

Biologically based strategies for overcoming in vivo barriers with functional nano-delivery systems

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Abstract

Nanomedicine has been developed to reduce or eliminate the side effects and toxicity upon systemic therapy of chemotherapeutic agents and to improve their therapeutic efficacy. However, the translation of non-sized or nano-encapsulated drugs is hampered by the low penetration and accumulation of engineered nanoparticles (NPs) in sites of tumors as well as their poor pharmacokinetics. This may be due to the synthetic structure of NPs and also complicated and unknown characteristics of the solid tumor microenvironment (TME). As a result, the TME is being better identified, and the interactions between NPs and the TME or human body are being discovered or predicted. These findings have led to the development of more biocompatible, intelligent, and controllable bio-based nanoformulations that could overcome current barriers and provide sufficient drug delivery to the TME, as discussed in this paper. These formulations are designed to (i) modify the surface of NPs to improve blood circulation while reducing their off-target accumulation and side effects in vivo, (ii) pass through the tumor vasculature by modulating or targeting angiogenesis, (iii) promote NPs distribution in solid tumor regions by applying biological/physical stimuli or extracellular matrix remodeling, and (iv) overcome the cell membrane barrier and other compartments of the cell by specific cell targeting to release the payload drug into the cytoplasm or nucleoplasm.

KEYWORDS

biological barriers, cancer, drug delivery, nanotechnology, tumor microenvironment

1 | INTRODUCTION

Cancer cells are located in a complicated tumor microenvironment (TME) consisting of cancer cells, stromal cells (fibroblast and mesenchymal stem cells [MSC]), tumor-supporting immune cells (i.e., regulatory T cells, M2 type macrophage, etc.), all linked to each other

in the domain of extracellular matrix (ECM).^[1] To survive they need to be nourished by preexisting blood vessels or require induction of angiogenesis.^[2] During cancer growth and progression, the ECM network is remodeled, resulting in impaired function of blood vessels that, unlike normal conditions, they lose ordered structure.^[3] All this leads to the formation of leaky tumor blood and an increase in

intratumoral fluid pressure and may also create a hypoxic area.^[4] Hypoxia is associated with angiogenic induction, immune suppression, tumor invasion and, importantly, reduction in the therapeutic efficacy of chemo agents in both free and nanoformulated forms.^[5]

Although the leaky tumor vessel and the enhanced permeation and retention (EPR) are expected to provide high penetration of nanoparticles (NPs) into the TME,^[6] the abnormal vasculature and a dense ECM limited the extravasation, delivery, and impact of NPs on cancer cells.^[7] In addition to the physicochemical properties of NPs (i.e., charge, size, surface chemistry, and shape), which affect their stability and function in vivo, their penetration into the cytoplasm or compartment of cells and release of entrapped drugs is faced with other barriers,^[8] all of which require consideration for efficient drug delivery to the solid tumors. Systemically injected NPs must cross the intravascular-, extracellular-, and cellular-barriers, which all affect the NP's half-life in the bloodstream, penetration and accumulation in tumor site, and uptake by cancer cells and even subcellular organelles to exert their therapeutic effects.^[9,10] The interaction mechanisms and factors determining the ability of NPs to open the paracellular pathway provide a better understanding of how NPs can open the paracellular pathway in a safe and controllable manner. The number of biological barriers varies according to target depth, and therefore, various multifunctional NPs are developed to cross the mentioned barriers, as presented in this study.

2 | ENGINEERED NPs TO CROSS TUMOR-ASSOCIATED BARRIERS

2.1 | Challenges within NP's half-life in circulation and how to solve them

Conventionally, the foreign particles and intravenously injected NPs in the bloodstream are cleared by (i) specific pathway identified by deposition of opsonins such as natural immunoglobulins and third complement component (C3) on NPs and then taken up by phagocytes having receptors for opsonin or induce proinflammatory responses and infusion-related adverse effects including allergic reactions,^[11,12] and (ii) nonspecific binding of lipids or lipoproteins and plasma proteins (or protein corona formation) (e.g., albumin, IgG) to the NPs to reach an acceptable size that can be taken up and premature cleared by the reticuloendothelial system (RES).^[13,14] Moreover, the spherical particles limit both active targeting strategies as well as effective accumulation by passive targeting mechanisms (e.g., EPR). In addition, the size of the nanocarrier also influences its in vivo fate, with evidence that larger particles (>200 nm) accumulate in the liver and spleen. On the other hand, NPs with a diameter of <~5 nm undergo rapid renal clearance after intravenous administration.^[15] These undesirable processes alter the original physicochemical identity of the NPs and significantly reduce their half-life in circulation, and also limit their identification and uptake by cancer cells by covering their targeting moieties (i.e., antibodies, aptamers,

lipoproteins).^[16] In summary, NPs with an average size of ~100 nm are generally long-lived in circulation. Long blood half-life increases the tendency of NPs to extravasate through fenestrations in the tumor vasculature, which range in size from 380 to 780 nm.^[15]

Alleviating these challenges and to reduce off-target uptake, and providing longer retention time in vivo, the surface of NPs precisely shielded or modified by (i) biologic materials such as hyaluronic acid,^[17] synthetic peptides,^[18] antibodies, clusterin (apolipoprotein J), CD47 proteins,^[19,20] plasma immunoglobulin/protein,^[21] tumor cell-derived peptides and membrane,^[22] and (ii) non-biologic compounds such as polyethylene glycol (PEG)^[23] and polyvinyl pyrrolidone, polyglycerol,^[24] polyphosphoester,^[25] methacryloyloxyethyl phosphoryl choline polymers^[26] (Figure 1A). Polymer-coated NPs inhibit some nonspecific interactions with proteins. This delays the NP opsonization process and reduces NP uptake in macrophage cells. This also results in an improvement in NP circulation time in the blood due to the avoidance of RES trapping and decreasing NP aggregation.^[27] CD47 molecules as cell surface receptors binding to the signal-regulatory protein α on the macrophage membrane leading regulator of phagocytosis, and this anti-phagocytic property of CD47 used to functionalization of NPs with whole or recombinant CD47 domains and peptides, which elevated the half-life of CD47-functionalized NPs, and provided delivery of paclitaxel in rats with A549 tumors^[28] (Figure 1B).

