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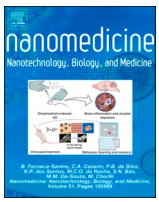

Review Article

Nanomedicine in oncology: Diagnostic breakthroughs and therapeutic Frontiers

Adeeb Shehzad [a,*], Júlia Alves [b,c], Mazhar Ul-Islam [d], Abdullah Khamis Al Saidi [d], Sofia O.D. Duarte [b,c], Mohammad Sherjeel Javed Khan [e], Pedro Fonte [b,c,f,g,**]

[a] Research Center, Dhofar University, Salalah 211, Oman
[b] iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisbon, Portugal
[c] Associate Laboratory i4HB–Institute for Health and Bio-economy, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal
[d] Department of Chemical Engineering, College of Engineering, Dhofar University, Salalah, Oman
[e] Institute of Energy Infrastructure (IEI), Universiti Tenaga Nasional (UNITEN), 43000, Selangor, Malaysia
[f] Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), Campus de Gambelas, Universidade do Algarve, 8005-139 Faro, Portugal
[g] Department of Chemistry and Pharmacy, Faculty of Sciences and Technology, University of Algarve, Gambelas Campus, 8005-139 Faro, Portugal

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ABSTRACT

Nanomedicine is a multidisciplinary field, offering significant promises for cancer detection and therapy. Nanoparticles (NPs), nanoprobes and nanobiosensors can be tailored to achieve highly sensitive tumor detection by contrast imaging techniques. The application of directed drug delivery for cancer therapies can be achieved via the formulation and tailoring of drug-loaded nanocarriers. NPs have been employed as carrier to transport drugs or biological molecules to tumor tissues via active or passive mechanisms, consequently improving treatment outcomes and minimize harmful effects. However, nanomedicine translation has been hindered by augmented permeability and retention and ICI of the TME, limiting improvement and potential outcomes of patients. TME, consisting of cancerous cells, CAFs or TAFs, specific immune cells, and the stroma, performs a crucial part in contributing to cancer resistance to nanotherapy. This review summarizes nanotechnology application in the identification and treatment of cancers by exploring pathophysiological features, mechanisms and limitation of nanomedicine in cancer.

Introduction

Cancer continues to be a primary cause of mortality globally, with approximately 20 million more cases diagnosed annually and around 10 million casualties.[1,2] The anticipated global prevalence of cancer survivors within five years of diagnosis is 53.5 million. Approximately 20 % of people will face a cancer diagnosis at some point in their lives, and around 11 % of men and 8 % of women will ultimately die from the illness.[1,2] Given the alarming rise in incidence and mortality rates, it is crucial to address this global health challenge by advancing research on tumor biology, enhancing diagnostic techniques, and developing innovative therapeutic approaches. While the cancer burden persists in developed countries, it continues to pose a major global challenge.[3] Advance therapeutic strategies are needed for more advanced innovations in diagnosis and treatments, along with the practical understanding and effective application of current cancer treatment strategies. Currently, several treatment modalities such as surgery, radiotherapy, and chemotherapy are in practice for the treatment of malignant tumors, either alone or synergistically. Surgery and radiation therapy are considered as the gold standard treatment method for most solid tumors followed by systematic treatment to prevent recurrence of metastasis.[4] Traditional chemotherapy has also reached the limit of ineffectiveness due to instability, drug resistance, absorption, distribution, insolubility, and rapid elimination.[5] However, nanotechnology aims to encapsulate and optimize existing drug formulations with advanced physicochemical properties, ensuring a relatively stable microenvironment that facilitates dynamic interactions within the body while enhancing bioavailability, extending half-life, and improving

* Corresponding author at: Research Center, Dhofar University, Salalah 211, Oman
** Corresponding author at: P. Fonte, iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal.
E-mail addresses: ashehzad@du.edu.om (A. Shehzad), prfonte@ualg.pt (P. Fonte).

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efficacy at the tumor site.

Nanomedicine is a rapidly developing field with great potential for addressing problems in the detection and therapy of cancer. By understanding the distinctive biological features of tumors, researchers can design targeted approaches for delivering chemotherapeutic agents, genes, and antibodies.[6] The nano-complex can circulate through the bloodstream and concentrate at the tumor location via active or passive targeting mechanisms. These nano-complexes utilize both active and passive methods to display extended half-life in systemic circulation with maximum bioavailability. Additionally, nanotechnology synergistically enhances the potential of photothermal therapy and immunotherapy by employing hyperthermia or triggering the immune system to destroy tumor malignant cells.

TME produces distinct variation in biomarkers, which can trigger the drug release from nanocarriers upon arriving at tumor site.[7,8] The diversity of tumors must always be considered during clinical treatment procedures. Innovative designs can achieve controlled drug-release, essential for targeted tumor treatment delivered at designated times and locations. Nano approaches can enhance therapeutic effectiveness with minimum side effects. Despite the approval of multiple therapeutic nanoparticle (NP) platforms, including liposomes, albumin-based carriers, and polymeric micelles, by drug regulatory agencies for therapeutic approaches for various cancer types, their clinical application remains an area of ongoing research and development. Table 1 presents a comprehensive overview of ongoing clinical trials utilizing various nanotechnology platforms across multiple cancer types, detailing the nanoformulations, clinical status, trial locations or centres, and corresponding NCT identifiers. Despite promising advancements in nanotechnology for cancer treatment, several issues such as potential toxicity, resource consumption, instability, and applicability need to be resolved before translation to the clinical settings.[9] Detailed pharmacokinetics and toxicology in vivo studies of nanomaterials are needed to optimize the pharmacokinetic (absorption, distribution, metabolism, elimination) parameters and strengthen the therapeutic effects. Clinical applications often show delayed responses when combining therapies and diagnostics, leading to possible drug overdoses and unintended effects. Monitoring the real-time distribution of drugs in vivo after intravenous administration remains a challenge. Thus, many obstacles need to be overcome to facilitate the transition of nanotechnology into clinical practice.[10]

This review consolidates the latest insights on the various types of nanomedicines and their diagnostic and therapeutic applications in the management of cancer and patient care. Various synthetic and natural types of nanomedicines such as NPs, liposomes, micelles, hydrogels, exosomes, viruses, and inorganic NPs have been discussed. This study also underpins the potential applications of nanotechnology in malignant tumors including digestive, respiratory, hematological, and skeletal cancers. Finally, the limitations and challenges of nanomedicine have been discussed in malignant tumors.

Role of Nanomedicine in cancer diagnosis

While conventional treatments like chemotherapy, radiotherapy, and surgery have enhanced cancer management outcomes, they often result in adverse effects, including damage to normal cells and drug resistance. Thus, there is a pressing need for innovative therapeutic approaches that can enhance efficacy while reducing side effects. Recently, nanotechnology has made tremendous progress, and nanomedicines such as NPs, liposomes, polymers, photon therapy, and adjuvant nano therapies are widely used for diagnosis and treatment of various cancers.[11–13] In this study, we enhance the understanding of novel nanotechnology applications in the diagnosis and treatment of gastrointestinal cancers (Fig.1).

Diagnosis

It is widely recognized that detecting and diagnosing malignancies typically rely on identifying cancer biomarkers and utilizing imaging technologies in clinical practice. The timely identification of tumors can be made possible from blood biomarkers, which are secreted in the circulation. However, the secreted biomarkers are diluted, which can lead to false positive and negative results. Imaging techniques such as X-rays, CT scan, MRI, and endoscopic ultrasonography (EUS) can also be hindered by low sensitivity.[14–17] Thus, nanotechnology-based strategies have enhanced the precision of biomarker detection, the accuracy and efficiency of imaging, and the ability to target and penetrate localized tumor clusters while minimizing non-specific interactions.

Nanotechnology in biosensor-based diagnosis

Numerous studies have been investigating nano-based approaches to overcome the challenges in cancer diagnosis. For example, multiprotease nanosensors, such as ultrasmall gold nanoclusters (AuNCs) have been developed as highly sensitive and specific probes for in vivo

Table 1
Applications of nanotechnology in cancer.

Cancer Type	Nanoformulation	Status	Location/Centre	Identifier/NCT number
Rectal Cancer	CNO (Carbon NPs)	Not Applicable	Peking University People's Hospital, China	NCT03550001
Head and Neck Cancer	Iron Oxide NPs	Early Phase 1	Center for Advanced Biomedical Imaging (CABI)	NCT01895829
Hepatic cancers	Iron oxide NPs	Phase I	Allegheny Singer Research Institute	NCT04682847
Colorectal Cancer	CNP	Not Applicable	Peking University People's Hospital	NCT03778268
Endometrial neoplasm	CNP	Not Applicable	Peking University People's Hospital	NCT03778255
Uterine Cervical neoplasm	CNP	Not Applicable	Peking University People's Hospital	NCT03778268
Brain cancer	Silica NPs with 89Zr-DFO-cRGDYPEG-Cy5-C′ dots	Phase I	Memorial Sloan Kettering Cancer Center	NCT03465618
Metastatic melanoma/malignant brain cancer	Silica nanomolecules with 124I-cRGDY-PEGdots	Not applicable -Interventional clinical trial	Memorial Sloan Kettering Cancer Center	NCT01266096
Pancreatic cancer	Hafnium oxide NPs	Phase II	NANOBIOTIX	NCT02465593
Head and Neck Cancer, Melanoma Breast Cancer, Colorectal Cancer	Fluorescent cRDGY-PEG-Cy5.5-C dots	Phase 1/ Phase 2	Memorial Sloan Kettering Cancer Center	NCT02106598
Lymphoma	Immuno-tethered lipoplex NPs	Not Applicable	Ohio State University Comprehensive Cancer Center	NCT03656835
Stomach Disease/ Gastric cancer	Nano sensors		Anhui Medical University	NCT01420588
Metastatic melanoma/malignant brain tumors	124I-cRGDY-PEG-dots	Not Applicable	Memorial Sloan Kettering Cancer Center	NCT01266096
Brain Cancer	89Zr-DFO-cRGDY-PEG-Cy5-C dots	Phase 1	Memorial Sloan Kettering Cancer Center	NCT03465618

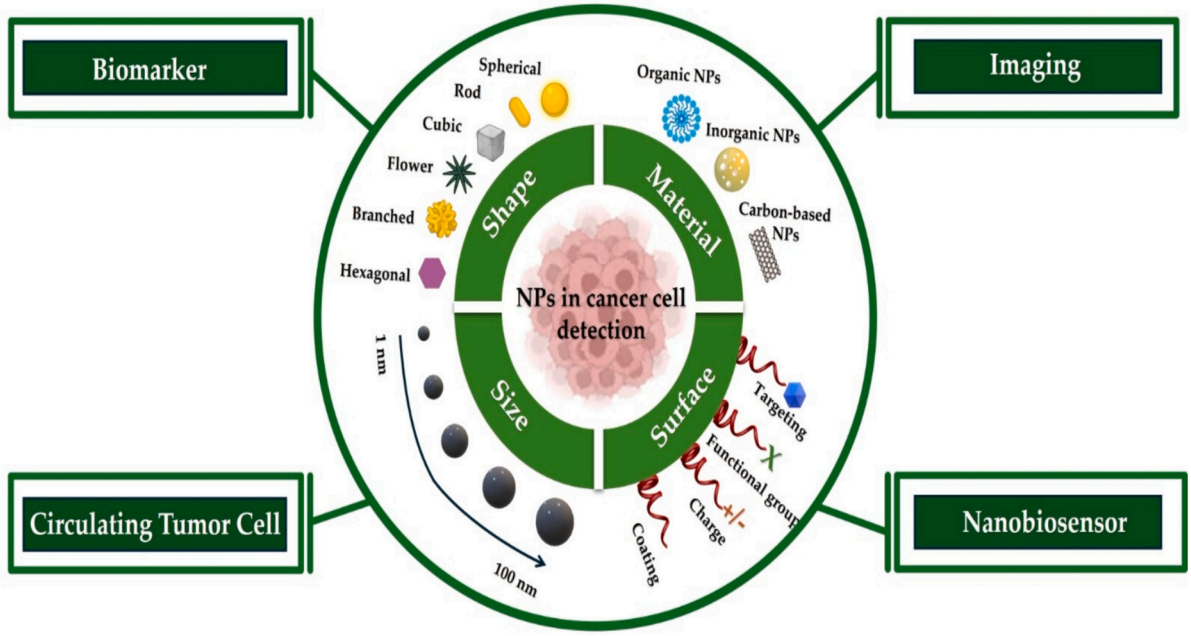

Fig. 1. The key parameters influencing nanoparticle (NP)-based cancer cell detection and their diverse biomedical applications are based on shape, size, surface characteristics, and material composition, all of which dictate their efficacy in targeting and detecting cancer cells. Surrounding panels illustrate major applications such as biomarker recognition, circulating tumor cell (CTC) capture, advanced imaging modalities, and nanobiosensing platforms. The integration of physico-chemical tuning with biological targeting enhances sensitivity and specificity in early cancer diagnostics.

