

Nanotechnology-Enabled Diagnosis and Treatment of Hepatocellular Carcinoma: Theranostics, Combination Regimens, and Translation

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Abstract: This manuscript explores the innovative application of nanotechnology in the diagnosis and treatment of hepatocellular carcinoma (HCC), a leading cause of cancer-related mortality worldwide. Emphasizing the synergistic potential of nanotechnology, the paper discusses advanced nanomaterials and techniques, such as targeted drug delivery systems, nanoparticle-based imaging, and multi-modal therapy, which enhance the precision and efficacy of HCC interventions. Nanotechnology offers significant improvements in early diagnosis through enhanced imaging capabilities and tumor-specific biomarkers, enabling more accurate detection of HCC at its early stages. Furthermore, it enables the development of novel therapeutic strategies, including the targeted delivery of chemotherapeutic agents, gene therapies, and immunotherapies, minimizing side effects and improving patient outcomes. The manuscript also highlights challenges such as bio-barrier penetration, biocompatibility, and the high production costs associated with nanomedicine. Despite these obstacles, the integration of nanotechnology with artificial intelligence and personalized medicine promises a transformative future for HCC treatment. This review underscores the pivotal role of nanotechnology in advancing both the diagnostic and therapeutic landscapes for HCC, offering a new frontier for improving survival rates and quality of life for patients.

Keywords: hepatocellular carcinoma, diagnosis, treatment, nanotechnology, multimodality

Introduction

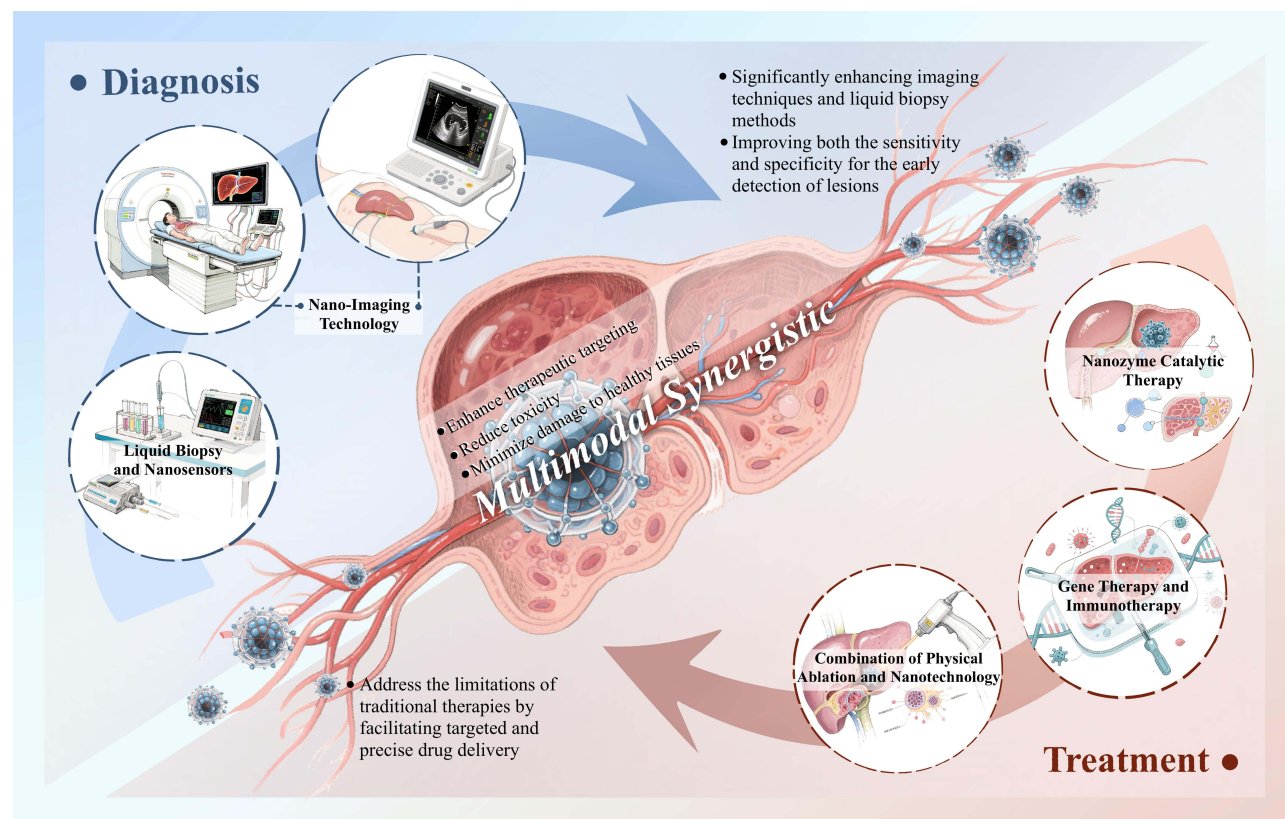
Global Epidemiology and Therapeutic Challenges of Hepatocellular Carcinoma (HCC)

Epidemiological Analysis of HCC

Primary liver cancer is a prevalent malignancy globally and is mainly composed of three pathological subtypes: HCC, intrahepatic cholangiocarcinoma (ICC), and combined hepatocellular-cholangiocarcinoma (CHC). Among these, HCC is the most common, accounting for approximately 85% of primary liver cancer cases and ranking as the sixth most prevalent cancer worldwide.^{1,2} More than half of all primary liver cancer cases and deaths occur in regions such as Southeast Asia, Oceania, and sub-Saharan Africa, where incidence rates exceed 30 per 100,000. In contrast, North America, Latin America, and Europe are considered low-incidence regions, with rates above 11 per 100,000.^{3,4}

HCC is one of the most lethal malignancies, ranking as the third leading cause of cancer-related deaths worldwide. According to the GLOBOCAN database, liver cancer is responsible for approximately 830,000 deaths worldwide, with HCC accounting for about 75% of these cases.^{1,5} The overall five-year survival rate for HCC is approximately 18%, making it the third leading cause of cancer-related mortality worldwide.⁶⁻⁸ Over recent decades, the incidence of HCC has either declined or stabilized in some Asian countries, while the number of combined liver cancer and HCC cases in

Graphical Abstract



Western nations has increased, with incidence rates steadily rising.^{9,10} Although the decline in HCC incidence in some Asian regions signals positive progress, these areas still bear a significant portion of the global liver cancer burden, with the global liver cancer incidence increasing at an annual rate of 7.9%.¹¹

Viral hepatitis infections, particularly hepatitis B (HBV) and hepatitis C (HCV), are key etiological factors in the development of HCC.¹² Regional variations in incidence patterns reflect geographical differences in the proportion of liver cancer cases attributable to HBV versus HCV. HBV remains the dominant risk factor for HCC in Asia and Africa, whereas HCV is more prevalent in the United States and other Western countries.² Additionally, factors such as obesity, metabolic syndrome-associated non-alcoholic fatty liver disease, alcoholic cirrhosis, liver fluke infections, aflatoxin exposure, trace element imbalances, and smoking further contribute to the elevated risk of liver cancer.^{2,13}

Current Treatment Modalities for HCC and Their Challenges

Clinically available treatments for HCC are primarily applicable to early- and intermediate-stage disease and include surgical therapy, ablation therapy, intravascular interventional therapy, and radiotherapy.¹⁴ For early-stage HCC, surgical and ablation therapies are the primary curative approaches.^{15,16} Surgical treatment typically involves two main modalities: hepatectomy and liver transplantation, though both have limited indications. Hepatectomy is primarily indicated for cases involving smaller tumors confined to one lobe of the liver, provided that sufficient residual liver volume and preserved liver function can be maintained post-resection.^{17,18} On the other hand, liver transplantation can be considered for patients with decompensated liver function but is restricted to those who meet the Milan criteria (ie, a single tumor ≤ 5 cm in diameter or up to three tumors, each ≤ 3 cm, without macrovascular invasion).¹⁹ Studies, however, suggest that post-hepatectomy mortality rates in HCC patients are nearly 50% higher than those following liver transplantation, with a threefold increased risk of recurrence.^{1,20} A study by Hong et al indicated that 266 out of 466 patients (57.1%) who

underwent initial resection required secondary treatments due to disease progression.²¹ The patient's postoperative residual liver functional reserve must meet physiological demands, which is a critical determinant of surgical success and a primary reason why surgery is often less effective for advanced-stage HCC.

Ablation therapy, which includes commonly used techniques such as radiofrequency ablation (RFA) and microwave ablation (MWA), is primarily indicated for early-stage HCC with smaller tumor diameters. RFA is generally effective for tumors ≤ 3 cm but is associated with higher local recurrence rates and does not eliminate the risk of microvascular invasion.²² A study by Xu et al found that HCC patients treated with RFA exhibited 3-year and 5-year overall survival rates of 78.6% and 60.8%, respectively, both of which are lower than those achieved with hepatectomy.²³

For intermediate-stage HCC (BCLC-B), intravascular interventional therapies are the primary treatment approach. These include transarterial chemoembolization (TACE), hepatic artery infusion chemotherapy (HAIC), and Y-90 selective internal radiation therapy (SIRT).¹ While these methods are generally safe for HCC patients ineligible for surgery, they are often non-curative and mainly serve to delay tumor progression. For instance, TACE has limited efficacy in preventing HCC recurrence and is applicable only to specific disease stages. It also has prolonged impacts on liver function and frequent side effects, such as nausea and vomiting. Furthermore, TACE may induce a hypoxic tumor microenvironment, which accelerates resistance through the vascular endothelial growth factor (VEGF) pathway.²⁴

For patients with advanced HCC, supportive palliative therapy is primarily used to alleviate symptoms.²⁵ Locoregional approaches, such as radiotherapy and chemotherapy, may be utilized. Radiotherapy offers a curative potential only for small-diameter tumors and has limited efficacy for larger or advanced-stage tumors. It is rarely considered a radical treatment for HCC due to frequent prognostic complications and drug resistance issues.^{26,27} Chemotherapy, in contrast, carries significant drug toxicity, which may adversely affect normal cells, and is prone to inducing drug resistance. First-line therapeutic agents are limited in their availability and efficacy, characterized by low objective response rates and marginal survival benefits. Following the failure of first-line treatments, second-line therapies are often prolonged, with poor drug accessibility, contributing to the low cure rate of HCC.²⁸

In contrast to patients who are eligible for curative therapies, typically at the early stages of liver cancer, statistical analyses indicate that approximately 80% of HCC patients are diagnosed at intermediate or advanced stages, when physical abnormalities are detected and confirmed as HCC.²⁹ At these stages, tumors progress rapidly, grow to larger sizes, and are prone to metastasis. Existing treatments for advanced HCC remain limited, with no effective radical therapies, and are often associated with significant post-treatment adverse effects.³⁰ Consequently, current therapeutic approaches fail to fully meet the clinical demands for HCC management, highlighting the urgent need for novel scientific and technological advancements in HCC treatment. This necessity has driven the integration of nanotechnology with medicine, promoting the development of more sensitive and rapid nanomedicine-based therapeutic strategies.

Nanomaterials, due to their small size, targeting capabilities, and catalytic activity, provide a unique opportunity to explore biological processes and mechanisms at a microscopic level.³¹ They also offer innovative approaches for the diagnosis and therapy of cancer. A critical focus of research and translation in this field is the application of nanotechnology and nanomaterials to improve the specificity, precision, and efficacy of HCC diagnosis and treatment across various disease stages. The medical application of nanotechnology began in the late 20th century, initially focusing on drug delivery systems. In recent years, however, nanotechnology has made substantial progress in the fields of biology, pharmacology, and medicine, evolving from drug delivery to the integration of multimodal theranostics and advancing into combination therapies, such as targeted therapy and immunotherapy. With the convergence of interdisciplinary fields, nanotechnology—particularly through the modification of diverse nanoparticles—has found extensive applications in biomedical research particularly in the early diagnosis and precision treatment of advanced HCC.³²

Advantages of Nanotechnology in Cancer Diagnosis and Treatment

Properties of Nanomaterials

Nanoparticles possess unique properties, such as small size effects, surface effects, quantum tunneling effects, and biocompatibility. These properties contribute to prolonged drug circulation time in the bloodstream, enhanced drug solubility in bodily fluids, and a broad range of applications in medicine, especially in cancer therapy.³³ Nanoparticles typically range from 1 to 100 nm in size, and their small diameter imparts distinct physical and chemical characteristics

that facilitate metabolic clearance from the body.³⁴ Their high surface-to-volume ratio provides numerous active sites, which enables the loading of therapeutic agents, thereby extending drug activity duration and enhancing reaction activity and catalytic efficiency.³⁵ For example, carbon-based nanomaterials, with their large surface area, are ideal for developing electrochemical sensors to detect HCC biomarkers.³⁶

Moreover, most nanomaterials exhibit excellent biocompatibility, which minimizes immune responses and systemic toxicity.³⁷ By designing and modifying nanomaterials, and selecting appropriate materials for nanoparticle formation—such as functionalizing nanomaterials with ligands like antibodies or aptamers—the surface effects can be harnessed to enable targeted drug delivery or catalytic activity. This strategy results in superior biocompatibility and biodegradability, allowing for gradual *in vivo* degradation, controlled drug release, and metabolic elimination, thereby reducing the risk of long-term toxic accumulation. Peptide-hydrogel nanocomposites are one example of such innovative materials.³⁸ Traditional HCC drugs often fail to effectively reach tumor sites due to issues such as instability, poor solubility, and drug resistance. In contrast, nanoparticles encapsulate drugs within their structure, and by optimizing factors like size, shape, and surface chemistry, nanoparticles enhance retention and accumulation at tumor sites.³⁹ This significantly improves drug delivery efficiency compared to conventional chemotherapeutic agents.

Multimodal Potential of Nanotechnology: Integration of Diagnosis and Treatment (theranostics) and Synergistic Enhancement Effects

Potential in Theranostics

Nanoparticles hold significant promise in diagnostics by serving as advanced imaging agents, leveraging their optical, electrical, and other unique properties to enhance the early detection of liver tumors and improve imaging contrast in modalities like MRI and CT.⁴⁰ Moreover, nanotechnology facilitates the detection of specific biomarkers in HCC cells by functionalizing nanoparticles with molecular probes, which in turn improves diagnostic sensitivity and accuracy.⁴¹ In cancer treatment, nanoparticles enable the targeted delivery of anticancer drugs through the incorporation of specific ligands (such as folic acid and glycyrrhizic acid).⁴² This targeted approach helps to bypass the body's natural defense mechanisms, enabling the drugs to specifically interact with tumor cell receptors, thereby enhancing therapeutic precision and reducing toxicity to healthy tissues. Based on this, nanotechnology can effectively combine diagnosis and treatment, allowing for the precise localization and imaging of tumors while simultaneously delivering therapeutic agents directly to cancer cells, improving drug bioavailability and efficacy.⁴³ In recent years, numerous high-impact studies have highlighted the transformative role of nanotechnology in HCC diagnosis and treatment. Emerging research shows that nanoprobe targeting novel biomarkers such as Vav1 offer significant advantages over traditional diagnostic markers like AFP and GPC3, enabling earlier and more sensitive detection through liquid biopsy or molecular imaging.⁴⁴ Furthermore, advanced nanocarrier systems and gene silencing tools targeting key proteins such as PRDX6, KIF5B, and other critical hub genes can specifically intervene in crucial oncogenic pathways like PI3K/Akt/mTOR, offering innovative strategies for achieving highly effective, low-toxicity treatments by simultaneously disrupting multiple signaling axes.^{45–47} Additionally, certain nanomaterials allow real-time monitoring during treatment, offering the ability to track tumor status, temperature changes at the treatment site, drug release kinetics, and therapeutic outcomes. This facilitates the optimization of treatment parameters and supports personalized therapy. For example, palladium nanosheets functionalized with radioactive iodine isotopes can be utilized for single-photon emission computed tomography (SPECT) imaging, while also exhibiting synergistic antitumor effects, thus enabling real-time monitoring of therapeutic efficacy during HCC treatment.⁴⁸

Advantages of Synergistic Enhancement Effects

Nanoparticles also possess the ability to co-deliver multiple therapeutic agents to tumor cells, enabling synergistic interactions between drugs. Some nanoparticles can carry both chemotherapeutic drugs and agents for photothermal or photodynamic therapy (PDT).⁴⁹ When combined with external energy sources such as light, these nanoparticles integrate PDT, photothermal therapy (PTT), and other therapeutic modalities with chemotherapy, creating reinforcing effects that enhance therapeutic outcomes. For instance, liposomes loaded with sensing components can release chemotherapeutic agents in response to external stimuli such as light, temperature, or ultrasound.⁵⁰ This precise control over drug release—

achieved by adjusting light intensity, wavelength, or exposure duration—allows for more effective treatments. These combinatorial approaches harness synergistic mechanisms to improve efficacy, enhance tumor cell apoptosis, minimize damage to normal tissues, and reduce the risk of drug resistance associated with monotherapy.