Another strategy for evading the NPs from the immune system prolonged their blood circulation, and specific recognition (due to the membrane antigens) is camouflaging of them with cell membranes of RBC, platelets, immune cells and cancer cells^[29] (Figure 1C), that albumin-based NPs coated with macrophage plasma membranes and loaded with paclitaxel showed prolonged blood circulation after 24 h and also induced antitumor efficacy after selective accumulation at the tumor site in B16F10 tumor-bearing mouse xenografts.^[30] Using the tumor-self components, the thermosensitive liposome vesicles, which were coated with the HCC cell membrane, and loaded with doxorubicin and indocyanine green as a photosensitizer, indicated its ability of homologous aggregation of HCC cell membranes and can target to recurrent HCC tumor regions.^[31] Regarding the peripheral macrophages, as the largest sequesters up to 70% of nanomaterials NPs, another strategy is macrophage depletion before NPs administration as the liposome-loaded clodronate 48 h before administration of AuNPs significantly depleted spleen and liver macrophages while prolonged AuNPs stability and increased their tissue accumulation and antitumor effect in vivo.^[32]

In addition, the outer membrane of erythrocytes expresses decay accelerating factors (DAF or CD55) and CD59 as well as complement receptor 1 (CR1), which protect erythrocytes from complement attack and fixation by complex mechanisms. CR1 and DAF regulate the early phase of the complement cascade by inhibiting C3 convertases, while CD59 inhibits the assembly of the membrane attack complex. Given these protective mechanisms, coating NPs with these proteins or derivatives could be used in the development of functional NPs^[33] (Figure 1D).

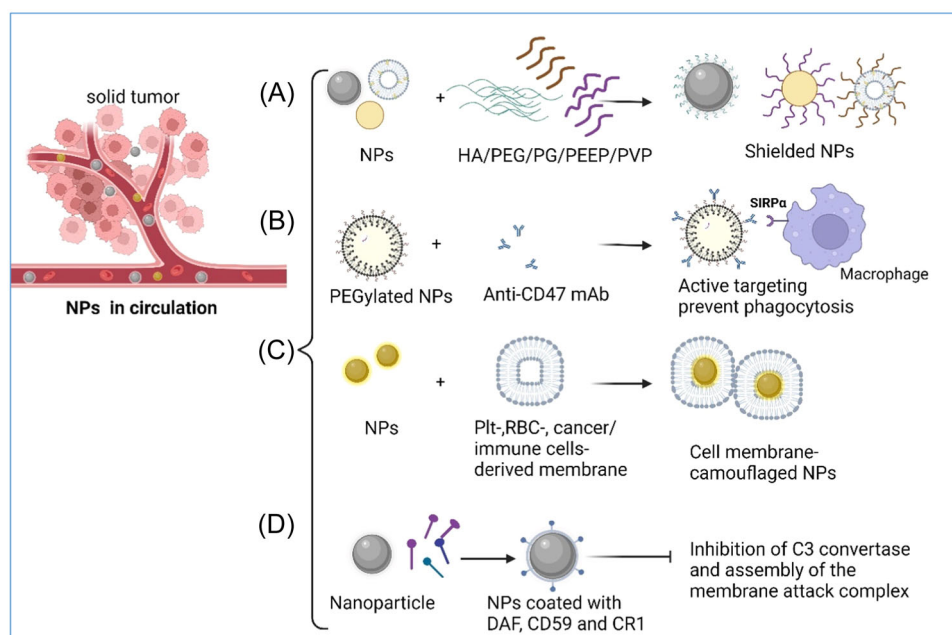


FIGURE 1 Conventional approaches include (A) shielding, (B) active targeting, (C) camouflaging with the cell membrane, and (D) coating with DAF, CD59, or CR1 to suppress complement attack and to increase the half-life of intervenes injected NPs in circulation. CR1, complement receptor 1; DAF, decay-accelerating factors; HA, hyaluronic acid; mAb, monoclonal antibody; NP, nanoparticle; PEEP, polyphosphoester; PEG, polyethylene glycol; PG, polyglycerol; Plt, platelet; PVP, polyvinyl pyrrolidone; RBC, red blood cell; SIRP α , signal-regulatory protein α .

2.2 | Challenges within NP's extraversion and how to solve them

Another important barrier that limits NPs localization and retention in the tumor region is a vascular wall, which demands consideration to present high delivery efficiency and permeability at the tumor site. Different approaches are developed to address this barrier, including vasodilation,^[34] vascular normalization,^[35] vascular disruption,^[36] vascular infarction,^[37] active vascular targeting,^[38] vascular component depletion,^[39] and using the Trojan system.^[40]

Vasodilation of tumor blood vessels is an approach performed by several methods such as local nitric oxide (NO) generation, ultrasound, high-intensity focused ultrasound (pHIFU), radiotherapy, photothermal therapy (PTT), photodynamic therapy (PDT), and their combination, to increased blood flow and permeability of NPs to the tumor tissue and even tissue penetration while providing oxygenation of hypoxic tumors.^[41] NO plays a role in regulating angiogenesis and maintaining vascular homeostasis and can be used to normalize tumor vasculature. As an example of vasodilation, under in situ ultrasound stimulation, the ultrasound-responsive liposomes loaded with PAMAM@ doxorubicin and nitrosoglutathione (GS) to generate NO showed that the low-dose NanoNO promoted tumor vasodilation and enhanced the permeation of NPs with the release and penetration of doxorubicin into the tumor depth.^[42]

Addressing the nonstructured vascular network in tumor condition, another strategy is tumor vessel normalization or neovascularization through the restoration of endothelial cell integrity, promotes pericyte coverage and increased VE-cadherin tight junctions by using

optimized doses of antiangiogenic agents (e.g., anti-VEGF/R and anti-TGF- β 1 antibodies, and angiopoietin-1)^[43] (Table 1). Vessel normalization promotes the delivery of radioactive agents and both free and nanoformulated of chemotherapeutic drugs, especially small NPs (12 nm).^[55] PEGylated gold (Au)NPs loaded with human recombinant endostatin (rhES) reduced vascular permeability and improved vascular normalization while increasing the intratumoral delivery of cytotoxic 5-FU in HCC-bearing mice.^[51] In addition, the combination of tumor blood vessel normalization chemotherapy using multifunctional NPs (CCM@LMSN/DOX-loaded sunitinib (a receptor tyrosine kinase inhibitor), resulted in neovascularization through the expression of junctional proteins Claudin-4 and VE-cadherin of endothelial cells and delivery of nanoformulated DOX inhibited breast cancer growth and metastasis in vivo.^[56]

Taking into account the tumor's acidic pH, pH-sensitive selenium NPs loaded with anti-VEGF siRNA (small interfering RNA) accumulated significantly in the tumor region and reduced tumor growth in vivo, mediated by the silencing of the VEGF gene.^[57] In addition, pH-sensitive switchable polymeric NP to delivery of siRNA targeting the Nogo-B receptor (NgBR) as a regulator of angiogenesis and EMT, resulted in suppression of NgBR expression in tumor-bearing tissues, followed by normalizing tumor vessels, EMT reduction, and suppressing the breast cancer cells metastasis in vivo (Figure 2).^[54]

More importantly and besides drug delivery, vascular normalization promotes ECM remodeling,^[58] reprograms the immunosuppressive TME,^[59] and provides durable antitumor immunity by tumor immune reprogramming and enhancing therapeutic effects of immune checkpoint blockers (ICBs) (i.e., antibodies targeting

TABLE 1 Application of nanomedicine for tumor vessel normalization and therapy.

