Trends in Cancer

CellPress

Review

Nanomedicine-based strategies to target and modulate the tumor microenvironment

Bárbara B. Mendes [1,2,3], Diana P. Sousa [1,2,3], João Conniot [1,2,3] and João Conde [1,2,*]

The interest in nanomedicine for cancer theranostics has grown significantly over the past few decades. However, these nanomedicines need to overcome several physiological barriers intrinsic to the tumor microenvironment (TME) before reaching their target. Intrinsic tumor genetic/phenotypic variations, along with intratumor heterogeneity, provide different cues to each cancer type, making each patient with cancer unique. This brings additional challenges in translating nanotechnology-based systems into clinically reliable therapies. To develop efficient therapeutic strategies, it is important to understand the dynamic interactions between TME players and the complex mechanisms involved, because they constitute invaluable targets to dismantle tumor progression. In this review, we discuss the latest nanotechnology-based strategies for cancer diagnosis and therapy as well as the potential targets for the design of future anticancer nanomedicines.

Highlights

The tumor microenvironment (TME) favors an intricated crosstalk between different cellular and non-cellular elements involved in metabolic, cellular, and tissue remodeling. This complex network of components has a crucial impact on tumor proliferation and metastasis.

The TME can act as a multicomponent barrier that limits the delivery of anticancer therapies. Understanding the dynamic of TME will further unravel potential therapeutic targets for anticancer therapies.

Several nanotechnology-based strategies have been developed to target the TME and to circumvent its intrinsic barriers, leading to innovative imaging approaches and improved therapeutic outcomes.

Nanotechnology-based systems can be designed to deliver their content through TME internal stimuli, such as pH, redox, and enzymes, or by external stimuli, including light, magnetic and electrical fields, and ultrasound.

Gene and immune modulation have been enabled and improved through nanotechnology-based approaches as therapeutic alternatives for emerging diseases with unmet needs.

Nanotechnology-based strategies for cancer diagnosis and therapy

The use of **nanotechnology** (see Glossary) for cancer diagnosis and therapy (**theranostics**) has increased over the past few years, especially compared with conventional strategies, such as chemotherapeutics and radiation [1,2]. The tunable surface functionalization of **nanoparticles (NPs)** along with their ability to carry a variety of therapeutic/imaging agents adds to their use as multifunctional and highly selective nanoengineered systems against cancer [2,3] Several **nanomedicines** are already approved by the FDA and many others are in preclinical and clinical trials [2]. These strategies show improved drug solubility, increased efficacy per dose, and reduced off-target effects.

As cancer **nanomedicine** research has evolved, different challenges have emerged, demanding different solutions. For example, nanoengineered materials need to overcome a variety of biophysical barriers (e.g., gastrointestinal tract and blood–brain barrier), as well as physiological barriers intrinsic to the **TME**, before reaching and acting on their target [4]. Tumor genetic and phenotypic variations along with intratumor heterogeneity (i.e., spatial and temporal heterogeneity in the same patient) provide additional challenges for the design of such nanomaterials [5]. Thus, it is crucial to design novel nanoengineered strategies with a safer, well-controlled, and fine-tunable manner. These features will enable their accumulation at the tumor site and enhance their active targeting efficiency to modulate the TME, while reducing toxic side-effects and off-target outcomes.

TME features

The TME is a major contributor to intertumor heterogeneity, because it comprises crosstalk between different cellular and non-cellular elements involved in metabolic, cellular, and tissue remodeling [6]; thus, it has a crucial impact on the maintenance of normal tissue homeostasis and is able to influence tumor proliferation [7]. Therefore, it is important to understand how these elements interact (Figure 1), how such interaction influences tumor progression, and the mechanisms involved, such that efficient therapeutic strategies can be developed.

[1]NOVA Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal
[2]Centre for Toxicogenomics and Human Health (ToxOmics), Genetics, Oncology, and Human Toxicology, NOVA Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal
[3]These authors contributed equally

*Correspondence:
joao.conde@nms.unl.pt (J. Conde).

The cellular TME

The cellular TME includes an array of cell types, which contact through cell–cell interactions, secreted soluble molecules (e.g., growth factors, cytokines), and vesicles (e.g., exosomes), altering **extracellular matrix (ECM)** properties and sometimes contributing to tumor cell proliferation and spread [7].

Solid tumors are generally infiltrated with immune cells that are recruited by the tumor and can be responsible for an immunosuppressive microenvironment that favors tumor growth [7,8]. **Tumor-associated macrophages (TAMs)** have pivotal interactions with tumor cells [7,9] and secrete chemokines, cytokines, and growth factors [e.g., C-C motif chemokine ligand 2 (CCL2) and granulocyte-macrophage colony-stimulating factor (GM-CSF)] that recruit monocytes, which can differentiate into macrophages [9]. TAMs have been categorized according to their functions and polarization state as M1 or M2 macrophages. M1 TAMs have antitumoral functions, secrete proinflammatory cytokines [e.g., interleukin (IL)-6, IL-12, and tumor necrosis factor-alpha (TNF-α)], and generate nitric oxide (NO) and reactive oxygen species (ROS) [8,10]. M2 TAMs have protumoral and immunosuppressive functions associated with tumor progression, and secrete anti-inflammatory cytokines [e.g., IL-10 and transforming growth factor-beta (TGF-β)], matrix metalloproteinases (MMPs; e.g., MMP-2 and MMP-9), and growth factors (e.g., vascular endothelial growth factor, VEGF) [8]. Depending on the type of cancer, and due to this M1/M2 dichotomy, TAMs can elicit both favorable and unfavorable outcomes [10–12]. TAMs also participate in ECM remodeling by secreting proteases, growth factors, and cytokines, which contribute to tumor progression [13].

Neutrophils are also found in the TME, being recruited by inflammatory signals, such as hydrogen peroxide, chemokines, and cytokines [e.g., C-X-C motif chemokine (CXCL)-5, CXCL8, TNF-α, and IL-17] [14–17]. Similar to TAMs, neutrophils have been characterized into N1 (antitumoral) or N2 (protumoral), associated with improved or poorer outcomes, respectively [18,19]. Neutrophils participate in tissue remodeling, tumor progression, and angiogenesis by secreting reactive nitrogen species (RNS), ROS, and MMP-9 [20,21], and can recruit other immune cells, influencing the immune heterogeneity in the TME and **drug resistance** [22].

Regulatory T cells (T_{regs}) are also recruited to the TME and are involved in tumor progression as a result of their immunosuppressive activity, hindering antitumor immunity by secreting anti-inflammatory cytokines (e.g., IL-10, IL-35, and TGF-β) [23], suppressing the functions of macrophages and antigen-presenting cells, reducing the activity of $CD4^+$ T helper and natural killer (NK) cells, and regulating cytotoxic ($CD8^+$) lymphocyte proliferation by promoting T cell exhaustion [24,25]. Additionally, they have been associated with enhanced epithelial-to-mesenchymal transition (EMT) [26].

Cancer-associated fibroblasts (CAFs) are a major component of the TME, being recruited and activated by growth factors [e.g., platelet-derived growth factor (PDGF) and TGF-β] secreted by immune infiltrating cells and tumor cells. CAFs are responsible for ECM protein synthesis [27] and are involved in the recruitment of circulating endothelial progenitor cells and in the immune and metabolic reprogramming of the TME by secreting chemokines, cytokines, and growth factors (e.g., CXCL10, IL-8, and VEGF-A) [28–30]. The secretion of such factors contributes to angiogenesis by stimulating and/or activating endothelial cells and, combined with proangiogenic factors secreted by the tumors, supports tumor progression [30]. Moreover, cytokine secretion by CAFs was reported to increase drug resistance in different types of cancer [31,32] and to induce and mediate EMT and the metastatic potential of tumors [31,33]. CAFs produce ROS, inducing oxidative stress in the TME and alterations in cells [20], and participate in ECM remodeling by activating different pathways [e.g., nuclear factor (NF)-κB and hypoxia-inducible

Glossary

Cancer-associated fibroblasts (CAFs): spindle-shaped cells engaged in the remodeling of the ECM; one of the most abundant cell types of the TME.
Dendrimer: regularly and hyperbranched macromolecule with unique 3D structures, shapes, and topological features with a high density of end groups.
Drug resistance: significant decrease in the capacity of a drug to effectively improve a condition or disease; a main cause of tumor recurrence.
Enhanced permeability and retention (EPR) effect: process in which small-sized particles and macromolecular drugs accumulate more in the tumor than in normal tissues.
Extracellular matrix (ECM): a highly dynamic and 3D network comprising extracellular macromolecules, such as proteins, glycosaminoglycans, and glycoconjugates, that provides scaffolding and biochemical support to the surrounding cells.
Hypoxia: condition in which a tissue is deprived of an adequate oxygen supply.
Immunosuppression: decrease in the activation and efficiency of the immune response that can be permanent or temporary, associated with certain conditions, and can increase susceptibility to disease.
Liposome: small artificial spherical vesicle comprising one or more phospholipid bilayers and cholesterol.
Nanomedicine: application of nanotechnology for the prevention and treatment of diseases; comprises several nanoscale materials, biological devices, nanoelectronic biosensors, among others, for diagnosis, drug delivery, and sensing.
Nanomedicines: small molecules or biologics with pharmaceutical activity delivered by nano-sized carriers comprising other elements such as lipids, polymers, proteins, or even metallic compounds.
Nanoparticles (NPs): small, nanometer-sized particles.
Nanotechnology: combination of science, engineering, and technology, conducted on the nanometer scale (10^{-9} m).
Polyethylene glycol (PEG): synthetic polymer that can be prepared using a range of sizes and terminal functional groups and used in different applications.

factor (HIF)-1], by secreting proteases (e.g., MMP-2, MMP-3, and MMP-9) and modulating protease secretion [33].