imaging, which show unique tumor growth and effective renal clearance features.[18] Utilizing the peroxidase-like activity of AuNCs and the kidney's precise nanometer-scale filtration capability, AuNCs were combined with a neutral avidin protein scaffold to create the AuNC-NAV complex. This complex remains stable in vivo without interference, while preserving its catalytic functionality. AuNCs were detected in the urine of a mouse model of colorectal cancer and disease stage was identified by the catalytic activity AuNC to catalyze peroxidase substrate compared to healthy mice, producing a quantifiable colorimetric urinary readout. Tumor-bearing mice showed a 13-fold increase in the colorimetric signal compared to healthy mice, with disease detection in less than 1 h. AuNC-P2 20-NAV nanosensor was cleaved by proteases including serine protease thrombin and zinc-dependent matrix metalloproteinase 9 (MMP9), which are abnormally higher during cancer. The nanosensors were entirely cleared through hepatic and renal excretion within four weeks of injection, without any evidence of toxicity. The findings demonstrated the swift identification of various diseases by detecting specific enzymatic colorimetric signal (Fig. 2).[18]

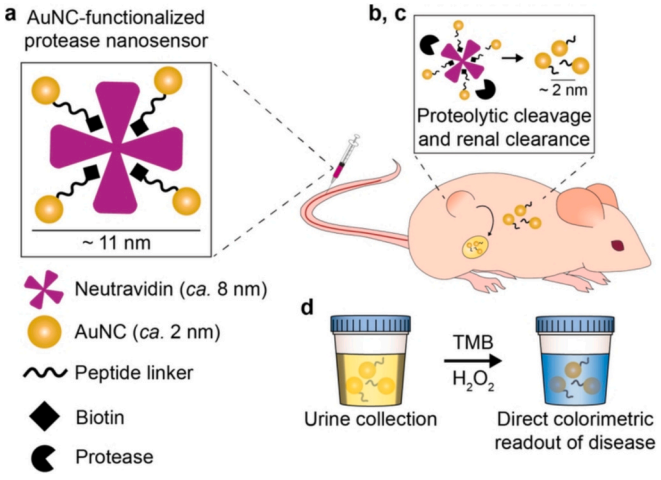

Fig. 2. AuNC-funcionalized protease nanosensors for in vivo disease monitoring. AuNCs are combined with a neutral avidin protein scaffold (a) through a biotinylated protease-cleavable peptide linker (b). The complex disassembles when exposed to the activity of dysregulated proteases at the site of disease (c), allowing the AuNCs to be filtered through the kidneys and into urine (d). *Reprinted with permission from.*[18]

Nanotechnology in biomarker-based diagnosis

Similarly to nanosensors, biomarkers are differentially expressed in hepatocellular carcinoma, and high-sensitive identification of different biomarkers is crucial for the early detection and diagnosis of hepatocellular carcinoma. A surface-enhanced Raman scattering (SERS) frequency-shift immunoassay was developed for early detection of hepatic cancer biomarkers α-fetoprotein and Glypican-3 down to sub-picomolar concentrations enhancing liver cancer detection sensitivity.[19] When bound with antibody biomarker, a shift in the reporters SERS spectrum were noted, which exhibit remarkable sensitivity and specificity and shown to function in fetal calf serum and in the serum of a patient with hepatocellular carcinoma.[19] In pancreatic cancer, serum level of mucin glycoprotein tumor marker CA19–9 is significantly increased.[20] However, it is not recommended for general screening, since it is elevated in non-malignant pancreatic conditions such as chronic pancreatitis and can produce false negatives. The concentrations of plasma thrombospondin-2 (THBS2) were noted in plasma in stage I pancreatic ductal adenocarcinoma (PDAC). THBS2 data enhanced the diagnostic performance of CA19–9 in differentiating PDAC from pancreatitis. With a specificity of 98 %, the combined use of THBS2 and CA19–9 achieved a sensitivity of 87 % for PDAC.[20] In a separate investigation, carbon nanotubes (CNTs) and polyethyleneimine (PEI) were employed to construct thin films on a gold substrate using a layer-by-layer (LBL) assembly method. The carboxyl groups on the CNTs were activated using NHS-EDC chemistry, allowing the immobilization of an anti-CA19–9 antibody onto the membrane surface, resulting in the development of a biosensor. The spectroscopic detection threshold for CA19–9 was determined to be 0.35 U/mL in a medium. Additionally, the

biosensor's selectivity was validated by testing samples containing glucose, ascorbic acid, and the p53 antigen.[21]

Nanotechnology in imaging-based diagnosis

Current CT and MRI imaging techniques aim to provide precise detection and staging of cancer. However, the integration of nanotechnology with endoscopic ultrasound (EUS), CT, and MRI for targeted imaging has significantly improved the feasibility of early detection and monitoring of tumors.[22] NPs were synthesized using diethylenetriamine pentaacetic acid (DTPA) through a solvent diffusion approach, followed by the creation of PLA-PEG-PLL-Gd NPs via the chelation of Gadolinium (Gd) ions with DTPA groups on the nanoparticle surface. These PLA-PEG-PLL-Gd NPs were subsequently functionalized with an antibody targeting vascular endothelial growth factor (VEGF), resulting in a novel multifunctional polymeric nano-contrast agent (anti-VEGF PLA-PEG-PLL-Gd NPs) with an average diameter of 69.8 ± 5.3 nm. In comparison to non-VEGF-modified NPs, the VEGF-modified NPs showed enhanced cellular uptake.[22] Both in vivo and in vitro MRI assessments revealed that this contrast agent considerably improved the relaxation characteristics of the chelating unit, thereby boosting imaging signals. Furthermore, the imaging duration significantly increased from under one hour to twelve hours, underscoring the potential of anti-VEGF PLA-PEG-PLL-Gd NPs as a promising nano-contrast agent for the early detection of liver cancer.[22] In another investigation, the urokinase-type plasminogen activator receptor (uPAR)-targeted nanoprobe dendron-grafted polylysine (DGL)-U11 was developed by conjugating dendron-grafted poly-L-lysine (DGL) with the uPAR-targeting peptide U11, gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA), and cyanine dye Cy5.5. This nanoprobe enabled dual-mode MR/near-infrared fluorescence (NIRF) imaging for the precise molecular detection of precancerous pancreatic intraepithelial neoplasia and PDAC lesions. The results showed that the targeted probe exhibited superior sensitivity in fluorescence imaging compared to MRI, enhancing its capabilities for accurate tumor detection.[23] Additionally, a tumor-targeted and matrix metalloprotease-2 (MMP-2)-activatable nanoprobe (T-MAN) was developed by covalently modifying Gd-doped CuS micellar NPs with cyclic RGD (cRGD) peptides and an MMP-2-cleavable fluorescent substrate. This study demonstrated that the combination of dual biomarker recognition by T-MAN and dual-modality imaging (MRI and NIR fluorescence) allowed for the detection of lymph node and gastric cancer metastases in mice.[24]

Role of nanomedicine in malignant cancer treatment

Modern innovations in nanotechnology make it possible to improve the performance of weakly soluble systemic drugs and reduce the toxicities in-vivo. Nanotechnology can deliver insoluble or unstable drugs enhancing their bioavailability and chemotherapeutic effectiveness.

Chemotherapy

Chemotherapy is being used as a primary option for the treatment of cancers, but their effectiveness is restricted because of insufficient specificity, and often high doses of drugs are required to cope with the low concentration of drugs in tumors.[25,26] Nanotechnology holds significant potential to modulate the pharmacokinetic profile of drugs by enhancing their absorption and distribution within the body, improving drug accumulation in the TME, and optimizing targeted delivery and efficacy of chemotherapeutic agents.[27–30] A stimuli-responsive mesoporous silica encapsulated superparamagnetic doped iron oxide ($MnFe_2O_4$@$CoFe_2O_4$) nanoparticle with doxorubicin was synthesized. The shell's cargo can be regulated by a thermoresponsive molecular gatekeeper and released via magnetic heating under an alternating magnetic field (AMF), effectively inhibiting pancreatic cancer cells in an AMF-dependent manner.[31]

Beyond optimizing targeted delivery, nanodrugs also have the potential to penetrate deep and accumulate in the tumor lesion site.[32] A study was conducted to address the drug resistance of PDAC to conventional treatments due to an insufficient penetration of nanosized therapies in the dense tumor stroma. A tailored aptamer (GBI-10) targeting the extracellular matrix (ECM) component tenascin-C, was conjugated to a stroma-permeable cell-penetrating peptide (CPP), and a disulfide-containing dimeric camptothecin prodrug (CPTD) was encapsulated, creating a sequentially triggered nanoparticle, aptamer/cell-penetrating peptide-camptothecin prodrug (Apt/CPP-CPTD NPs). In this design, tenascin-C selectively binds to GBI-10, enabling the detachment of CPP, which enhances PDAC penetration through tumor cell endocytosis, thereby improving drug delivery and therapeutic efficacy.[33]

Apt/CPP-CPTD NPs can penetrate tumors due to increased intracellular redox potential and accumulation in the in-vitro 3D PDAC spheroids and in-vivo tumor sections.[33] In another study, a novel estrogen-targeted PEGylated liposome with oxaliplatin (ES-SSL-OXA) was developed to enhance binding affinity with estrogen receptors, metabolic behavior, anti-tumor efficacy and safety of conventional oxaliplatin formulations. The fluorescence microscopy and in-vivo imaging system revealed enhance targeting effect of ES-SSL-OXA in gastric cancer cells (SGC-7901) and athymic tumor-bearing mice,[34] and the acute toxicity study showed that ES-SSL-OXA could reduce toxicity caused by oxaliplatin.[34] Furthermore, a study aimed to enhance the hepatocellular carcinoma (HCC) treatment was performed, focusing on the therapeutic efficacy of Sorafenib, a first line drug with limited therapeutic effect. To address this issue, butyrate-modified NPs were separately encapsulated with sorafenib and salinomycin. After oral administration, this multifunctional ligand butyrate improves transepithelial transport effectively by enhancing intracellular iron and lipid peroxidation as well as depleting glutathione peroxidase 4 and glutathione by onset of ferroptosis. This formulation causes effective elimination of tumors and establishment of systemic immune memory in the orthotopic HCC model.[35]

Nanotechnology can enhance poor bioavailability, boost therapeutic effectiveness, and minimize toxicity of conventional chemotherapy and poor soluble drugs.[36] Previously, a mixture of folic acid (FnA), 5-fluorouracil (5-FU), and oxaliplatin (OxP) was incorporated into the conventional FOLFOX regimen for treating colorectal cancer, however, patients still suffer from drawbacks such as low efficacy, high toxicity, and long course of treatment. PEGylated lipid NPs (Nano-Folox) ($C_{26}H_{35}N_9O_7Pt$) were developed by combining the active form of OxP ($[Pt(DACH)(H_2O)_2]^{2+}$) with FnA through a nanoprecipitation method. The PEGylated NPs significantly enhanced the circulation time and tumor accumulation of both the platinum drug and FnA, achieving a roughly 10-fold increase in blood circulation in an orthotopic colorectal cancer (CRC) mouse model. The NPs exhibited an encapsulation efficiency (EE) of approximately 99 % and a loading capacity (LC) of 67 wt%, indicating excellent chemo-immunotherapeutic potential without inducing toxicity in CRC mice. Furthermore, the incorporation of an anti-PD-L1 monoclonal antibody further augmented the Nano-Folox/5-FU formulation, leading to a marked reduction in liver metastases in mice.[37] The same group have further synthesized Nano-FdUMP with the PEGylated aminoethyl anisamide (AEAA, a targeting ligand for sigma-1 receptor overexpressing on CRC and HCC). Second, for Nano-FdUMP, FdUMP (the active metabolite of 5-Fu) is entrapped inside the amorphous $Ca_3(PO_4)_2$ nanoprecipitate, and the resultant $Ca_3(PO_4)_2$*FdUMP nanoprecipitate is encapsulated into the AEAA-targeted PEGylated lipid NPs.[46]

