG. This PEG linker is then covalently attached to the gold nanoparticle surface via sulfur−gold interactions at a density of <0.1 antibodies per square nanometer of nanoparticle surface area. The remaining nanoparticle surface is then backfilled with shorter 2-kDa methoxy-terminated PEG. This PEG backfilling strategy significantly reduces nonspecific serum protein adsorption and maximizes specific antibody—cell surface receptor interactions.[81]

Other strategies to reduce nanoparticle protein corona formation include the use of zwitterionic polymers as surface ligands.[115,116] A different and highly innovative approach to reduce MPS uptake of nanoparticles is by covering the nanoparticles with cell membranes derived from erythrocytes, leukocytes, or thrombocytes.[117,118] This camouflaging strategy can help in the reduction of nanoparticle MPS clearance from the bloodstream and increase nanoparticle blood circulation times.[119] Discher *et al.* reported a biologically inspired nanoparticle surface engineering strategy to minimize MPS recognition and clearance of nanoparticles. They demonstrated that nanoparticles showed reduced uptake by phagocytic cells for improved drug delivery when the nanoparticle surface was decorated with minimal "self"-peptides, such as CD47 peptides.[120]

In summary, nanoparticle surface engineering strategies allow reduction in protein corona formation and MPS clearance by reducing nonspecific protein adsorption onto the nanoparticle surface. However, these strategies cannot completely passivate the nanoparticle surface and may not fully prevent protein adsorption. This opens up many opportunities for researchers to focus on better chemical and biological nanoparticle surface engineering strategies. The goals of these strategies may be manifold, including (1) minimizing nanoparticle protein corona formation; (2) reducing nanoparticle MPS interactions; (3) controlling nano−bio interactions; and (4) improving nanoparticle delivery to targeted tissues and cells in the body.

4.8 Clinical potential of passive nanoparticle targeting

Passive and active nanoparticle targeting strategies are extensively used in preclinical research. In contrast to clinical research, which involves human subjects, preclinical research involves studies in tissue culture and animal models. While the concept of "active nanoparticle targeting" has generated significant interest with >1500 preclinical publications on this topic between 2007 and 2017, there are currently no FDA-approved active targeting cancer nanotherapeutics.[82,121] In 2016, seven active targeting nanoparticle cancer formulations were in early clinical trials (phase I and phase II) according to a survey by Chan *et al.*[121] These data highlight that, while active targeting is an attractive idea, significant challenges exist with delivering nanoparticles to tumors and cancer cells. One of these challenges is to deliver nanoparticles *in vivo* in adequate quantities to their intended diseased tissue destinations.[122] Research by Chan *et al.* has demonstrated that overall median nanoparticle delivery efficiencies to solid tumors in preclinical studies reach only approximately 1% of the injected nanoparticle dose.[38] Importantly, the nanoparticle delivery efficiency to malignant cells in a solid tumor has been reported to be about 500 times lower (<0.002%ID) with similar efficiencies for passive and active targeting nanoparticles.[82] This indicates that the majority of nanoparticles that reach a tumor do not interact with tumor cells. In addition, intratumoral nanoparticles are more likely to interact with TAMs and other off-target cells in the tumor rather than malignant cells.[82,83]

Active targeting strategies have not yet translated into FDA-approved cancer nanotherapeutics. The current list of FDA-approved cancer nanotherapeutics comprises five liposome-based and one protein-based formulations (Table 4.1).[15,123] These formulations have been designed with the goal to exploit passive nanoparticle targeting strategies and do not exhibit active targeting surface ligands. The first cancer nanotherapeutic received FDA approval in 1995 and was introduced into the market as Doxil.[124,125] Doxil is a liposomal formulation of the small-molecule cancer drug doxorubicin. Doxil liposomes have been formulated with PEG surface ligands to increase blood circulation times by reducing MPS interactions. Therefore, Doxil is a typical example of a cancer nanotherapeutic formulation that exploits the fundamental concepts of passive nanoparticle targeting for improved nanoparticle tumor delivery.[17]

In summary, passive targeting has high clinical relevance in nanomedicine. All FDA-approved nanoformulations are based on passive targeting design principles. While active targeting nanoparticles are intensively explored and applied in preclinical research, only few of them have entered clinical trial phases. It is important to note that none of the current FDA-approved cancer nanotherapeutics uses active targeting strategies for enhanced tumor accumulation or cell targeting specificity.

TABLE 4.1 FDA-approved cancer nanotherapeutics.

Generic name and/or proprietary name	Nanoparticle type	Active pharmaceutical ingredient (API)	Type of cancer	Year of FDA approval	Refs
Liposomal doxorubicin (Doxil)	Liposome	Doxorubicin	HIV-related Kaposi sarcoma, ovarian cancer, and multiple myeloma	1995	17
Liposomal daunorubicin (DaunoXome)	Liposome	Daunorubicin	HIV-related Kaposi sarcoma	1996	126
Nab-paclitaxel (Abraxane)	Albumin nanoparticles	Paclitaxel	Breast, lung, and pancreatic cancer	2005	127
Liposomal vincristine (Marqibo)	Liposome	Vincristine sulfate	Acute lymphoblastic leukemia	2012	128
Liposomal irinotecan (Onivyde or MM-398)	Liposome	Irinotecan	Postgemcitabine metastatic pancreatic cancer	2015	129
Liposomal cytarabine-daunorubicin (Vyxeos or CPX-351)	Liposome	Cytarabine and daunorubicin (5:1)	High-risk acute myeloid leukemia	2017	123