Nanomaterial–Tumor Microenvironment (TME) Interactions

Mechanistic Basis of Nanomaterial–TME Interactions in HCC

Nanomaterials influence HCC progression not only by enhancing drug delivery but also by actively remodeling the TME/TIME via defined molecular and cellular processes. After systemic administration, nanoparticles rapidly acquire a protein corona, which can alter their biological identity, receptor engagement, and clearance by the mononuclear phagocyte system.⁵¹ In the liver, Kupffer cells may sequester circulating nanoparticles, thereby regulating the fraction that reaches tumor parenchyma and immune niches. These early nano–bio interactions are therefore critical determinants of downstream signaling effects, immune modulation, and therapeutic performance in HCC.⁵²

Immune-Cell Modulation and TIME Reprogramming

Nanomaterials improve therapeutic responses by reprogramming immunosuppressive TIME components that are prominent in HCC. Specifically, nanoplateforms designed to relieve hypoxia or normalize local conditions can attenuate hypoxia-driven immunosuppressive programs, thereby limiting Treg expansion/function (eg, suppression of Foxp3-associated pathways) and enhancing effector T-cell activity.⁵³ Moreover, engineered nanoparticles can reduce MDSC-mediated immune suppression through selective targeting and functional inhibition, promoting restoration of cytotoxic T lymphocyte (CTL) responses and supporting synergy with immune checkpoint blockade. Collectively, these TIME-directed mechanisms help counteract liver tumor immune tolerance and shift the immune balance toward antitumor activity.^{54,55}

Signal Transduction, Stress Pathways, and Immunometabolic Regulation

Beyond altering immune-cell composition, nanomaterials can induce pathway-level responses in tumor and immune cells via microenvironment-responsive activation and intracellular trafficking. ROS-generating or ROS-amplifying nanoplateforms can activate oxidative-stress signaling and damage-response pathways, increasing tumor cell apoptosis/necrosis and sensitizing tumors to radiotherapy, chemotherapy, and photodynamic/photothermal modalities.⁵⁶ In addition, nanocarriers delivering nucleic acids or small molecules that modulate tumor metabolism (eg, inhibition/silencing of glycolysis-associated enzymes such as LDHA) can reduce lactate accumulation, alleviate acidosis (a key suppressor of T-cell function), and thereby improve CTL activity and immune-mediated tumor control.⁵⁷ Together, these examples highlight an immunometabolic mechanism, whereby metabolic normalization of the TME restores immune competence and enhances combination efficacy.⁵⁸

Receptor-Mediated Uptake and Subcellular Delivery as Determinants of Pathway Engagement

The biological effects of nanotherapeutics depend on efficient cellular entry and subcellular trafficking. Ligand-directed nanoparticles engaging hepatocyte/tumor-associated receptors (eg, ASGPR or transferrin receptor) undergo receptor-mediated endocytosis and subsequent processing through endosomal–lysosomal pathways. For nucleic-acid therapeutics and certain protein/peptide cargos, endosomal escape and cytosolic release are mechanistically required to achieve target knockdown/translation and downstream pathway modulation.⁵⁹ Accordingly, nanoengineering parameters (surface chemistry, charge, ligand density, and responsiveness to pH/enzymes/redox conditions) directly determine intracellular bioavailability and the resulting signal-transduction outcomes.⁶⁰

Application of Nanotechnology in HCC Diagnosis

Nano-Imaging Technology

Application of Magnetic Nanoparticles in MRI Enhancement

Magnetic Resonance Imaging (MRI) is a non-invasive, radiation-free imaging modality that offers high resolution and multi-parameter imaging capabilities, making it a crucial tool in the diagnosis of HCC.⁶¹ MRI plays an essential role in visualizing liver anatomy, lesion size, morphology, quantity, and their relationships with surrounding tissues, thereby

aiding in the early detection and accurate diagnosis of HCC.^{1,62} Compared to traditional imaging techniques, MRI offers superior soft tissue contrast, allowing for clearer visualization of liver lesions and enhancing diagnostic precision.⁶³

Magnetic nanoparticles, particularly superparamagnetic iron oxide nanoparticles (SPIONs), have been extensively used in both the diagnosis and treatment of HCC.⁶⁴ Due to their superparamagnetic properties, SPIONs offer unique advantages in MRI contrast enhancement. By modifying SPIONs with specific ligands and adjusting factors such as surface coatings (eg, SMG and SHP) and core size, active targeting of tumor biomarkers can be achieved, thereby improving the specificity and sensitivity of imaging.⁶⁵

Early-stage HCC lesions, which are often small and exhibit low contrast with surrounding normal tissues, are difficult to detect using traditional contrast agents.⁶⁶ To address this challenge, Lu et al developed an i-motif DNA-assisted, pH-responsive iron oxide nanocluster assembly, termed RIA. Under the acidic pH conditions of the tumor microenvironment, RIA dissociates rapidly, leading to a sharp decrease in relaxivity (r_2/r_1). This alteration switches RIA from a T2 to a T1 contrast agent. In T1-weighted imaging mode, HCC lesions become significantly brighter, while normal liver tissues darken, which substantially enhances the sensitivity and accuracy of early HCC detection.⁶⁷ Additionally, Xu et al introduced a novel targeted contrast agent, Fe₃O₄-PEG-RGD nanoparticles, which were created by conjugating ultra-small superparamagnetic carboxylated Fe₃O₄ nanoparticles with polyethylene glycol (PEG)-linked arginine-glycine-aspartic acid (RGD) peptides. In vitro cellular experiments, MR imaging of cells, and in vivo murine studies demonstrated that these nanoparticles specifically bind to HepG2 cells, with the tumor's MR signal intensity significantly higher than that of non-targeted magnetic particles. These nanoparticles also showed excellent biocompatibility and ultra-high r_1 relaxivity.⁶⁸

Nanotechnology holds considerable promise in enhancing MRI diagnostics. The use of magnetic nanoparticles as MRI contrast agents enables targeted imaging, thereby increasing the sensitivity and specificity of MRI. This innovation offers the potential for improved monitoring of HCC development, providing valuable support for early detection and precision therapy. However, further optimization of magnetic nanoparticle surface modifications and preparation processes is necessary to improve their in vivo stability and biocompatibility. Furthermore, additional clinical trials are needed to validate the safety and efficacy of magnetic nanoparticles in the diagnosis of HCC.

Nanomaterial-Mediated Imaging Contrast Techniques

Nanomaterial-Enhanced Photoacoustic Imaging

Photoacoustic imaging (PAI) is a non-invasive hybrid imaging technique that utilizes the photothermal effect to provide high optical contrast and deep tissue imaging with excellent depth resolution.^{69,70} Endogenous chromophores in vivo, such as hemoglobin, lipids, and melanin, absorb light strongly within specific optical windows, generating significant photoacoustic signals.⁷¹ By analyzing hemoglobin oxygenation and distribution, PAI facilitates the diagnosis of various diseases, including HCC, and has proven effective in detecting both structural and functional abnormalities in cancerous tissues.⁷² The use of nanomaterials as contrast agents enhances imaging signals due to their unique optical properties and excellent biocompatibility, enabling precise manipulation. For instance, inorganic metal nanoparticles, such as copper sulfide, amplify photoacoustic signals, while organic polymer nanoparticles, like semiconducting polymer nanoparticles, combine both photoacoustic and fluorescent properties to detect reactive oxygen species (ROS).⁷³

Studies show that nanomaterial-enhanced PAI can detect microlesions in early-stage HCC tumors, improving diagnostic accuracy through multimodal imaging techniques. Deng et al developed a targeted multifunctional nanoprobe for PAI that allows non-invasive imaging of tissues several centimeters deep. This technology targets HCC by recognizing the biomarker glypican-3 (GPC3) on HCC cell membranes, offering a high spatial resolution for distinguishing the progression of micro-HCC lesions originating from cirrhosis. This innovation addresses the diagnostic challenge of differentiating HCC from cirrhotic nodules.⁷⁴ The integration of nanotechnology with PAI presents a promising pathway for enhancing HCC detection, characterization, and theranostics. By combining photoacoustic imaging with nanotechnology, it is possible to amplify photoacoustic signals, and through the integration of advanced imaging techniques with targeted nano-theranostics, researchers aim to improve both the accuracy and efficacy of HCC diagnosis and management.

Contrast-Enhanced Ultrasound (CEUS)

Ultrasound imaging generates tissue visualizations by analyzing reflected signals from acoustic wave pulses. CEUS further enhances imaging quality by intravenously administering ultrasound-specific contrast agents, which amplify backscattered echoes.⁷⁵ The liver's dual blood supply, originating from both the hepatic artery and portal vein, presents unique opportunities for CEUS to evaluate liver function and detect abnormalities.

Following the injection of ultrasound contrast agents, CEUS imaging of the liver reveals three overlapping vascular phases. Different types of liver lesions exhibit distinct enhancement and attenuation patterns across these phases, aiding in lesion characterization.⁷⁶ CEUS enhances the vascular features of liver lesions using ultrasound contrast agents, thus improving diagnostic accuracy and reliability (Figure 1).⁷⁷ Nano-based ultrasound contrast agents typically offer excellent biocompatibility, prolonged circulation times, and tunable acoustic properties.

They amplify ultrasound signal scattering, enhancing imaging contrast and resolution to better visualize tissue/lesion morphology, structure, and blood perfusion.⁷⁸ For instance, Qiu et al developed an ATO/PFH NPs@Au-cRGD nano-

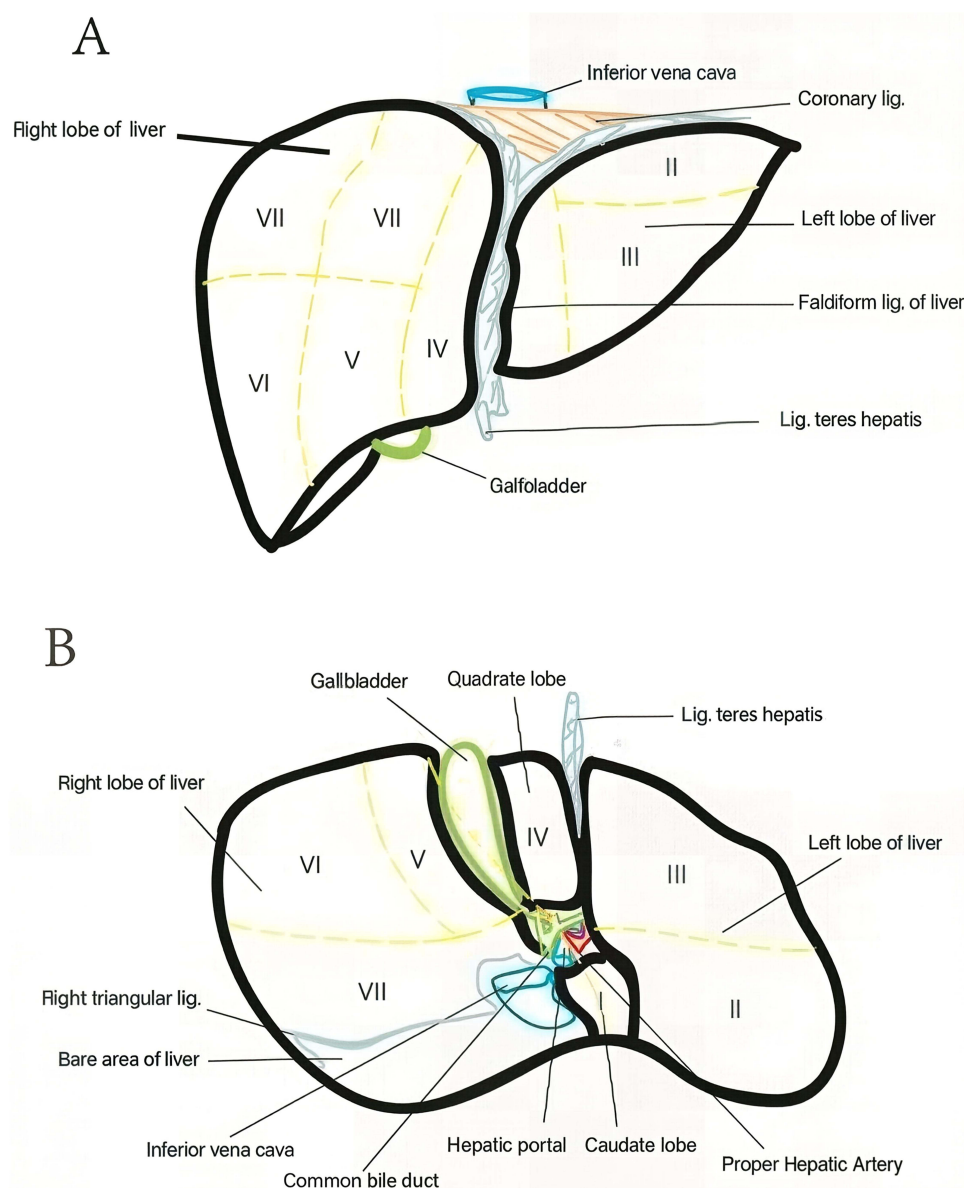


Figure 1 Liver anatomy (lobed and segmented). (A) Diaphragmatic surface of the liver. (B) Visceral surface of the liver.⁷⁷ (I) Caudate lobe. (II) Left lateral superior segment. (III) Left lobe of liver (Left lateral inferior segment). (IV) Quadrate lobe. (V) Right anterior inferior segment. (VI) Right lobe of liver (Right posterior inferior segment). (VII) Right lobe of liver (Right posterior superior segment).

contrast agent for HCC theranostics. Results demonstrated that ATO/PFH NPs@Au-cRGD significantly improved contrast under ultrasound exposure, achieving imaging effects comparable to those of commercial contrast agents in tumors. This allowed clear visualization of contrast enhancement and dynamic tumor blood perfusion, facilitating precise molecular imaging during treatment.⁷⁹

Although the materials for nano-ultrasound contrast agents are widely selected and demonstrate excellent performance in ultrasound imaging, most research is confined to tumor-bearing mouse models and has not yet been scaled up for clinical application.⁸⁰ With ongoing advancements in nanotechnology and materials science, future nano-ultrasound contrast agents are expected to offer higher imaging resolution and specificity, enabling more precise detection of small tumors and early lesions, thereby providing strong support for early disease diagnosis.

Liquid Biopsy and Nanosensors

In addition to imaging examinations, liquid biopsy plays a crucial role in the early diagnosis of HCC. Current research in precision medicine emphasizes the development of advanced biomarkers.⁸¹ Liquid biopsy and nanosensors facilitate early cancer detection and minimize patient trauma by analyzing overexpressed surface proteins (eg, alpha-fetoprotein, AFP), bloodborne tumor markers (eg, circulating tumor cells [CTCs] and cell-free DNA [cfDNA]), and other indicators.⁸² These methods not only promise early detection with minimal patient discomfort but also allow for the study of tumor mutations and biological tissues, addressing the gap in molecular-level data for HCC.⁸³ Biomarkers for HCC diagnosis are currently categorized into five classes: nucleic acids, proteins, CTCs and extracellular vesicles, serum metabolites, and gut microbiota.⁸⁴ The timely identification of suitable HCC biomarkers in the early stages of disease, combined with the design of highly specific nanomaterials tailored to their unique properties, holds significant potential for personalized detection and medical intervention.