In the blood vessels, endothelial cells (ECs) are essential for tumor growth, supporting the transport of nutrients, oxygen, and metabolites, as well as metabolic waste and chemical mediators [34]. ECs are involved in tumor angiogenesis and are associated with tumor progression, having effects on drug resistance and metastasis, as well as on the stromal cell arrangement of the tumor and immune cell infiltration [34,35]. The secretion of chemokines (e.g., CCL2 and CXCL5) and angiogenic factors (e.g., VEGF) by tumor cells activates ECs, favoring angiogenesis and increasing vascular permeability. This influences tumor cell intravasation and is associated with the metastatic process [36]. Pericytes, which surround ECs, also support angiogenesis via TGF-β signaling promoted by CAFs [37].

The non-cellular TME

The non-cellular elements of the TME comprise its physicochemical characteristics, soluble molecules, ECM components, and extracellular vesicles. These elements contribute to cell–cell communication, and cell adhesion and differentiation processes, and are often involved in loss of tissue integrity, EMT, and tumor heterogeneity [38].

The ECM is a complex 3D network of macromolecules, including proteoglycans, glycoproteins, and collagens, among others. Besides physically supporting cells, the ECM participates in the maintenance of tissue homeostasis and modulates cell–matrix and cell–cell interactions. In cancer, these processes are often deregulated, and the ECM may correspond to half of the tumor mass [39]. The reorganization of the collagen and elastin network induced by proteinases, such as MMPs, along with increased cell motility and expression of laminin, contribute to tumor cell growth, proliferation, migration, and invasion [39,40]. Additionally, the ECM contributes to therapy resistance by creating a physical barrier that shields tumor cells and negatively influences drug delivery and NP diffusion [39].

Extracellular vesicles (EVs), such as exosomes, contain diverse biomolecules (e.g., lipids, proteins, and nucleic acids) and mediate cell–cell interactions [41]. This can be associated with tumor progression and invasion, because the EVs and their cargo modulate different signaling pathways, alter the stem-cell properties of tumor cells, increase drug resistance, induce **immunosuppression**, and participate in angiogenic and EMT processes [41,42].

Physicochemical properties of the TME

Rapidly growing tumors may have fibrotic areas that can coincide with an acidic, hypoxic, and nutrient-deficient environment, because the neighboring vessels cannot meet the nutrient and oxygen requirements. These physicochemical properties are also critical players in tumor development [38]. **Hypoxia** is a primary driver of angiogenesis, and tumor cells adapt to these conditions by increasing the transcriptional activity of HIFs (HIF-1 and HIF-2). This stimulates the expression of VEGF and basic fibroblast growth factor (FGF2), which promote the proliferation of vascular ECs and pericytes. Neovascularization and the metabolism of fatty acids and glucose is also increased, which enhances tumor invasiveness and metastasis, and can lead to upregulation of multidrug-resistance proteins [38,43,44].

The extracellular pH of most tumors is generally more acidic (pH_e ~6.5–6.8) compared with normal tissue (pH ~7.4) [38,45]. This acidity is associated with the increased oxygen-independent glycolysis of tumor cells, which damages the ECM, decreasing adhesion between tumor cells and contributing to chemotherapy resistance, and tumor progression and metastasis.

Polymers: synthetic or natural materials comprising macromolecules, formed by chemical bonding of small molecules or repeating units called monomers.
Prodrug: compound that is only metabolized into a pharmacologically active drug after administration to improve its pharmacokinetics.
Superparamagnetic: property of small ferrimagnetic or ferromagnetic NPs whereby their magnetization can flip direction randomly when under the influence of temperature.
Theranostics: treatment strategies that combine therapeutic and diagnostic techniques.
Tumor microenvironment (TME): dynamic milieu with a heterogeneous tumor cell population, fibroblasts, infiltrating immune cells, stromal cells, as well as signaling molecules, blood vessels, and ECM.
Tumor-associated macrophages (TAMs): type of immune cell population that is present in the TME and is involved in tumor-associated inflammation, aiding tumor cell proliferation and metastasis.

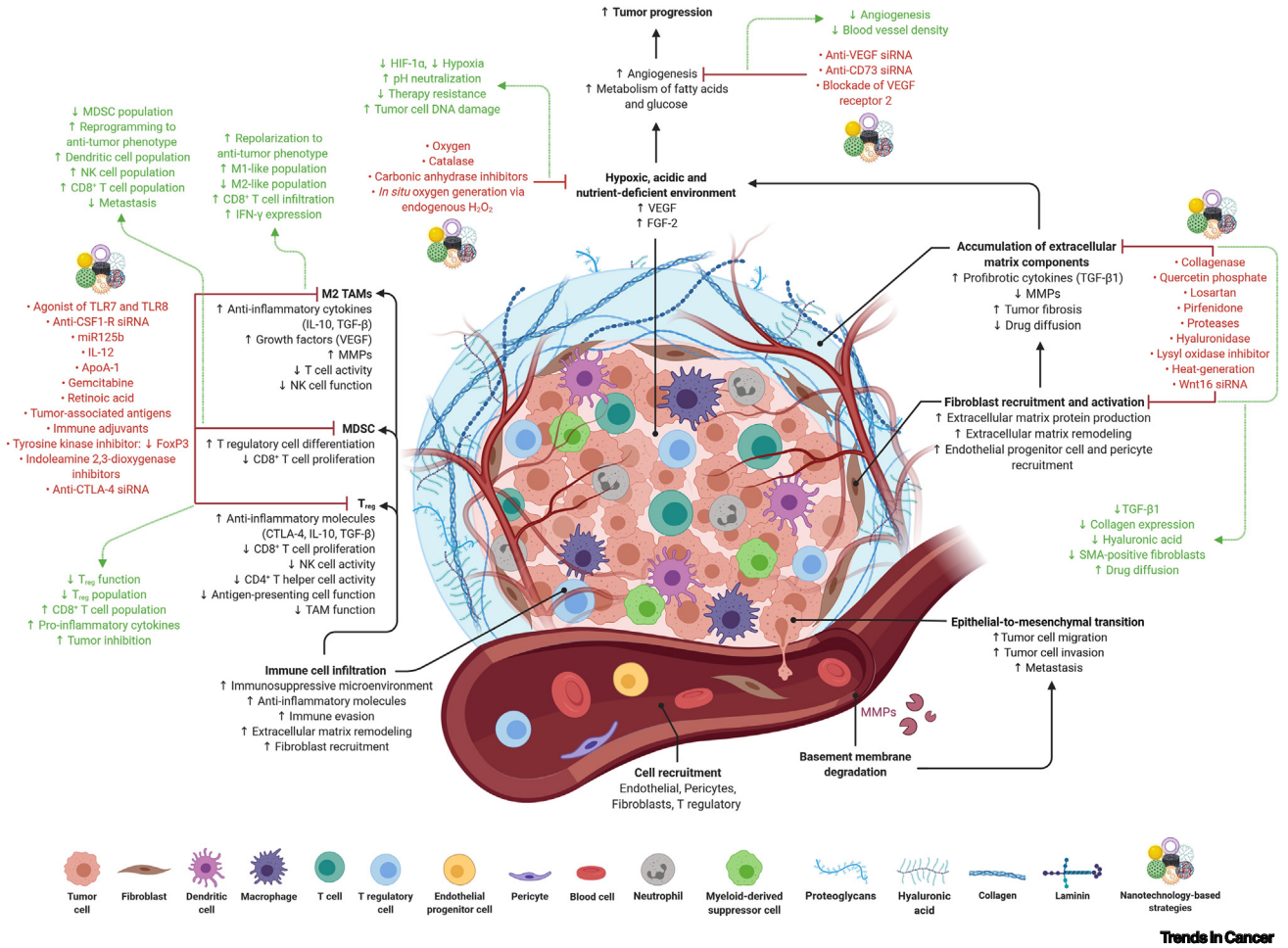

Figure 1. Schematic representation of the tumor microenvironment (TME), which comprises stromal and immune cells and extracellular matrix components, among others, involved in metabolic, cellular, and tissue remodeling. In the context of cancer, these TME elements often have alterations (in black) that promote tumor progression. Over the past few years, nanotechnology-based strategies (in red) have been developed to target and/or modulate these alterations, aiming at reversing them (in green). Figure created with BioRender (BioRender.com). Abbreviations: CSF1-R, colony stimulating factor 1 receptor; CTLA, cytotoxic T lymphocyte antigen; FGF2, fibroblast growth factor 2; HIF, hypoxia-inducible factor; IFN, interferon; IL, interleukin; MDSC, myeloid-derived suppressor cell; MMP, matrix metalloproteinase; NK, natural killer; SMA, smooth muscle actin; TAM, tumor-associated macrophage; TGF, transforming growth factor; TLR, Toll-like receptor; Treg, regulatory T cell; VEGF, vascular endothelial growth factor.

Differences in interstitial fluid pressure are also observed in the TME, promoted by fluid leakage and accumulation in the interstitium possibly induced by changes in the ECM, impaired lymphatic and venous drainage, and increased vascular permeability and cell density, which has been associated with decreased therapeutic delivery to tumors [38]. Excessive accumulation of ECM components, such as collagen, stimulates the transition of the tumor stroma to fibrosis; this results from, among other causes, decreased collagen degradation caused by MMP imbalance and from increased collagen synthesis promoted by profibrotic cytokines (TGF-β1) [46]. The fibrotic environment may compress blood vessels affecting the vascular transport of the therapeutic agents and create a physical barrier to molecular movement [47].

Nanotechnology-based approaches to target and modulate the TME

The tumor stroma is crucial for tumor development, progression, and metastasis, and acts as a multicomponent barrier that limits treatment outcomes [5,38]. Consisting of a complex synergism

between blood and lymphatic vascular networks, ECM, stromal fibroblasts, and tumor-infiltrating immune cells, the TME offers many potential targets to halt and dismantle tumor progression. In this section, we highlight the latest developments in TME-targeting nanotechnology-based strategies, which have been designed as promising platforms to improve targeting and modulation efficiencies, and therapeutic outcomes (Figure 2 and Table 1).