Nanocarrier	Antiangiogenic agents	Combined with	Tumor model/major finding	References
Mesoporous silicon nanoparticle (MSN)	Dopamine	Doxorubicin	Mouse tumor model/the blood flow penetration and DOX effect are much higher in normalized vessels compared to abnormal vessel structures.	[44]
Low-density lipoprotein (LDL)-based NPs	Vandetanib	-	4 T1 breast cancer model/long-term normalized vasculature improves tumor perfusion, reverses hypoxia and heterogeneity, and also inhibits tumor progression.	[45]
Enzyme responsive AuNPs	Cediranib	-	4T1 tumor model/cediranib pretreatment markedly increased tumor vascular permeability and tumor oxygenation.	[46]
Nanodroplets (NDs)	Physical US stimulation	Doxorubicin	DOX-NDs + US a large amount of drug extravasation, vascular disruption, and immune cell infiltration at the tumor center.	[47]
HSA-PTX nanoparticles	Erlotinib	Paclitaxel/anti-PD-L1 antibody	4T1 breast tumors, CT26 colorectal tumors, and SCC7 carcinoma tumors/erlotinib treatment lead to downregulated VEGF expression increased tumor accumulation of HSA-PTX nanoparticles in mice models.	[48]
Thermosensitive nanocarriers	Light irradiation	Doxorubicin	A pharmacodynamics model/responsive NPs supply more than 2.1 times more drugs than traditional chemotherapy in extracellular space.	[49]
Doxil nanomedicine	Tranilast	ICB therapy	Tranilast-Doxil-ICB decrease in ECM levels that followed by an increased number of CD3 + T cells/decrease in the levels of pro-tumor M2-like TAMs and reduction of metastatic burden in metastatic models, 4T1 and E0771.	[50]
Gold nanoparticles (AuNPs)	Recombinant human endostatin (Endostar/rhES)	5-FU	rhES-AuNPs-PEG induce transient tumor vascular normalization and enhance the antitumor efficacy of cytotoxic 5-FU.	[51]
Gold nanoparticles (AuNPs)	-	-	Reversed EMT in B16F10 cells correlated with reductions in melanoma metastases.	[52]
PAMAM dendrimers-anti-EGFR Apt	Chloroquine (CQ)	Erlotinib/survivin-shRNA	Promoted drug delivery and enhanced drug efficacy in Erlotinib-resistant NSCLC cells in vitro and in vivo.	[53]
Polymeric nanoparticle	siRNA against Nogo-B receptor (NgBR)	-	Orthotopic breast cancer model/NP-NgBR siRNA significantly increased supply of oxygen, disturbs the function of TEC, reverse the EMT, and suppress distant metastasis.	[54]

Abbreviations: ECM, extracellular matrix; ICB, immune checkpoint blockers; PEG, polyethylene glycol; siRNA, small interfering RNA; TEC, tumor endothelial cells.

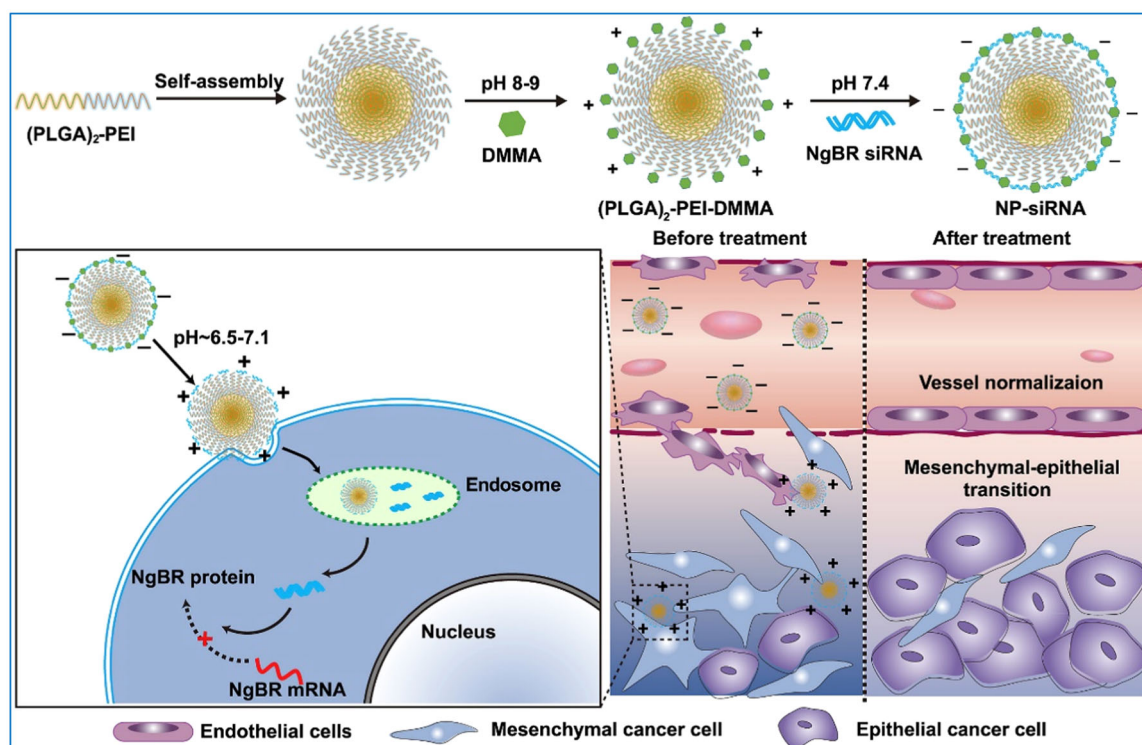


FIGURE 2 Combination of nanomedicine and siRNA therapy for tumor vessel normalization. NP-NgBR siRNA was surface-absorbed and conjugated to polymeric NPs containing 2,3-dimethylmaleic anhydride (DMMA) molecules and poly(lactic-co-glycolic acid)-poly(ethylenimine) ((PLGA)₂-PEI) copolymers. The negatively charged NPs at pH 8–9 were converted to positive surface charge upon arrival at the acidic TME (pH 7.4). Thus, NP-NgBR siRNA was effectively taken up by tumor cells, followed by NgBR downregulation in both tumor cells and endothelial cells, resulting in vascular normalization and reversal of the process of epithelial–mesenchymal transition (EMT). NgBR, Nogo-B receptor; siRNA, small interfering RNA; TME, tumor microenvironment. Reproduced from Wang et al.^[54]