4.9 Perspective and conclusion

Effective and efficient delivery of drugs and imaging contrast agents to tumors in the body is one of the major challenges in cancer treatment and diagnosis. Strategies that allow specific delivery of chemotherapeutic drugs to diseased tissues while avoiding healthy organs and cells are needed. Current nanomedicines are designed to address these clinical needs. Researchers have been engineering nanoparticles with active and passive targeting capabilities. However, the current list of FDA-approved cancer nanotherapeutics comprises only passive targeting nanoparticles (Table 4.1). Most of these formulations have received FDA approval because they showed favorable toxicity profiles in comparison with administration of free drug rather than improved overall survival.[130]

One of the potential reasons for why it is so challenging to translate cancer nanomedicines into the clinic is the current gap between preclinical animal models and human subjects.[130a] While preclinical animal tumor models may exhibit EPR, its prevalence in tumors of human subjects may be substantially different. The extent of EPR for human tumors is a controversial topic due to significant intratumoral and interpatient variability.[29,49,131] This variability makes it challenging to select cancer patients who may benefit from treatment with cancer nanomedicines. To address this challenge, a recent clinical trial quantified tumor accumulation of administered ferumoxytol (iron oxide nanoparticle) as a marker to predict tumor treatment response to a liposomal irinotecan formulation (MM-398). Preliminary results indicate positive correlation between tumor size reduction (i.e., tumor shrinkage) as a result of MM-398 treatment and ferumoxytol levels in tumors for a small group of patients.[132] These results are encouraging as they may suggest that researchers could preselect patients who are more likely to benefit from nanomedicine to improve overall therapeutic outcomes.[133]

Another challenge in preclinical research is that commonly used fast-growing animal tumor models do not recapitulate the majority of solid tumors in humans in terms of pathophysiology and EPR.[24] Therefore, animal tumor models that better resemble human tumor pathology are needed.[134,135] The variability of tumors is a major concern when developing nanoparticles for cancer applications. A recent study by Sykes *et al.* demonstrated that physicochemical properties of nanoparticles can be optimized according to a tumor's pathophysiology to improve nanoparticle tumor accumulation and therapeutic effects.[136] While this study used preclinical animal models, it suggests that there may be a need to tailor nanoparticle design to a patient's individual cancer characteristics, including cancer type and stage. This may open opportunities for personalized cancer nanomedicine, which may be combined in the future with companion diagnostics and imaging to preselect patient groups with improved likelihood for nanomedicine-based treatment response.

The development of personalized nanomedicine requires deep understanding of nano–bio interactions.[136a] Biological processes and mechanisms involved in the biodistribution and intratumoral distribution of nanoparticles need to be further investigated to guide the design of nanomedicines.[4] To this end, Chan *et al.* have applied 3D optical microscopy as a new tool with the ultimate goal to

image the intratumoral distribution of nanoparticles on a whole-tissue level with subcellular resolution.[137–139] High-resolution volumetric optical imaging results can be further supported by single cell analytical approaches, including flow cytometry, fluorescence-activated cell sorting, and single-cell elemental analysis (for example, single-cell inductively coupled plasma mass spectrometry, SC-ICP-MS[140]). Quantitative imaging studies and single-cellbioanalysis may be used to inform the engineering of nanoparticles with optimal intratumoral distribution and cellular interaction.[140a]

While EPR has become a blanket term that incorporates a large number of complex biological processes,[4] engineering of nanomedicines with EPR as the only nanoparticle design rationale may be outdated. As discussed in this chapter, passive and active targeting nanoparticles rely on passive paracellular transport across the endothelium for tumor accumulation. This is a fundamental paradigm of the EPR effect. However, studies have emphasized the high intratumoral and intertumoral variability that may raise concerns about its significance.[27] While paracellular extravasation of nanoparticles has been the key concept for designing nanomedicines over the past few decades, recent research suggests the possibility for transcellular extravasation pathways.[141,142] Specifically, caveolae-mediated pathways across tumor endothelium as reported by Schnitzer *et al.* may be intriguing new concepts of nanoparticle delivery to tumors.[39,143] In addition, the use of iRGD peptides as shown by Ruoslahti and coworkers may open transcytosis pathways for nanomedicines to cross the tumor endothelial barrier.[144–146] Research by Dvorak *et al.* suggests a different transcellular pathway for nanoparticles. A network of grouped and interlinked cytoplasmic vesicles and vacuoles, termed vesiculovacuolar organelle may be exploited for efficient nanoparticle delivery into tumors.[38,147,148] These transcellular pathways may open up new strategies for nanoparticle tumor delivery and may shift the EPR paradigm toward a transcellular nanoparticle tumor delivery mechanism. However, more research is needed to better evaluate the significance and impact of these transcellular pathways for cancer nanomedicine.[38]

In summary, the concept of passive nanoparticle targeting has allowed the design and clinical translation of nanomedicines for systemic use in patients. The current collection of FDA-approved cancer nanotherapeutics exploits the fundamental concepts of passive targeting, which highlights the clinical potential of this technology. However, the true promise of nanomedicine has yet to be fulfilled. Current nanoparticle targeting strategies do not allow full control over biodistribution and cellular interactions of nanomedicines.[148a] More research is needed to better understand nano–bio interactions, systemic nanoparticle transport, and delivery of nanoparticles to targeted sites in the body. Further fundamental research studies with the aim to elucidate biological mechanisms and processes of nanoparticles *in vivo* may guide the design and development of future nanomedicines. This improved fundamental understanding may enable researches to achieve the true goal of nanomedicine, that is, substantial improvements in patient survivals and significant clinical benefits.

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