TME-responsive approaches

Nanotechnology-based systems can be designed to deliver their content in a spatiotemporally controlled manner in response to tumor internal stimuli, including pH, redox, and enzymes [48]. pH-sensitive nanomaterials are only 'activated' when reaching the acidic TME, facilitating the selective release of their content [45]. These nanomaterials have been designed with pH-responsive properties via acid-triggered protonation of the functional groups (e.g., micellization association and/or dissociation), acid-triggered cleavage of linkers [e.g., 2,3-dimethylmaleic amide (DMMA) linker], acid-triggered decomposition (e.g., calcium carbonate or calcium phosphates)

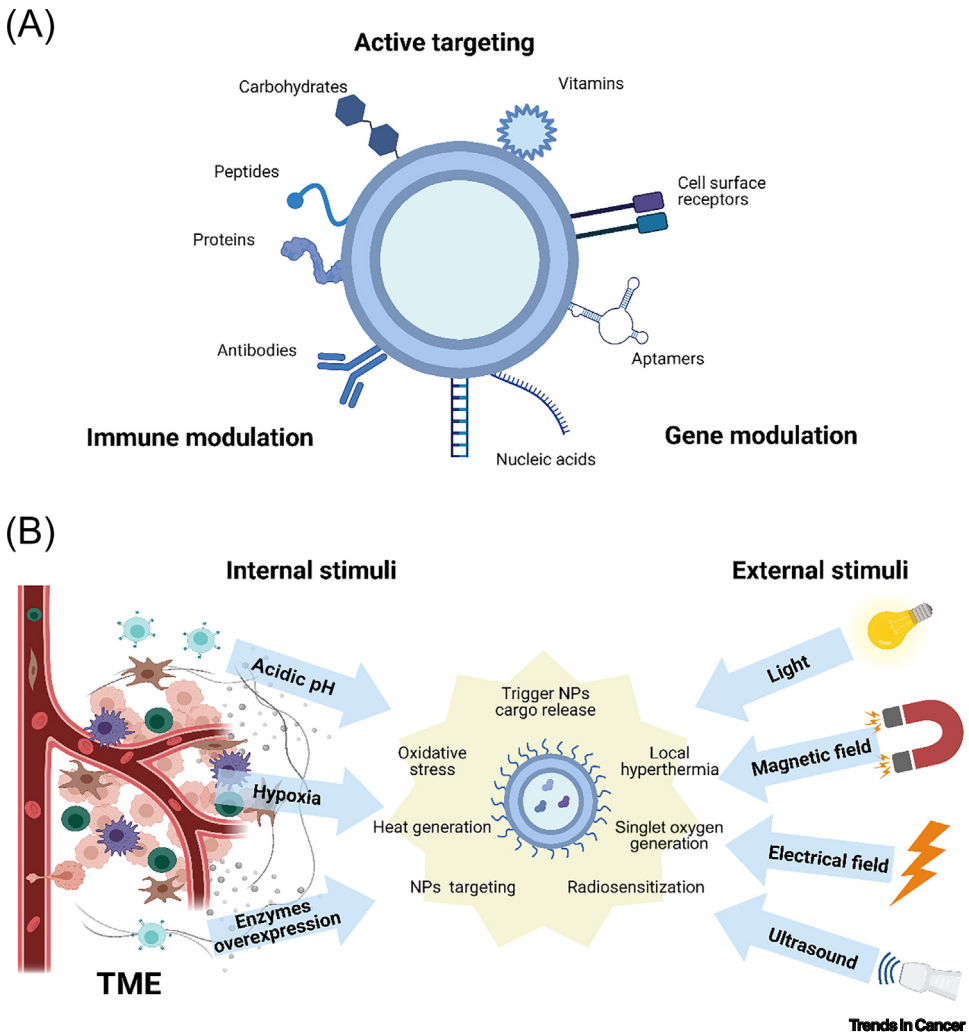

Figure 2. Schematic illustration of (A) commonly engineered nanoparticle (NP) surface functionalizations to target and modulate the tumor microenvironment (TME) and (B) internal and external stimuli for triggering the therapeutic effects of nanotechnology-based systems. Figure created with BioRender (BioRender.com).

Table 1. Examples of preclinical studies over the past 5 years of NPs engineered to target or modulate the TME

NP	Therapeutic payload	Target molecule	Animal model	Therapeutic outcome	Refs
Acidic pH					
Inorganic	N/A	N/A	Melanoma-bearing BALB/c mice	Exhibited excellent catalase-like and oxidase-like activity in acidic TME; significantly inhibited tumor growth via multienzyme stepwise cascade catalysis; under physiological pH, NPs lost their enzymatic activity via pH-responsive biodegradation, keeping normal tissues unharmed	[130]
Polymeric	$NaGdF_4$-calcium carbonate nanoconjugates	HELA cell membranes	Cervical adenocarcinoma-bearing BALB/c mice	Encapsulated calcium carbonate NPs were highly responsive to mildly acidic TME; triggered rapid nanoassembly disintegration via gas diffusion reaction, releasing $NaGdF^4$ NPs; exhibited tumor-targeting capabilities due to cell membrane coating; greatly enhanced tumor imaging contrast	[131]
	DOX, boron-dipyrromethene dye	Poly(2-lactobionamidoethyl methacrylate-b-poly (3-acrylamidophenylboronic acid)	Hepatocellular carcinoma/ ADR-bearing BALB/c mice	Acidic pH triggered zwitterionic shell detachment and activated surface ligands to decrease drug leakage into circulation and enhance tumor accumulation; showed superior antitumor therapeutic efficacy and enabled *in vivo* imaging to trace drug distribution	[132]
Enzyme overexpression					
Inorganic	N/A	Prostate-specific membrane antigen (PSMA)	Prostate tumor- bearing mice	Cathepsin-induced linker cleavage to promote photosensitizer release, enabling fluorescence and photoswitchable photodynamic activity; showed excellent selectivity for PSMA-positive tumor cells; greatly enhanced NP uptake and therapeutic efficacy	[133]
	Tirapazamine, $TPPS_4$, gadolinium-III	HA	Squamous cell carcinoma-bearing BALB/c mice	Hyaluronidase promoted payload release. HA-targeting moiety (CD44 receptor) dramatically increased endocytosis of tumor cells; achieved superior synergistic antitumor effect due to oxygen depletion process (PDT) and activation of bioreductive chemotherapy under NIR fluorescence/magnetic resonance imaging guidance	[134]
Hypoxia					
Inorganic	Chlorin e6	N/A	Breast tumor-bearing BALB/c mice	Acted as oxygen generators and produced hydroxyl radicals to enhance photo- and chemodynamic therapy via Fenton-like reaction; light-triggered ROS production under H_2O_2-rich conditions, inhibiting tumor growth	[135]
	NA	HA	Glioma-bearing Wistar rats	Triggered rapid generation of oxygen and release of Mn^{2+}; attenuated tumor hypoxia via downregulation of HIF-1α and VEGF expression to inhibit tumor progression	[136]
Light irradiation					
Polymeric	5′-Deoxy-5-fluorouridine and silicon 2,3-naphthalocyanine bistrihexylsilyloxide	N/A	Breast tumor-bearing mice	Activated H_2O_2-responsive afterglow prodrug and afterglow initiator in response to tumor-upregulated H_2O_2. Upon laser irradiation, generated singlet oxygen to react and transform uncaged afterglow substrate to produce a self-luminescent substrate, allowing real-time monitoring of prodrug activation	[137]

	DOX, indocyanine green	Folic acid	Cervical adenocarcinoma-bearing BALB/c mice	Overproduced ROS by reacting with endogenous H_2O_2; triggered NP shrinkage and decomposition under laser irradiation and acidic pH; enhanced NP intratumoral permeability and controlled cargo release, achieving imaging-guided synergetic therapy	[138]
Inorganic	N/A	Zoledronic acid	Breast tumor-bearing NUNU/nude mice	Showed bone-targeting ability; increased toxicity to cancer cells and antibone resorptive effect; in combination with NIR lasers, enhanced inhibition of tumor growth and significantly reduced bone pain	[139]
Magnetic field					
Inorganic	*N*-[*N*-(3,5-difluorophenacetyl-L-alanyl)]-*S*-phenylglycinet-butylester	HA	Breast tumor-bearing BALB/c mice	HA enhanced NPs solubility and improved NPs uptake in $CD44^+$ cells; allowed high loading efficiency and induced hyperthermia under alternating current magnetic field; effectively eliminated cancer stem cells and inhibited tumor growth	[140]
Ultrasound actuation					
Polymeric	DOX, sodium carbonate and perfluorocarbon	N/A	Hepatocellular carcinoma-bearing BALB/c mice	Exhibited sensitive response to ultrasound stimulation and rapidly released Na_2CO_3 to neutralize lactic acidosis in controlled manner; inhibited tumor growth with minimal adverse effects and enhanced DOX susceptibility of tumors	[141]
Immune modulation					
Inorganic	N/A	Anti-CD3, anti-CD28, and anti-PD-L1 antibodies	Breast tumor-bearing mice	Localized NPs via magnetic navigation to tumor, improving therapeutic efficacy; induced T cell activation and blocked immunosuppressive PD-L1 pathways; increased tumor-specific interferon (IFN)-γ^+CD44^+ T cells at tumor with central memory potential; achieved significant tumor suppression and extended median survival	[142]
		Anti-PDL-1 and anti-4-1BB antibodies	Melanoma-bearing C57BL/6 mice	Enhanced effector–target cell conjugation, resulting in increased density, specificity, and functionality of tumor-infiltrating $CD8^+$ T cells; delayed tumor growth and extended survival compared with soluble antibodies	[143]
Lipid based	Dinaciclib	Anti-PD-L1 antibody	Glioblastoma- bearing C57BL/6 mice	Exhibited high targeting payload efficiency to tumor-associated myeloid cells (TAMCs). Robust TAMC depletion and attenuation of immunosuppressive functions extended mouse survival; able to synergize with radiation therapy	[144]
Polymeric	Melanoma-associated peptides and Toll-like receptor ligands	Mannose receptor on dendritic cells	Melanoma-bearing C57BL/6	Increased infiltration of $CD8^+$ T cells; synergism with immune checkpoint modulators, inhibiting tumor growth and prolonging overall survival	[145]
Gene modulation					
Polymeric	Apoptosis-inducing siRNA encoding polo-like kinase 1	N/A	Pancreatic adenocarcinoma-bearing BALB/c mice	Precisely regulated chain length; charge-matching enabled dynamic and selective ion-pairing, generating a dynamically equilibrated unit polyion complex to stable blood circulation; efficiently delivered cargo to tumor site, exerting significant antitumor activity	[146]
	siRNA against bromodomain 4	Tumor cell-targeting- and penetrating peptide-amphiphile (TCPA)	Prostate tumor-bearing mice	Efficiently accumulated in tumor tissue; TME pH induced rapid NP disassembly, releasing their cargo; enhanced cytosolic transport; achieved efficient gene silencing, inhibiting tumor growth	[147]