Irinotecan is an important clinical drug for the treatment of colorectal and pancreatic cancer, but its toxicity has been detected in the gastrointestinal tract (GIT) and bone marrow.[38] Previously, PDAC patients have been treated with liposomal formulation of irinotecan (Onivyde), but toxicity evaluation of Onivyde is still awaiting in other cancers. In line with this, silicasome, a lipid bilayer encapsulated

irinotecan, was coated with MSNPs, and demonstrated enhanced therapeutic efficacy while minimizing bone marrow and gastrointestinal toxicity in patients with PDAC. An ongoing clinical trial (NCT02551991) is exploring the potential of Onivyde as a first-line alternative to irinotecan in FOLFIRINOX; however, the liposomal formulation is currently utilized as a second-line treatment, in combination with 5-fluorouracil and leucovorin, for patients with metastatic PDAC who have failed gemcitabine therapy.[39] In another study, it has been demonstrated that the hydrophobic anticancer agent docetaxel (Dtxl) was successfully incorporated into polyethylene glycol silica nanotubes (SN-PEG) via electrostatic absorption. At 72 h, the half-maximum inhibiting concentration (IC50) of silica nanorattle-encapsulated docetaxel (SN-PEG-Dtxl) was only 7 % of that of free Dtxl, indicating superior antitumor efficacy in human liver cancer Hep-G2 cells and minimal systemic toxicity in healthy ICR mice. When administered intravenously at 20 mg/kg, SN-PEG-Dtxl exhibited significantly enhanced antitumor effects, with a tumor inhibition rate approximately 15 % greater than that of Taxotere in a murine hepatocarcinoma 22 subcutaneous model.[40]

Traditional chemotherapeutic drug effect is reduced by drug resistance, but nanocarriers have the potential to overcome drug resistance, enhance the pharmacokinetics of drugs, and augment the anticancer efficacy. In metastatic pancreatic adenocarcinoma, albumin-bound paclitaxel (nab-paclitaxel) and gemcitabine improved survival and response rates but increased peripheral neuropathy and myelosuppression (NCT00844649).[41] To enhance the penetration of antitumor chemotherapeutics in the central stroma whereas protecting the external stroma, a micelle was coloaded with paclitaxel and phosphorylated gemcitabine based on the ethylene glycol-polyarginine-polylysine (PEG-pArg-pLys). Upon changes in pH, micelles released paclitaxel and phosphorylated gemcitabine to alter the tumor's immunosuppressive microenvironment by increasing the population of cytotoxic T cells, disrupting the internal tumor structure, and eliminating pancreatic cancer cells.[42] In another study, a tumor stroma-targeted nanovehicle (FH-SSL-Nav) was synthesized to precisely ruin cancer-associated fibroblasts (CAFs), enhance tumor invasion of nanomedicines and cut off the stroma's provision to cancer cells. The FH-SSL-Nav efficiently infiltrated hepatocellular carcinoma cells and modulated TME. Additionally, microenvironment-induced drug resistance was relatively changed by FH-SSL-Nav. In a human Hep G2 xenograft nude mouse model, FH-SSL-Nav significantly increased the anti-cancer effect of liposomal doxorubicin (7pep-SSL-DOX) with small dose and minimal toxicity. There is no synergistic effect of Nav and DOX against Hep G2 cells, thus improved antitumor efficacy of FH-SSL-Nav was due to the comprehensive TME penetration.[43]

Gene therapy

Tumor gene therapy has been the present focal point of research. It encompasses either gene function restoration or elevating gene expression, knocking out abnormal genes, boosting immunity, and reducing disease risk factors. Various gene therapy strategies are investigated for treating gastric tumors.[44,45] mRNA-based therapy exhibits numerous advantages compared to DNA-based therapies and makes significant improvements in cancer treatment. Invitro-transcribed mRNA serves as an alternative therapeutic agent to plasmid DNA and plays an important role in this process. In vitro transcribed (IVT) mRNA encodes the survivin-T34A gene, targeting colon tumors. Liposome-protamine lipoplex (CLPP) NPs have been used as the delivery agent for IVT mRNA (Fig. 3).[44] CLPP/MSur-T34A NPs demonstrated effective delivery of mRNA responsible for the expression in C26 tumor cells via lipid raft-mediated endocytosis, making these NPs a promising therapeutic candidate for colon cancer therapy.[44]

Nanotechnology further enhances gene therapy by enabling RNA manipulation for targeted therapeutic purposes. For instance, researchers achieved specific ligand display on extracellular vesicle (EV) membranes and regulated RNA loading into EVs by altering the integration of cholesterol on RNA NPs. Incorporating cholesterol at the tail allowed ligand expression aimed at targeted delivery, whereas it's positioning at the arrowhead enhanced RNA nanoparticle loading. Ligand-expressing EVs effectively delivered siRNA to target cells, inhibiting notable tumor growth in prostate, breast, and colorectal cancer models. The results highlight the promise of RNA-engineered

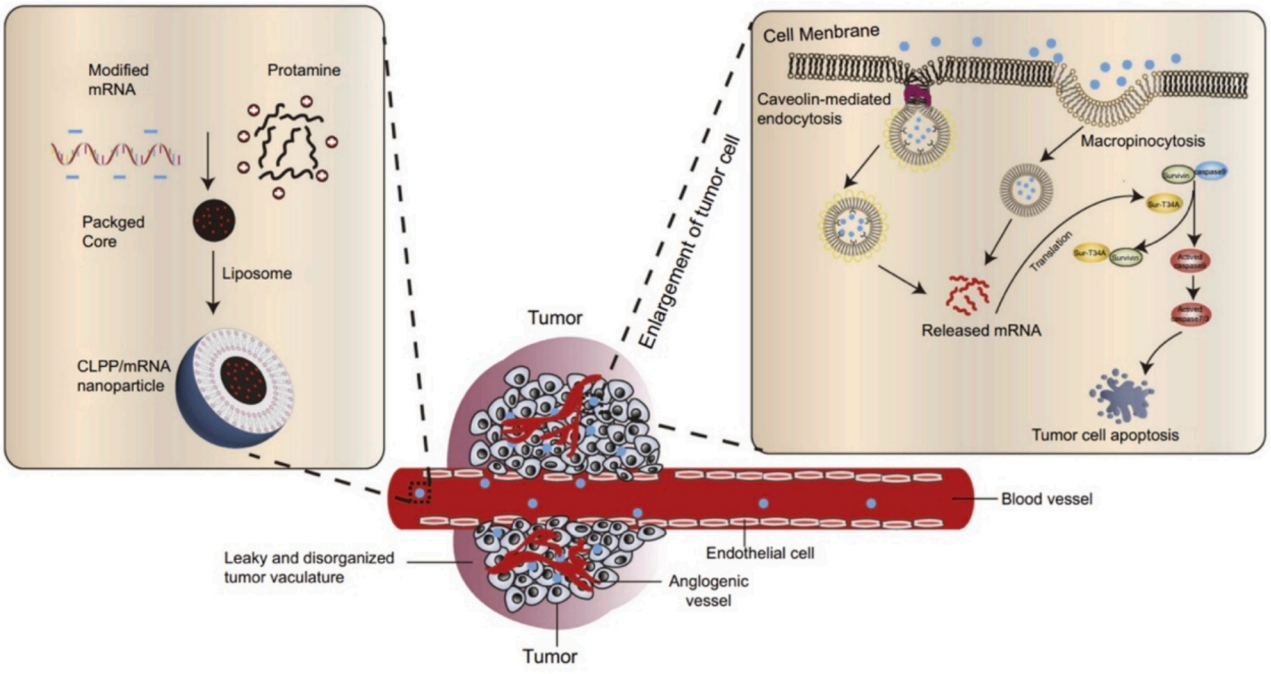

Fig. 3. CLPP-based mRNA delivery. CLPP/mRNA NPs are formed by encapsulating modified mRNA and protamine into a liposomal structure. The NPs are internalized in the tumor cells through lipid raft-mediated pathway (Caveolin-mediated endocytosis and macropinocytosis). Once inside, the mRNA is released and translated, inducing tumor cell apoptosis. *Reprinted with permission from.*[44]

extracellular vesicles for targeted and efficient cancer treatment.[45]

The conventional virus vector has issues, for instance immunogenicity and mutation. The non-viral vector utilizing nanotechnology addresses the limitations of viral vectors and can attain enhanced therapeutic efficacy through modification. Gene repair can be safely and effectively accomplished via nano-mediated delivery of tumor suppressor genes, resulting in a consistent anti-tumor outcome. Kim et al. developed a galactosylated poly(ethylene glycol)-chitosan-*graft*-spermine (GPCS) copolymer via amide bond formation with melamine to enhance PDCD4 gene loading. In vitro and in vivo studies showed superior transfection efficiency of the GPCS/DNA complex over PCs/DNA. Increased green fluorescent protein (GFP) expression in liver tissue suggests that asialoglycoprotein receptors (ASGPR)-mediated uptake facilitates PDCD4 gene delivery to hepatoma cells.[47]

Considering the marked overexpression of epidermal growth factor receptor (EGFR) in hepatocellular carcinoma (HCC), the strong correlation between elevated acetylcholinesterase gene (AChE gene) levels and enhanced apoptotic activity, targeted therapeutic strategies leveraging this relationship hold significant potential for improving HCC treatment outcomes. Liu et al. developed the epidermal growth factor receptor (EGFR)-targeting gene vector YPC by conjugating YC21, which targets EGFR, to a polycationic vector made of β-cyclodextrin and PEI_{600}. The YPC vector demonstrates a strong capacity for gene transfer to EGFR-positive hepatoma cells, enhancing AChE gene expression and markedly suppressing liver cancer both in vivo and in vitro through the inhibition of p-ERK and cyclin D1.[48]

Activated hepatic stellate cells (AHSCs) facilitate the activation of immunosuppressive cells, such as M2 macrophages and myeloid-derived suppressor cells (MDSCs), while forming a dense extracellular matrix that restricts T cell infiltration and function through CXCL12/CXCR4 signaling and growth factors like TGF-β, thereby fostering tumor progression. In contrast, intrahepatic relaxin (RLN), an antifibrotic peptide, suppresses AHSC activity and alleviates liver fibrosis.[49] Hu et al. developed aminoethyl anisamide (AEAA)-modified liposome calcium phosphate NPs (LCPs) targeting the sigma-1 receptor to efficiently deliver the RLN gene into tumors, disrupting the immunosuppressive microenvironment. In colorectal and pancreatic liver metastasis models, pRLN-loaded LCP nanotherapy significantly inhibited tumor progression, extended survival, and restored immune activity.[50]

Gene therapy exerts therapeutic effects by suppressing oncogenes or downregulating genes linked to tumor overexpression. Research has focused on utilizing nanocarriers for siRNA delivery, particularly in pancreatic cancer. Since nervous microenvironment has a big impact during the growth and metastasis of pancreatic cancer, the suppression of nerve growth factor (NGF) gene expression presents a promising strategy for treatment.[51] Lei et al. developed a novel fluorescent gold nanocluster (GNC) via a one-step reaction, designed for siRNA delivery. The GNC-siRNA complex has shown considerable effectiveness in protecting siRNA molecules from degradation by serum nucleases, enhances cellular uptake, and facilitates escape from the lysosome into the cytoplasm, thereby achieving effective siRNA-mediated NGF gene silencing. GNC can prolong the circulation time of siRNA in the bloodstream and enhance the concentration of siRNA specifically in tumor tissues through the enhanced permeation and retention (EPR) effect. In the subcutaneous pancreatic cancer model, mice treated with GNC-siRNA exhibited a 52 % reduction in tumor volume compared to the