Nanozyme Catalytic Technology for High-Sensitivity Detection of HCC Biomarkers

Nanozymes are nanomaterials with enzyme-mimicking catalytic activity. Their unique physicochemical properties confer advantages in biosensing and bioanalysis, enabling their widespread use in biosensor development.⁸⁵ These materials enhance the sensitivity, stability, and selectivity of biosensors, expanding their utility across various fields. Traditional methods for detecting HCC biomarkers, such as enzyme-linked immunosorbent assays (ELISA), although clinically prevalent, face limitations, including restricted sensitivity and specificity, as well as prolonged detection times.^{86,87} HCC biomarkers often manifest as overexpressed surface receptors, and the design of nanozymes specifically tailored to these biomarkers offers innovative solutions to these challenges (Figure 2).⁸⁸

Currently, two primary protein biomarkers for HCC—AFP and GPC3—can be detected with increased sensitivity through enzyme-mimicking catalytic reactions, offering significant value for the early diagnosis of HCC.⁸⁹

AFP, one of the most widely studied and clinically utilized biomarkers for HCC, shows markedly elevated serum concentrations in HCC patients compared to healthy individuals.⁹⁰ Nanozyme catalytic technology enhances detection capabilities by designing nanozyme-antibody complexes that specifically bind to AFP and catalyze substrate reactions, generating detectable signals. For example, Li et al developed a nanozyme-linked immunosorbent surface plasmon resonance (nano-ELISPR) biosensor based on nanozymes and an oxidized 3,3',5,5'-tetramethylbenzidine (oxTMB) etching reaction. This nano-ELISPR platform modulates the absorption spectral peak by adjusting the thickness of noble metals via target molecule adsorption on the substrate surface. This system achieves a lower detection limit for AFP compared to commercial ELISA kits, requires thinner gold layers, reduces manufacturing costs, and demonstrates ultrasensitivity and high specificity.⁹¹ Additionally, Foluke et al designed a highly sensitive electrochemical immunosensor for AFP detection using a carbon nanofiber (CNF)/gold nanoparticle (AuNP) nanocomposite platform. The CNF/AuNP platform significantly improves analytical performance, amplifying the electrochemical response of the AFP antigen-antibody interaction. This immunosensor exhibits a wide concentration range, low detection limit, excellent reproducibility, and high selectivity.⁹²

GPC3, a glycosylphosphatidylinositol-anchored oncofetal proteoglycan expressed on the cell surface, is virtually undetectable in adult liver tissue but is overexpressed in HCC, making it a highly specific biomarker for the qualitative and quantitative analysis required to detect and monitor HCC progression.⁹³ For example, Li et al synthesized H-rGO-Pd

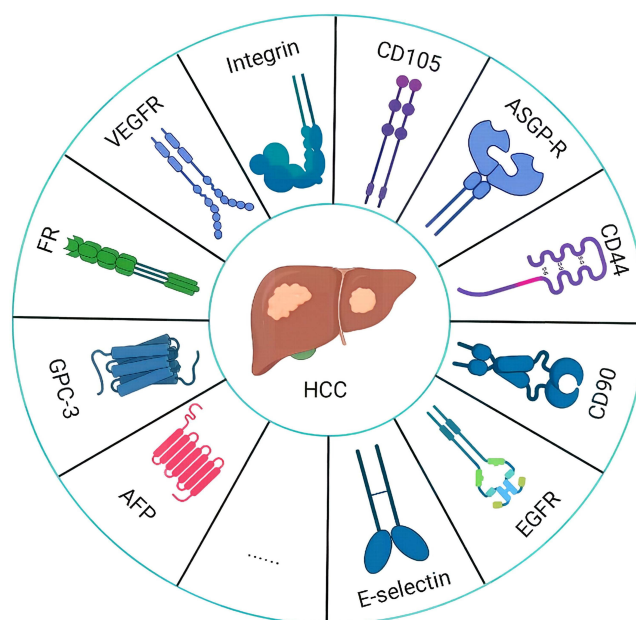


Figure 2 The above is a list of the receptors that are overexpressed in HCC cells.⁸⁸

NPs nanozymes with peroxidase-like catalytic activity. By labeling GPC3 aptamers onto their binding sites to form detection probes and immobilizing GPC3 antibodies as capture probes on Au NP@rGO-modified screen-printed electrodes (SPEs), they constructed a novel electrochemical nanobiosensor based on a nanozyme signal amplification strategy. This sensor enables quantitative analysis of GPC3 with strong specificity, short-term stability, and high recovery rates. While its detection limit is slightly higher than some existing methods, the antibody-antigen-aptamer sandwich structure and enzyme-catalyzed silver deposition signal amplification enhance stability and sensitivity, making it an ideal design for high-sensitivity clinical assays.⁹⁴

As an emerging biosensing technology, nanozyme catalysis has made significant strides in the high-sensitivity detection of HCC biomarkers. Its advantages—including superior sensitivity, stability, and rapid detection for AFP and GPC3—offer novel tools for early HCC diagnosis and therapeutic monitoring. However, the technology is still in the developmental stage, and several challenges remain to be addressed. The preparation processes and quality control of nanozymes require further optimization. Specificity in complex biological samples must be improved to reduce nonspecific binding and interference, thereby enhancing stability and accuracy. Moreover, most nanozyme-based detection methods are still in the laboratory research stages, necessitating extensive clinical validation and evaluation before large-scale clinical adoption.

Nanomaterial-Based Detection of Circulating Tumor Cells and Exosomes

Nanomaterial-enabled liquid biopsy is an emerging cancer diagnostic technology that combines the unique properties of nanomaterials with the advantages of liquid biopsy, offering novel pathways for early cancer diagnosis, treatment monitoring, and prognosis assessment.^{82,95} Nanomaterials possess a high specific surface area and abundant surface-active groups, enabling specific binding to tumor biomarkers for efficient enrichment. Simultaneously, nanosensors exhibit high sensitivity and specificity, facilitating effective capture and detection of circulating tumor cells (CTCs) or exosomes (EXOs), while leveraging their inherent optical, magnetic, or physical properties for multimodal visualization of tumor biomarkers.

Current Research on Nanomaterial-Based CTC Detection

CTCs are tumor cells shed from primary lesions into the bloodstream, driving metastasis and serving as real-time markers for disease progression and survival.⁹⁶ The presence of CTCs correlates closely with tumor invasion, metastasis,

and recurrence. Their quantity and specific phenotypes can act as biomarkers for early HCC recurrence, aiding in prolonging survival and reducing mortality.⁹⁷ Due to the extremely low abundance of CTCs in blood, high-purity and high-sensitivity isolation techniques are required to extract CTCs from samples for detection and analysis.⁹⁸ Magnetic nanobeads, with their magnetic responsiveness and biocompatibility, enable CTC separation and enrichment via magnetic fields. Compared to traditional CTC isolation methods, nanomagnetic bead-based separation achieves higher efficiency and purity, significantly improving detection sensitivity.⁹⁹ For instance, Mao et al developed supramolecular immuno-magnetic nanoparticles via a π - π stacking-driven supramolecular layer-by-layer self-assembly strategy. These nanoparticles demonstrated high sensitivity, specificity, and biocompatibility for model CTCs, with low nonspecific adsorption to negative cells. Captured cells remained viable for subsequent reculturing, highlighting their potential and applicability for clinical in vitro diagnostics.¹⁰⁰

Optical Nanotechnology-Based Exosome Detection

EXOs are 30–120 nm extracellular vesicles involved in intercellular communication and diverse physiological/pathological processes.¹⁰¹ In cancer, their biogenesis is regulated by cancer cells, promoting metastasis and modulating the tumor microenvironment. EXO surface markers hold significant potential for early cancer diagnosis, metastasis monitoring, and therapeutic evaluation (Figure 3).

For instance, Zhang et al introduced a fluorescent biosensor based on inorganic nanoflakes, integrated with DNzyme-mediated detection. This system specifically targets exosomal microRNA, generating fluorescent signals that enhance the accuracy and sensitivity of exosomal microRNA as a biomarker. Their study demonstrated that nucleic acids are robust biomarkers for exosome-based tumor detection, offering a promising method for HCC diagnosis.¹⁰³ Additionally, Saman et al summarized various optical techniques—such as colorimetry, surface plasmon resonance (SPR), fluorescence, and Raman scattering—used to quantify cancer exosome biomarkers, including lipids, proteins, RNA, and DNA. The integration of platforms like microchip systems, magnetic nano-platforms, and optical nano-platforms facilitates the detection of specific proteins, aptamers, and lipid molecules on exosome surfaces, thereby enhancing the sensitivity and accuracy of biosensors.¹⁰⁴

Nanomaterials present unique advantages in the detection of CTCs and exosomes, offering innovative tools for early HCC diagnosis, treatment monitoring, and prognosis assessment.^{105,106} However, current technologies for CTC and

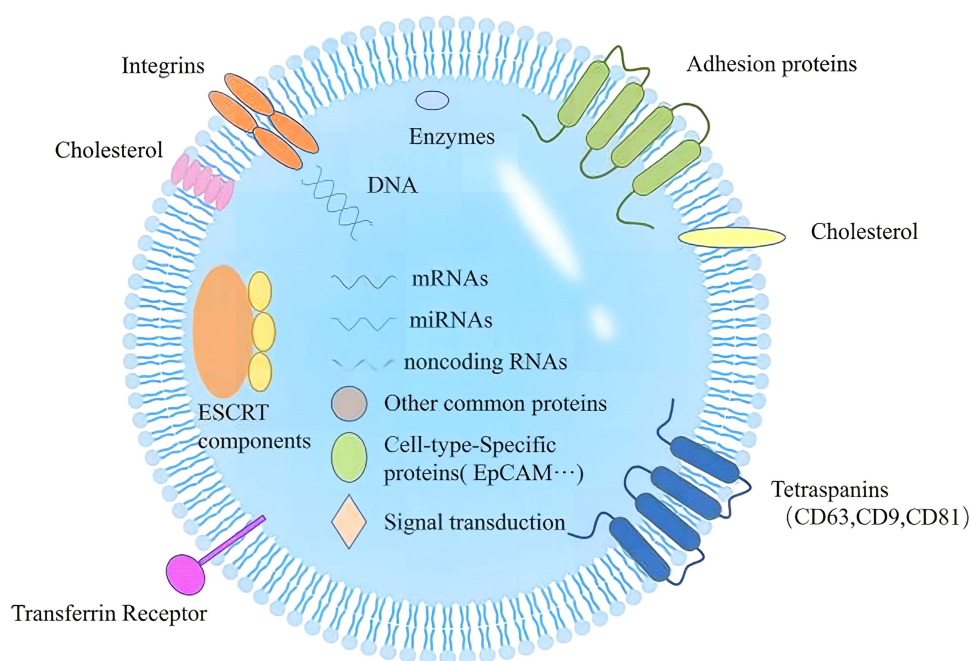


Figure 3 Exosomes contain various important biomarkers, such as proteins, lipids, and miRNAs.¹⁰²

exosome detection face challenges in separation and analysis. Nanotechnology-enabled liquid biopsy is still largely confined to laboratory research, with limited studies focusing on HCC diagnosis. Future efforts must address issues such as nanomaterial biocompatibility and technical standardization to facilitate their clinical application in HCC detection.

Innovative Nanotechnology Strategies in HCC Treatment

Targeted Drug Delivery Systems

Passive Targeting: EPR Effect-Based Nanocarriers

The passive targeting of nanodrugs is primarily achieved through the differential retention of particles by tissues or organs based on their size, which exploits the enhanced permeability and retention (EPR) effect—an essential mechanism in drug delivery. This phenomenon is driven by the unique anatomical and pathophysiological characteristics of tumor vascular networks, which feature an abundance of capillaries with structural defects, enlarged endothelial gaps, and impaired lymphatic drainage. These features enable macromolecular drugs (typically ranging from 1–100 nm in size) to preferentially accumulate and remain within tumor tissues after systemic administration. As a result, drug concentrations in tumors exceed those in normal tissues, leading to precise drug delivery, sustained therapeutic action, and reduced off-target toxicity to healthy tissues.^{107,108}

Passive targeting serves as the fundamental mechanism by which nanomedicines selectively deliver their payloads to HCC tumors. Nanodrug delivery systems are typically composed of nanocarriers and therapeutic agents, with nanocarriers being small, spherical, or cylindrical structures made from one or more materials that ensure chemical stability and biocompatibility. These nanocarriers are usually nanoparticles or microparticles that are ≤ 200 nm in diameter, such as liposomes, lipid-based carriers like micelles, lipid emulsions, and lipid-drug complexes^{109–111} (Figure 4). Research suggests that nanoparticles with more hydrophobic surfaces are cleared more efficiently by the liver, spleen, and lungs, while hydrophilic surfaces tend to evade macrophage capture, thereby improving efficacy.¹¹² Therefore, the use of nanomaterials with hydrophilic surfaces as carriers, such as PEGylated liposomes,¹¹³ not only protects the drug from environmental degradation and evades macrophage uptake but also improves passive delivery to the target site—liver

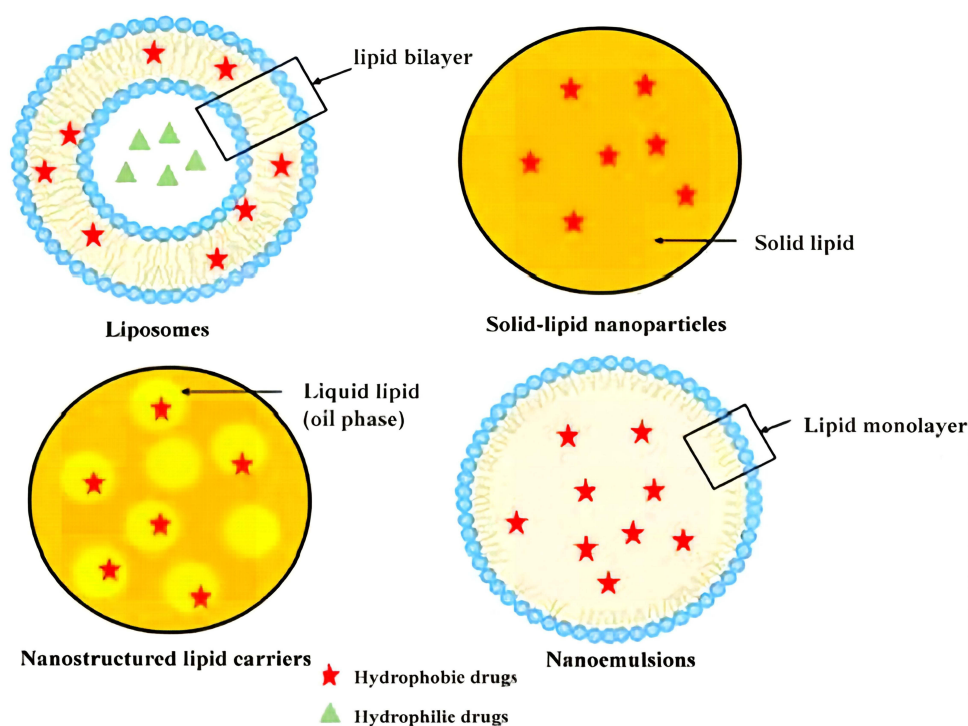


Figure 4 The classification of nanocarriers based on lipids or phospholipids.¹¹¹

tumor cells—through modifications in nanotechnology. The drugs are encapsulated within these nanoscale carriers, allowing for the selection of various drug types and concentrations based on therapeutic requirements.