as well as shielding–deshielding transitions [45,48]. For example, co-assembled NPs comprising platinum **prodrug**-conjugated poly(amidoamine)-graft-polycaprolactone (PCLCDM-PAMAM/Pt), a PCL homopolymer, and a poly(ethylene glycol)-b-poly(e-caprolactone) copolymer were developed [49] in which the amide bond between the amino groups from PAMAM and the CDM anhydride residue is cleaved at pH_e, which triggers the release of the PAMAM **dendrimer** prodrugs (~5 nm) at tumor sites. Recently, TME-sensitive **liposomes** modified with slightly acidic pH-sensitive peptides were used to reduce the expression levels of integrin and collagen to weaken the adhesion between cancer cells and ECM, increasing tumor penetration [50]. With the purpose of modulating the acidic TME, several studies have targeted pH-regulating membrane transporters and proteins. In the TME, overexpression of carbonic anhydrases, which are enzymes that catalyze CO_2 hydration to bicarbonate (HCO_3^-) and protons (H^+), can be correlated with increased acidosis, tumor progression, and metastasis [51]. Porous silicon NPs carrying a specific carbonic anhydrase inhibitor and doxorubicin (DOX) have inhibited acidification *in vitro* [52]. Calcium carbonate NPs have also been used to neutralize the acidic pH of the TME, leading to tumor growth inhibition [53].

The hypoxic TME regions generate ROS and exhibit a high redox potential difference between intracellular and extracellular spaces, which provided the basis for designing hypoxia-responsive NPs (e.g., reductive-sensitive **polymers** and conjugates and/or prodrugs) [54,55]. In this regard, carboxymethyl dextran was chemically modified with black hole quencher 3 (BHQ3) [56]. Under hypoxia, the release rate of DOX increased due to reduction of the azo bond in BHQ3 (i.e., hypoxia-sensitive moieties). Decreasing hypoxia can constitute an advantage to overcome the resistance to chemotherapeutic agents and enhance the therapeutic outcomes of other antitumor strategies [57,58]. Hypoxia can be mitigated by oxygen nanocarriers that deliver oxygen directly into tumors (e.g., using perfluorocarbon nanodroplets) [59,60] or by *in situ* oxygen generation, decomposing endogenous H_2O_2 with catalytic NPs [58]. Hybrid MnO_2 NPs have been developed to produce oxygen through the reaction with H_2O_2 and H^+ in the TME. Local and systemic administration of hybrid **polyethylene glycol (PEG)**ylated lipid derivative-MnO_2 NPs reduced the expression not only of HIF-1α, a hypoxia marker, but also of P-glycoprotein, associated with drug resistance [57]. Albumin-based NPs with MnO_2 have also been designed for the same purpose [58,61]. Tantalum oxide nanoshells have also been investigated to carry catalase, which breaks down H_2O_2 to O_2. The mesoporous tantalum oxide nanoshells protect catalase from proteases and increase contact with endogenous H_2O_2, alleviating hypoxia in the TME and overcoming resistance to radiotherapy. Administration of these catalase-loaded nanoshells relieved hypoxia in the TME and led to increased DNA damage in tumor cells and tumor ablation following X-ray radiation [62]. Tumor hypoxia was also relieved using human serum albumin-paclitaxel NPs, which improved tumor blood flow and oxygenation through reduction of interstitial fluid pressure and normalization of tumor vasculature [63]. Co-encapsulation of metformin and chlorin-e6 in liposomes has also shown promising results by decreasing the oxygen consumption of tumor cells to mitigate hypoxia [64].

Several nanomedicine strategies have explored the overexpression of enzymes in the TME, such as cathepsin B, legumain [65], hyaluronidase [66], and MMPs [67], to develop enzyme-activable drug delivery NPs. Dual stimuli-responsive clustered NPs were developed based on PEG-block-poly(β-benzyl-L-aspartate) polymers, acid-sensitive octadecylamine-p(Asp-API)10 polymers (OAPI), and legumain-cleavable linkers containing DOX-carbon quantum dot conjugations (CD-C9-AANL-DOX) [68]. Once the nanosystem reached the tumor tissue, the cargo was released in intracellular acidic conditions, mainly due to imidazole groups in the side chains of OAPI, and with legumain digestion, which further promoted the controlled release of drug-coupled carbon dots (CDs). Similar approaches explored the use of these enzymes to modulate the TME, such

as polystyrene NPs modified with collagenase on the surface that increased tumor penetration [69]. PEG-ylated NPs comprising coordination polymers of Mn^{2+} and benzoic-imine linkers have been used to encapsulate collagenase [70]. These TME-responsive NPs selectively degraded collagen and loosened the ECM. Mesoporous silica NPs modified with bromelain, a mixture of cysteine and sulfhydryl proteases, enhanced NP permeation by digestion of tumor ECM [71]. The surface of PLGA NPs was modified with an inhibitor of lysyl oxidase, an enzyme in the TME that catalyzes the cross-linking of elastin and collagen, slowing tumor growth with reduced systemic side-effects compared with the free inhibitor [72]. Hyaluronidase has also been embedded into PEGylated PLGA NPs to improve tumor accumulation and enhance the therapeutic efficacy [73].

Physical stimuli strategies

Tumor-specific imaging and therapeutic nanomaterials have been designed in response to external/physical stimuli, including light, magnetic and electrical fields, and ultrasound [48]. Strikingly, the intrinsic properties of the nanomaterials (e.g., silver and gold metals) can also improve cancer theranostic strategies.

Light has been explored extensively to develop stimuli-responsive systems, which include its use to achieve photochemical reactions/groups (i.e., photoisomerization, photocleavable groups, or 'caging' groups), to produce cytotoxic agents in the presence of a photosensitizer (i.e., photodynamic therapy), or to generate heat (i.e., photothermal therapy) [74]. Recently, hyaluronic acid (HA)-coated D–A-conjugated polymer NPs reduced tumor growth by photothermal therapy with 1064-nm near-infrared (NIR) laser irradiation [75]. The NPs efficiently adsorbed the NIR light energy and converted it into local heat due to the optical absorption of the conjugated polymer. NIR lasers have become attractive light stimuli due to their high biocompatibility, deep tissue penetration, and *in situ* polymerization capability [76]. In another study, the denaturation of collagen fibers by heat generated from carbon nanotubes by NIR irradiation led to softening and remodeling of the TME [77].

Magnetic fields have been applied to guide magnetic NPs under a permanent magnetic field (e.g., core-shell NPs or porous metallic nanocapsules) and/or to increase the local temperature under an alternating magnetic field (AMF), known as magnetic hyperthermia [48]. AMF led to hysteresis loss or Néel relaxation in the magnetic or **superparamagnetic** NPs, generating heat at the surrounding tumor area [78]. The properties of thermosensitive block copolymers on the surface of magnetic NPs, thermo-sensitive linkers, and magnetic molecularly imprinted polymers can be fine-tuned to trigger drug release both temporally and spatially [78]. Gold NPs exhibit strong light absorption upon excitation of surface plasmon oscillations and heat generation through thermal relaxation [79]. Kwon and coworkers developed superparamagnetic gold NP-clusters (SPAUNCs) synthetized on a virus-like particle with tumor cell receptor-binding peptides on the surface [80]. Interestingly, SPAUNCs have a small size (<2 nm) that minimizes NP retention *in vivo*, and demonstrate excellent magnetic hyperthermia effects in both subcutaneous and deep-tissue tumors. Heat-generating gold nanorods have been also used to increase the temperature in the TME and alter the collagen matrix, enhancing the diffusion of inorganic NPs inside the tumor tissue [81].

Electrical fields promote the burst or sustained release of nanosystem cargoes through a variety of mechanisms, such as electrochemical reduction–oxidation, electric field-driven movement of charged molecules, and reversible scission of supramolecular polymers [82]. In this context, porous platinum NPs loaded with DOX were combined with electrodynamic therapy and chemotherapy to achieve a more effective cancer therapy [83]. Under an alternating current electric field,

the proposed system generates high amounts of cytotoxic ROS due to the electro-driven catalytic reaction of platinum NPs that triggers water-molecule decomposition, while enhancing the accumulation of DOX inside tumor cells.

Finally, upon ultrasound actuation, several thermal and/or mechanical effects can occur, such as cavitation, acoustic fluid streaming, pressure variation, and local hyperthermia, which have been widely explored to develop ultrasound-responsive strategies [84]. For example, ultrasound and glutathione dual-responsive vesicles of Janus gold-manganese oxide NPs (JNPs) grafted with PEG and a ROS-sensitive polymer were developed [85]. Gold NPs were used as potent cavitation inducers (i.e., sonosensitizer) and producers of ROS, single oxygens, and hydroxyl radicals. After ultrasound irradiation, the vesicles first disassembled into small JNPs that promoted penetration, and then into smaller gold NPs and Mg^{2+} ions induced by the Fenton-like reaction in the tumor area.

Targeting approaches

Passive targeting

Passive targeting relies on the encapsulation of small molecules within the NPs to prolong their systemic circulation and decrease side-effects without specifically targeting a certain tissue. The **enhanced permeability and retention (EPR) effect** allows NP accumulation and retention through the increased permeability of the tumor vasculature and poor lymphatic drainage.

Despite the significance of the EPR effect in the passive targeting of nanomaterials, the simplistic perspective of the EPR has been a matter of debate [86]. For example, it has been hypothesized that eruption sites at the leaky blood vessels enhance NP extravasation into the tumor interstitial space [87]. It has been also discussed that EC gaps (~2000 nm) in the tumor blood vessels rarely occur and passive extravasation contributes to only a fraction of NP entry into mouse solid tumors [88]. Recently, certain NPs (e.g., 10–30-nm gold NPs) induced micrometer-sized gaps between ECs even in the absence of any EPR effect, known as 'nanoparticle-induced endothelial leakiness' (NanoEL) [89].