programmed death-ligand 1 [PD-L1], and PD-L2 in tumor cells and cytotoxic T-lymphocyte-associated protein 4 [CTLA-4] and programmed cell death protein 1 [PD-1] expressed on T cells.^[60] In addition, vascular normalization improves the infiltration of effector immune cells (CD4/8T cells) that subsequently, the activated CD4 + T lymphocytes increase vessel normalization upon treatment with ICBs^[61] while restricting tumor metastasis by closing vascular gaps^[62] (see examples in Table 1).

Disruption of preexisting tumor vascular by free (e.g., CA4, combretastatin A4) and nanoformulated vascular disrupting agents provide a long-lasting effects followed by tumor necrosis and even apoptosis due to reducing blood flow and interruption of cell proliferation. Regards to the low bioavailability and short half-life of free CA4, conjugation of CA4 agent with the poly (L-glutamic acid) (PLGA)-based NPs, improved its *in vivo* circulation and enhanced accumulation at the tumor site while induced vascular disruption as well as reduced the growth of aggressive murine colon C26 tumor *in vivo*.^[63] Addressing robust antitumor effect, nanoformulated CA4 associated with cytotoxic payload such as doxorubicin and docetaxel, that sequentially released CA4 provide vascular disruption toward tumor malnourishment and then, the cytotoxic effects of drugs induced cell apoptosis *in vivo*.^[64] Moreover, pretreatment with tumor-vascular disrupting agents, including microtubule destabilizing

drugs (e.g., combretastatin A-4 disodium phosphate [CA4P, CA1P], Plinabulin, and CDK-516), combretastatins (e.g., combretastatin A-4, CA4), and flavonoids such as Vadimezan (or DMXAA) provided accumulation of small fibrinogen-conjugated AuNPs (fAuNPs) in tumor site for following PTT under near-infrared irradiation as well as inhibition the tumor metastasis^[65] (Figure 3).

However, wide vascular disruption may lead to hypoxia to surrounding normal tissue, which could be limited to the tumor area by targeting tumor endothelial cells (TECs) and induction of vascular thrombosis in the tumor-feeding endothelium. NPs decorated with TECs-specific markers provide targeted homing to the tumors, as iron oxide NP (SPIO) decorated with CREKA peptide specific for fibrin clots or fibrin–fibronectin complexes provided aggregation and binding of SPIO to TECs.^[66] In combination with chemotherapy and tumor-infarction therapy, the chitosan-based polymer NPs loaded with thrombin as a coagulation factor and doxorubicin and surface functionalized with CREKA peptide. *In vivo* results indicated this targeted nanoplateform significantly accumulated in the tumor and improved the prolonged antitumor therapeutic index in an animal model.^[67] Anyway, more than the EPR effect, it seems that trans-endothelial pathways in the TECs play a vital role in the extravasation and delivery of NPs into tumorous tissues.^[68] Addressing this criteria, targeting NPs within the TEC-specific marker or activator of

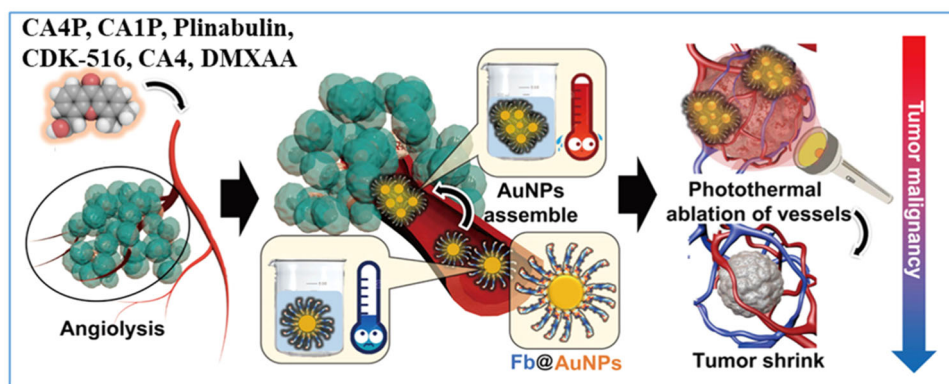


FIGURE 3 Combination of tumor-vascular disrupting agents (e.g., CA4P, CA1P, Plinabulin, CDK-516, CA4, and DMXAA) and photothermal therapy (PTT) Ps. The administration of DMXAA leads to the disruption of tumor vessels, which induces the aggregation of fibrinogen-conjugated gold nanoparticles (Fb@AuNPs) in tumor vessels. This is followed by near-infrared irradiation (NIR) for further photothermal ablation of the tumor vasculature. NPs, nanoparticles. Reproduced from Hong et al.^[65]

transendothelial and transcellular transport by coated NPs with iRGD as known tumor-penetrating peptides is another strategy that enhanced uptake of NPs by tumor cells vesicular transport pathway.^[69]

Finally, another biomimetic strategy for crossing the vascular barrier and accumulation of drugs or nanodrugs in the tumors is employing cellular or natural biocarriers with the capability of extravasation and tumor homing to deliver specific cargoes. MSCs are examples of natural carriers with intrinsic homing property and tropism to both primary and metastatic human tumors, thus, making them promising candidates for tumor-targeted therapy.^[70] MSC-loaded with silica NPs-Pp-18 photosensitizer demonstrated a naturally high tumor affinity in vivo, and the retention of PS-SiO₂NPs-loaded MSCs after injection led to significant inhibition of tumor growth by PDT in the breast tumor model.^[71] In addition, human MSC-loaded with poly (lactic-co-glycolic acid) (PLGA) microparticles containing the G114 prodrug provided sustained release of G114 in prostatic tumor xenografts in vivo of nude athymic mice and displayed an antitumor effect.^[72]

Regarding to poor penetration of NPs to some micrometastases and hypoxic tumors by nature, exploiting inflammatory immune cells such as monocytes, macrophages, and neutrophils with natural metastasis-homing abilities and loaded with therapeutic NPs (e.g., SMA-AANK-Mertansine conjugate, doxorubicin-loaded polymer vesicles, and paclitaxel-PLGA NPs) resulted in efficient drug deliver tumor-tropic monocytes and inhibition of metastatic cancer cells in vivo.^[73–75] However, the limitation of tumor infiltration capability of neutrophils may reduce their location in the core of solid tumors, which could be alleviated using bacteria-secreted outer membrane vesicles (OMVs)-coated PEG-b-PLGA NPs encapsulating PBIBDF-BT (PBT) NPs (OMVs are naturally sensed and ingested by neutrophils) for PTT pretreatment as resulted in 60% complete tumor eradication in mice model^[76] (Figure 4).