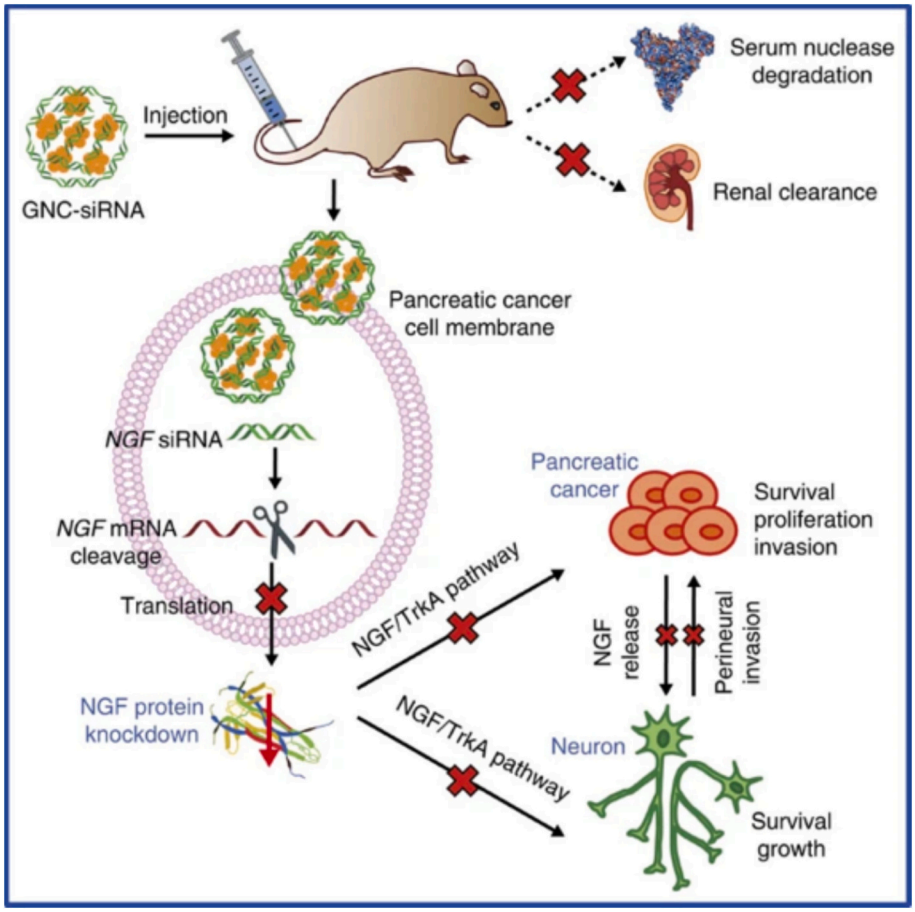

Fig. 4. GNC-siRNA complex is injected and remains stable against serum nucleases degradation and renal clearance. It facilitates siRNA accumulation in cancer cells, leading to NGF mRNA cleavage and downregulation of NGF expression. This process inhibits pathways involved in pancreatic cancer cell growth and neurogenesis in the tumor environment. *Reprinted with permission from.*[51]

saline control group, while NGF mRNA levels in these mice decreased by 69 %. The results indicate that the GNC siRNA complex enhances the knockdown of a specific NGF gene in pancreatic tumors, effectively inhibiting tumor growth in the pancreatic tumor model without adverse effects or toxicity (Fig. 4).[51]

CAFs serve a pivotal function within the malignant microenvironment. Through the secretion of growth factors, stimulation of angiogenesis, amendment of the extracellular matrix, and suppression of the anti-tumor immune response, CAFs facilitate tumorigenesis. Interleukin-6 (IL-6) has been shown to regulate interactions among gastrointestinal malignancy cells and CAFs by facilitating fibroblast activation.[52] The TME significantly influences the pathogenesis of various cancer types, particularly upper-gastrointestinal (GI) cancers, emphasizing the significance of CAFs in tumor progression which presently has limited therapeutic options. IL-6 is identified as a crucial mediator in the interaction between tumor cells and CAF, promoting tumor growth and the activation of fibroblasts. Targeting the IL-6 receptor (IL6Rα) represents a viable therapeutic strategy. In preclinical models, the downregulation of IL-6 resulted in decreased tumor growth, while the anti-IL6Rα antibody, tocilizumab, inhibited tumor growth in vivo by influencing signaling pathways such as STAT3 and MEK/ERK. Analysis of a pan-cancer dataset revealed that elevated levels of IL-6 and IL6Rα are associated with reduced survival rates.[52] The research validated the efficacy of tocilizumab in xenografts of head and neck squamous cell carcinoma and gastric adenocarcinoma. Salimifard et al. administered IL6-specific siRNA via hyaluronic acid PEG chitosan lactate (H-PCL) NPs, demonstrating the ability to inhibit the proliferation and metastasis of CT26 cells and impede cancer progression.[53]

Immunotherapy

Nanotechnology-enhanced immunotherapeutic strategies

The efficacy of tumor immunotherapy is hindered by the limited immunogenicity of tumor cells and the immunosuppressive nature of TME. Combining nanotechnology with immunotherapy enhances tumor responsiveness to immunotherapeutic strategies. The resistance of solid tumors, such as CRCs, to immunotherapies, is due to the immunosuppressive TME, which shields malignant cells from cytotoxic T lymphocytes (CTLs).[54,55] For example, research has demonstrated the role of the immunosuppressive TME in the resistance of solid tumors, with a focus on CRCs, to immunotherapy treatments. The result represented the downregulation of the type I interferon receptor chain IFNAR1 in human CRC and mouse models, which is associated with increased tumor development and growth, as well as the establishment of an immune-privileged niche, resulting in poor patient prognosis. The study indicates that genetic stabilization of IFNAR1 improves the survival of CTLs and increases the efficacy of chimeric antigen receptor T cell therapy and PD-1 inhibition.[53]

Tumor-associated macrophage (TAM)-targeted immunotherapy offers a promising approach to reprogram the immunosuppressive TME using the immunomodulator imiquimod (R837) to enhance cancer treatment efficacy. However, the clinical potential of R837 is hindered by its poor water solubility and suboptimal targeting efficiency. To overcome these limitations, two distinct polymeric micelle systems were engineered to co-deliver R837 and the chemotherapeutic agent doxorubicin (DOX) to TAMs and tumor cells via intratumoral and intravenous administration, respectively, optimizing breast cancer chemo-immunotherapy. Upon tumor accumulation, immune-stimulating micelles released R837, activating Toll-like receptor 7 (TLR-7) on the lysosomal membrane of TAMs, thereby promoting their maturation, triggering an antitumor immune response, and reversing immunosuppression within the TME. Simultaneously, chemotherapeutic micelles released DOX into tumor cell cytoplasm, directly inducing apoptosis. This nanomedicine-based strategy synergistically integrates immunotherapy and chemotherapy, enhancing immune activation while suppressing tumor growth. As a result, the combination of chemo-immunotherapy presented the most significant anti-tumor effect, achieving the highest tumor inhibition rate (85 %) and survival rate (80 %) of mice after 46 of treatment. The development of such targeted nanotherapeutics presents an innovative platform for advancing combination chemo-immunotherapy in cancer treatment.[56]

Personalized cancer vaccines and immunomodulation

Neoantigen vaccines show promise for personalized cancer immunotherapy, though many neoantigens lack sufficient immunogenicity. A adjuvant neoantigen nanovaccine (banNV) was developed to co-deliver the peptide Adpgk with TLR7/8 agonist R848 and TLR9 agonist CpG, enhancing immune responses.[57] BanNVs were synthesized via nano-templated concatemer CpG, cationic polypeptide condensation, and loading with R848 and Adpgk. This approach significantly boosted immunogenicity and reduced systemic toxicity. BanNVs also sensitized T cells by targeting the PD-1 pathway and combining them with anti-PD-1 antibodies led to complete regression of 70 % of neoantigen-specific tumors.[57] These results highlight the potential of banNVs in advancing personalized cancer immunotherapy.

Checkpoint blockade therapy has shown promise in cancer treatment but remains effective in only 10–40 % of patients and is costly. This study introduces a rapid, low-temperature self-assembly method to integrate immunological molecules into metal-organic-framework (MOF)-gated mesoporous silica (MS) for cancer vaccines. MS NPs act as immunopotentiators, storing antigens and immune modulators, while the MOF gate prevents off-target release. Combining MOF-gated MS vaccines with PD-1 blockade therapy enhances antitumor immunity, reducing the anti-PD-1 antibody dose by up to 90 % in E.G7-OVA tumor models. This strategy boosts adaptive CD8+ T-cell responses, overcomes immune suppression, and achieves lasting tumor control.[57,58] The research investigates the systemic toxicity of IL-12, a powerful anti-cancer cytokine, by creating a targeted delivery method utilizing gold NPs modified with isoDGR, a peptide that binds to αvβ3-integrin. The resultant nano-drug, Iso1/Au/IL12, is stable, monodisperse, and efficiently administers low-dose IL-12 (18–75 pg) to tumors in murine cancer models, demonstrating notable antitumor efficacy without harm. In contrast to free IL-12 or non-targeted NPs, Iso1/Au/IL12 augments tumor infiltration by innate immune cells, especially NK cells, which are essential for its effectiveness. It expands the effectiveness of adoptive T-cell treatment, presenting a promising method to augment IL-12's therapeutic index.[59]

Immunostimulatory agents, including agonistic anti-CD137 and interleukin (IL)-2, induce robust anti-tumor immunity; however, they also produce significant toxicities that hinder their clinical use. Research demonstrates that integrated therapy utilizing anti-CD137 and an IL-2-Fc fusion results in considerable preliminary anti-cancer efficacy yet also induces severe immunotoxicity due to the activation of circulating leukocytes. Anchoring IL-2 and anti-CD137 to liposome surfaces enables targeted tumor accumulation of immune agonists while minimizing systemic toxicity. Immunoliposomes significantly improved tumor infiltration by cytotoxic lymphocytes, fostering enhanced cytokine secretion and upregulation of granzyme expression. These liposomes demonstrated immunostimulatory effects comparable to those of free drugs, effectively activating immune responses within the localized TME.[60] The targeted delivery of immune agonists via immunoliposomes not only promotes stronger antitumor immunity but also ensures a more controlled and efficient therapeutic response, minimizing off-target effects while amplifying the immune response at the tumor site.[61] Previously, studies have employed tripolyphosphate (TPP) as a coagulated crosslinking agent to synthesize chitosan (CS) NPs, encapsulating IL-12 (CS-TPP/IL-12). The NPs average diameter varied between 178 and 372 nm as the chitosan to TPP weight ratio increased. Mice treated with CS-TPP/IL-12 showed a notable reduction in liver enzymes, particularly alanine aminotransferase (ALT) and aspartate aminotransferase (AST), compared to those receiving IL-12 treatment alone. Additionally, no

significant pathological alterations were observed in the heart, liver, spleen, lungs, or kidneys of these mice. These findings suggest that CS-TPP effectively alleviates the toxicity of IL-12 during systemic circulation, providing a safer and more efficient delivery method for therapeutic applications. This approach holds potential for minimizing adverse effects while enhancing the therapeutic efficacy of cytokine-based treatments. In vivo studies have demonstrated that the CS-TPP/IL-12 complex significantly inhibits liver metastasis in CRC by facilitating NK cell infiltration and recruiting specific T cell populations. The NPs markedly reduced liver metastases by enhancing NK and T-cell infiltration, underscoring chitosan's promise as a cytokine delivery system for cancer immunotherapy.[62]

Physical forces play a crucial role in regulating tumor growth, progression, and metastasis. One study, for instance, looks into the creation of polymeric mechanical amplifiers that use physical forces both in vivo and in vitro to enhance the apoptosis of tumor cells brought on by immune cytokines. These mechanical amplifiers, consisting of biodegradable polymeric particles linked to tumor cell surfaces via polyethylene glycol, boost the apoptotic response to immune cytokines subjected to fluid shear forces by up to 50 % compared to static conditions. In vivo, targeted polymeric particles enhance the apoptotic effect of immune cytokine therapy, reduce circulating tumor cells in the bloodstream by more than 90 %, and significantly suppress overall tumor cell burden. When combined with the antioxidant resveratrol, this approach further inhibits solid tumor growth, offering a promising strategy for enhanced cancer immunotherapy. It provides novel applications for various micro-, and NPs aimed at enhancing receptor-mediated signaling and functionality under physical forces.[63–66] The cytokine milieu plays a crucial role in determining whether T cells differentiate into effector (Teff) or regulatory (Treg) subsets, both of which influence cancer progression and autoimmune disorders. Treg survival and function rely heavily on cytokines like IL-2 and TGF-β. In autoimmune conditions, reduced production of these cytokines can promote Treg conversion into aggressive effector cells in a proinflammatory environment. However, therapeutic use of soluble IL-2 and TGF-β is limited due to their toxicity and poor bioavailability to CD4(+) T cell targets. To address this, a strategy that corrects the cytokine environment and improves Treg stability and expansion is needed. A recent study demonstrated that biodegradable NPs loaded with TGF-β and IL-2, when directed at CD4 (+) cells, can promote Treg induction in vitro and boost their proliferation in vivo. Cytokine-loaded NPs enhanced Treg stability, preserving their suppressive phenotype even in the presence of proinflammatory cytokines.[64] These findings highlight the potential of nanocarrier-based approaches for enhancing Treg functionality in immunotherapies for autoimmune diseases and inflammation.