Active targeting

Over the past few decades, nanomedicine strategies have been reported to target biological agents of the TME by covalently conjugating tumor-specific targeting moieties (e.g., antibodies, peptides, aptamers, and small molecules) onto the surface of the nanosystems, which can bind to receptors overexpressed in cells, thus guiding the nanosystems and improving their affinity to their targets, increasing the amount of cargo delivered to the target site [90]. Several NPs have targeted vital cellular components of the TME, including CAFs, ECs, myofibroblasts, pericytes, stem cells, ECM, and immune cells [2,90].

CAFs can contribute to form a barrier with the ECM, which limits drug and NP diffusion. Therefore, CAF depletion could disrupt the positive synergism between CAFs and tumor cells while facilitating access of therapeutics to the TME. Docetaxel (DTX) conjugated to a PEGylated-acetyl-carboxymethylcellulose polymer was used to produce NPs that accumulated preferentially in smooth muscle actin (SMA)-positive CAFs and led to considerable depletion of these cells [91], with increased tumor blood perfusion and significant antimetastatic effects in several tumor models [92]. Dextran-coated cerium oxide NPs reduced the expression of SMA in fibroblasts and led to decreased tumor invasion [93]. Liposomes functionalized with anisamide, a ligand of the sigma receptor overexpressed in CAFs, have higher internalization in CAFs by enhanced receptor mediated endocytosis compared with nonfunctionalized liposomes [94].

Anisamide-coated liposomes loaded with cisplatin showed enhanced antitumor effect compared with nontargeted liposomes, by decreasing the percentage of CAFs. Chronic exposure of fibroblasts to cisplatin NPs led to upregulation of damage-response program molecules, such as Wnt16, which resulted in tumor cell resistance. Co-delivery of cisplatin NPs with Wnt16 siRNA NPs, to decrease Wnt16 expression, inhibited tumor resistance and increased the antitumor effect [95].

VEGF binds to VEGF receptor-2 (VEGFR2), stimulating the proliferation of ECs to maintain the oxygen and nutrient supply to rapidly proliferating tumor cells. An acrylamide-based hydrogel polymer with high affinity for a key VEGF was used to prepare NPs that prevented the binding of VEGF to VEGFR2, limiting angiogenesis [96]. Inhibition of CD73, a cell surface protein involved in tumor angiogenesis and VEGF expression, has also been used as an antitumor strategy. Chitosan lactate NPs loaded with anti-CD73 siRNA resulted in reduced CD73 mRNA levels and blood vessel density in a breast cancer model [97].

TAMs present in the TME are mostly polarized to a M2 phenotype, which suppress NK and T cell-killing activity [98]. Lipid NPs loaded with the siRNA anti-colony stimulating factor 1 receptor (CSF1-R), the receptor of a cytokine involved in TAM differentiation and proliferation, have been used to target TAMs in the TME. These lipid NPs showed preferential targeting to M2 TAMs, reducing their population in the TME. The reduction of M2 TAMs increased the infiltration of $CD8^+$ T cells and the expression of interferon-gamma in a melanoma mouse model [99,100]. HA-poly(ethylenimine)-based NPs encapsulating miR-125b, a miRNA that can reprogram TAMs into an antitumor M1 phenotype, have been used to target macrophages. Intraperitoneal injection of these NPs induced significant repolarization toward the M1 phenotype in a non-small cell lung cancer mouse model [101].

In the biological environment, NPs are rapidly covered with a layer of biomolecules (i.e., protein corona) and only proteins with high NP affinity are maintained (i.e., those with a hard corona) [102]. Therefore, NPs will show different protein corona composition and binding dynamics that largely define their chemical and biological functionality, such as their biological identity and lifetime [103]. Recently, unmodified gold NPs (20 nm) incubated with conditioned medium from a pancreatic cancer cell line were reported to impair the secretion of several factors (e.g., cytokines and growth factors) and transform pancreatic stellate cells toward a lipid-rich quiescent phenotype, thus reprograming the TME [104].

Immune modulation strategies

Immunotherapy is recognized as a new pillar of cancer treatment due to its ability to enlist the immune response of the patient to search for, and destroy, cancer cells [105]. These nanomedicine-based strategies comprise immunotherapeutic agents to target T cells, immune cytokines, immune checkpoint blockers, and cancer vaccines [105]. Recently, Moon's group developed high-density lipoprotein-like nanodiscs comprising an apolipoprotein A1 mimetic peptide and phospholipids, which were loaded with DOX [106]. This chemoimmunotherapy delivery platform potentiated antitumor T cell responses and the therapeutic efficacy of anti-programmed death 1 (anti-PD1) immunotherapy. Besides the efficient elimination of established tumors, the proposed system also prevented tumor recurrence and metastasis to the liver.

Poly(β-amino ester) polymer particles carrying IL-12 have been used to induce the repolarization of macrophages from an immunosuppressive to a proinflammatory phenotype [107]. Both intravenous and intratumoral administration of IL-12-loaded NPs resulted in greater antitumor effects compared with free IL-12. By contrast, cyclodextrin-based NPs have been used to deliver

resiquimod, an agonist of Toll-like receptors 7 and 8, as a strategy to repolarize TAMs towards an M1 antitumor phenotype [108]. Intravenous injection of resiquimod-loaded cyclodextrin NPs increased the population of M1 macrophages in the TME and restricted tumor growth in colorectal cancer-bearing mice. The combination of these cyclodextrin NPs with resiquimod and anti-PD1 therapy resulted in enhanced antitumor efficacy compared with anti-PD1 therapy alone.

Multicomponent PLGA-based polymeric NPs have been used to deliver imatinib, a tyrosine kinase inhibitor that suppresses T_{reg} function by downregulating the expression of the transcription factor Foxp3 [109]. Indoleamine 2,3-dioxygenase (IDO) inhibitor-loaded polymeric oxaliplatin-based micelles have also reduced T_{reg} function. Intravenous administration of polymeric micelles carrying IDO inhibitors induced a significant decrease in the T_{reg} population and the highest $CD8^+$ T cell:T_{reg} ratio in the TME compared with the control groups, in a breast cancer mouse model [110]. Cationic PEGylated poly(D,L-lactide) and cationic lipid NPs have been used to deliver anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) siRNA, decreasing the number of T_{regs} in the TME [111]. Repeated administration of these NPs increased tumor-infiltrating $CD8^+$ T cells as well as the levels of proinflammatory cytokines, inhibiting tumor growth.

Gene modulation strategies

Over the past decade, gene therapy has become a versatile platform to target the TME, relying on effective gene carriers that deliver functional genes to their intracellular target and avoid nucleic acid degradation [90]. RNAi therapeutics, such as siRNAs, miRNAs, and short hairpin RNAs, can be used to achieve sequence-specific gene silencing for cancer therapy [112]. Gold NPs were designed for the therapeutic delivery of siRNA against Kras, a key oncogene driver [113]. The local delivery of a triple-therapy patch (i.e., hydrogel for drug/gene delivery and local hyperthermia) in a colon cancer model synergistically abrogated the tumor, facilitating complete tumor remission when applied to nonresected tumors and preventing tumor recurrence following resection. Strikingly, *in vitro*-transcribed mRNA encoding M1-polarizing transcription factors was loaded into targeted nanocarriers to reprogram TAMs to a phenotype that induced antitumor immunity and promoted tumor regression [114].

Cationic liposomes and NPs comprising polymers and/or inorganic materials, such as calcium carbonate and calcium phosphate, have been used to deliver siRNA for VEGF inhibition [115]. Lipid and polyamine hybrid NPs loaded with siRNA have shown high delivery efficiency to tumor ECs, enabling the silencing of multiple endothelial genes *in vivo* [116]. Nanoplexes of siRNA with thiolated glycol chitosan have been used to efficiently knockdown the gene encoding VEGF, reducing blood vessel formation in the TME [117]. The synergistic combination of VEGF-targeted chitosan NPs with chemotherapy led to promising tumor growth inhibition. Similarly, nanocomplexes of linear copolymer poly[bis(ε-Lys-PEI)Glut-PEG] carrying VEGF siRNA reduced VEGF expression in triple-negative breast cancer, controlling tumor growth [118]. Polyetherimide-grafted magnetic silica NPs have also shown promising results for the simultaneous delivery of siRNA anti-VEGF and magnetic resonance imaging [119].

Concluding remarks and future perspectives

Despite the great advances of nanomedicine, developing nanotechnology-based strategies to modulate the TME and support anticancer therapies still faces many challenges (see Outstanding questions). The reproducible synthesis and assessment of optimal physicochemical properties of these nanotechnology-based strategies are still difficult. Recently, Particle Replication In Non-wetting Template (PRINT) and microfluidic technologies were proposed for the synthesis of NPs with improved reproducibility, narrower size distribution, as well as more tunable physicochemical properties, compared with traditional bulk methods [120,121].

Outstanding questions

What barriers must be overcome for nanoengineered materials to reach their target in the tumor?

What are the main challenges for the development of novel nanotechnology-based approaches in cancer?

What are the advantages of nanomedicine for cancer therapy and diagnosis? What are the latest innovations?

What are the main features of the TME that can limit cancer therapy?

What are the main strategies to improve cancer therapy and diagnosis through nanoengineered materials?

How can we modulate the TME with nanotechnology-based strategies? What is the role of nanomedicine in gene and immune modulation for cancer therapy?

What are the main obstacles to the clinical translation of cancer nanomedicines?

Even though the scale-up of simple NPs with small molecules that have optimal physicochemical properties can be achieved through manufacturing unit operations widely used in the pharmaceutical industry, complex nanomedicines require changes in the existing unit operations or the development of alternative manufacturing processes. For instance, multifunctional nanomedicines, involving biological targeting or combining several therapeutic agents, pose additional manufacturing challenges that can hinder the transition from preclinical to clinical development. Thus, scale-up considerations should accompany NP design and engineering from the early stages of development [2].