Despite promising preclinical results in animal models, the clinical translation of these therapies remains limited, with only a few nanomedicines currently approved for cancer treatment. Approximately 0.7% of passive-targeting nanodrugs have been approved for clinical cancer therapy.¹¹⁴ While the EPR effect is often used to improve tumor-specific drug delivery, its applicability in liver tumors is highly variable. The heterogeneous vasculature and poor lymphatic drainage in HCC make passive targeting via the EPR effect unstable and inefficient. Additionally, the liver's reticuloendothelial system (RES), particularly Kupffer cells, rapidly clears most circulating nanoparticles. This limitation arises from the EPR effect being dependent on the molecular weight of the drugs. Molecules with a molecular weight lower than the renal clearance threshold are rapidly eliminated from the bloodstream, which restricts the efficacy of passive targeting to large molecules with a molecular size greater than 40–50 kDa. Clinical studies also confirm that the EPR effect in human HCC is less pronounced than in preclinical models, with significant individual variability, leading to suboptimal efficacy of EPR-based nanomedicines.¹¹⁵ Therefore, to further realize passive targeting nanotherapies, it is necessary to optimize nanoparticle size, encapsulating therapeutic molecules into sizes exceeding the renal clearance threshold but smaller than those prone to excessive hepatic capture, or to develop physically or biologically regulated nano-systems, such as combining nanoparticles with sonosensitizers, etc., to temporarily increase vascular and cell membrane permeability. Consequently, there are strict requirements for the drug size,¹¹⁶ and it is necessary to optimize the size and develop environment-responsive nanoparticles that release therapeutic agents specifically in the tumor microenvironment (TME) to enhance targeting efficiency.¹¹⁷

Active Targeting: Ligand-Modified Precision Delivery

Active targeting in nanodrug delivery involves the direct modification of nanoparticles to exploit the unique characteristics of tumor tissues or cells, such as specific receptor expression or microenvironmental features (Figure 5).¹¹⁸ This enables precise drug delivery and release through ligand-receptor interactions. Current active targeting strategies include

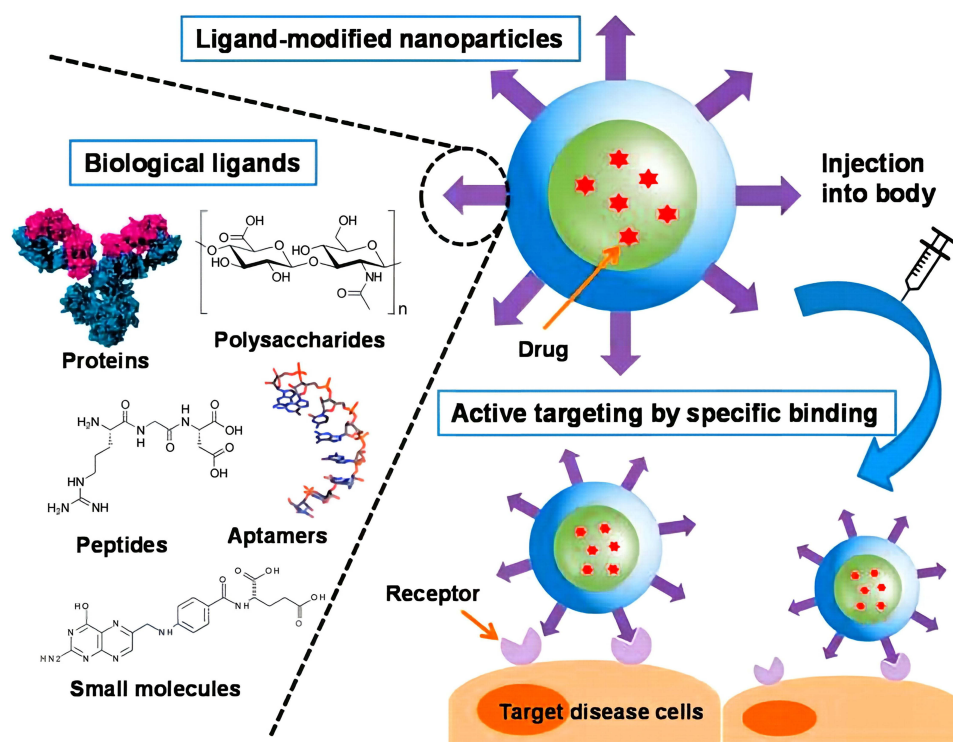


Figure 5 Illustration of biological ligands for active targeting of nanoparticle drug carriers.¹¹⁸

the use of secreted proteins, carbohydrate receptors, tumor vasculature targeting, and tumor microenvironment-responsive targeting.¹¹⁹

Cancer cells often exhibit upregulated glycans attached to surface glycoproteins, which makes sugars or polysaccharides potential ligands for liver-targeted drug delivery. Additionally, vitamins that bind to hepatoma cell receptors, antibodies targeting tumor surface antigens, and aptamer conjugation (single-stranded DNA, RNA, or unnatural oligonucleotides with molecular weights of 10–15 kDa) can also mediate active targeting.¹²⁰ The efficiency and specificity of active targeting depend on direct, point-to-point recognition and interaction between ligands on nanoparticles and receptors on the tumor cell membrane. As such, dual-ligand-modified nanosystems have been shown to enhance targeting capabilities compared to single-ligand systems, improving specificity and internalization within HCC cells.¹²¹

In recent years, as the demand for higher HCC cure rates has grown, passive targeting strategies have proven insufficient for both basic and clinical research. Consequently, there has been an increasing focus on developing active-targeting nanodrugs tailored to the characteristics of HCC cells, with many strategies advancing from laboratory research to clinical trials.^{122,123} For instance, Xiaoran Zhang et al developed transferrin-modified self-assembled polymer nanoparticles for the co-delivery of doxorubicin and cisplatin, aiming to achieve synergistic tumor therapy.¹²⁴ Additionally, Xin Zhang et al proposed a carrier-free, self-assembled nanodrug based on celastrol and galactose, targeting HCC through ferroptosis induction, thus further diversifying HCC-targeted therapeutic approaches.¹²⁵

Compared to passive targeting, active targeting not only enhances drug accumulation in tumor tissues but also reduces off-target toxicity. Although modifying targeting ligands on nanoparticle surfaces can enhance enrichment in tumor tissue, this gain is sometimes only around 1.5-fold.¹²⁶ In the complex liver TME, this advantage is often diluted. Additionally, target expression is highly heterogeneous within tumors and among different patients, leading to ineffective recognition and killing of some cancer cells. Modifying two or more ligands targeting different antigens on a single nanoparticle, or designing TME-responsive targeting, can further improve the enrichment efficiency of actively targeted nanoparticles. Furthermore, living cells with natural tumor-homing abilities, such as mesenchymal stem cells (MSCs), macrophages, or neutrophils, can be used as biomimetic delivery carriers for nanomedicines.¹²⁷ Ongoing efforts focus on translating these laboratory-scale innovations into clinical applications, aiming to fully realize the potential of targeted therapies in HCC management.

Environment-Responsive Nanocarriers: PH/Hypoxia/Enzyme-Triggered Drug Release

Certain nanodrug delivery systems are designed to exploit the unique tumor microenvironment of HCC, which includes a variety of cell types such as aneuploid cancer cells, stromal cells, immune cells, and bioactive factors. These systems enable precise drug delivery to HCC tissues, cells, mesenchymal stromal cells, or subcellular organelles.^{128,129} Environment-responsive smart nanocarriers, including pH-responsive and hypoxia-responsive drug release platforms, are capable of adapting to changes in the tumor-associated environment (Figure 6).¹³⁰ These systems modulate drug metabolic pathways, improve pharmacokinetics and biodistribution, enhance tumor-specific drug accumulation, and amplify local therapeutic efficacy, all while minimizing systemic side effects.

For example, Duan et al developed a hyaluronic acid-geraniol-based, multi-bioresponsive, self-assembled nanodrug delivery system for the treatment of HCC. This system demonstrated high internalization efficiency, biocompatibility, and safety in laboratory studies, underscoring the potential of innovative delivery platforms in HCC therapy.¹³¹ Sun et al explored the use of *Angelica sinensis* polysaccharide (ASP) as a natural liver-targeting carrier for oridonin (ORI). ASP was esterified with deoxycholic acid (DOCA) to form an ASP-DOCA conjugate, which achieved an ORI loading capacity of up to 9.2%. The conjugate rapidly degraded in the acidic tumor microenvironment, facilitating accelerated drug release and significantly increasing ORI accumulation in liver tumors. This study confirmed the feasibility of using natural compound-based targeted delivery systems for HCC treatment.¹³²

The interaction between drugs and nanocarriers prolongs the drug's half-life in vivo, reduces dosing frequency and dosage, and minimizes the distribution of the drug in healthy tissues, thus mitigating unnecessary side effects.¹³³ While a few nanocarrier systems, such as those based on taxane drugs, have reached clinical application, their efficacy and toxicity profiles remain suboptimal.¹³⁴ Most nanodelivery platforms are still undergoing preclinical testing in murine models. Additionally, different nanomaterials exhibit distinct pharmacokinetic effects.¹³⁵ For example, liposomes or

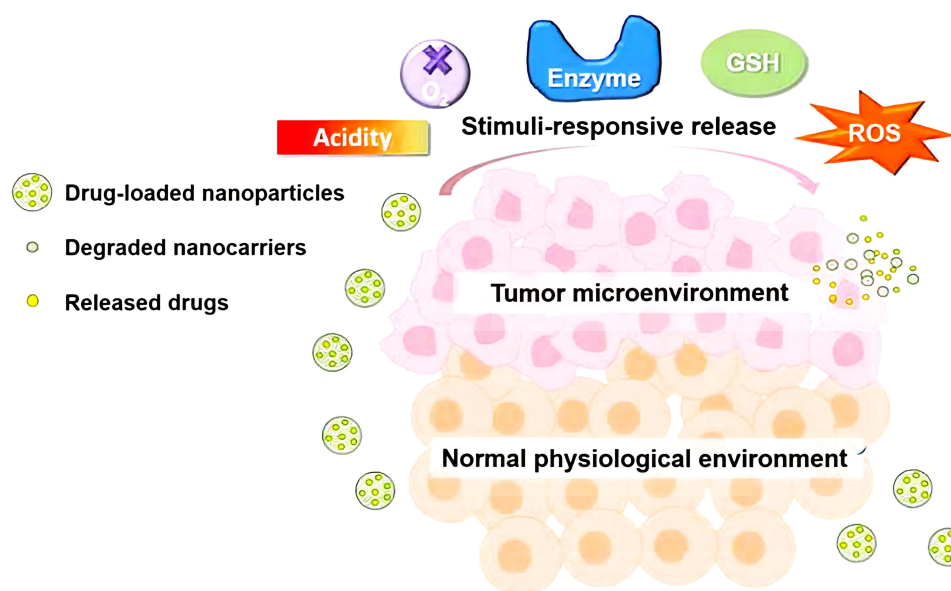


Figure 6 Schematic illustration of srNPs for controlled drug release in cancer treatment.¹³⁰

micelles, favored for their biocompatibility, protect drugs from enzymatic degradation and enhance stability. Metal-organic frameworks (MOFs), with their controllable porous structures, enable sustained drug release. Polymeric nanoparticles, which often use pH- or enzyme-responsive materials, allow for site-specific drug release. Compared to liposomal carriers, polymeric nanoparticles exhibit similar targeting specificity and low toxicity, while offering superior stability and controlled release capabilities.¹¹² Consequently, the personalized application of nanocarriers and the tailored design of anticancer drugs require further exploration. Meanwhile, the development of nanodelivery systems faces challenges such as optimizing carrier design to improve drug-loading capacity, target cell distribution efficiency, bioavailability, tumor targeting, biomembrane permeability, and enabling co-delivery with other active agents.¹³⁶

Integration of Physical Ablation and Nanotechnology

Nanoknife (Irreversible Electroporation) in Hepatic Hilar Tumors

Nanoknife technology, approved for clinical tumor ablation applications in 2012 and also known as irreversible electroporation (IRE), involves the insertion of probes into lesions to deliver high-voltage direct current pulses. These pulses generate nano-sized irreversible pores in the cell membranes, altering membrane permeability and inducing apoptosis, which leads to tumor ablation.^{137,138} Over time, the treated areas are gradually replaced with healthy tissue. In contrast, traditional physical ablation techniques for HCC—such as radiofrequency ablation (RFA), microwave ablation (MWA), and cryoablation—lack selectivity, often resulting in the damage of normal tissues, blood vessels, nerves, and bile ducts within the ablation zone. Furthermore, temperature-dependent ablation methods suffer from heat-sink effects, particularly in highly vascularized regions like the hepatic hilum, where blood flow dissipates thermal energy, thus reducing the efficacy of tumor eradication and increasing the risk of local recurrence.¹³⁹

As a novel nanoscale ablation technology, Nanoknife offers significant advantages, particularly for tumors located in challenging areas, such as the hepatic hilum or near critical structures like blood vessels or bile ducts.¹⁴⁰ Unlike traditional techniques, it operates without generating heat or relying on heat, effectively bypassing the heat-sink effects caused by adjacent vasculature.¹⁴¹ This feature allows for successful ablation of high-risk lesions, induces apoptosis while preserving tumor-associated antigens, and activates antitumor immune responses.¹⁴² Furthermore, Nanoknife is minimally invasive, and the procedure is associated with short recovery times, facilitating rapid postoperative recovery. In a study by Shi et al, the combination of IRE and immunotherapy in an orthotopic HCC mouse model resulted in enhanced tumor necrosis in both treated and off-target lesions, while promoting T-cell recruitment and reducing tumor-associated inflammatory infiltration.¹⁴³ Clinically, HCC patients typically undergo Nanoknife ablation under ultrasound

guidance using percutaneous, laparoscopic, or open surgical approaches. An early study on percutaneous IRE demonstrated lower early recurrence rates in HCC tumors compared to other ablation methods.¹⁴⁴

Despite its promising outcomes, the widespread use of IRE is limited by the complexity of preoperative anesthesia protocols and procedural workflows. To address these challenges, high-frequency irreversible electroporation (H-FIRE) has been developed. Preclinical studies suggest that H-FIRE offers simplified clinical operations, although it results in smaller ablation volumes compared to conventional IRE.¹⁴⁵ Therefore, optimizing Nanoknife protocols, reducing recurrence rates, streamlining treatment procedures, and advancing clinical translation remain crucial challenges.

Magnetic Nanoparticle-Mediated Magnetic Hyperthermia

Hyperthermia is a therapeutic approach that uses localized high temperatures to damage or kill tumor cells, often enhancing the efficacy of radiotherapy or chemotherapy.¹⁴⁶ Among various hyperthermia techniques, magnetic fluid hyperthermia is one of the most widely studied. Recently, magnetic nanoparticles (MNPs) have gained significant attention for their role in HCC hyperthermia.

Under the influence of an alternating magnetic field (AMF), MNPs absorb the externally applied energy and efficiently convert it into thermal energy, thereby raising the temperature of tumor cells loaded with these nanoparticles and inducing thermal damage.¹⁴⁷ Maintaining the environmental temperature within the coil area below physiological temperature (ie, under 37 °C) during operation is a critical design consideration (Figure 7).¹⁴⁸

Chang Pej et al explored a novel dual-targeted magnetic nanodrug combined with hyperthermia therapy for HCC. Their study employed thermoresponsive polymer-coated nanoparticles, showcasing the potential of magnetic nanoparticles for dual therapeutic targeting in HCC.¹⁴⁹ Similarly, Gharibkandi et al synthesized (198)Au-coated SPIONs for combined magnetic hyperthermia and radionuclide therapy in HCC. These nanoparticles demonstrated high saturation magnetization, enabling a temperature rise to 43°C under a 386 kHz magnetic field. This facilitated the generation of both radiotoxic effects and heat, effectively damaging HepG2 HCC cells.¹⁵⁰ Notably, multicore iron oxide magnetic nanoparticles, referred to as Sarah nanoparticles, are currently in a first-in-human clinical trial. Following intravenous

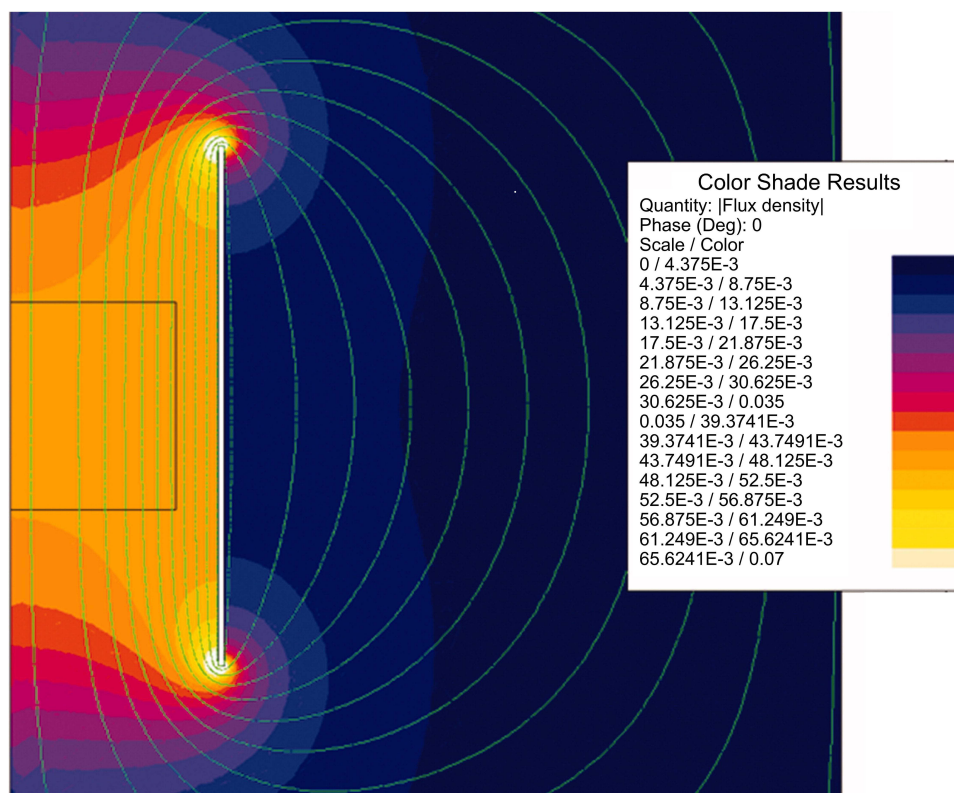


Figure 7 Computer simulation of magnetic field strength distribution in a single turn induction coil for magnetic nanoparticle hyperthermia.¹⁴⁸

administration, Sarah nanoparticles accumulate in tumor tissues, and their exposure to an external alternating magnetic field generates heat, selectively targeting and destroying cancer cells while stabilizing disease progression in patients with advanced liver cancer, and importantly, without incurring significant toxic side effects.¹⁵¹