Advances in immunotherapy for specific cancers

Various immunotherapeutic strategies have been utilized in anti-tumor research, focusing on the unique characteristics of digestive system cancers. Microsatellite-stable CRC demonstrates resistance to immunotherapy. Studies suggest that quercetin (Q) and malonate lactone (A) in a 1:4 M ratio (Q:A) can synergistically trigger immunogenic cell death (ICD). Zhang et al. developed QA-M micelles for the targeted delivery of Q and A in colon cancer therapy, incorporating 1,2-distearyl-sn-glycerin-3-phosphate ethanolamine-methoxy-poly (ethylene glycol 2000) (DSPE-PEG2000) and D-α-tocopherol polyethylene glycol succinate (TPGS). The micelles displayed an average diameter of 20 ± 0.6 nm, with a relatively narrow distribution. The micelles exhibited an entrapment efficiency exceeding 90 %. QA-M micelles significantly inhibited in situ colon cancer tumors in mice. Introduction of immunogenic cell death (ICD) successfully reactivated antitumor immunity, leading to cytotoxic effects and the modulation of the immunosuppressive TME.[67]

Researchers have employed lipid calcium phosphate (LCP) NPs to co-deliver a phosphorylated adjuvant 5'pppdsRNA, a RIG-I ligand, and a phosphorylated tumor-specific peptide antigen (p-AH1-A5) in a CRC model. In the CT-26 FL3 in-situ CRC liver metastasis model, the treatment significantly suppressed primary colon cancer growth and inhibited the formation of liver metastases. The incorporation of the 5'pppdsRNA adjuvant notably expanded the CD8+ T cell population, promoting an effective anti-tumor immune response. Importantly, the vaccine did not lead to an increase in immunosuppressive cell types, such as T regulatory cells or myeloid-derived suppressor cells, suggesting that the approach selectively enhances immune activation while minimizing immune suppression. This strategy holds potential for improving therapeutic outcomes in CRC by enhancing the immune response without triggering unwanted immunosuppressive pathways.[68] Lipid calcium phosphate NPs are an effective platform for encapsulating various phosphorylated molecules, from nucleotides to plasmid DNA (pDNA), and have shown promise as immunotherapeutic vaccine carriers. Three vaccine formulations were tested for anti-cancer efficacy, co-encapsulating phosphorylated adjuvants, such as CpG, 2'3'cGAMP, and 5'pppdsRNA, with the tumor-specific peptide antigen p-AH1-A5. In a colorectal liver metastasis model using aggressive CT-26 FL3 cells, the co-encapsulation of 5'pppdsRNA with p-AH1-A5 significantly inhibited primary tumor growth and liver metastasis, outperforming other formulations and controls.[68] The liver functions as a natural immune tolerance organ, with its effects exacerbated in the presence of liver disease and inflammation.

Ceramide, a metabolite of sphingolipids, plays a crucial role in T cell signaling and facilitates the apoptosis of cancer cells. However, its hydrophobic nature and poor cellular uptake limit its therapeutic potential. To overcome these challenges, Li et al. engineered C6-ceramide-loaded nanoliposomes (LipC6), which significantly enhance ceramide's permeability across cell membranes. This innovative approach not only improves ceramide delivery but also strengthens its anti-cancer effects, offering a promising strategy for improving cancer immunotherapy. The results indicated that LipC6 administration significantly inhibited tumor growth through the reduction of tumor cell proliferation and Akt phosphorylation, while also enhancing tumor cell apoptosis. LipC6 may enhance CD8+ T cell activity, decrease tumor-associated macrophage populations, and mitigate tumor-associated macrophages (TAM) tolerance.[69]

Liver sinusoidal endothelial cells (LSECs) are vital in maintaining hepatic immune tolerance and are potential targets for cancer immunotherapy. However, their targeted modulation has not been achieved. Research shows that melittin NPs (α-melittin-NPs) specifically target and activate LSECs, as demonstrated by intravital imaging, which reveal fluorescence within 20 s post-injection. α-melittin-NPs alter the liver's cytokine and chemokine environment, shifting it to an activated immune state, and effectively reduce metastatic lesion formation. In a liver metastatic tumor model, α-melittin-NPs improved survival to 80 %, highlighting their potential for modulating LSEC-mediated immune tolerance to control liver metastasis.[70]

Pancreatic cancer is known for its low immunogenicity, making effective treatment challenging. To address this, Li et al. developed a mixed micellar delivery system targeting M2 tumor-associated macrophages, which selectively inhibits the PI3K-γ and colony-stimulating factor-1 receptor (CSF-1R) signaling pathways. This micellar system enhances anti-tumor immunity through a dual mechanism: it suppresses tumor inhibitory signals and reduces myeloid-derived suppressor cell (MDSC) infiltration, while simultaneously reshaping the immunosuppressive TME into one that supports immune activation. Consequently, the approach significantly improves the immunotherapeutic response, offering a promising strategy for treating pancreatic cancer. Furthermore, this system holds the potential to be combined with other therapies to further augment its efficacy in overcoming the immunosuppressive barriers in pancreatic tumors.[71]

Most reported research utilizes multifunctional drugs for tumor treatment to enhance therapeutic efficacy and reduce toxicity. Nanotechnology offers a versatile platform with high specificity for incorporating diverse drug delivery strategies. Among these, polymeric

micelles have gained attention not only for cancer therapy but also for systemic delivery of biopharmaceuticals, such as insulin, via non-traditional routes like pulmonary administration.[72] For instance, polymeric micelle-based powders demonstrated favorable aerosolization characteristics, improved pharmacological availability, and reduced toxicity in vivo, highlighting their potential to enhance therapeutic outcomes while minimizing systemic side effects (e.g., inflammation and cytotoxicity).[73,74] Leveraging such delivery systems aids in improving drug targeting, utilizing complementary delivery advantages, and enhancing overall efficacy—particularly in combating tumors of the digestive system. Small interfering RNA siRNA-based gene therapy holds promise for cancer treatment, but challenges like enzymatic degradation, poor transfection, nonspecific distribution, and uncontrolled release hinder its clinical use. Zeolitic imidazolate frameworks-8 (ZIF-8) have potential as drug carriers but are underexplored for siRNA delivery. This study introduces a multifunctional PDAs-ZIF-8 (PZ) nanoparticle platform for siRNA delivery, combining photothermal therapy (PTT) and gene therapy (GT) with noninvasive photoacoustic (PA)/near-infrared (NIR) dual-modal imaging. The PZ NPs enabled targeted siRNA delivery, minimizing leakage and damage. ZIF-8 degraded in the acidic tumor environment, releasing siRNA for gene silencing. Polydopamine NPs (PDAs) served as a contrast agent for PA/NIR imaging and a photothermal agent for therapy. In vitro and in vivo studies showed that the PDAs-siRNA-ZIF-8 (PSZ) system outperformed photothermal therapy (PTT) or gene therapy (GT) alone, offering promising potential for advanced cancer diagnostics and therapies.[75]

FOLFOX, a combination of folinic acid (FnA, FOL), fluorouracil (5-Fu, F), and oxaliplatin (OxP, OX), has long been the standard treatment for CRC and hepatocellular carcinoma. Recent advances in nano delivery methods may improve FOLFOX antitumor activity and reduce adverse effects. Nano-Folox, a nanoformulation containing OxP derivative and FnA, utilizing nanoprecipitation, when given with free 5-Fu, nano-Folox triggered OxP-mediated immunogenic cell death (ICD)-associated antitumor immunity, which greatly reduced tumor development in orthotopic CRC mice. Nano-FdUMP, a nanoformulation containing FdUMP (5-Fu active metabolite), was produced by nanoprecipitation and utilized with Nano-Folox to treat CRC and HCC. Orthotopic CRC and HCC mouse models showed synergy. The key reason was that Nano-FdUMP formed reactive oxygen species (ROS), which enhanced Nano-Folox-induced ICD. Nano-Folox/Nano-FdUMP and anti-PD-L1 antibodies also decreased CRC liver metastasis, resulting in long-term mouse survival.[37] The study proves that two nano-delivery methods can treat FOLFOX-associated CRC and HCC. Optimizing dose and timing will improve this combination strategy's therapeutic prospects for patients.

Combined therapy

Combination therapy is a promising strategy to improve cancer treatment, but differing drug pharmacokinetics limit its effectiveness. NPs can enhance the synchronized delivery of combinatorial therapies to tumor cells, but challenges in clinical translation remain. These include difficulties in precisely controlling drug loading and optimizing NP properties. For example, a novel redox-responsive poly-prodrug nanoplatform was developed for targeted siRNA delivery and synergistic cancer treatment. The platform features a redox-sensitive 10-hydroxy-camptothecin (HCPT)-based polyprodrug core, an amphiphilic lipid-poly (ethylene glycol) shell, and lactobionic acid (LA) for targeting hepatoma cells via asialoglycoprotein (ASGP) receptors. Elevated glutathione (GSH) levels reduce disulfide bonds in polyHCPT, releasing HCPT and Bcl-2 siRNA, promoting synergistic tumor growth inhibition through apoptosis and gene silencing (Fig. 5).[76]

The poor prognosis of esophageal cancer is largely due to the lack of effective targeted therapies. A promising solution lies in combination therapy using nano-targeted delivery systems. In this context, Jun et al. developed an innovative nanocarrier, EYLN, loaded with the chemotherapy drug doxorubicin (DOX) and a small interfering RNA (siRNA) targeting LPCAT1, a gene commonly overexpressed in esophageal cancer. This led to the creation of EYLN-DOX/siLPCAT1. Further modification with a proinflammatory leukocyte membrane (mEYLNs-DOX/siLPCAT1) enabled enhanced targeting and internalization by esophageal cancer cells, significantly reducing cell proliferation and migration. The therapeutic effect of EYLNs-DOX was found to outperform that of the free DOX formulation, and the addition of siLPCAT1 greatly improved the tumor inhibition. This combination of gene silencing and chemotherapy not only enhanced anti-cancer efficacy but also offered a novel strategy for improving treatment outcomes in esophageal cancer. Moreover, this approach shows potential for broader applications in targeting other malignancies with similar molecular profiles.[77]

Combine therapy with immunotherapy

Immunogene therapy has the potential to inhibit immune escape mechanisms, ameliorate the immunosuppressive TME, and augment tumor immunotherapy efficacy. Chen et al. demonstrated that the administration of chemotherapeutic agents resulted in the upregulation of Xkr8 gene expression at the transcriptional level in both in vitro and in vivo models. Xkr8, a protein with scramblase activity, is known for its