Another challenge is the development systems that mimic the human TME to evaluate the efficacy of nanomaterials. Several 3D-culture models have been used to simulate the TME, including the ECM and tumor vasculature, supporting cells that can affect penetration and diffusion [122]. Using tumor spheroids in microfluidic channels could provide further insights into NP diffusion and accumulation [123]. However, to assess the *in vivo* safety and efficacy of nanotechnology-based strategies, the use of animal models will be crucial. The biodistribution and pharmacokinetics are some of the parameters with clear discrepancies between preclinical and clinical trials [124]. Despite the availability of several animal models, they all fail to fully replicate human cancers. For instance, the EPR is more consistent in animal models than in human tumors [125]. The development of animal models that reproduce more closely the histology and heterogeneity of human tumors, including high-fidelity patient-derived xenografts [126] and humanized mouse models [127], will improve the translation of these nanotechnology-based strategies.

As observed in the current coronavirus disease 2019 (COVID-19) global pandemic, nanotechnology can provide exceptional solutions quickly to emergent situations. The first two vaccines approved by the FDA and the European Medicines Agency (EMA) rely on NPs to induce immunity against severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) with remarkable outcomes[i] [128]. mRNA strategies have also been studied to restore tumor suppressors (e.g., p53-mRNA), resulting in an efficient antitumor strategy [129]. Thus, it is envisioned that this concept can be widely applied to modulate the TME in the near future, leading to their clinical translation. An integrated knowledge of NP interactions with the TME and their *in vivo* performance would improve the efficacy of the next generation of cancer nanomedicines, which are expected to carry novel therapeutic entities, including, kinase inhibitors, siRNAs, and mRNA.

Acknowledgments

The authors acknowledge financial support from Fundação para a Ciência e a Tecnologia/ Ministério da Ciência, Tecnologia, e Ensino Superior in the framework of PhD grant 2020.06638.BD (to D.P.S.), and from the European Research Council grant agreement No 848325 (J.C. for the ERC Starting Grant).

Declaration of interests

None declared by authors.

Resources

[i]https://clinicaltrials.gov/ct2/show/NCT04283461

References

1. Prasad, R. *et al.* (2020) Localized nanotheranostics: recent developments in cancer nanomedicine. *Mater. Today Adv.* 8, 100087
2. Shi, J. *et al.* (2017) Cancer nanomedicine: progress, challenges and opportunities. *Nat. Rev. Cancer* 17, 20–37
3. Conde, J. *et al.* (2014) Revisiting 30 years of biofunctionalization and surface chemistry of inorganic nanoparticles for nanomedicine. *Front. Chem.* 2, 48
4. Li, H. *et al.* (2018) Cancer nanomedicine: mechanisms, obstacles and strategies. *Nanomedicine* 13, 1639–1656
5. Swetha, K.L. and Roy, A. (2018) Tumor heterogeneity and nanoparticle-mediated tumor targeting: the importance of delivery system personalization. *Drug Deliv. Transl. Res.* 8, 1508–1526
6. Tlsty, T.D. and Coussens, L.M. (2006) Tumor stroma and regulation of cancer development. *Annu. Rev. Pathol. Mech. Dis.* 1, 119–150

7. Burgos-Panadero, R. *et al.* (2019) The tumour microenvironment as an integrated framework to understand cancer biology. *Cancer Lett.* 461, 112–122
8. Lorenzo-Sanz, L. and Muñoz, P. (2019) Tumor-infiltrating immunosuppressive cells in cancer-cell plasticity, tumor progression and therapy response. *Cancer Microenviron.* 12, 119–132
9. Lin, Y. *et al.* (2019) Tumor-associated macrophages in tumor metastasis: biological roles and clinical therapeutic applications. *J. Hematol. Oncol.* 12, 76
10. Miguel, R.D.V. *et al.* (2010) CTL induction of tumoricidal nitric oxide production by intratumoral macrophages is critical for tumor elimination. *J. Immunol.* 185, 6706–6718
11. Strachan, D.C. *et al.* (2013) CSF1R inhibition delays cervical and mammary tumor growth in murine models by attenuating the turnover of tumor-associated macrophages and enhancing infiltration by CD8+ T cells. *Oncoimmunology* 2, e26968
12. Gordon, S.R. *et al.* (2017) PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature* 545, 495–499
13. Yao, R.-R. *et al.* (2018) M2-polarized tumor-associated macrophages facilitated migration and epithelial-mesenchymal transition of HCC cells via the TLR4/STAT3 signaling pathway. *World J. Surg. Oncol.* 16, 9
14. Coffelt, S.B. *et al.* (2016) Neutrophils in cancer: neutral no more. *Nat. Rev. Cancer* 16, 431–446
15. Carnevale, S. *et al.* (2020) The complexity of neutrophils in health and disease: Focus on cancer. *Semin. Immunol.* 48, 101409
16. Coffelt, S.B. *et al.* (2015) IL-17-producing γδ T cells and neutrophils conspire to promote breast cancer metastasis. *Nature* 522, 345–348
17. Capucetti, A. *et al.* (2020) Multiple roles for chemokines in neutrophil biology. *Front. Immunol.* 11, 1259
18. Mukaida, N. *et al.* (2020) Two-faced roles of tumor-associated neutrophils in cancer development and progression. *Int. J. Mol. Sci.* 21, 3457
19. Finisguerra, V. *et al.* (2015) MET is required for the recruitment of anti-tumoural neutrophils. *Nature* 522, 349–353
20. Kotsafti, A. *et al.* (2020) Reactive oxygen species and antitumor immunity-from surveillance to evasion. *Cancers (Basel)* 12, 1748
21. Deryugina, E.I. *et al.* (2014) Tissue-infiltrating neutrophils constitute the major in vivo source of angiogenesis-inducing MMP-9 in the tumor microenvironment. *Neoplasia* 16, 771–788
22. Zhou, S.-L. *et al.* (2016) Tumor-associated neutrophils recruit macrophages and T-regulatory cells to promote progression of hepatocellular carcinoma and resistance to sorafenib. *Gastroenterology* 150, 1646–1658
23. Li, C. *et al.* (2020) Regulatory T cells in tumor microenvironment: new mechanisms, potential therapeutic strategies and future prospects. *Mol. Cancer* 19, 116
24. Ihara, F. *et al.* (2019) Regulatory T cells induce CD4–NKT cell anergy and suppress NKT cell cytotoxic function. *Cancer Immunol. Immunother.* 68, 1935–1947
25. Sawant, D.V. *et al.* (2019) Adaptive plasticity of IL-10+ and IL-35+ Treg cells cooperatively promotes tumor T cell exhaustion. *Nat. Immunol.* 20, 724–735
26. Oh, E. *et al.* (2019) Regulatory T cells induce metastasis by increasing TGF-β and enhancing the epithelial–mesenchymal transition. *Cells* 8, 1387
27. Grinnell, F. *et al.* (1981) Distribution of fibronectin during wound healing in vivo. *J. Invest. Dermatol.* 76, 181–189
28. Cheng, Y. *et al.* (2018) Cancer-associated fibroblasts induce PDL1+ neutrophils through the IL6-STAT3 pathway that foster immune suppression in hepatocellular carcinoma. *Cell Death Dis.* 9, 422
29. Sahai, E. *et al.* (2020) A framework for advancing our understanding of cancer-associated fibroblasts. *Nat. Rev. Cancer* 20, 174–186
30. Wang, F.-T. *et al.* (2019) Cancer-associated fibroblast regulation of tumor neo-angiogenesis as a therapeutic target in cancer. *Oncol. Lett.* 17, 3055–3065
31. Wang, L. *et al.* (2018) CAFs enhance paclitaxel resistance by inducing EMT through the IL-6/JAK2/STAT3 pathway. *Oncol. Rep.* 39, 2081–2090
32. Yi, Y. *et al.* (2018) Cancer-associated fibroblasts promote epithelial-mesenchymal transition and EGFR-TKI resistance of non-small cell lung cancers via HGF/IGF-1/ANXA2 signaling. *Biochim. Biophys. Acta Mol. basis Dis.* 1864, 793–803
33. Lugo-Cintrón, K.M. *et al.* (2020) Breast fibroblasts and ECM components modulate breast cancer cell migration through the secretion of MMPs in a 3D microfluidic co-culture model. *Cancers (Basel)* 12, 1173
34. Hida, K. *et al.* (2018) Contribution of tumor endothelial cells in cancer progression. *Int. J. Mol. Sci.* 19, 1272
35. Cao, Z. *et al.* (2017) Molecular checkpoint decisions made by subverted vascular niche transform indolent tumor cells into chemoresistant cancer stem Cells. *Cancer Cell* 31, 110–126
36. Salazar, N. and Zabel, B.A. (2019) Support of tumor endothelial cells by chemokine receptors. *Front. Immunol.* 10, 147
37. Zonneville, J. *et al.* (2018) TGF-β signaling promotes tumor vasculature by enhancing the pericyte-endothelium association. *BMC Cancer* 18, 670
38. Wei, R. *et al.* (2020) Cellular and extracellular components in tumor microenvironment and their application in early diagnosis of cancers. *Anal. Cell. Pathol.* 2020, 6283796
39. Henke, E. *et al.* (2020) Extracellular matrix in the tumor microenvironment and its impact on cancer therapy. *Front. Mol. Biosci.* 6, 160
40. Lederle, W. *et al.* (2010) MMP13 as a stromal mediator in controlling persistent angiogenesis in skin carcinoma. *Carcinogenesis* 31, 1175–1184
41. Choi, J.U. *et al.* (2020) The biological function and therapeutic potential of exosomes in cancer: exosomes as efficient nanocommunicators for cancer therapy. *Int. J. Mol. Sci.* 21, 7363
42. Chen, W. *et al.* (2014) Exosomes from drug-resistant breast cancer cells transmit chemoresistance by a horizontal transfer of microRNAs. *PLoS One* 9, e95240
43. Lin, E.Y. *et al.* (2007) Vascular endothelial growth factor restores delayed tumor progression in tumors depleted of macrophages. *Mol. Oncol.* 1, 288–302
44. Baltazar, F. *et al.* (2002) Lactate beyond a waste metabolite: metabolic affairs and signaling in malignancy. *Front. Oncol.* 10, 231
45. Feng, L. *et al.* (2018) The acidic tumor microenvironment: a target for smart cancer nano-theranostics. *Natl. Sci. Rev.* 5, 269–286
46. Yamauchi, M. *et al.* (2018) The fibrotic tumor stroma. *J. Clin. Invest.* 128, 16–25
47. Hauge, A. and Rofstad, E.K. (2020) Antifibrotic therapy to normalize the tumor microenvironment. *J. Transl. Med.* 18, 207
48. Wang, S. *et al.* (2016) Hierarchical targeting strategy for enhanced tumor tissue accumulation/retention and cellular internalization. *Adv. Mater.* 28, 7340–7364
49. Li, H.-J. *et al.* (2016) Stimuli-responsive clustered nanoparticles for improved tumor penetration and therapeutic efficacy. *Proc. Natl. Acad. Sci. U. S. A.* 113, 4164–4169
50. Suzuki, S. *et al.* (2017) Tumor microenvironment-sensitive liposomes penetrate tumor tissue via attenuated interaction of the extracellular matrix and tumor cells and accompanying actin depolymerization. *Biomacromolecules* 18, 535–543
51. Mboge, M.Y. *et al.* (2018) Carbonic anhydrases: role in pH control and cancer. *Metabolites* 8, 19
52. Janoniene, A. *et al.* (2017) A versatile carbonic anhydrase IX targeting ligand-functionalized porous silicon nanoplatform for dual hypoxia cancer therapy and imaging. *ACS Appl. Mater. Interfaces* 9, 13976–13987
53. Som, A. *et al.* (2016) Monodispersed calcium carbonate nanoparticles modulate local pH and inhibit tumor growth in vivo. *Nanoscale* 8, 12639–12647
54. Brown, J.M. and Wilson, W.R. (2004) Exploiting tumour hypoxia in cancer treatment. *Nat. Rev. Cancer* 4, 437–447
55. Shim, M.S. and Xia, Y. (2013) A reactive oxygen species (ROS)-responsive polymer for safe, efficient, and targeted gene delivery in cancer cells. *Angew. Chem. Int. Ed.* 52, 6926–6929
56. Son, S. *et al.* (2018) Carboxymethyl dextran-based hypoxia-responsive nanoparticles for doxorubicin delivery. *Int. J. Biol. Macromol.* 110, 399–405