Similar to whole live cells, NPs could be loaded into cell-derived membranes and exosomal vesicles as extracellular nano-shuttles, and exploiting cancer cell-sourced exosomes and then loaded the

anticancer therapeutics (e.g., Doxil) or NPs (ZnO nanocrystal) indicated their homing to their original tumor cells/tissues and eradicated them in vitro^[77] and in vivo in HT1080 tumor-bearing mice while avoided off-target cardiotoxicity.^[78] Unlike synthetic vesicles and liposomes, exosomes have excellent in vivo stability, display don't eat me signal by nature, and also express transmembrane and membrane-anchored proteins that can promote endocytosis, thereby enhancing the delivery of their internal cargo.^[78]

2.3 | Challenges within NP's tissue distribution and how to solve them

Over the vascular wall, NPs faced to the overexpressed fibrotic ECM with a dense structure due to high collagen content and lack of efficient lymphatic drainage, which limited the penetration and distribution of NPs in solid tumor regions. Moreover, the interstitial fluid pressure within the core of tumorous tissue may pump out the NPs from the tumor, which demands consideration upon drug delivery. Therefore, penetration of NPs into deep sites of solid tumors demands physical or biochemical degradation or remodeling of ECM (Table 2), and applying collagosome, a 100 nm liposome encapsulating collagenase, resulted in a reduction of fibrotic tissue in pancreatic ductal adenocarcinoma (PDAC) which followed by enhancing tissue permeability and penetration of paclitaxel micelles as well as tumor growth inhibition by 87% in vivo.^[92] Regard to overexpression of hyaluronan (HA) as a typical glycosaminoglycans (GAGs) in ECM, exosome-mediated co-delivery of PH20 hyaluronidase and doxorubicin was evaluated in PC3 (HA^{high}) tumor-bearing mice. Results have shown that Exo-PH20-Doxorubicin effectively depleted the HA from ECM and increased the subsequently enhanced tumor penetration of Dox through increased relative vascularization that was associated with ≈83% inhibition tumor growth in PC3 xenograft mouse models and confirmed the combination treatment resulted in improvement of antitumor efficacy.^[93] Addressing the sufficient proteolytic

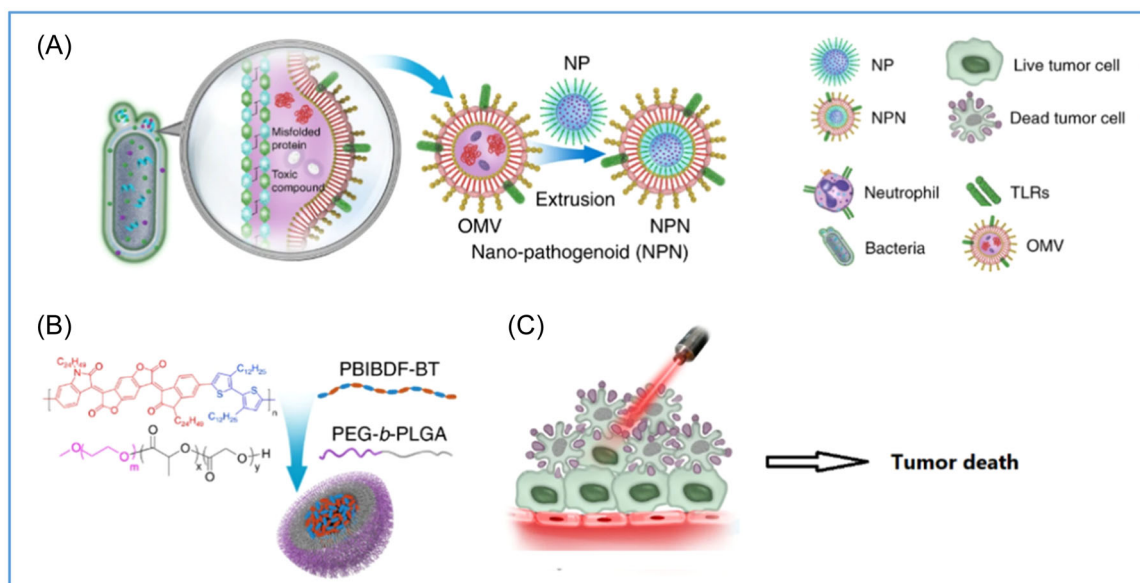


FIGURE 4 (A) Preparation of nano-pathogenoid (NPN) by coating OMVs on NPs. (B) Preparation of PEG-b-PLGA NPs encapsulating PBIBDF-BT as a photothermal transducer. (C) Neutrophils sensed NPNs with the recognition of LPS and lipoprotein by TLRs and subsequently engulfed them. Neutrophils laden with NPNs were recruited into the tumor site in response to laser light used in PTT, which causes NPNs to be released from neutrophils to kill tumor cells. NPNs, nanoparticles; PEG, polyethylene glycol; PTT, photothermal therapy. Reproduced from Li et al.^[76]

enzyme stability, recombinant human hyaluronidase PH20 (rHuPH20) was embedded in the PEG layer and functionalized onto the surface of PLGA-NPs containing doxorubicin that resulting in the enhancement of serum half-life of rHuPH20 in vivo that improved the penetration of nanoplatforms into tumors region while inhibited the growth of aggressive 4T1 mouse breast tumors.^[94]