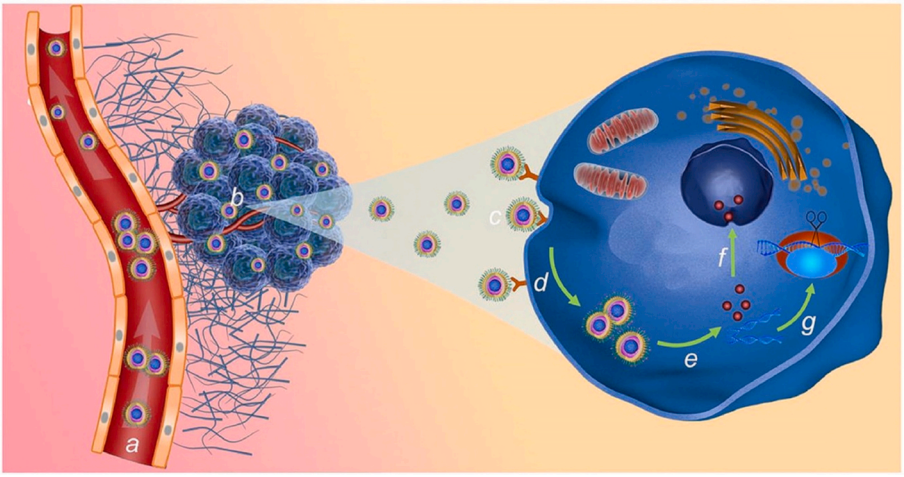

Fig. 5. The siRNA loaded NPs are intravenously injected (a) and can accumulate in tumor cells (b) by targeting ASGP receptors (c). Once inside de cell (d) elevated GSH levels reduce disulfide bonds in polyHCPT, releasing HCPT and the encapsulated siRNA (e). This leads to synergistic tumor growth inhibition through apoptosis (f) and anti-apoptotic gene silencing (g). *Reprinted with permission from.*[76]

activation during apoptosis. Leveraging this insight, the researchers developed a novel nanocarrier designed to deliver both Xkr8-specific short interfering RNA (siRNA) and FuOXP, a prodrug combining 5-fluorouracil and oxoplatin. Upon intravenous administration, the nanocarrier effectively suppressed tumor progression in pancreatic and colon cancer models. Furthermore, it significantly enhanced the immune response against the tumors, suggesting a promising strategy for improving the therapeutic efficacy of chemotherapeutic agents while modulating apoptotic pathways.[78]

Immunotherapy, leveraging the immune system, is pivotal in cancer treatment, though its efficacy is limited by tumor immunogenicity and the immunosuppressive TME. A study introduces host–guest prodrug nanovectors for targeted tumor treatment and immune tolerance mitigation. These nanovectors combine hyaluronic acid (HA) for tumor targeting and a reduction-labile heterodimer of Pheophorbide A (PPa) and NLG919. PPa acts as a photosensitizer, while NLG919 inhibits IDO-1. NIR laser irradiation activates ROS release, enhancing antitumor immunity and CTL infiltration. IDO-1 inhibition alters the immunosuppressive tumor environment. This combined approach effectively eliminates CT26 colorectal tumors, suggesting potential for broader use in immunotherapy.[79]

Microbiome role in immunotherapy

The formation of the TME in colon cancer is strongly linked to the intestinal microbiome and chronic inflammation. This microbiome plays a crucial role in modulating the effectiveness of tumor immunotherapy. Dietary and regional factors affect cancer patients' immune checkpoint inhibitor (ICI) responses, although prospective trials have not examined them. Baseline fecal microbiota profiles and dietary patterns were analyzed in 103 trial participants from Australia and the Netherlands receiving neoadjuvant immune checkpoint inhibitors (ICIs) for high-risk resectable metastatic melanoma, with integrated data from 115 patients in the US. Distinct microbiome signatures linked to therapeutic response and immune-related adverse events were identified across different. Overall, Ruminococcaceae-dominated microbiomes had higher response rates than Bacteroidaceae-dominated ones. At baseline, poor response was associated with reduced fiber and omega-3 fatty acid consumption and increased peripheral C-reactive protein. These findings suggest that native gut microbial profiles, dietary intake, and systemic inflammation affect ICI responsiveness and toxicity.[80] Lipopolysaccharide (LPS), produced by intestinal Gram-negative bacteria, has the capacity to activate oncogenes associated with colon cancer, contributing to an immunosuppressive microenvironment. According to Wang et al. elevated levels of LPS in orthotopic CRC tissue has been associated with low responses to anti-PD-L1 mAb therapy. To address this, nanotechnology was used to develop an LPS trap system, designing a LPS-targeting fusion protein, and loading its coding sequence into a nanoparticle system, measuring 140 nm in size and exhibiting a surface charge of +40.5 mV, to block LPS inside the tumor. Following injection into mice with CT26-FL3 tumors via the caudal vein, LPS trap exhibited high expression within the tumor. Simultaneously, there was an increase in CD8+, CD4+ T cells, MHCII+, and CD86+ dendritic cells, while myeloid-derived suppressor cells decreased. These findings suggest that targeting LPS may significantly improve dendritic cell function, promote T-cell infiltration, and reduce the presence of immunosuppressive cells. When combined with anti-PD-L1 therapy, there was an increase in the production of CD8+ and CD4+ T cells, which prolonged the survival of mice and improved the efficacy of anti-PD-1/PD-L1 checkpoint blockade therapy, despite no response observed in CRC.[81]

The microbiome has recently garnered recognition as a critical factor in the initiation and progression of cancer. Conventional strategies for modulating the microbiome such as the administration of antibiotics, probiotics, and microbiota transplants have, in some instances, enhanced the efficacy of cancer therapies.[82] However, the potential for unintended disruption of beneficial microbial populations and the inherent variability of these interventions highlight the need for more precise and innovative technologies tailored to the microbiome–cancer interface.

Nanotechnology has profoundly reshaped the landscape of cancer diagnostics and therapeutics. Emerging technologies capable of modulating interactions at the microscopic and molecular levels within both the microbiome and the tumor microenvironment (TME) present novel opportunities for therapeutic innovation. At the convergence of nanotechnology, microbiome science, and oncology, a new frontier is unfolding—one that holds significant promise for the development of transformative cancer treatment strategies.[83] (See Fig. 6.)

Immunotherapy shows significant potential in cancer treatment; however, its limited effectiveness stemming from an immunosuppressive TME, and systemic toxicity restricts its wider implementation. A unique tumor-targeted lipid-dendrimer-calcium-phosphate (TT-LDCP) nanoparticle system has been created to improve the effectiveness of cancer immunotherapy while reducing systemic toxicity. These NPs, designed with thymine-functionalized dendrimers, demonstrate dual functionality by integrating efficient gene transport with immunological adjuvant capabilities via the activation of the stimulator of interferon genes (STING)-cGAS pathway. TT-LDCP NPs simultaneously transport siRNA targeting PD-L1 and an IL-2-encoding plasmid DNA to hepatocellular carcinoma (HCC), leading to increased CD8+ T-cell infiltration, reconfiguration of the immunosuppressive TME, and higher efficacy of cancer vaccine immunotherapy. This novel method emphasizes the promise of nanotechnology-driven platforms for precise, tailored cancer immunotherapy.[85]

Combine therapy with phototherapy

Phototherapy, the use of light for therapeutic interventions, has been extensively utilized in the treatment of numerous diseases, including cancer. While phototherapy offers non-invasive benefits, it faces several challenges, including difficulties in administering photosensitive agents, managing phototoxicity, and ensuring effective light delivery.[86] To address these limitations, the combination of nanomaterials and bacteria in phototherapy has emerged as a promising approach, capitalizing on the unique properties of both components. Nano-bacteria biohybrids have shown enhanced therapeutic efficacy compared to individual components, suggesting a synergistic effect that improves treatment outcomes. The integration of photoelectric nanomaterials with genetically modified bacteria, although still in the early stages of development, holds significant potential as a novel biosystem for tumor-targeted phototherapy. This innovative combination could revolutionize cancer treatment, offering improved precision and minimizing side effects. Further research is needed to optimize the design and application of nano-bacteria biohybrids in clinical settings, with the aim of advancing cancer therapies to more effective and personalized levels.[86]

In biological tissue, limited light penetration results in reduced therapeutic efficacy, and the precise delivery of photosensitizers presents additional challenges. Nanotechnology offers an effective method for the delivery of photosensitizers and the integration of photodynamic therapy (PDT) with additional treatments such as chemotherapy, gene therapy, and immunotherapy to improve therapeutic efficacy. Photodynamic therapy (PDT) induces apoptosis by stimulating the production of reactive oxygen species (ROS), offering a selective space-time effect with minimal systemic toxicity. Its application in treating gastrointestinal tumors is particularly advantageous due to the ease of access through gastrointestinal endoscopy and the unique tubular structure of these organs. Despite these benefits, one of the key challenges lies in the precise regulation of antitumor immune activation.[87] The ability to control the immune response during PDT remains an area of ongoing research, as an uncontrolled immune reaction could lead to undesirable side effects. To optimize PDT's therapeutic potential, strategies to enhance immune modulation and tumor-targeting specificity are essential. Additionally, exploring combination therapies that integrate

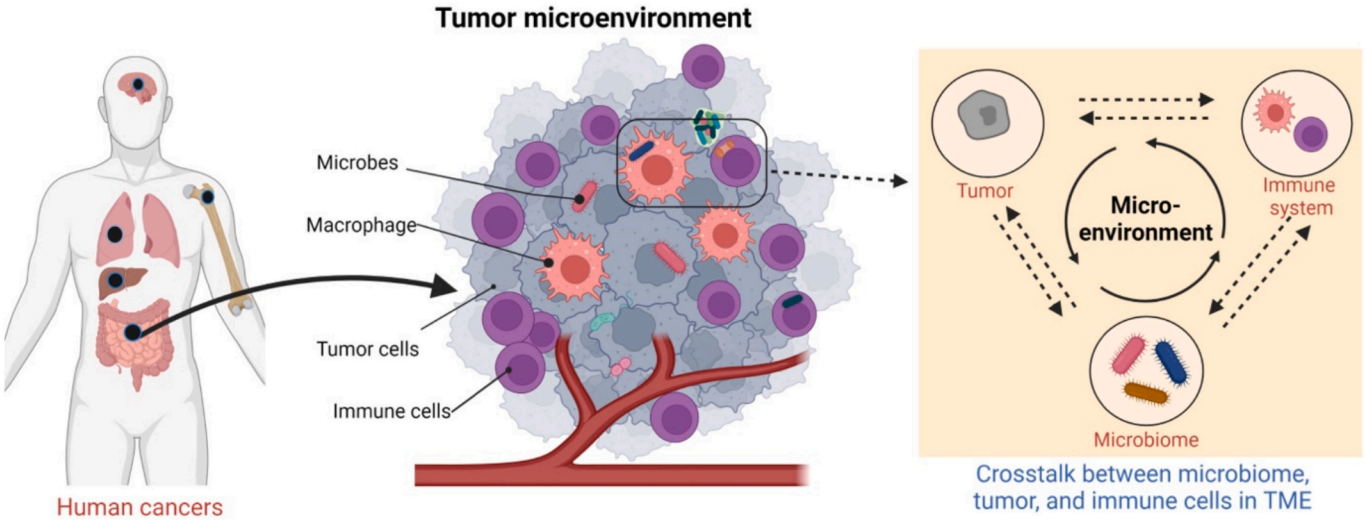

Fig. 6. The intestinal microbiome influences TME, modulating the effectiveness of immunotherapy. Emerging nanotechnological strategies offer new opportunities to manipulate the microbiome and improve therapeutic outcomes. *Reprinted with permission from.*[84]

PDT with immune checkpoint inhibitors or other immunotherapies could further improve the efficacy of cancer treatments, offering new avenues for clinical advancements in gastrointestinal oncology.[88–90]