57. Gordijo, C.R. *et al.* (2015) Design of hybrid MnO2-polymer-lipid nanoparticles with tunable oxygen generation rates and tumor accumulation for cancer treatment. *Adv. Funct. Mater.* 25, 1858–1872
58. Chen, Q. *et al.* (2018) Intelligent albumin-MnO2 nanoparticles as pH-/H2O2-responsive dissociable nanocarriers to modulate tumor hypoxia for effective combination therapy. *Adv. Mater.* 30, 7129–7136
59. Cheng, Y. *et al.* (2015) Perfluorocarbon nanoparticles enhance reactive oxygen levels and tumour growth inhibition in photodynamic therapy. *Nat. Commun.* 6, 8785
60. Gao, M. *et al.* (2017) Erythrocyte-membrane-enveloped perfluorocarbon as nanoscale artificial red blood cells to relieve tumor hypoxia and enhance cancer radiotherapy. *Adv. Mater.* 29, 1701429
61. Prasad, P. *et al.* (2014) Multifunctional albumin–MnO2 nanoparticles modulate solid tumor microenvironment by attenuating hypoxia, acidosis, vascular endothelial growth factor and enhance radiation response. *ACS Nano* 8, 3202–3212
62. Song, G. *et al.* (2016) Catalase-loaded TaOx nanoshells as bionanoreactors combining high-Z element and enzyme delivery for enhancing radiotherapy. *Adv. Mater.* 28, 7143–7148
63. Tian, L. *et al.* (2017) Radionuclide I-131 labeled albumin-paclitaxel nanoparticles for synergistic combined chemoradioisotope therapy of cancer. *Theranostics* 7, 614–623
64. Song, X. *et al.* (2017) Liposomes co-loaded with metformin and chlorin e6 modulate tumor hypoxia during enhanced photodynamic therapy. *Nano Res.* 10, 1200–1212
65. Liu, Z. *et al.* (2014) Legumain protease-activated TAT-liposome cargo for targeting tumours and their microenvironment. *Nat. Commun.* 5, 4280
66. Shi, K. *et al.* (2020) Tumor microenvironment targeting with dual stimuli-responsive nanoparticles based on small heat shock proteins for antitumor drug delivery. *Acta Biomater.* 114, 369–383
67. Gao, H. *et al.* (2014) Angiopep-2 and activatable cell-penetrating peptide dual-functionalized nanoparticles for systemic glioma-targeting delivery. *Mol. Pharm.* 11, 2755–2763
68. Li, Y. *et al.* (2020) Tailor-made legumain/pH dual-responsive doxorubicin prodrug-embedded nanoparticles for efficient anticancer drug delivery and in situ monitoring of drug release. *Nanoscale* 12, 2673–2685
69. Goodman, T.T. *et al.* (2007) Increased nanoparticle penetration in collagenase-treated multicellular spheroids. *Int. J. Nanomedicine* 2, 265–274
70. Liu, J. *et al.* (2018) Collagenase-encapsulated pH-responsive nanoscale coordination polymers for tumor microenvironment modulation and enhanced photodynamic nanomedicine. *ACS Appl. Mater. Interfaces* 10, 43493–43502
71. Parodi, A. *et al.* (2014) Bromelain surface modification increases the diffusion of silica nanoparticles in the tumor extracellular matrix. *ACS Nano* 8, 9874–9883
72. Kanapathipillai, M. *et al.* (2012) Inhibition of mammary tumor growth using lysyl oxidase-targeting nanoparticles to modify extracellular matrix. *Nano Lett.* 12, 3213–3217
73. Zhou, H. *et al.* (2016) Hyaluronidase embedded in nanocarrier PEG shell for enhanced tumor penetration and highly efficient antitumor efficacy. *Nano Lett.* 16, 3268–3277
74. Rwei, A.Y. *et al.* (2015) Photoresponsive nanoparticles for drug delivery. *Nano Today* 10, 451–467
75. Wei, Z. *et al.* (2020) A thieno-isoindigo derivative-based conjugated polymer nanoparticle for photothermal therapy in the NIR-II bio-window. *Nanoscale* 12, 19665–19672
76. Cho, H.J. *et al.* (2015) Engineered photo-responsive materials for near-infrared-triggered drug delivery. *J. Ind. Eng. Chem.* 31, 15–25
77. Marangon, I. *et al.* (2017) Tumor stiffening, a key determinant of tumor progression, is reversed by nanomaterial-induced photothermal therapy. *Theranostics* 7, 329–343
78. Cazares-Cortes, E. *et al.* (2019) Recent insights in magnetic hyperthermia: from the "hot-spot" effect for local delivery to combined magneto-photo-thermia using magneto-plasmonic hybrids. *Adv. Drug Deliv. Rev.* 138, 233–246
79. Kennedy, L.C. *et al.* (2011) A new era for cancer treatment: gold-nanoparticle-mediated thermal therapies. *Small* 7, 169–183
80. Kwon, K.C. *et al.* (2017) Superparamagnetic gold nanoparticles synthesized on protein particle scaffolds for cancer theragnosis. *Adv. Mater.* 29, 1701146
81. Raeesi, V. and Chan, W.C.W. (2016) Improving nanoparticle diffusion through tumor collagen matrix by photo-thermal gold nanorods. *Nanoscale* 8, 12524–12530
82. Zhao, Y. *et al.* (2016) Nano-engineered electro-responsive drug delivery systems. *J. Mater. Chem. B* 4, 3019–3030
83. Chen, T. *et al.* (2020) Porous Pt nanoparticles loaded with doxorubicin to enable synergistic chemo-/electrodynamic therapy. *Biomaterials* 255, 120202
84. Sirsi, S.R. and Borden, M.A. (2014) State-of-the-art materials for ultrasound-triggered drug delivery. *Adv. Drug Deliv. Rev.* 72, 3–14
85. Lin, X. *et al.* (2020) An ultrasound activated vesicle of Janus Au-MnO nanoparticles for promoted tumor penetration and sono-chemodynamic therapy of orthotopic liver cancer. *Angew. Chem. Int. Ed.* 59, 1682–1688
86. Danhier, F. (2016) To exploit the tumor microenvironment: since the EPR effect fails in the clinic, what is the future of nanomedicine? *J. Control. Release* 244, 108–121
87. Matsumoto, Y. *et al.* (2016) Vascular bursts enhance permeability of tumour blood vessels and improve nanoparticle delivery. *Nat. Nanotechnol.* 11, 533–538
88. Sindhwani, S. *et al.* (2020) The entry of nanoparticles into solid tumours. *Nat. Mater.* 19, 566–575
89. Setyawati, M.I. *et al.* (2017) Gold nanoparticles induced endothelial leakiness depends on particle size and endothelial cell origin. *ACS Nano* 11, 5020–5030
90. Thakkar, S. *et al.* (2020) Tumor microenvironment targeted nanotherapeutics for cancer therapy and diagnosis: A review. *Acta Biomater.* 101, 43–68
91. Ernsting, M.J. *et al.* (2011) Synthetic modification of carboxymethylcellulose and use thereof to prepare a nanoparticle forming conjugate of docetaxel for enhanced cytotoxicity against cancer cells. *Bioconjug. Chem.* 22, 2474–2486
92. Ernsting, M.J. *et al.* (2015) Targeting of metastasis-promoting tumor-associated fibroblasts and modulation of pancreatic tumor-associated stroma with a carboxymethylcellulose-docetaxel nanoparticle. *J. Control. Release* 206, 122–130
93. Alili, L. *et al.* (2011) Combined cytotoxic and anti-invasive properties of redox-active nanoparticles in tumor–stroma interactions. *Biomaterials* 32, 2918–2929
94. Guo, S. *et al.* (2014) Unmodified drug used as a material to construct nanoparticles: delivery of cisplatin for enhanced anti-cancer therapy. *J. Control. Release* 174, 137–142
95. Miao, L. *et al.* (2015) Nanoparticle modulation of the tumor microenvironment enhances therapeutic efficacy of cisplatin. *J. Control. Release* 217, 27–41
96. Koide, H. *et al.* (2017) A polymer nanoparticle with engineered affinity for a vascular endothelial growth factor (VEGF165). *Nat. Chem.* 9, 715–722
97. Ghalamfarsa, G. *et al.* (2018) Anti-angiogenic effects of CD73-specific siRNA-loaded nanoparticles in breast cancer-bearing mice. *J. Cell. Physiol.* 233, 7165–7177
98. Noy, R. and Pollard, J.W. (2014) Tumor-associated macrophages: from mechanisms to therapy. *Immunity* 41, 49–61
99. Lin, E.Y. *et al.* (2001) Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J. Exp. Med.* 193, 727–740
100. Qian, Y. *et al.* (2017) Molecular-targeted immunotherapeutic strategy for melanoma via dual-targeting nanoparticles delivering small interfering RNA to tumor-associated macrophages. *ACS Nano* 11, 9536–9549
101. Parayath, N.N. *et al.* (2018) Repolarization of tumor-associated macrophages in a genetically engineered nonsmall cell lung cancer model by intraperitoneal administration of hyaluronic acid-based nanoparticles encapsulating microRNA-125b. *Nano Lett.* 18, 3571–3579
102. Monopoli, M.P. *et al.* (2012) Biomolecular coronas provide the biological identity of nanosized materials. *Nat. Nanotechnol.* 7, 779–786
103. Huai, Y. *et al.* (2019) Nanoparticle interactions with the tumor microenvironment. *Bioconjug. Chem.* 30, 2247–2263