A major challenge in the development of magnetic fluid hyperthermia is the lack of a robust theoretical model to describe energy conversion by magnetic particles in alternating magnetic fields.^{152,153} Specifically, quantifying the hysteresis loss power among interacting particles embedded in viscous media under alternating fields remains difficult, thereby limiting the reduction of energy conversion losses.¹⁵⁴ Additionally, the delivery of magnetic nanoparticles requires further refinement. When iron-based agents are administered intravenously, only a small fraction reaches the liver tumor sites. This is due to nanoparticle sequestration by the reticuloendothelial system (RES) and vascular barriers that hinder intratumoral delivery, thus impeding the clinical translation of magnetic hyperthermia.¹⁵⁵

Gene Therapy and Immunotherapy siRNA/CRISPR-Cas9 Nanodelivery Systems

Gene therapy is a treatment approach that involves modifying or replacing abnormal genes. In the context of HCC, nanotechnology facilitates the delivery of gene-editing tools or anticancer genes, overcoming the limitations of traditional gene therapy by improving delivery efficiency and targeting. Current nanocarriers used for HCC gene delivery—such as liposomes, polymeric nanoparticles, and exosomes—encapsulate siRNA, plasmid DNA, or CRISPR systems, protecting nucleic acids from enzymatic degradation. Surface modifications allow for active targeting of overexpressed receptors on HCC cells, significantly enhancing transfection efficiency.¹⁵⁶

Tumor Suppressor Gene Restoration and Oncogene Silencing

p53 Gene Delivery

More than 30% of HCC patients exhibit mutations in the tumor suppressor gene *TP53*. Nanocarrier-mediated delivery of the wild-type p53 gene can directly correct these mutations. Studies have demonstrated that cationic liposomes encapsulating p53 plasmids, such as DDC/pp53-EGFP complexes, target the HCC cell surface receptor CD44, thereby enhancing gene transfection efficiency and inhibiting tumor growth by more than 60% in murine ovarian cancer models.^{157,158}

RNA Interference Technology

RNA interference (RNAi) is an evolutionarily conserved gene-silencing mechanism that is activated by double-stranded RNA (dsRNA), leading to the degradation of target mRNA. A key component of RNAi, small interfering RNA (siRNA), integrates into the RNA-induced silencing complex (RISC) to guide the sequence-specific degradation of mRNA. SiRNA has demonstrated efficacy in inhibiting viral infections and suppressing tumor cell growth in vitro.¹⁵⁹ In the context of HCC, siRNA targeting vascular endothelial growth factors or c-Myc has been shown to impede tumor angiogenesis and cellular proliferation. In 2016, Oh et al developed galactoliposomes to target and co-deliver doxorubicin and vimentin siRNA to liver tumor cells. This breakthrough demonstrated for the first time that inhibiting vimentin protein expression via siRNA could synergistically enhance the induction of apoptosis in tumor cells when combined with doxorubicin.¹⁶⁰

RNA activation (RNAa), which involves dsRNA targeting gene promoters to induce transcription, utilizes small activating RNAs (saRNAs) as effector molecules. These molecules, chemically similar to siRNA, function in the nucleus and are designed with complementary sequences to promoter regions, thereby inducing long-term, sequence-specific gene expression.¹⁶¹ Setten et al optimized a CEBP α saRNA and encapsulated it into Marina Biotech's liposomal carrier (MTL-CEBPA) to enhance saRNA drug delivery efficiency. Studies in various animal models, including DEN-induced cirrhotic HCC rats and CCl₄-induced liver failure rats, demonstrated that MTL-CEBPA therapy reduced tumor size, improved liver function, and enhanced survival.¹⁶²

Nanotechnology-Mediated Gene Editing

CRISPR-Cas9 Gene Editing

CRISPR-Cas9 is a versatile genome-editing tool used in human cells to cleave double-stranded DNA at specific target sites. This process involves CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA), which are combined to

form a single-guide RNA (sgRNA). When the Cas9 endonuclease binds to the sgRNA and recognizes the target DNA sequence, its catalytic domains induce site-specific cleavage.¹⁶³ In cancer therapy, CRISPR/Cas9-mediated gene editing has been utilized to knock out cancer-associated genes, driving the need for enhanced targeting of gene-editing tools. Currently, multiple gene nanocarriers are under investigation to enhance this process (Figure 8).¹⁶⁴

For instance, Qi Liu et al reported a multistage nanocarrier (MDNP) that facilitates efficient CRISPR-Cas9 release for tumor-targeted delivery and restoration of endogenous microRNA expression in vivo. Their study demonstrated the simultaneous disruption of multiple signaling pathways linked to cancer proliferation in murine models, leading to significant tumor growth retardation.¹⁶⁵

Genetically Encoded DNA Origami

DNA origami is an advanced nanotechnology that exploits complementary base-pairing principles to precisely fold DNA molecules into predetermined shapes, creating unique DNA nanostructures with enhanced functionalities^{166,167} (Figure 9).

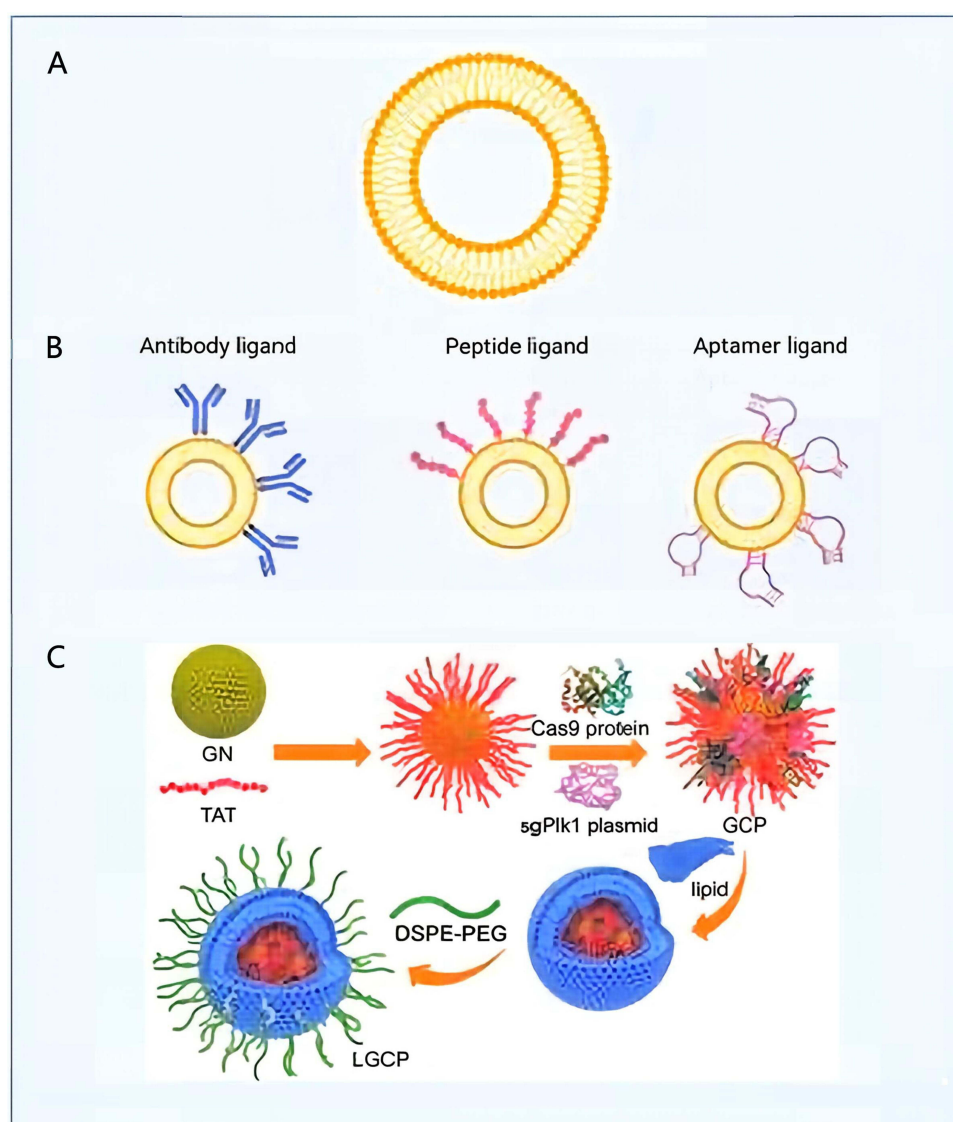


Figure 8 Lipid-based strategies for the delivery of CRISPR/Cas9.¹⁶⁴ (A) Lipid-based delivery approaches. (B) Surface modification of lipid-based delivery systems for cell targeting. (C) An example lipid-based CRISPR/Cas9 carrier in the literature.

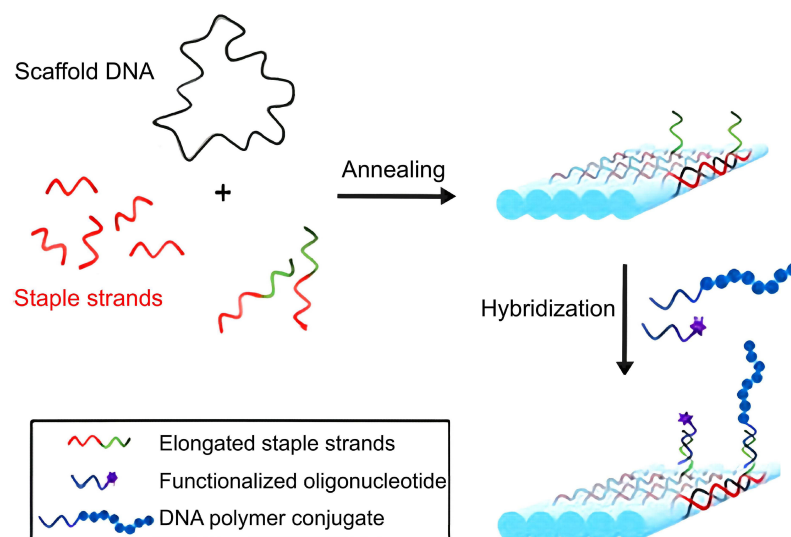


Figure 9 Schematic diagram of the principle of DNA origami.¹⁶⁷

The design and fabrication of DNA origami nanostructures (DONs) with specific targeting capabilities can reduce harm to healthy cells. DONs serve as protective shells, shielding antitumor drugs from degradation in the hepatocellular tumor microenvironment and under physiological conditions, thereby improving drug stability and extending circulation half-life.¹⁶⁸ Additionally, the customizable nature of DNA origami allows for the creation of carriers that integrate multiple therapeutic agents, such as chemotherapeutics and targeted drugs, enabling combinatorial treatment regimens that include immunotherapy and gene therapy. For example, loading chemotherapeutic and targeted drugs onto DONs has the potential to address drug-resistant hepatocellular tumors.¹⁶⁹

In Zhang et al's study, DNA origami, gold nanorods, and molecularly targeted drugs were co-incorporated into pH-responsive calcium phosphate nanoparticles to enhance the drug-loading capacity. A thin phospholipid coating was applied to improve biocompatibility. This resulting nanocomposite not only protected DNA origami from degradation but also facilitated the synergistic co-delivery of DNA origami nanostructures and anticancer drugs, thereby inducing apoptosis and reducing multidrug resistance.¹⁷⁰

Nanovaccines Combined with Immune Checkpoint Inhibitors

Cancer immunotherapy aims to boost the immune system's antitumor response while minimizing off-target effects, in contrast to conventional chemotherapy. Immunotherapeutic agents typically activate or amplify immune responses to counteract cancer's immune evasion mechanisms.¹⁷¹ Nanovaccines, which employ nanomaterials to deliver specific antigens and adjuvants, offer unique advantages in targeted tumor immunotherapy, including prolonged drug circulation, enhanced therapeutic efficacy, and improved prophylactic potential. Zhang et al developed a self-assembled, carrier-free multi-component nanovaccine (SVMAV) and established the HLS@SVMAV platform for neoantigen-targeted personalized cancer vaccines. In orthotopic HCC mouse models, SVMAV treatment significantly reduced tumor size, whereas anti-PD-1 therapy showed limited efficacy. Notably, SVMAV treatment did not significantly alter M1 macrophage levels, highlighting its potential as an innovative strategy for HCC treatment.¹⁷²

Immunotherapy can also be combined with gene therapy, wherein RNA molecules activate innate and adaptive immune responses by silencing or upregulating immune-related genes, thus enabling highly selective modulation of targets to minimize off-tissue effects. Various nanomaterials have been developed to enhance RNA delivery to tumors and immune cells (Figure 10).¹⁷³

Potential siRNA targets for HCC-specific nanocarriers include FA-PEI polymers targeting PD-L1 for immune checkpoint blockade and ROS-responsive nanoparticles targeting TGF- β to reprogram the immunosuppressive microenvironment.¹⁷⁴ Additionally, for HCC cases with reduced T-cell infiltration due to PTEN-related defects, nanoparticle-loaded mRNA can enhance PTEN expression in tumors, thereby promoting CD8⁺ T-cell infiltration. For

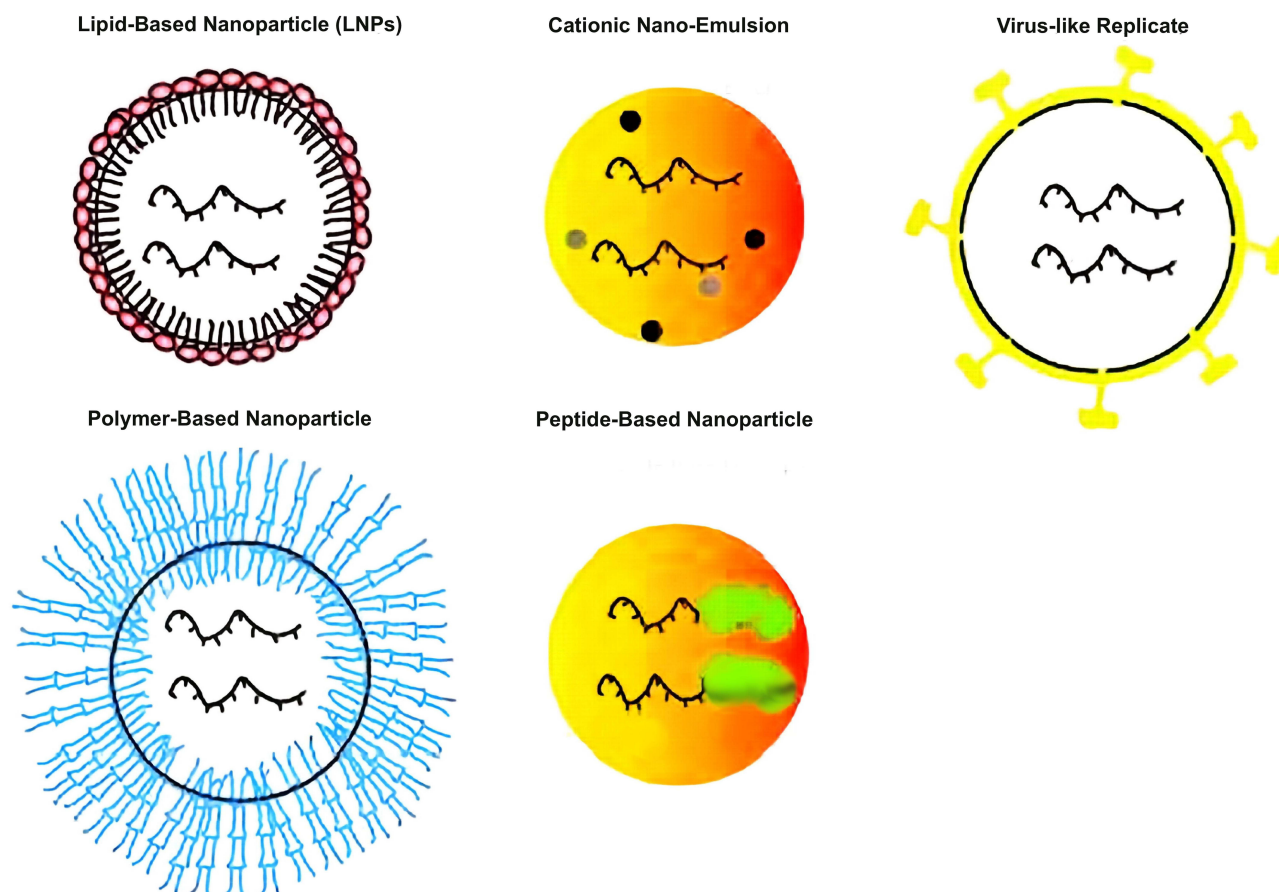


Figure 10 Various of nano-delivery vectors for mRNA vaccines.¹⁷³

example, CLAN nanoparticles (cationic lipid-assisted nanoparticles) deliver siRNA to silence lactate dehydrogenase A (LDHA), reversing tumor acidosis and restoring T-cell antitumor function.¹⁷⁵