In addition to ECM degradation, targeting the stroma cells (fibroblasts) associated with the desmoplastic TME contributes to the ECM remodeling,^[95] that lipid-coated protamine DNA complexes loaded with sTRAIL (soluble Tumor necrosis factor-related apoptosis-inducing ligand) resulted induction of apoptosis in tumor cells and dormancy in associated fibroblasts as well as remodeling the TME in a murine xenograft model of human desmoplastic bladder carcinoma.^[96] Moreover, applying physical methods and external stimulus such as pulsed high-intensity focused ultrasound (pulsed-HIFU) R and ultrasound boosts the EPR effect and triggers the cleavage of the S-S linkage in the ECM network that results in deep penetration and cellular internalization of NPs.^[97] External stimulus such as light NIR or an alternating magnetic field induce hyperthermia in delivered gold NPs, carbon nanotubes, and magnetic iron oxide NPs, resulting in the irreversible denaturation and disruption of the collagen fibrils followed by increasing the internalization of theranostic NP.^[98] Interestingly such bacteria *Salmonella typhimurium* express hyaluronidase that could be employed in combination with nanomedicine for degrading human HA deposited within PDAC tumors and enhanced NP diffusion within tumor tissue.^[99] Finally, blocking local ECM synthesis through targeting crosslinkers (e.g., LOX, lysyl oxidase), ECM-maintain homeostasis, (e.g., MMPs, matrix metalloproteinases), and cathepsins have been developed to disrupt tumor ECM. For example,

the decoration of PLGA-PEG NPs with the anti-LOX antibody showed the suppression of ECM enhancement and tumor expansion in vivo.^[100] Overall, ECM limits NP transport in tumor tissues, and targeting or remodeling ECM is an important factor for improving the efficacy of drug delivery and cancer therapies.^[101]

2.4 | Challenges within NP's uptake by target cell and how to solve them

If the administrated NPs passed the above-mentioned journeys, then they faced within the cell membrane barrier and other compartments of the cell to release the payload drug into the cytoplasm or nucleoplasm. This transporting is affected by both the physicochemical/mechanical properties of NPs and also targeted tumor cells. For example, the rod-shaped NPs showed better cell binding and internalization upon endocytosis than the spherical NPs,^[102] and the nanodiscs and the stiff nanocarrier (e.g., Silica Nano capsules) are more accumulated in the cell than soft ones may due to elasticity and transformability limitation exerted by membrane wrapping during cellular uptake.^[103] The shape also has a strong influence on NP architecture, and discoidal particles exhibit unique tumbling and margination dynamics that are significantly more conducive to interaction with the vessel wall than spherical particles, with implications for particle binding and adhesion to the endothelium.^[104] Particle circulation half-life is also strongly influenced by the shape, with filamentous polymer micelles having a long circulation life (>1-week post-dose) compared to spherical counterparts (2–3 days). Regarding particle shape at first cell contact, particles with a

TABLE 2 Targeting ECM to improve NPs penetration and distribution.

Approach/treatment	Therapeutic system	Target	Major effect	References
<i>Physical method</i>				
Pulsed high-intensity focused ultrasound (Pulsed-HIFU)	Cy5.5-labeled glycol chitosan nanoparticles (Cy5.5-CNPs)	Collagen	Decreased collagen contents, and enhanced penetration of CNPs. Dye in treated A549 tumor tissues	[79]
Local hyperthermia	Gold nanorods	Collagen (I)	Improve the transport of large nanoparticles through collagen (I) matrix	[80]
Magnetic hyperthermia	Magnetic NPs	Collagen matrix	Facilitated the migration of the particles from the outer areas of the 3D structure to the inner parts	[81]
<i>Biochemical method</i>				
Coinjection with oncolytic HSV vector	Bacterial collagenase	Collagen	Vector distribution areas increased nearly threefold	[82]
Abraxane1 and GEM	PEGPH20	HA	Survival improved in patients with PDAC	[83, 84]
Red blood cell membrane-coated NPs	Decorated with rHuPH20	HA	Enhanced RBCM-NPs diffusion in matrix gels of PC3 prostate cancer cells	[85]
<i>Blocking ECM synthesis</i>				
Doxil	LOX _{ab} NPs	LOX	Increased L-MU-P positive tumor areas and overall survival in mice treated with Doxil	[86]
MU-P in a liposome (L-MU-P)	Phosphorylated prodrug of MU	Hyaluronan (HA)	Improves the tumor distribution of liposomes	[86]
Pegylated liposomal doxorubicin (Doxil)	Losartan	Collagen I	Enhancement of the efficacy of nanotherapeutics in patients with desmoplastic tumors	[87]
FOLFIRINOX (F-NOX)	Losartan	TGF-β1	Remodeling desmoplastic stroma and improving vascular perfusion	[88]
<i>Stromal cell depletion</i>				
Nanoparticle-based photodynamic therapy (nano-PIT)	Ferritin conjugated with FAP-specific scFv	CAFs	Suppressed ECM deposition, CAF depletion, and enhanced CD8 + T cell infiltration	[89]
NP loaded with DOX	Amphiphilic peptide decorated with mouse mAb to target human FAP-alpha on the surface	Fibroblast-activation protein (FAP) on CAFs	CAF depletion and breakdown of the stromal barrier	[90]
Encapsulating losartan and DOX	α-PD1	M2 TAMs	Cause ECM reduction, facilitating enhanced delivery of Dox-L and further DC maturation	[91]

Abbreviations: ECM, extracellular matrix; NPs, nanoparticles; PDAC, pancreatic ductal adenocarcinoma.