The combination of cancer nanomedicine and immunotherapy has emerged as a highly promising approach for cancer treatment, offering enhanced safety and efficacy. A smart semiconducting polymer nano-immunomodulator (SPNI) enables precision photodynamic immunotherapy by responding to the acidic TME. The SPNI self-assembles via a NIR-absorbing polymer and an amphipathic polymer linked to a TLR7 agonist through an acid-labile linker. The SPNI undergoes hydrolysis upon arriving at the tumor site, which in turn causes an effective release of the TLR7 agonist in reaction to the acidic TME, which is necessary for the activation of dendritic cells within the body. In addition, SPNI can produce photodynamic effects, which include the direct elimination of tumors and the death of immunogenic cancer cells when exposed to NIR photoirradiation. The combination impact of acidic-TME-activated TLR7 agonist and released immunogenic components has the potential to function as an in situ-generated cancer vaccine that can trigger powerful anticancer activity. Specifically, this type of localized immune activation enhances systemic anti-tumor immune responses, which in turn leads to increased cytotoxic CD8+ T infiltration, which in turn inhibits the growth of tumors and their ability to metastasize. It is for this reason that this work proposes a general strategy for the development of immunotherapeutic prodrugs to precisely regulate cancer immunotherapy.[88]

Combine therapy with gene therapy

RISPR-Cas9 is a primary focus of the burgeoning area of gene editing, while photodynamic therapy (PDT) is a clinical-stage ablation technique that combines photosensitizers with light irradiation. Both technologies are related to the field of gene editing. Metal coordination biomaterials, on the other hand, have only seldom been examined for their potential use in both fields. Chlorin-e6 (Ce6) Manganese (Mn) coordination micelles loaded with Cas9 were produced to enhance the effectiveness of combined anti-cancer treatment. These micelles were given the name Ce6-Mn-Cas9. The delivery of Cas9 and single guide RNA (sgRNA) ribonucleoprotein (RNP), the Fenton-like effect, and the improved endonuclease activity of RNP were all facilitated by manganese, which played many roles in various processes. Using a straightforward admixture, it was possible to associate histidine (His)-tagged RNP with Ce6 that was encapsulated in Pluronic F127 (F127) micelles. Cas9 was released by Ce6-Mn-Cas9, which was triggered by ATP and the acidic pH of endolysosomes. This release did not affect the structure or function of the protein. Dual guide RNAs were developed to target the antioxidant regulator MTH1 as well as the DNA repair protein APE1. This led to an increase in oxygen levels and an enhanced PDT impact. Using a mouse tumor model, the combination therapy of PDT and gene editing caused Ce6-Mn-Cas9 to suppress the growth of the tumor. The combination of Ce6-Mn-Cas9 constitutes a novel biomaterial that possesses a high degree of adaptability, making it possible to employ phototherapy and gene therapy techniques.[89]

The incorporation of nanomaterials in photosensitizers improves solubility, bioavailability, targeting capability, stability, and decreases toxicity. Photosensitizers (PSs) are significant in the management of CRC. Phototherapy presents significant potential in the treatment of CRC due to its minimal invasiveness and low toxicity. Most PSs are associated with limitations such as poor solubility, low selectivity, and high toxicity. The incorporation of nanomaterials in PSs offers several benefits, such as enhanced solubility, improved bioavailability, targeted delivery, increased stability, and reduced toxicity.[90,91]

Combine therapy with co-delivery of drugs

Polymeric micelles with varying ratios of doxorubicin (DOX) and zinc(II) phthalocyanine (ZnPc) have been developed for dual chemotherapy and photodynamic therapy (PDT). These micelles, composed of methoxypolyethylene glycol (PEG) and poly(β-benzyl-L-aspartate) (PBLA), are conjugated with DOX and ZnPc via acid-labile and redox-responsive linkers, respectively. The micelles, approximately 160–180 nm in size, exhibit stability and neutral surface charges. In vitro studies show internalization by HepG2 cells, with DOX localized in the nucleus and ZnPc in the cytoplasm. The micelles demonstrated both dark- and photo-cytotoxicity, with a synergistic effect at a specific DOX/ZnPc ratio. The DOX-ZnPc-micelles-2 formulation induced apoptosis and showed enhanced tumor retention in mice, suggesting their potential for dual therapy applications.[92]

Checkpoint inhibitors, such as anti-PD-1/PD-L1 antibodies, show notable efficacy, but their sustained response rate is limited in CRC. Recent studies suggest that photodynamic therapy (PDT) may enhance PD-L1 blockade effects, though the mechanisms remain unclear. This study investigates multifunctional NPs (mTHPC@VeC/T-RGD NPs) in PDT to boost PD-L1 blockade efficacy in CRC. mTHPC@VeC/T-RGD NPs, activated by a 660-nm near-infrared laser, induce tumor cell apoptosis and necrosis, while also triggering a systemic immune response. PD-L1 blockade further inhibits both primary and metastatic

tumor growth and enhances long-term immunity. PDT-induced hypoxia activates HIF-1α, increasing PD-L1 expression, thereby amplifying the therapeutic effect. These findings suggest that mTHPC@VeC/T-RGD NP-mediated PDT can improve the response to anti-PD-L1 therapies in CRC.[93]

Tumor molecular heterogeneity hampers treatment outcomes, highlighting the need for novel approaches. Metal-organic framework MIL-100 (Fe) NPs were synthesized via a microwave-assisted method, and oxaliplatin (OXA) and indocyanine green (ICG) were incorporated into hyaluronic acid (HA)-modified MIL-100 NPs to form multifunctional NPs (OIMH NPs). These NPs enabled effective photoacoustic imaging (PAI) and demonstrated synergistic effects combining chemotherapy and photothermal therapy (PTT) for tumor eradication. Chemo-photothermal therapy triggered immunogenic cell death (ICD) and T cell activation, enhancing the response to immune checkpoint blockade (aPD-L1) and promoting systemic antitumor immunity. The combination therapy inhibited tumor growth, suggesting a promising new strategy for CRC treatment.[94]

Combine therapy and diagnosis

Nanotechnology enables the integration of diagnostic and therapeutic functions, facilitating simultaneous loading of therapeutic agents and contrast materials for imaging and treatment. Gastric cancer, a leading cause of cancer-related deaths, lacks sufficient diagnostic biomarkers and screening methods, particularly for early gastric cancer (EGC).[95] A dual-target, cooperatively responsive fluorescent nanomachine was developed for the simultaneous detection of miR-5585-5p and PLS3 mRNA, key biomarkers identified via next-generation sequencing and RT-qPCR. This RNA extraction-free, PCR-free, nonenzymatic biosensor allows tumor cell imaging and serum diagnostics with just a 20 μL blood sample and a 20-min incubation. The nanomachine demonstrates femtomolar sensitivity and a wide linear detection range. It outperforms the clinically used biomarker CA 72–4 with an AUC value of 0.884 and shows strong potential for EGC diagnosis with an AUC of 0.859. This study introduces a functionalized DNA nanomachine for combined gastric cancer diagnosis, offering a novel, practical approach to serum biomarker translation in clinical setting.[95]

Programmed death ligand-1 (PD-L1), which is overexpressed in stomach malignancies, facilitates T-cell immunological resistance through its interaction with programmed death-1 (PD-1) on T cells. A multifunctional theranostic nanoparticle system was created for targeted PD-L1 knockdown and MRI-based diagnostics. This technique utilizes folic acid (FA) and disulphide (SS) polyethylene glycol (PEG) conjugated polyethylenimine (PEI) complexed with superparamagnetic iron oxide (Fe_3O_4) NPs (SPIONs) for the delivery of siRNA. The FA-PEG-SS-PEI-SPION polyplex exhibited significant transfection efficiency and cellular uptake in folate receptor-overexpressing gastric cancer cells (SGC-7901), low cytotoxicity at reduced nitrogen-to-phosphate (N:P) ratios, and T2-weighted MRI contrast properties. Functional analyses demonstrated successful PD-L1 knockdown and re-established T-cell activity, as indicated by modified cytokine output in a coculture system. This method underscores the promise of multifunctional NPs for precise gene therapy and imaging in the treatment of gastric cancer.[96]

The co-delivery of two drugs with distinct physicochemical properties and a specific administration sequence is crucial for overcoming drug resistance and minimizing side effects in cancer theranostics. An investigation of novel amphiphilic PCL-AuNC/Fe(OH)3-PAA Janus nanoparticle (JNP) designed to concurrently retain the hydrophilic drug (doxorubicin) and the hydrophobic drug (docetaxel) within separate domains. Due to their unique heterostructure and independent pH and NIR sensitivity, sequential drug release from a single inorganic JNP was achieved for the first time. The findings indicated that the synchronous release of two drugs resulted in a 5 % improvement in therapeutic effect. The advanced computed X-ray tomography/magnetic resonance (CT/MR) imaging capabilities of AuNC and $Fe(OH)_3$ indicate that JNPs may effectively facilitate cancer therapy. The mice treated with dual drug-loaded PCL-AuNC/$Fe(OH)_3$-PAA JNPs under near-infrared (NIR) laser irradiation exhibited superior tumor inhibition compared to groups receiving solo drug, cocktail, and dual drug treatments, highlighting the efficacy and importance of combined cancer therapy.[97]

Improving patient outcomes and reducing overall healthcare costs necessitates highly sensitive and precise tumor detection for accurate diagnosis and effective therapy. However, this remains challenging with conventional single-mode imaging techniques. A study developed a near-infrared (NIR)-responsive photothermal therapy (PTT) platform (Au@MSNs-ICG) for the localization, diagnosis, and NIR/computed tomography (CT) bimodal imaging-guided PTT of tumor tissues. This platform utilizes gold (Au) nanospheres coated with indocyanine green (ICG)-loaded mesoporous silica NPs (MSNs), ensuring superior sensitivity and accuracy. The NPs demonstrated excellent monodispersed, fluorescence permanence, biocompatibility, and NIR/CT signaling, along with an advantageous temperature response when subjected to NIR laser irradiation, both in vitro and in vivo. The tumor was accurately localized and effectively eliminated in vivo through the injection of Au@MSNs-ICG, utilizing a combination of NIR/CT imaging and PTT treatment. Thus, multifunctional NPs may significantly contribute to the precise treatment of tumors in forthcoming clinical applications.[98]

Advanced liver cancer remains one of the most lethal malignancies, with poor treatment outcomes due to its complexity and heterogeneity. Combination therapies offer improved efficacy by activating multiple pathways and modulating the tumor immune microenvironment. Nanodrug delivery systems have emerged as promising strategies for integrated liver cancer treatment. In order to improve liver cancer treatment and ultrasound imaging, this study presents arsenic trioxide (ATO)/PFH NPs@Au-cRGD, a nano ultrasound contrast agent that combines therapeutic and diagnostic properties. The system triggers tumor-associated antigen release via ATO-induced ferroptosis and photothermal-induced immunogenic cell death, optimizing the effects of ATO and photothermal therapy in Huh7 and Hepa1–6 cells. This delivery system activates the antitumor immune response, promoting macrophage M1 polarization in the TME, with minimal side effects in both subcutaneous and orthotopic liver cancer models. Furthermore, combining this system with anti-programmed death-ligand 1 therapy inhibits metastasis and induces long-term immunological memory in orthotopic liver cancer.[99] This nanodrug delivery system enhances antitumor therapy, inhibits lung metastasis, and allows for visual assessment of therapeutic efficacy, demonstrating significant potential for clinical applications in liver cancer.

Finally, genetically programmable cell membrane-coated nanoparticles loaded with glycolysis inhibitors like 3-bromopyruvate (3BP) and cloaked with SIRPα-displaying membranes demonstrate selective targeting to CD47-overexpressing colorectal cancer cells while promoting macrophage-mediated phagocytosis and M1 polarization, enhancing both metabolic and immune therapeutic outcomes.[100] Similarly, recent work has highlighted the integration of T cell and NK cell membranes to endow nanoparticles with prolonged systemic circulation, immune evasion, and specific cytotoxic targeting capabilities in cancer and viral therapy contexts.[101] Concurrently, cell membrane coating strategies have evolved toward homologous tumor targeting using cancer cell membrane (CCM)-coated platforms, which leverage tumor-recognition motifs to enhance selective delivery and drug accumulation.[102] These systems are further augmented by engineering and tailoring of lipid insertion, membrane hybridization, and glycan amination customize surface functionality, extend circulation time, and optimize tumor localization.[103] Furthermore, stimuli-responsive delivery systems designed to react to pH, enzymes, or redox gradients within the tumor microenvironment enable on-demand drug release, minimizing off-target effects and increasing therapeutic precision.[104] Together, these innovations underscore the trajectory of nanomedicine toward multifunctional platforms that integrate immune modulation, metabolic interference, and smart targeting to overcome longstanding

barriers in cancer therapy.