Metal-Immunotherapy

Since HCC is known for abnormal angiogenesis and severe hypoxia-induced immunosuppression, the hypoxic conditions of the TME are also a critical barrier to the effectiveness of HCC immunotherapy. Hypoxia not only inhibits the degree of T cell infiltration and functional response but also promotes immunosuppression. Therefore, targeting the hypoxic immunosuppressive microenvironment of HCC using oxygen-supplying nanomaterials for re-oxygenation is a highly promising strategy to enhance the efficacy of immunotherapy.¹⁷⁶ Hu et al studied a metal-immunotherapy, utilizing Co +Len@OVA nanoclusters (ovalbumin nanocarrier) to co-deliver cobalt ions and lenvatinib. Cobalt ions induce ROS generation and activate the cGAS-STING pathway, affecting redox status, while lenvatinib normalizes tumor vasculature and promotes the penetration of anti-PD1 antibodies into tumors, providing a clinically translatable strategy for unresectable HCC.¹⁷⁷

Nanozyme Catalytic Therapy

As previously discussed, nanozymes are synthetic materials that combine the unique properties of nanomaterials with enzyme-mimicking catalytic functions. By integrating the advantages of nanomaterials and natural enzymes, nanozymes exhibit tunable catalytic activity, low cost, and high stability, effectively circumventing challenges such as enzymatic degradation and the storage/transport difficulties associated with natural enzymes.^{178,179} Consequently, nanozymes have attracted significant research attention. Nanozymes applied in biotherapy primarily mimic the activity of oxidoreductases, including peroxidases, catalases, superoxide dismutases, and oxidases. Recent advancements have also introduced

nanozymes with glucose oxidase-like (GOD) and NADH oxidase-like (NOX) properties. Nanozymes with diverse enzyme-like activities have demonstrated significant efficacy across a range of tumor therapies.¹⁸⁰

Biomimetic Dual-Inorganic Nanozymes Inducing ROS Cascade Reactions

Gao et al developed a biomimetic dual-inorganic nanozyme platform based on ultra-small gold nanoparticles (Au NPs) and Fe_3O_4 nanoparticles co-loaded onto dendritic mesoporous silica nanoparticles (DMSNs). The Au NPs mimic glucose oxidase (GOD) to catalyze glucose into hydrogen peroxide (H_2O_2), while the Fe_3O_4 NPs function as peroxidase mimics, converting H_2O_2 into highly toxic hydroxyl radicals ($\cdot\text{OH}$) via the Fenton reaction, thereby inducing tumor cell death. Both in vitro and in vivo experiments demonstrated robust tumor suppression, achieving a tumor suppression rate of 69.08% (Figure 11).¹⁸¹

In a similar study on biomimetic dual-inorganic nanozymes, Wang et al reported a tumor-specific precision therapeutic strategy using CD44-targeted mesoporous silica/AuNP nanozymes (MMSN/AuNPs). By synthesizing nanocatalysts with glucose oxidase-like (GOD) and peroxidase-like (POD) activities, they triggered cascade reactions within the tumor microenvironment to induce apoptosis. Preliminary in vivo experiments in murine models demonstrated that these nanocatalysts, through CD44-specific tumor targeting, exhibited excellent biocompatibility, enhanced catalytic cascade efficacy in HCC treatment, and effectively inhibited hepatoma cell growth.¹⁸²

Ferroptosis and Calcium Overload Pathways Activated by Nanozyme Strategies

Nanozymes can also induce regulated cell death by activating various signaling pathways, such as ferroptosis, calcium overload, pyroptosis, and autophagy, to eliminate liver tumor cells.¹⁸⁰ For example, Fan et al developed nitrogen-doped porous carbon nanospheres (N-PCNSs) as nanozymes for tumor catalytic therapy. Through ferritin-mediated targeting,

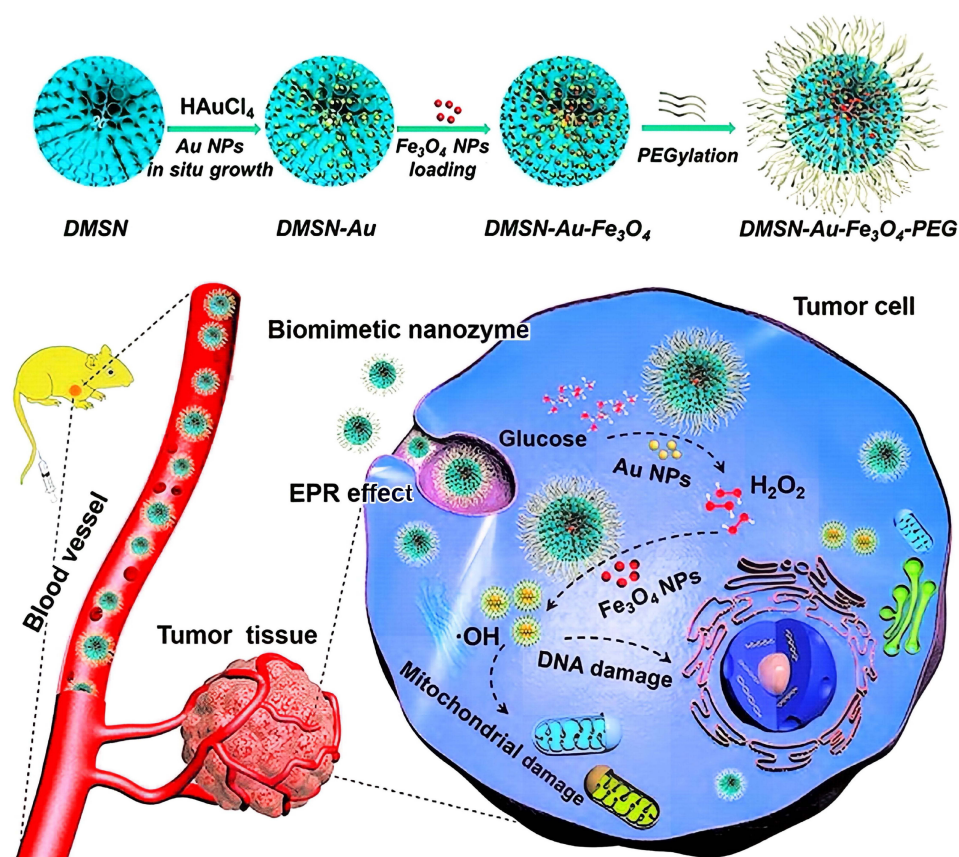


Figure 11 Schematic illustration of “toxic-drug-free” nanocatalytic tumor therapy by biomimetic inorganic nanomedicine-triggered cascade catalytic reactions.¹⁸¹

N-PCNSs localized to tumor cell lysosomes, exerting oxidase-like and peroxidase-like activities in acidic microenvironments to elevate ROS levels, induce cell death, and improve survival in tumor-bearing mice.¹⁸³

Currently, nanozyme design predominantly focuses on functional optimization from a materials science perspective, but interdisciplinary gaps hinder clinical translation, with most applications remaining confined to experimental models. Given the complex pathological features and individual heterogeneity of HCC, future nanozyme development must prioritize precise targeting of these characteristics to enhance clinical applicability.

Classification Framework for Synergistic Nanomedicine in HCC: Single-Platform Multimodality vs Regimen-Level Combinations

To avoid redundancy, this section classifies synergistic HCC nanomedicine by the level at which integration occurs. “Single-platform multimodality” refers to co-integrating two or more diagnostic/therapeutic functions within one nanoplatform (eg, dual-modal imaging plus photo- or chemo-therapy). In contrast, “regimen-level combinations” refer to combining distinct treatment modules (eg, TACE, radiotherapy, immunotherapy, gene therapy, PDT/PTT/SDT) where nanotechnology primarily acts as a delivery, sensitization, or microenvironment-reprogramming enabler. Each subsection below is therefore organized around distinct technical characteristics and therapeutic goals.

Single-Platform Multimodal Theranostic Nanoplatfoms

Integrated theranostic nanoplatfoms combine both diagnostic and therapeutic functions within a single system, enabling simultaneous monitoring, treatment, and prognosis of HCC. These platfoms typically exhibit targeting specificity, multifunctionality, and synergistic effects.

Nanomaterials with Dual Imaging and Therapeutic Functions

Chen et al introduced PEGylated melanin nanoparticles (MNPs), a multifunctional biocompatible nanoreagent conjugated with the near-infrared (NIR) dye IR820. This system enhances photoacoustic (PA) imaging and photothermal therapy (PTT), achieving a photothermal conversion efficiency of 40.2%. It allows for selective tumor ablation under PA/MR imaging guidance in orthotopic HCC mouse models, demonstrating potential for in vivo detection and ablation of micro-HCC lesions.¹⁸⁴

In a recent study, Sun et al developed a multifunctional nanodelivery carrier, LPSD-DOX/siRNA, loaded with oleic acid-modified superparamagnetic iron oxide nanoparticles (OA-SPIONs) and the antineoplastic drug doxorubicin (DOX), and modified with DOTAP to deliver GPC3-targeting siRNA. This integrated nanotechnology system facilitates combined HCC therapy and real-time tumor imaging. The nanocarrier extends DOX circulation time, enhances tumor-specific drug accumulation, and exhibits high drug-loading capacity, high gene transfection efficiency, and low toxicity, showcasing significant potential for HCC theranostics.¹⁸⁵ Lai et al designed and synthesized a Zn(II)-Schiff base complex-based nanotheranostic platform (Zn-MTDH) that combines real-time fluorescence imaging with pH-responsive drug release, specifically targeting lysosomes. This platform demonstrates synergistic imaging and therapeutic effects in cancer cells, with enhanced drug release in acidic environments, providing a promising new strategy for theranostic integration in HCC. This approach exemplifies how targeted nanomedicines can enhance both the diagnostic and therapeutic capabilities of nanotechnology in HCC treatment.¹⁸⁶ Integrated nanoplatfoms leverage nanotechnology to combine imaging and therapeutic modalities, enabling real-time monitoring of tumor dynamics during targeted drug delivery or treatment. This approach allows for dynamic optimization of therapeutic strategies.

Nanoprobe Imaging for Intraoperative Navigation

During HCC surgery, precise preoperative tumor localization and intraoperative real-time monitoring are critical due to the high vascularity of HCC, its propensity for microvascular invasion, and the metastatic risk. Nanoparticles with external responsiveness (eg, light, ultrasound, and magnetic fields) offer tumor-targeting, fluorescence imaging, and real-time monitoring capabilities, facilitating accurate intraoperative detection and resection with improved visualization and efficiency.¹⁸⁷

Since liver tumors are prone to metastasize to surrounding tissues via blood vessels and lymphatic channels, injecting near-infrared (NIR) nanoscale imaging agents during surgery can effectively identify the extent of tumor invasion and determine the necessary surgical resection margins. NIR fluorescence imaging, a non-invasive, real-time, cost-effective, and highly sensitive technique, is widely studied for surgical navigation. Advances in nanoimaging optimize spatial resolution, penetration depth, and reduction of optical interference from biological substrates, enhancing surgical precision.¹⁸⁸ A key application involves indocyanine green (ICG) fluorescence imaging for real-time navigation and resection line planning in hepatectomy. This method ensures complete tumor excision and has proven valuable in HCC management.¹⁸⁹

Fang et al integrated ICG molecular fluorescence imaging with a 3D laparoscopic augmented reality (AR) surgical navigation system for liver resection. By projecting preoperative 3D models onto the surgical field alongside ICG fluorescence, their system reduced intraoperative blood loss and complication rates, demonstrating the clinical potential of multimodal imaging-guided surgery.^{190,191}

Additionally, a fluorescent nanoemulsion serves as a novel contrast agent to illuminate HCC cells during surgical navigation. By employing self-emulsifying nanotechnology to combine indocyanine green (ICG) and lipophilic alcohols, this system enables optical surgical navigation, allowing for more precise delineation of tumor tissue margins.¹⁹²

Postoperative/Local Control Enabled by Multifunctional Biomaterials and Magnetic Hyperthermia

Monosurgical treatment for HCC is often limited by postoperative recurrence, intraoperative blood loss, and poor patient prognosis. Therefore, the development of multifunctional biomaterials is critical to improving therapeutic outcomes in HCC. In 2023, Gong et al developed an injectable magnetic nanocomposite hydrogel loaded with mica nanosheets and magnetic iron oxide nanoparticles. This hydrogel seals bleeding liver margins after minimally invasive hepatectomy and prevents recurrence via magnetic hyperthermia ablation, while also suppressing unresectable HCC tumors through magnetothermal therapy.¹⁹³

Regimen-Level Combination Strategies Enabled by Nanotechnology

Chemo-Radio-Immunotherapy Trimodal Nanosystems

Nanotechnology-Enhanced TACE

TACE blocks the proximal blood supply to tumors using embolic agents, depriving them of essential nutrients and oxygen while simultaneously delivering sustained chemotherapy to induce necrosis and apoptosis.¹⁹⁴ For patients resistant to conventional TACE, the use of sorafenib (SOR)—an oral multi-kinase inhibitor—has proven effective in suppressing tumor proliferation and angiogenesis, while preserving liver function and mitigating disease progression, such as extrahepatic spread or vascular invasion.¹⁹⁵ Despite its widespread use in improving survival rates for advanced HCC, sorafenib faces certain limitations, including side effects such as rash, diarrhea, and others. Additionally, its poor solubility and rapid metabolism result in low absorption efficiency in tumor tissues. Furthermore, some patients either have an inherent resistance to sorafenib or develop resistance after treatment.¹⁹⁶

Recently, various types of nanoparticles—such as polymeric, lipidic, silica, and metallic nanoparticles—have been extensively explored to enhance the efficacy of sorafenib. These nanoparticles can improve the pharmacokinetic properties of sorafenib, increase its solubility in tumor tissues, and enhance its bioavailability (Figure 12).¹⁹⁷ Furthermore, nanoparticles can control the drug release rate, achieve sustained drug release at the tumor site, and prolong the duration of the drug's action. For example, Zheng et al synthesized thermosensitive composite nanoparticles as an effective carrier for sorafenib and combined them with radiotherapy for local and sustained treatment of liver cancer. This approach not only enables continuous sorafenib release but also exhibits site-specificity and long-term anti-cancer effects.¹⁹⁸

By enhancing drug targeting and efficacy, nanoparticles can reduce the dosage and frequency of sorafenib administration while maintaining its therapeutic effects. This approach not only minimizes the drug's side effects but also improves patient compliance. Although nanotechnology has not yet been integrated into the clinical standard protocol for TACE, experimental studies have demonstrated its potential to improve the targeting of embolic agents and reduce liver toxicity.