104. Saha, S. *et al.* (2016) Gold nanoparticle reprograms pancreatic tumor microenvironment and inhibits tumor growth. *ACS Nano* 10, 10636–10651
105. Nam, J. *et al.* (2019) Cancer nanomedicine for combination cancer immunotherapy. *Nat. Rev. Mater.* 4, 398–414
106. Kuai, R. *et al.* (2018) Elimination of established tumors with nanodisc-based combination chemoimmunotherapy. *Sci. Adv.* 4, eaao1736
107. Watkins, S.K. *et al.* (2007) IL-12 rapidly alters the functional profile of tumor-associated and tumor-infiltrating macrophages in vitro and in vivo. *J. Immunol.* 178, 1357–1362
108. Rodell, C.B. *et al.* (2018) TLR7/8-agonist-loaded nanoparticles promote the polarization of tumour-associated macrophages to enhance cancer immunotherapy. *Nat. Biomed. Eng.* 2, 578–588
109. Ou, W. *et al.* (2018) Combination of NIR therapy and regulatory T cell modulation using layer-by-layer hybrid nanoparticles for effective cancer photoimmunotherapy. *Theranostics* 8, 4574–4590
110. Feng, B. *et al.* (2018) Binary cooperative prodrug nanoparticles improve immunotherapy by synergistically modulating immune tumor microenvironment. *Adv. Mater.* 30, 1803001
111. Li, S.-Y. *et al.* (2016) Restoring anti-tumor functions of T cells via nanoparticle-mediated immune checkpoint modulation. *J. Control. Release* 231, 17–28
112. Conniot, J. *et al.* (2021) Revisiting gene delivery to the brain: silencing and editing. *Biomater. Sci.* 9, 1065–1087
113. Conde, J. *et al.* (2016) Local triple-combination therapy results in tumour regression and prevents recurrence in a colon cancer model. *Nat. Mater.* 15, 1128–1138
114. Zhang, F. *et al.* (2019) Genetic programming of macrophages to perform anti-tumor functions using targeted mRNA nanocarriers. *Nat. Commun.* 10, 3974
115. Li, F. *et al.* (2019) Co-delivery of VEGF siRNA and etoposide for enhanced anti-angiogenesis and anti-proliferation effect *via* multi-functional nanoparticles for orthotopic non-small cell lung cancer treatment. *Theranostics* 9, 5886–5898
116. Dahlman, J.E. *et al.* (2014) In vivo endothelial siRNA delivery using polymeric nanoparticles with low molecular weight. *Nat. Nanotechnol.* 9, 648–655
117. Kwak, G. *et al.* (2017) Synergistic antitumor effects of combination treatment with metronomic doxorubicin and VEGF-targeting RNAi nanoparticles. *J. Control. Release* 267, 203–213
118. Zhao, Z. *et al.* (2019) Development of a biocompatible copolymer nanocomplex to deliver VEGF siRNA for triple negative breast cancer. *Theranostics* 9, 4508–4524
119. Li, T. *et al.* (2015) Polyetherimide-grafted Fe_3O_4@SiO_2 nanoparticles as theranostic agents for simultaneous VEGF siRNA delivery and magnetic resonance cell imaging. *Int. J. Nanomedicine* 10, 4279–4291
120. Rhee, M. *et al.* (2011) Synthesis of size-tunable polymeric nanoparticles enabled by 3D hydrodynamic flow focusing in single-layer microchannels. *Adv. Mater.* 23, 79–83
121. Xu, J. *et al.* (2013) Future of the particle replication in nonwetting templates (PRINT) technology. *Angew. Chem. Int. Ed.* 52, 6580–6589
122. Le, Q.-V. *et al.* (2019) Nanomaterial-Based Modulation of Tumor Microenvironments for Enhancing Chemo/Immunotherapy. *AAPS J.* 21, 64
123. Albanese, A. *et al.* (2013) Tumour-on-a-chip provides an optical window into nanoparticle tissue transport. *Nat. Commun.* 4, 2718
124. Choi, S.Y.C. *et al.* (2014) Lessons from patient-derived xenografts for better in vitro modeling of human cancer. *Adv. Drug Deliv. Rev.* 79–80, 222–237
125. Bertrand, N. *et al.* (2014) Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology. *Adv. Drug Deliv. Rev.* 66, 2–25
126. Lin, D. *et al.* (2014) High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. *Cancer Res.* 74, 1272–1283
127. Rongvaux, A. *et al.* (2014) Development and function of human innate immune cells in a humanized mouse model. *Nat. Biotechnol.* 32, 364–372
128. Polack, F.P. *et al.* (2020) Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N. Engl. J. Med.* 383, 2603–2615
129. Kong, N. *et al.* (2019) Synthetic mRNA nanoparticle-mediated restoration of p53 tumor suppressor sensitizes p53-deficient cancers to mTOR inhibition. *Sci. Transl. Med.* 11, eaaw1565
130. Hu, X. *et al.* (2020) Biodegradation-mediated enzymatic activity-tunable molybdenum oxide nanourchins for tumor-specific cascade catalytic therapy. *J. Am. Chem. Soc.* 142, 1636–1644
131. Yi, Z. *et al.* (2019) In vivo tumor visualization through MRI off-on switching of NaGdF4–CaCO3 nanoconjugates. *Adv. Mater.* 31, 1901851
132. Guo, Q. *et al.* (2019) In situ real-time tracing of hierarchical targeting nanostructures in drug resistant tumors using diffuse fluorescence tomography. *Chem. Sci.* 10, 7878–7886
133. Luo, D. *et al.* (2020) Nanoparticles yield increased drug uptake and therapeutic efficacy upon sequential near-infrared irradiation. *ACS Nano* 14, 15193–15203
134. Chen, W.-H. *et al.* (2017) Mesoporous silica-based versatile theranostic nanoplatform constructed by layer-by-layer assembly for excellent photodynamic/chemo therapy. *Biomaterials* 117, 54–65
135. Wu, X. *et al.* (2019) Ferric hydroxide-modified upconversion nanoparticles for 808 nm nir-triggered synergetic tumor therapy with hypoxia modulation. *ACS Appl. Mater. Interfaces* 11, 385–393
136. Fu, C. *et al.* (2019) Targeted magnetic resonance imaging and modulation of hypoxia with multifunctional hyaluronic acid-MnO2 nanoparticles in glioma. *Adv. Healthc. Mater.* 8, 1900047
137. He, S. *et al.* (2019) An organic afterglow protheranostic nanoassembly. *Adv. Mater.* 31, 1902672
138. Wang, Z. *et al.* (2019) Near-infrared light and tumor microenvironment dual responsive size-switchable nanocapsules for multimodal tumor theranostics. *Nat. Commun.* 10, 4418
139. Sun, W. *et al.* (2019) Bone-targeted nanoplatform combining zoledronate and photothermal therapy to treat breast cancer bone metastasis. *ACS Nano* 13, 7556–7567
140. Tang, J. *et al.* (2017) Dual-mode imaging-guided synergistic chemo- and magnetohyperthermia therapy in a versatile nanoplatform to eliminate cancer stem cells. *ACS Appl. Mater. Interfaces* 9, 23497–23507
141. Meng, X. *et al.* (2020) Ultrasound-responsive alkaline nanorobots for the treatment of lactic acidosis-mediated doxorubicin resistance. *Nanoscale* 12, 13801–13810
142. Chiang, C.-S. *et al.* (2018) Combination of fucoidan-based magnetic nanoparticles and immunomodulators enhances tumour-localized immunotherapy. *Nat. Nanotechnol.* 13, 746–754
143. Kosmides, A.K. *et al.* (2017) Dual targeting nanoparticle stimulates the immune system to inhibit tumor growth. *ACS Nano* 11, 5417–5429
144. Zhang, P. *et al.* (2019) Therapeutic targeting of tumor-associated myeloid cells synergizes with radiation therapy for glioblastoma. *Proc. Natl. Acad. Sci. U. S. A.* 116, 23714–23723
145. Conniot, J. *et al.* (2019) Immunization with mannosylated nanovaccines and inhibition of the immune-suppressing microenvironment sensitizes melanoma to immune checkpoint modulators. *Nat. Nanotechnol.* 14, 891–901
146. Watanabe, S. *et al.* (2019) In vivo rendezvous of small nucleic acid drugs with charge-matched block catiomers to target cancers. *Nat. Commun.* 10, 1894
147. Xu, X. *et al.* (2017) Tumor microenvironment-responsive multistaged nanoplatform for systemic RNAi and cancer therapy. *Nano Lett.* 17, 4427–4435