Limitations and challenges

Cancers exhibit specific TME that causes variation in biomarkers, which can be used for the drug-release of the nanocarriers upon reaching the tumor niche. Sophisticated drug delivery systems, particularly those utilizing advanced formulations, enable the precise control of drug release kinetics, thereby enhancing targeted cancer therapies at specific times and locations within the tumor. However, it is important to address all the problems shown below to achieve maximum therapeutic efficacy of nanomedicines with lower side effects (Fig. 7). Beyond biodistribution, the immunogenicity of lipid-based nanocarriers introduces another level of complexity. Lipid nanoparticles (LNPs), which are now widely used in clinical mRNA delivery, are increasingly recognized as immunostimulatory. They can activate innate immune sensors including Toll-like receptors (TLRs), trigger complement pathways, and cause inflammatory responses such as cytokine release and complement activation-related pseudoallergy (CARPA).[105] In addition, exposure to some nanomaterials has been shown to cause oxidative stress, mitochondrial dysfunction, and excessive reactive oxygen species (ROS) production, which can further amplify immune activation and tissue damage.[105,106]

It is generally accepted that TME contributes significantly to the underwhelming outcomes of nanomedicine therapy.[107,108] TME harbor malignant cells, TAFs, specific types of immune cells, and stroma, play a key role in drug resistance by affecting drug delivery and immune cell movement, resulting in abnormal angiogenesis, desmoplasia, and hypoxia. Secondly, safety concerns in pharmacokinetic studies have resulted in an approval rate of less than 10 % for new nano-drugs, drawing increased attention to biosafety.[108,109] Nanomedicines are primarily metabolized in the liver and kidneys and excreted in the form of feces and urine through the biliary duct and kidneys. However, several reports have suggested that specific nanomaterials may persist in the body, potentially leading to long-term damage due to their accumulation in normal organs and the inability to biodegrade.[110] The accumulation of nanomaterials in healthy organs may lead to physical damage by obstructing microcirculation, which initiates a cascade of toxic reactions, including cellular dysfunction and inflammatory responses. This adverse effect is primarily driven by the poor biodegradability of nanomaterials, coupled with their capacity to aggregate and migrate within bodily tissues. Such interactions can result in prolonged and persistent physical harm, highlighting the need for improved biocompatibility and controlled degradation mechanisms in nanomedicine.[107,108] Consequently, the design of biodegradable and biocompatible nano-drug delivery systems has become a central focus in the field of nanomaterials research.

Beyond the challenges posed by the TME, numerous non-biological barriers continue to hinder the successful clinical translation of nanomedicines. These include issues with large-scale manufacturing, such as difficulties in scale-up synthesis and maintaining batch-to-batch consistency—factors that critically affect product quality and reproducibility.[111] Regulatory hurdles remain significant due to the lack of standardized evaluation criteria specific to nanotherapeutics, which complicates approval processes and lengthens development timeline.[111] Furthermore, patient heterogeneity, particularly variability in tumor biology and the Enhanced Permeability and Retention (EPR) effect across individuals, reduces the predictability of therapeutic responses.[111] A lack of comprehensive understanding of long-term toxicity, accumulation in non-target organs, and immunogenic effects further restricts widespread clinical adoption.[112] However, successful examples such as Doxil® and Abraxane® illustrate how these challenges can be navigated. Doxil® utilized PEGylated liposomes to improve doxorubicin's safety and pharmacokinetics, ultimately gaining FDA approval in 1995.[111] Similarly, Abraxane®, an albumin-bound paclitaxel formulation, eliminated the need for toxic solvents like Cremophor, enhancing tolerability and therapeutic index.[112] Thirdly, appropriate pre-clinical research models that accurately represent human conditions and establishing validated methods to characterize NPs are critical challenges hindering nanodrug development.[105] Furthermore, future research should focus on creating effective biodegradable carriers, standardized analytical methods, safety monitoring protocols, and understanding the structure-property relationships of nanomedicines. As we establish effective targeted drug delivery systems for cancer treatment, optimizations are also needed regarding size, surface properties, charge, drug loading/encapsulating efficiency, drug distribution, metabolism and elimination of nano-carriers.[113] During clinical translation, nanomedicines encounter challenges in pharmacokinetics, formulation, and biological assessment. Rational design of physicochemical properties is crucial for immune evasion, tumor

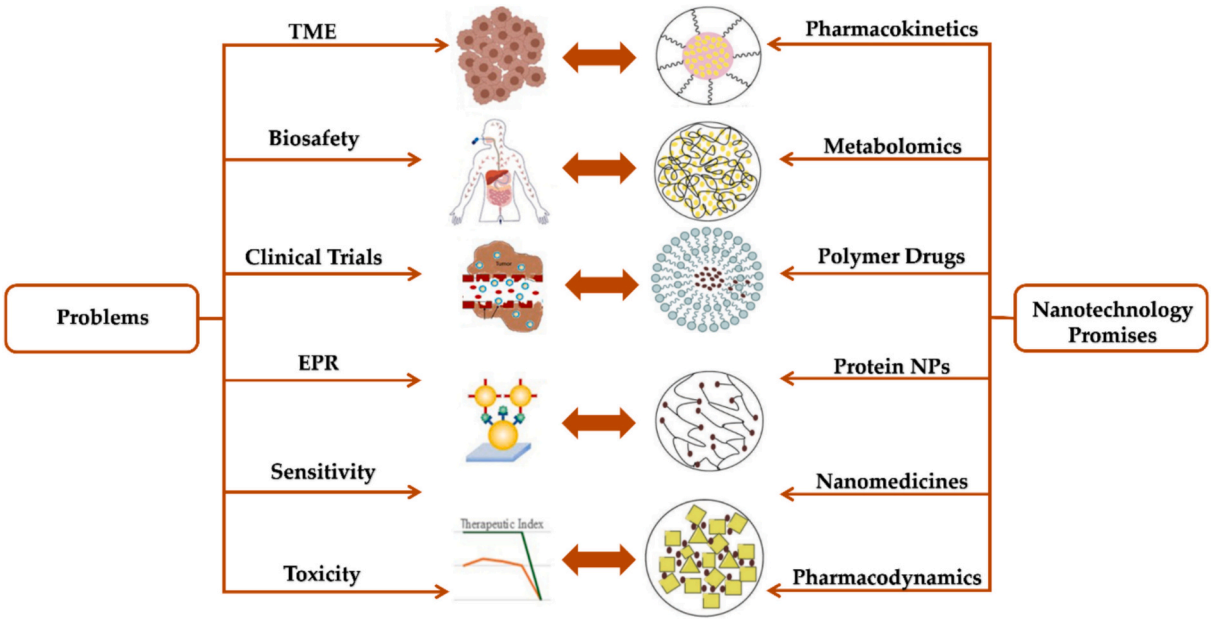

Fig. 7. Promises and problems of nanomedicines.

targeting, and controlled drug delivery.[114]

Despite successful results in cellular and animal models, significant challenges remain in clinical trials due to the substantial differences between human and animal physiology. Biocompatibility represents a significant challenge in the clinical translation of nanomedicines. While nanotechnology-based drug delivery systems often show promising results in cell and animal models, clinical trials face greater hurdles due to the biological differences between humans and animals.[114] The enhanced permeability and retention (EPR) effect, a contentious concept, suggests that molecules of specific sizes (e.g., liposomes, NPs, and macromolecular drugs) accumulate more in tumor tissue than in normal tissues. Thus, new and reliable evidence and techniques are needed to advance drug delivery systems.

The fifth challenge is to develop highly sensitive, reproducible nanoprobes with long shelf lives at reasonable costs. Most nanoprobes were synthesized in academic laboratories, with many techniques unworkable for clinical settings. For example, most studies used advanced spectroscopy that is rarely present in hospitals or clinical laboratories. The successful creation of point-of-care devices using NPs would greatly enhance the clinical application of nanotechnology in cancer diagnosis.

Finally, the clinical translation of nanomedicine is frequently hindered by safety concerns, particularly those related to nanoparticle biodistribution, immune interactions, and long-term toxicity. One of the most significant challenges is the non-specific accumulation of nanoparticles in off-target organs, especially the liver and spleen driven by recognition and clearance by the mononuclear phagocyte system (MPS), primarily liver-resident Kupffer cells.[115] This leads to high hepatic nanoparticle retention regardless of formulation, significantly limiting delivery to intended disease sites while raising the risk of hepatotoxicity and chronic inflammation.[115,116] Moreover, systemically administered nanoparticles also accumulate in secondary organs such as spleen, kidneys, lungs, and heart, as shown in preclinical biodistribution studies.[117] To reduce toxicity and improve safety, recent advancements have focused on surface functionalization and material selection. PEGylation, as well as coatings with zwitterionic or bioinspired polymers, has been shown to reduce protein corona formation and MPS uptake, thus prolonging systemic circulation.[118] Furthermore, the development of biodegradable and cholesterol-free lipid nanoparticle formulations is now helping to mitigate hepatic accumulation and promote more specific organ targeting. For instance, Su et al. demonstrated that exclusion of cholesterol and tuning of lipid architecture can significantly reduce liver accumulation while enhancing mRNA translation in the lungs.[116] In line with this, dose optimization is also critical, particularly for RNA-based therapeutics. Studies on self-amplifying mRNA (saRNA) vaccines have shown that reducing nanoparticle dose while preserving immunogenicity can effectively lower systemic exposure and associated toxicological risk. High-dose formulations may improve antigen expression, but they also correlate with broader organ distribution and increased inflammation.[117]

Altogether, these findings underscore that minimizing nanotoxicity requires a multi-pronged strategy: understanding biodistribution dynamics, engineering immune-evasive and biodegradable surfaces, reformulating nanoparticle compositions to enhance clearance, and tailoring dosing regimens to achieve therapeutic efficacy with minimal systemic toxicity.

The physicochemical characteristics of NPs, including shape, size, surface charge, composition, targeting ligands, and surface chemistry, play a critical role in determining their toxicity, biodistribution, and pharmacokinetic behavior. These properties not only influence the therapeutic efficacy but also the safety profile, highlighting the need for precise design in nanoparticle-based drug delivery systems.

Conclusion

While nanomedicine continues to evolve as a promising approach for cancer treatment, it is important to recognize that the integration of nanotechnology into clinical practices remains a developing field. Nanotechnology holds significant potential due to its ability to meet the requirements of effective tumor treatment, but the tumor heterogeneity must be carefully considered during clinical therapies. Additionally, the pharmacokinetics and toxicology of nanomaterials in vivo need thorough analysis and optimization to improve their therapeutic outcomes while minimizing adverse effects. One major challenge is the often delayed or inconsistent effects seen when combining therapies and diagnostic approaches, potentially leading to drug overdose or unintended side effects. Furthermore, real-time monitoring of drug distribution in vivo after intravenous administration remains a difficult task. Nanotechnology-based vectors show promise in providing theranostic information in a spatial-temporal context, yet more research is required to refine principles, optimize material design, develop animal models, and conduct large-scale clinical trials. These efforts should be guided by a deep understanding of tumors' biological characteristics. Ultimately, for nanotechnology-based vectors to reach their full therapeutic potential, they must be capable of precisely delivering their payloads to tumors and biodegrading without causing off-target effects.

CRediT authorship contribution statement

Adeeb Shehzad: Writing – review & editing, Validation, Supervision, Resources, Project administration. **Júlia Alves:** Writing – review & editing, Methodology. **Mazhar Ul-Islam:** Writing – original draft, Methodology, Investigation. **Abdullah Khamis Al Saidi:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Sofia O.D. Duarte:** Writing – review & editing, Visualization, Validation. **Mohammad Sherjeel Javed Khan:** Writing – original draft, Software, Resources, Investigation. **Pedro Fonte:** Writing – review & editing, Validation, Supervision, Funding acquisition.

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Declaration of competing interest

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