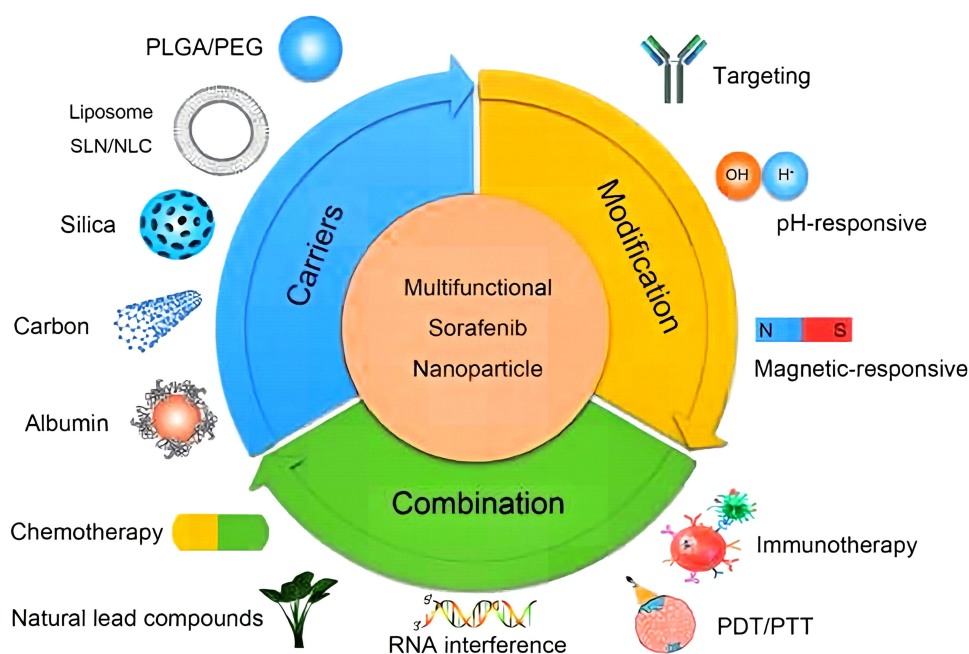


Figure 12 Research directions in the development of SOR-NPs for HCC.¹⁹⁷

Nanotechnology Combined with Radiotherapy

Nanotechnology-based radiotherapy aims to enhance the efficacy of conventional radiotherapy by utilizing the unique physical and chemical properties of nanomaterials to overcome the radioresistance of cancer cells.^{199,200} Radiotherapy (RT) uses high doses of electromagnetic radiation to directly kill tumors or cancer cells, or to inhibit their ability to proliferate by damaging their genetic material.²⁰¹ However, conventional radiotherapy poses a significant risk to normal tissue cells. Additionally, due to the unique characteristics of liver tumors—such as acidic pH and high levels of ROS—tumors may develop resistance to radiotherapy.

Nanomaterials can act as radiosensitizers, enhancing the effectiveness of radiotherapy through various mechanisms. These nanoparticles can preferentially penetrate and accumulate in cancer tissues, thereby increasing the radiation dose at the tumor site and reducing radiation exposure to normal tissues. This improves the specificity of radiotherapy, while simultaneously reducing toxicity and damage to healthy tissue.²⁰⁰

High atomic number metal nanoparticles, when exposed to radiation, undergo photoelectric absorption, releasing photons or electrons. The Auger electrons generated during this process can induce the radiation decomposition of surrounding water molecules, producing ROS such as oxygen radicals.^{202,203} These ROS enhance the sensitivity of cancer cells to radiation, leading to cellular damage and eventual cell death. In a report by Saeed et al, gold nanoparticles were found to play a radiosensitizing role in cancer cells, with the main biological responses including the production of ROS, oxidative stress, DNA damage induction, cell cycle disruption, and potential interference with the bystander effect.²⁰⁴ Additionally, nanoparticles can serve as carriers for radioactive isotopes, such as Y-90 glass microspheres, which can be locally delivered through hepatic artery infusion for embolization. In a study by Christopher et al, the combination of Y-90 microspheres and nano photosensitizers significantly enhanced oxidative stress in both cells and mitochondria, promoting the death of liver tumor cells. The inclusion of nano photosensitizers reduced the required Y-90 radiation dose, thereby minimizing damage to normal tissues while demonstrating good safety for healthy liver tissue.²⁰⁵

Application of Nanotechnology in Immunotherapy

Immunotherapy for cancer aims to boost the immune system's ability to target and destroy tumor cells while minimizing off-target effects, in contrast to conventional chemotherapy and other drugs that directly kill cancer cells.^{206,207} In

immunotherapy, drugs are often employed to activate or enhance the immune response, enabling it to attack cancer cells through natural mechanisms that are often evaded during disease progression.¹⁸²

Nanotechnology-mediated immunotherapy leverages the unique properties of nanomaterials to modulate the tumor immune microenvironment (TIME) and stimulate the immune system, thereby amplifying the therapeutic effect on liver tumors.^{208,209} Regulatory T cells (Tregs) are known to suppress anti-tumor immune responses in the TIME of HCC. The development of fluorine-assembled nanoparticles, which contain perfluorocarbon to alleviate hypoxia in the TIME, has been shown to inhibit Treg proliferation and infiltration, while also releasing chemical prodrugs that reduce Foxp3 expression in Tregs, reversing the immunosuppressive effect in liver tumors.^{210,211} Myeloid-derived suppressor cells (MDSCs) also play a significant immunosuppressive role in the tumor microenvironment of HCC. High-density lipoprotein nanoparticles synthesized to target scavenger receptor B-1, which is expressed on MDSCs, can significantly inhibit the activity of MDSCs, thereby suppressing tumor growth.¹⁷⁵ Furthermore, nanomedicines can be engineered to sense stimuli within tumors, activating specific immune responses, or equipped with ligands to increase drug concentrations in tumor tissues. These nanoparticles can achieve subcellular delivery, trigger immune signals, and promote immune cell proliferation, selectively exerting immunomodulatory functions, thus avoiding systemic immunosuppressive toxicity and mitigating adverse immune reactions.^{212,213}

CAR-T Cell Therapy and Nanotechnology

Nanomaterials have been utilized to enhance CAR-T cell delivery, persistence, and function within the immunosuppressive HCC microenvironment. For example, nanoparticles loaded with cytokines or engineered to modulate the TME can improve CAR-T cell tumor homing, activation, and longevity. Specifically, nanoformulations have shown the ability to counteract barriers such as poor T-cell infiltration and antigen heterogeneity, common challenges in solid tumors like HCC.^{214,215}

Current research is focused on optimizing nanobody-based CAR-T constructs that target GPC3, a tumor-associated antigen that is overexpressed in approximately 70% of HCC cases. These constructs, through engineering of hinge and transmembrane domains, achieved robust antitumor activity, with even complete tumor eradication in preclinical models.²¹⁶ Additionally, CAR-T cells engineered to secrete bispecific T-cell engagers (BiTEs) targeting HCC antigens like AFP have shown enhanced proliferation, persistence, and cytotoxicity when combined with nanopatforms.²¹⁷

Bispecific Antibodies and Nanotechnology

The integration of bispecific antibodies with nanotechnology represents a promising advancement in the therapeutic management of hepatocellular carcinoma. Bispecific antibodies that engage CD3 on T cells and GPC3 on HCC cells have demonstrated potent antitumor activity in preclinical models. To improve their pharmacokinetics and tumor accumulation, these bispecific antibodies have been conjugated with nanocarriers, which enhance bioavailability, tumor retention, and circulatory half-life.^{60,218}

Notably, GPC3-targeted bispecific antibodies, constructed using formats such as knob-into-hole or scFv-hFc-scFv, have shown efficient killing of GPC3-positive HCC cells in vitro and in vivo.²¹⁹ When combined with chemotherapeutic agents like irinotecan, or delivered via multifunctional nanovesicles targeting both GPC3 and immune receptors (eg, CD16A on NK cells), these bispecific antibodies elicit stronger immune activation and tumor suppression.²²⁰ Furthermore, bispecific antibodies targeting immunosuppressive elements in the TME, such as CXCL12 and PD-1, have shown promise in reprogramming the HCC microenvironment to support T-cell function.²²¹

Regimen-Level Synergy in Nano-Enabled Immunotherapy Combinations

Multimodal approaches combining nanotechnology, CAR-T cells, and bispecific antibodies hold immense potential. Nanoparticles can co-deliver chemotherapeutics, immune checkpoint inhibitors (eg, anti-PD-1), or immunomodulators to synergistically enhance CAR-T or bispecific antibody efficacy.²²² For instance, nanopatforms enabling combined photothermal therapy (PTT) and immunotherapy have achieved >90% tumor inhibition in liver cancer models while activating systemic immunity with minimal toxicity.²²³

Furthermore, locoregional delivery strategies—such as intra-tumoral or hepatic artery infusion of nanocarrier-formulated immunotherapies—are being explored to overcome systemic limitations and increase intratumoral CAR-T

or bispecific antibody concentrations.²²⁴ These strategies address key challenges in HCC, including heterogeneous antigen expression and the immunosuppressive TME.^{225,226}

Tri-Modal Nanotherapy System

The tri-modal nanosystem, which combines chemotherapy, radiotherapy, and immunotherapy, targets HCC tumor cells from multiple pathways, thereby improving treatment efficacy. Nanoanticancer drugs can target and eliminate rapidly proliferating tumor cells, while nanoradiotherapy sensitizers enhance radiotherapy by amplifying the ionizing radiation effect, leading to direct DNA damage in tumor cells and their subsequent death.^{173,227,228} Immunotherapy, on the other hand, activates the body's immune system and specifically regulates the tumor immune microenvironment, allowing immune cells to recognize and destroy tumor cells. This multi-pathway treatment approach offers a comprehensive attack on tumor cells, specifically eliminating drug-resistant cancer cells, synergistically enhancing the anti-tumor effect, and reducing tumor cell escape and recurrence.^{229–232} Furthermore, chemotherapy and radiotherapy can stimulate immunotherapy by releasing tumor antigens and immune-stimulating signals, which are recognized by the immune system, providing more targets for immunotherapy and enhancing antigen presentation and T cell activation. Nanotechnology ensures the effective delivery, release, and accumulation of these drugs and therapeutic components at the tumor site, thereby further boosting the immune response.²³³

Synergistic Mechanisms of Nanophotodynamic Therapy and Gene Silencing

PDT is a minimally invasive, low-toxicity clinical treatment option for advanced HCC that relies on the interaction between photosensitizers, light sources, and oxygen molecules.²³⁴ The core mechanism of PDT is the activation of photosensitizers by specific wavelength light, leading to the generation of ROS, which ultimately kill tumor cells.²³⁵ With advancements in nanotechnology, PDT can be enhanced by utilizing nanocarriers to encapsulate or attach photosensitizers, thereby increasing their stability and improving their targeting efficiency within the body, making photosensitizer delivery more effective.

In recent years, numerous studies have investigated the use of various nanoscale compounds or nanoparticles to enhance the efficacy of PDT in targeting HCC cells. Da Zhang et al developed a smart Cu(II)-aptamer complex-based nanoplatform designed for tumor microenvironment-triggered, programmable prodrug release, and targeted PDT for HCC. This platform utilizes PDT activation based on the redox reactivity of tumor cells and low pH-triggered chemotherapy drug release, positioning it as a promising co-therapy for HCC treatment.²³⁶ In Li et al's study, titanium dioxide nanoparticles were employed as nanoscale photosensitizer materials, inducing endoplasmic reticulum stress in liver cancer cells through ROS generation. This mechanism inhibited liver cancer cell growth, increased apoptosis, elevated intracellular ROS levels, and arrested the cell cycle at the G1 phase, thereby mediating PDT to reduce the size of liver tumor cells.²³⁷

Nanotechnology has significantly enhanced the efficacy of PDT in treating HCC by optimizing photosensitizer delivery, regulating the tumor microenvironment, and integrating multimodal therapies while also silencing the expression of oncogenes. However, PDT is limited by its reliance on visible light with poor tissue penetration, necessitating the use of fiber optic intervention and the integration of imaging techniques for real-time monitoring of photosensitizer distribution and oxygen concentration. Therefore, further research is required to improve PDT through nanotechnology, particularly in reducing the reliance on external light sources in medical applications and enabling intelligent, triggerable systems.

Nano-Based Immunotherapy Combined with Gene Therapy

Immunotherapy can be combined with gene therapy for more effective treatment. RNA molecules can activate both innate and adaptive immune responses by silencing or upregulating immune-related genes.^{174,238} This approach offers high selectivity for specific targets and reduces the risk of off-target effects. Various nanomaterials have been developed to enhance the delivery of RNA to tumor cells and immune cells.^{239,240} In the context of HCC, if issues such as PTEN gene-related dysfunction lead to reduced T cell infiltration, mRNA can be loaded onto nanoparticles to enhance its expression within tumors and promote CD8⁺ T cell infiltration.^{241,242} Additionally, vascular CLAN nanoparticles,

developed as siRNA delivery systems, can silence lactate dehydrogenase A (LDHA), thus reversing the acidic tumor environment and restoring the anti-tumor function of T cells.¹⁷⁵

Defining and Quantifying Synergy Across Modalities

In the context of HCC nanotheranostics, “synergy” transcends the simple coexistence of multiple therapeutic modalities; it embodies a comprehensive closed-loop paradigm that integrates diagnostics, targeted delivery, therapy, feedback monitoring, and prognostication. Within this framework, nanoparticle imaging facilitates precise localization and phenotyping of lesions, while stimulus-responsive delivery systems allow for both spatial and temporal control of drug release in the tumor microenvironment. Collectively, multimodal therapies target tumor cell death, and the identification of post-treatment biomarkers—such as circulating tumor DNA (ctDNA), exosomes, and metabolites—can inform subsequent therapeutic adjustments and recurrence monitoring. This nuanced interaction underscores that true synergy must surpass mere additive benefits.

Sonodynamic therapy has the potential to synergize effectively with other therapeutic strategies. For instance, in a study by Ma et al, cationic lipid nanobubbles (miR-15a-5p/CUR-NBs) were engineered to co-load a sonosensitizer, curcumin, alongside the microRNA miR-15a-5p. Upon ultrasound activation, this system not only facilitated sonodynamic cell death but also released miR-15a-5p, which effectively suppressed the translation and expression of PD-L1 protein in tumor cells. Preclinical investigations indicated that this dual approach significantly increased the infiltration of cytotoxic T lymphocytes (CTLs) in the tumor microenvironment and reprogrammed immunosuppressive M2 macrophages into the anti-tumor M1 phenotype, thereby fostering an enhanced anti-tumor immune response.²⁴³

Translational Considerations for Synergistic HCC Nanomedicines

Qi et al developed the ICG/Mn-PDA-PEG-CXCR4 (IMPP-c) multifunctional nanoplatform enabling PA/MR dual-modal imaging for high-resolution localization of early micro-HCC, followed by precise photothermal therapy, while simultaneously allowing in vivo tracking of nanoparticle disposition; treated animals showed no abnormal signs or weight loss, supporting favorable tolerability in addition to theranostic coupling.²⁴⁴ Furthermore, chemotherapy resistance poses a significant challenge in the management of HCC. Synergistic nanotheranostic strategies have been shown to ameliorate chemoresistance to a considerable extent. To illustrate quantitative enhancement in multimodal treatment, Chen et al designed a nanoco-delivery system for sorafenib + PD-L1 siRNA, achieving >90% tumor growth inhibition in animal models with enhanced antitumor immune activation, consistent with a mechanistically coupled chemo–gene immunomodulatory strategy.²⁴⁵ Similarly, Bai et al reported a metalloimmunotherapy–chemotherapy strategy using an iron(III)-covalent organic framework nanoplatform loaded with sorafenib, reaching a 93% tumor inhibition rate in mouse models.²⁴⁶ However, improvements in efficacy over single-agent therapies alone are insufficient to substantiate claims of synergistic interactions. It is imperative to quantitatively assess synergy against a predefined additivity model. Employing methodologies such as the CI and Bliss or Loewe analyses derived from dose–response matrices in rigorously controlled experimental designs will provide a more comprehensive understanding of the synergistic potential of multimodal treatment strategies.