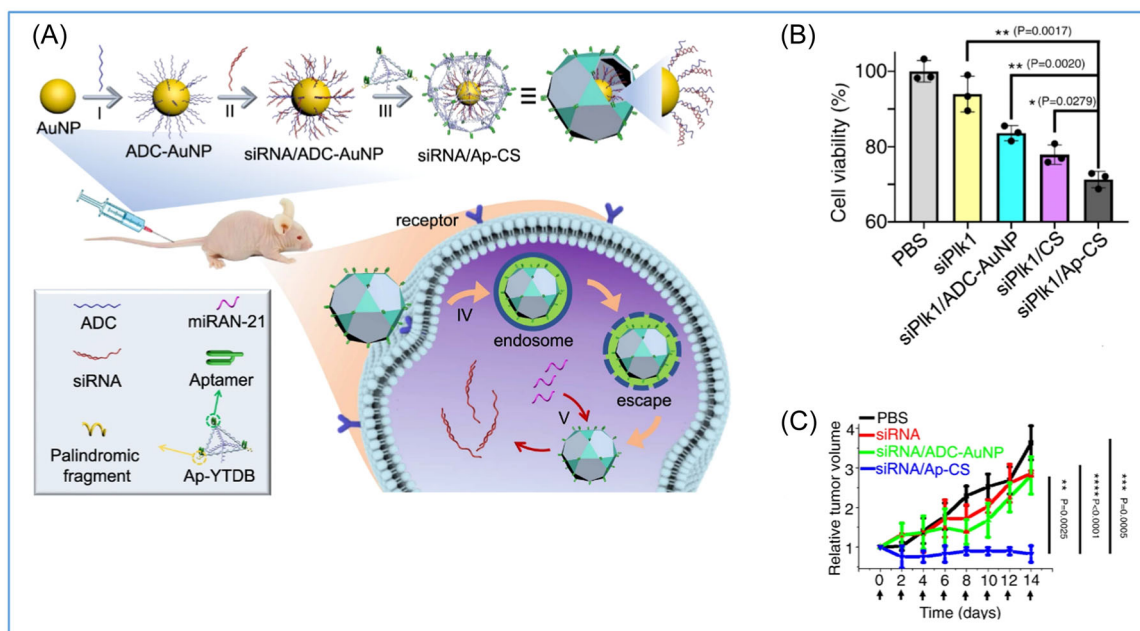


FIGURE 5 (A) Preparation of siRNA delivery and release inside tumor cells using multifunctional AuNP/aptamer core/shell-type siRNA delivery nanosystem, siRNA/Ap-CS. (B) cell viability and (C) tumor growth curves with different amounts of siRNA-targeting PIK1 (siPIK1)/Ap-CS. siRNA, small interfering RNA. Reproduced from Xue et al.^[120]

normalized curvature length, denoted Ω , less than or equal to 45° (spherical particles) undergo faster internalization than those with $\Omega \geq 45^\circ$. Interestingly, the aforementioned design parameter of size largely determined the successful completion of phagocytosis.^[105] These findings provided the rationale for the exploration of ellipsoidal, cylindrical, and discoidal particle shapes, all of which are constructs with high aspect ratios and minimal regions of curvature, such as worm-like particles and the aforementioned filomicelles, for the enhanced accumulation of therapeutics within tumors. Upon intravenous administration, paclitaxel-loaded nanoform and filomicelles showed higher tumor accumulation than spherical micelles.^[106,107]

Moreover, small and positively charged NPs showed more penetrate through the membrane than larger particles with negative charges,^[108,109] which could be enhanced upon functionalization of NPs with monoclonal antibodies (mAbs) or antibody binding fragment (Fab) targeting cancer cells specific receptors^[110] and this conjugation need be optimized according the distribution of these receptors in various cancers and also considered their stages.^[111] The neutral and negatively charged NPs have been shown to reduce serum protein adsorption, resulting in longer circulating half-lives and reduced liver and spleen accumulation.^[112] In contrast, positively charged NPs such as cationic liposomes show a higher rate of unspecific uptake in the majority of cells and facilitate endosomal release through mechanisms.^[113] Therefore, we may need to have a neutral or slightly negative surface charge on the NPs when they are administered intravenously, but a switch to a positive charge when they arrive at the tumor site.

In addition to NPs properties, the cancer cells can selectively uptake NPs, and these criteria must be considered to minimize uptake and effect of payload drug by off-target normal cells.

Meanwhile, tumor-specific targeting moieties (mAb or immune fragments) are decorated on the surface NPs that regard to the over-expression of Trop2 glycoprotein in the TNBC patients, the anti-Trop2 antibody-conjugated NPs (ST-NPs) loaded with doxorubicin and results have shown selective uptake by Trop2-positive MDA-MB-231 cancer cells.^[114] With regard to nonspecific NP delivery and toxicity on off-target cells and tumor cell heterogeneity, another approach to enhancing drug-to-cell delivery is the development of multivalent NPs or dual-receptor targeted NPs that target more than one receptor on cancer cells or co-targeting cancer cells and associated tumor-associated endothelial/stromal/immune cells in vivo. The K237 peptide (anti-EGFR) and Ep23 aptamer (anti-EpCAM) dual-functionalized NPs contained paclitaxel developed and showed a ligand density-dependent behavior for cell uptake in vitro in 4T1 cells, exhibited longer half-life blood circulation time as well as highest tumor accumulation in T1-GFP cell-derived CTCs in lung metastasis model while histological analysis no significant toxicity of various PTX formulations in vivo.^[115] In addition, polymer-lipid hybrid NPs co-loaded with doxorubicin and mitomycin C and decorated with Arg-Gly-Asp peptide (RGD), which target $\alpha v \beta 3$ integrin receptors on TEC and cancer cells, showed the highest accumulation in the lung metastasis model, showed significantly low toxicity to normal organ, and resulted in 4.7- and 31-fold reduction in lung metastasis burden compared to nontargeted NPs or free drugs, respectively.^[116]

However, after crossing the cell membrane, transported nanocarriers may be trapped and neutralized by lysosomal contents, which development of ones to prevent endo/lysosomal escape. For example, Dong et al.^[117] developed a pH-responsive metal-organic framework-based nanoplateform (doxorubicin@ZIF-8@AS1411) that

degraded under the acidic environment of lysosomes (pH 5.0) plus ZIF-mediated ROS generation, resulting in lysosomal membrane rupture and release of doxorubicin drug in the cytosol to induce anticancer effects. In addition, self-ROS-generable NPs (SRNs) act as a source of ROS upon PTT and irradiation of the amplified anticancer effect of payload drugs in vivo.^[118] Another strategy is a pre-occupation of endo-/lysosomes by the empty nanocarriers, for example, mesoporous silica NPs, and then treatment with main NPs containing anticancer doxorubicin.^[119]

With regard to the susceptibility of siRNAs to degradation by endogenous nuclease, their poor cell permeability, and their lack of tumor-targeting ability, functional oligonucleotide-modified AuNPs were conjugated with anti-*polo-like kinase-1* (PLK1) siRNA and then tiled with nuclease-resistant aptamer-incorporated Y-shaped backbone-rigidified triangular DNA bricks (Ap-YTDB) to interact with cell receptor.^[120] The designed multifunctional 3D DNA shell (Figure 5A) showed delivery loaded siPLK1 into MCF-7 cells in vitro that resulted in effective silencing of the target gene and cell proliferation (Figure 5B) and suppression of tumor growth in vivo (Figure 5C).

Besides lysosomal escape, some agent must have entered the cell nucleus to present anticancer function and, thus, require designing multifunctional NPs to cross many barriers, for example, covered by pentapeptide to protect from RES and conjugated the RGD and nuclear localization sequence (NLS) peptides to target cancer cells and to gain nuclear entry, respectively.^[121] In addition, aims to combine PTT/PDT and chemotherapy, the conjugation of PTT/PDTI-derived R780 dye with the HIV Tat-derived peptide and then loaded TID NPs containing doxorubicin that TAT peptide provide cellular internalization of IR780 in breast cancer cells and then upon laser irradiation at 785 nm the TID NPs guided photo-chemotherapy to induce cell apoptosis in vitro and in vivo in mice bearing breast cancer.^[122]

Over the past years, the designed nuclear-targeted nano-delivery systems have been developed into efficient platforms for targeted cancer therapy. However, the function of NLS depends on its density and occupation of importin α NLS binding pocket, and the total charge of NPs that low-intermediate NLS density shows better nuclear delivery than high NLS density due to an excess of NLS saturates the importin α and reduces the number of importins available for nuclear delivery, and also hydrophobic negatively charged cargoes have a greater change in crossing the nuclear pore complex.^[123]

3 | FUTURE PERSPECTIVES & CONCLUSION

An efficient nanodrug delivery system depends on the details of NPs (i.e., size, adhesion, molecular release, molecular loading, cytotoxicity, and cellular uptake) and tumors (acidity, rate of growth, tumor inter- and intratumor heterogeneity, hypoxia, physiology, tumor vasculature, tumor dormancy, and metabolic/population dynamics) and their interaction in vivo, each of which must be precisely controlled. For example, tumor heterogeneity must be assessed by single-cell techniques before NP design and formulation to achieve

optimal results. Addressing the lack of efficient models for evaluating nanoformulations and the inability of animal model, 3D spheroid, and organoid models to represent the native tissue-specific micro-environment, tumor-on-a-chip (TOC) is a novel platform that can link the nanosystem loaded with anticancer drug and personalized medicine.^[124,125] TOC can integrate 3D tumor spheroids, ECM and vasculature, and dynamic administration into one system to assess extravasation and interstitial diffusion, cellular uptake and penetration depth, delivery efficiency, toxicity, and drug resistance levels, and ultimately achieve clinical translation (reviewed by ref ^[126]).

In addition, the translation of NPs-based cancer treatments into clinical practice has so far been modest. The development of any new drug requires safety and efficacy testing in a variety of models, including cell culture and small animal studies. In an environment of only cultured cells, "tumor-targeted" drug delivery is meaningless. Moreover, none of the nanoformulations that showed some efficacy in mouse models met clinical expectations, and tumor targeting, which may have been the case in some mouse models, cannot be demonstrated in the clinic, or the mouse data showed that this is irrelevant for clinical applications. More importantly, because of the heterogeneous nature of cancer and the wide variability between individuals, it is not practical to assume that NPs will improve treatment for all patients.^[127] Formulation-driven research must, therefore, be transformed into disease-driven development for each patient. Irrelevance of mouse data to humans for clinical use could be due to (i) differences in the body or tumor size between a mouse and a human, (ii) exaggerating results from xenograft mouse models, (iii) inadequate selection of the control formulation in mouse studies, (iv) not applicability of some index such as EPR, (v) non-validation of NPs properties and behavior when experimental conditions are changed.^[128] Thus, the development of novel animal models capable of mimicking the heterogeneity and physiological characteristics of human cancers may enhance the translation of therapeutic nanosystems. The development of translatable nanomedicines through rational nanocarrier design also requires an understanding of the general principles and concerns of the pharmaceutical industry and the clinical development of nanotherapeutics.

Given the limited half-life of NPs in the circulation and their interaction with functional proteins (e.g., immune proteins, complement proteins, and apolipoproteins), by conducting meta-analyses of existing data, researchers can uncover key factors that drive protein corona formation, allowing for the development of predictive models for NP composition. This approach will ultimately facilitate the design of safe and effective NPs that can accurately target specific cells within the body.^[129] In addition, under hypercholesterolemic conditions, NPs interacted more with apolipoproteins, leading to their recognition by lipoprotein receptors in liver cells. Thus, the metabolomic profile may be an untapped factor influencing the target efficacy and in vivo fate of NPs, providing a way to develop personalized nanomedicines by exploiting disease-related metabolites.^[130] Regards to physicochemical properties of loaded drugs and NPs, multiple machine learning and artificial intelligence methods showed that zeta potential and core material were more important

than other properties like type, shape, and targeting,^[131] and factors like release rate and molecular weight negatively affected brain targeting. Therefore, it is crucial to consider these factors when designing drug delivery systems for brain targeting to improve the efficacy of treatments. Additionally, understanding the specific challenges presented by the blood–brain barrier can lead to the development of more effective and targeted therapies for neurological disorders.^[132]

The formation and evolution of the protein corona are influenced by the NP chirality, size, shape, surface properties, surface coatings, time of interaction, protein layer organization and compositions, binding affinity, and different abundances of proteins.^[133] Thus, deep understanding of corona evolution during transfer between different fluids and is powerful for the study of biological effects of nanomaterials and the development of nanomedicine. For example, by artificially modulating surface proteins and turning off their dynamic dissociation, we can present the moieties that prevent irreversible blocking of the surface and allow higher affinity interactions between cell membranes and transiently bare NP surfaces.^[134]

Considering the inability of anticancer drugs and NPs is to cross the plasma membrane, these algorithm was used and improved cellular uptake in human lung and kidney cell cancers while the cytotoxicity of utilized metal oxide NP could be detected by machine learning.^[135] Overall, data mining can be used to predict the structural and functional properties of NPs, provide the nano/bio interaction data, and subsequently optimize treatment methods, and bridge the gap between the information provided in the lab environment and the patient. In conclusion, the combination of anticancer nanomedicines, TOC, and machine learning provides an accurate and reliable approach for promising preclinical nanomedicines, not only for predicting overall therapeutic efficacy but also for evaluating each barrier of drug delivery.

AUTHOR CONTRIBUTIONS

Roya Ahmadzadeh, Seyed Alireza Taheri, Neda Mohammadi, Ahmed Hjazi, Soumya V. Menon, Wesam R. Kadhum, Abhinav Kumar, and Maha Noori Shakir: Investigation; writing—original draft. **Farid Karkon Shayan and Nahal Shirinkami:** Conceptualization; investigation; writing—original draft writing—review and editing; visualization; supervision; and project administration. All authors reviewed the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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How to cite this article: R. Ahmadzadeh, S. A. Taheri, N. Mohammadi, A. Hjazi, S. V. Menon, W. R. Kadhum, A. Kumar, M. N. Shakir, F. K. Shayan, N. Shirinkami, *J. Biochem. Mol. Toxicol.* **2024**, 38, e23782. <https://doi.org/10.1002/jbt.23782>