Clinical Translation of Nanomedicines for HCC

Nanoparticle-based drug delivery systems have shown promise in improving the treatment of hepatocellular carcinoma (HCC) by enhancing drug targeting, reducing systemic toxicity, and overcoming drug resistance.^{52,55} For example, liposomal formulations encapsulating doxorubicin (DOX) have been developed as smart nanomedicines for HCC therapy. One such system involves DOX encapsulated in ferric oxide-incorporated liposome nanovesicles, which demonstrated improved therapeutic efficacy in mimicking tumor microenvironments.²⁴⁷ Although conventional chemotherapy for HCC is limited by poor drug delivery and an immunosuppressive microenvironment, nanomedicine strategies—such as metal-drug self-delivery systems or multifunctional nanoplatforms—offer potential solutions to enhance precision and reduce off-target effects.^{248,249}

Ongoing Clinical Translation Efforts

While clinical approval of HCC-specific nanomedicines remains limited, preclinical studies are actively exploring advanced platforms. These include all-in-one therapeutic nanoplateforms (eg, FTY720@AM/T7-TL) designed for synergistic therapy,²⁵⁰ siRNA-loaded micelles targeting cancer stem cell markers like CD24 to overcome lenvatinib resistance,²⁵¹ and aptamer/peptide-modified lipid nanoparticles (eg, A54-PEG-SLN/OXA) that exhibit strong tumor cell targeting and antitumor efficiency in vitro.²⁵² Furthermore, decellularized HCC-on-a-chip models using 3D co-cultures of HepG2 and L02 cells on decellularized extracellular matrix (dECM) have been proposed to better predict nanomedicine efficacy by recapitulating the in vivo tumor microenvironment.²⁴⁷ Transarterial delivery approaches, such as TACE, provide a favorable route for localized nanomedicine administration, though challenges like postoperative residual tumor and recurrence persist.²⁵³

Challenges and Prospects

Current Challenges

After years of in-depth research, numerous studies on nanomedicine delivery systems have been published in the field of medical nanotechnology. Some targeted nanomedicines for HCC have entered clinical trials. However, most research has been conducted using cell models or laboratory mouse models, and successful translation into large-scale clinical applications remains elusive. Due to the heterogeneity of human diseases and the inherent differences between animal models and humans, there are discrepancies in the efficacy and toxicity of nanoparticles between preclinical animal studies and human clinical trials. These differences hinder accurate simulation of the tumor microenvironment of human HCC. Therefore, overcoming biological barriers and addressing the liver's shielding effect, based on the liver's characteristics and the mechanisms underlying HCC, is critical for guiding the rational design of targeted nanotherapies for HCC.²⁵⁴

Biological Barriers and the Liver's Shielding Effect

Special Shielding Effect of the Liver

The liver is known for its high tolerance to antigens and its unique immune microenvironment. This immune tolerance can be considered a form of immune shielding, helping to resist pathogens and tumor cells while preventing excessive immune responses to harmless antigens, particularly those originating from the intestine. However, in certain cases, the liver's immune system may become dysregulated. For example, chronic liver inflammation caused by viral hepatitis can lead to immune tolerance, allowing tumor cells to evade immune surveillance and promoting cancer development.²⁵⁵ Concurrently, the infiltration of immunosuppressive cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells, increases in the liver. The imbalance between effector T cells and regulatory T cells can suppress tumor immune responses, creating an immunosuppressive microenvironment (Figure 13).²⁵⁶ This imbalance interferes with the ability of immunotherapy drugs to activate the immune system to recognize and eliminate tumor cells during the treatment of HCC.²⁵⁷

In some instances, the liver's high metabolic activity can serve as a "shield." Studies have demonstrated that most orally or intravenously administered drugs are first concentrated in the liver and subsequently metabolized and excreted, leading to reduced bioavailability and therapeutic effects that fall short of expectations. In cancer treatment, the liver can metabolize certain chemotherapy drugs, potentially impacting both their efficacy and toxicity. In contrast, for liver cancer patients with impaired liver function, drug metabolism may be disrupted, which could trigger adverse drug reactions.

Mechanism of Nanoparticle Entry into the Liver

Although nanoparticles possess superior tumor enrichment properties compared to traditional drugs, the liver's barrier function against foreign substances remains a significant challenge. To explore the potential of nanotechnology in the treatment of HCC, it is essential to understand the mechanisms through which nanoparticles enter the liver.

Nanoparticles reach the liver through the bloodstream, where the EPR effect plays a critical role. Upon entering the liver, nanoparticles are absorbed by the reticuloendothelial system (RES), which is comprised of Kupffer cells responsible for clearing foreign substances from the body. This presents a challenge, as it prevents nanoparticles from entering

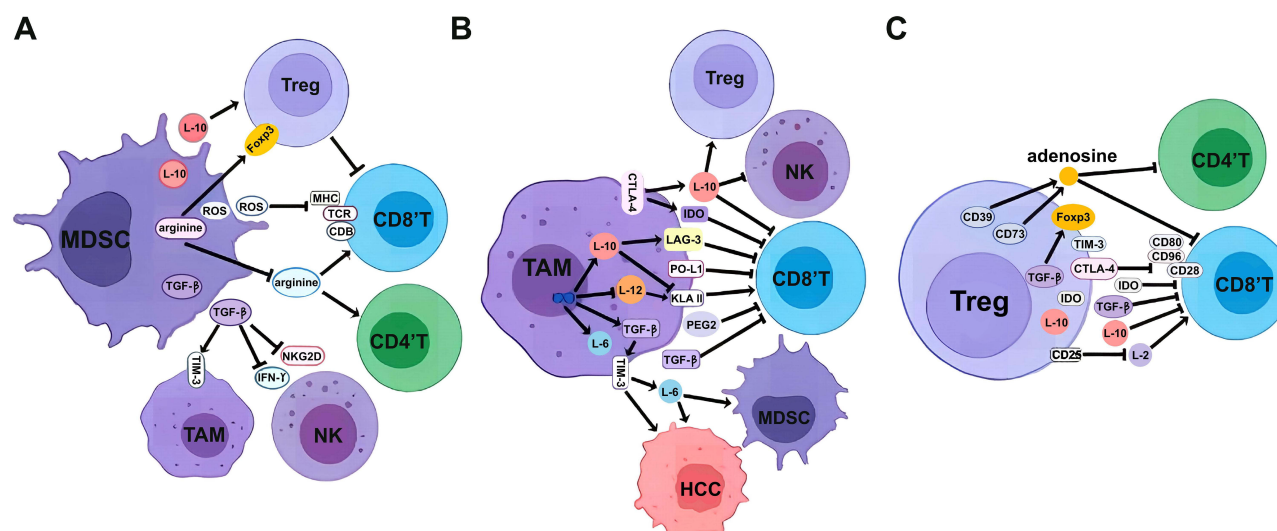


Figure 13 The mechanism of suppressive immune cells in the tumor microenvironment to promote HCC formation and the potential targets in these cells for HCC immunotherapy.²⁵⁶ (A) Regulatory mechanism centered on MDSCs. (B) Immunosuppressive mechanism mediated by TAMs. (C) Immunosuppressive mechanism driven by Treg cells.

liver cells. To overcome this, nanoparticles must evade Kupffer cells, penetrate the fenestrated endothelium, and be taken up by the RES before they can reach liver cells.²⁵⁸ Once nanoparticles enter the bloodstream, plasma proteins adsorb to their surface, and nanoparticles then bind to specific receptors on the surface of phagocytic cells. These nanoparticles are internalized, transported to phagosomes, and fuse with lysosomes, a process that rapidly conditions the nanoparticles and mediates macrophage endocytosis. This process causes macrophages to accumulate in the liver and prevents nanoparticles from re-entering the bloodstream.²⁵⁹

Tumor blood vessels are often abnormal, characterized by large pores and poor lymphatic drainage, which allows nanoparticles to extravasate from leaking tumor blood vessels and accumulate in the tumor tissue due to the EPR effect. Furthermore, due to the liver's unique characteristics, including its rich blood supply and large blood flow exchange area, there is slow blood flow, making it an ideal site for nanoparticle deposition.

Moreover, some nanoparticles can interact with specific receptors on liver cells and be actively delivered into these cells. By modifying nanoparticles with ligands that can bind to specific receptors on liver cells—such as secreted proteins (eg, GRP78 protein, transferrin receptor) and carbohydrate receptors on cancer cell surfaces (eg, mannose receptor, ASGPR)—the nanoparticles can enter liver cells via receptor-mediated endocytosis pathways. As a result, microenvironment-responsive nanoparticle drug delivery systems can be designed to precisely deliver drugs to HCC tissues, liver cancer cells, mesenchymal stromal cells, or subcellular organelles. This enables controlled and targeted drug release, significantly increasing the effective concentration of drugs at the site of liver cancer, which substantially enhances the therapeutic effect.

HCC-Specific Design Principles for Nanotechnology

Unlike many solid tumors, hepatocellular carcinoma arises within the highly specialized structural and immunological milieu of the liver, which imposes distinct biological constraints on the design of nanotheranostic systems. Liver sinusoidal endothelial cells (LSECs) are fenestrated and can, in principle, facilitate nanoparticle transport; however, the liver's highly active reticuloendothelial system—particularly Kupffer cells—efficiently phagocytoses and clears foreign nanomaterials, meaning that a substantial fraction of systemically delivered nanocarriers may be intercepted before reaching the tumor.²⁶⁰ Moreover, HCC commonly develops on a background of cirrhosis or chronic inflammation, which promotes LSEC capillarization and reduces or abolishes fenestrations, further altering transport behavior and intratumoral accessibility.²⁶¹ In addition, HCC vasculature is heterogeneous and chaotic, reflecting a dual blood supply via the portal vein and hepatic artery and uneven vascular leakage within tumors. This hemodynamic heterogeneity

makes reliance on passive EPR-based targeting particularly unstable in HCC, with marked variability among patients and even among lesions within the same patient.²⁶² Finally, the liver's baseline immune tolerance is frequently co-opted during HCC progression to create an immunosuppressive microenvironment characterized by impaired dendritic-cell function, T-cell exhaustion, and regulatory T-cell-mediated suppression, thereby limiting immunotherapy and constraining nano-immunomodulation strategies.²⁶¹

Accordingly, HCC nanotheranostic design should follow multidimensional, constraint-driven rules. Because hepatic phagocytic clearance is a primary barrier, material choice and engineering should prioritize strategies that reduce Kupffer-cell uptake and improve clearance profiles, including size control (eg, kidney-clearable ultrasmall nanoparticles),²⁶³ surface modification (eg, PEGylation), and biomimetic coatings, which have been shown to reduce Kupffer uptake and prolong circulation.^{264,265} Given the instability of passive targeting in HCC, active targeting is often required to improve localization and uptake. Leveraging HCC-overexpressed receptors such as ASGPR, GPC3, and CD44 can enhance delivery and partially overcome heterogeneous perfusion and variable permeability.^{266,267} Finally, because HCC is highly heterogeneous and dynamically evolving, integrated platforms that combine diagnostic imaging, stimulus-controlled drug release, and immunomodulation may be particularly advantageous for coordinating multiple biological processes and adapting to changing signals in the liver tumor microenvironment.

Safety Analysis of Nanomaterials

Safety Issues

Designing nanoparticles with the appropriate physical, chemical, pharmacokinetic, and pharmacodynamic properties remains a significant challenge. As anti-tumor drugs continue to evolve, even minor alterations in composition or properties—such as changes in size, mass, specific shape, surface functionalization, or biocompatibility—can lead to significant variations in pharmacology and toxicity. The current nanotechnology treatments for HCC mainly focus on nanodrug delivery systems. Therefore, the nanocarriers used, along with the anticancer drugs they carry, must be designed to achieve reversible binding, ensuring that they are not disrupted by the surrounding environment during transport and can be precisely released at the target cells.

The way nanoparticles enter the body also depends on their inherent properties. Nanoparticles intended for oral administration must exhibit high stability in the gastrointestinal tract, ability to penetrate the intestinal epithelium, and maintain high bioavailability after overcoming multiple barriers to reach liver cancer cells. For intravenous administration, nanoparticles must be able to penetrate various biological barriers. However, their rapid uptake by organs such as the spleen and liver may reduce their passive targeting to tumors, and prolonged exposure to these organs could lead to chronic toxicity.

Ensuring the long-term safety and tolerance of nanoparticles in the human body presents additional challenges. Nanomaterials are not entirely non-toxic, and their distribution, toxicity, and metabolic pathways within human tissues and organs remain unclear, which raises concerns about potential neurotoxicity. Beyond the toxicity inherent in the nanoparticles themselves—such as excessive acidic or alkaline substances that could damage liver vascular walls or heavy metals that might cause liver and kidney toxicity—the long-term retention of nanoparticles could also result in chronic toxicity. Some nanomedicines may accumulate in the liver, causing hepatotoxicity, while others may provoke immune responses that accelerate their clearance. Based on this, priority should be given to using biodegradable materials, ie, materials proven to be metabolized into harmless small molecules (like water, carbon dioxide) in the body, such as poly (lactic-co-glycolic acid) (PLGA), liposomes, specific structured silica, etc., and conducting long-term safety evaluations.^{268,269} Any new nanoparticle developed using different metals, polymers, or other materials requires extensive preclinical testing to determine its tolerance and safety thresholds.

Comparative Analysis of Inorganic and Organic Nanomaterials

Toxicity Comparison

Inorganic nanomaterials (eg, gold nanoparticles, iron oxide nanoparticles, silica nanoparticles) are often favored for their

high stability and versatile functions in imaging and drug delivery. However, they can accumulate in organs such as the liver, spleen, and lungs, leading to potential long-term toxicity. Gold nanoparticles (AuNPs), despite their promising therapeutic effects, may cause liver toxicity due to prolonged retention, resulting in inflammation and cytotoxicity at higher doses.²⁷⁰ Similarly, iron oxide nanoparticles (IONPs), widely used for imaging, can induce oxidative stress, leading to inflammation and cellular damage.²⁷¹ Surface modifications with biocompatible polymers like PEG can mitigate some of these issues, improving their biocompatibility.^{272,273}

Organic nanomaterials (eg, liposomes, polymeric nanoparticles, dendrimers) generally exhibit better biocompatibility and biodegradability compared to inorganic materials. Liposomes, extensively studied for drug delivery in HCC, are considered safe, with low systemic toxicity, particularly when encapsulating hydrophilic drugs. Polymeric nanoparticles can be designed to degrade into nontoxic byproducts, but their long-term stability and degradation products must be monitored to avoid potential immune responses or toxicity.^{274,275}

Metabolic Pathways and Elimination

Inorganic nanomaterials are primarily cleared via the reticuloendothelial system (RES), particularly by Kupffer cells in the liver, which can result in prolonged retention and potential toxicity in the liver and spleen. For instance, iron oxide nanoparticles are metabolized by macrophages and excreted primarily via feces. However, their slow clearance may pose a risk for chronic toxicity, particularly if they accumulate in organs over time.^{271,276}

Organic nanomaterials, particularly liposomal carriers, tend to be cleared more efficiently. Surface modifications like PEGylation enable longer circulation times and slower clearance from the body. Organic nanoparticles typically undergo enzymatic degradation, followed by renal or hepatic excretion of metabolites, which reduces the likelihood of accumulation and toxicity in organs.^{277,278}

Clinical Safety Limitations

Inorganic nanomaterials present significant clinical safety challenges, including immune activation and long-term accumulation in organs. For example, gold nanoparticles are associated with liver toxicity at higher doses, limiting their clinical utility. Silica nanoparticles, unless adequately surface-modified to prevent aggregation, may induce inflammatory responses, particularly in the liver and kidneys. These factors limit their clinical translation, especially for long-term use.^{270,279}

Organic nanomaterials, while generally more biocompatible, are not without limitations. Cytotoxicity in certain organic nanoparticles may increase with particle size or surface charge. Additionally, some polymeric nanoparticles may provoke immune responses, especially if their degradation products accumulate in tissues. Optimizing nanoparticle surface modifications is crucial to balance effective drug delivery with minimal immunogenicity, as certain surface modifications (eg, cationic groups) may trigger inflammatory responses.^{280,281}

Scaling Production and Costs

In addition to safety concerns, the production and cost of nanomaterials present significant challenges. The high ligand specificity and variability of nanoparticles make it difficult to ensure large-scale, unbiased production and adaptive characterization. As a result, only a limited number of nanoparticles are available on the market, and the costs associated with their production and characterization are generally high. Furthermore, many of the nanocarriers or nanoradiosensitizers currently under development or in use—such as high atomic number gold—are based on expensive and scarce elements, severely restricting their broader clinical application.

The storage and stability of nanomaterials also pose considerable challenges. Both during transportation and storage, as well as within the body, nanomaterials must meet stringent stability requirements. Researchers must account for the storage environment and in vivo half-life when developing nanodrugs for HCC. These factors place substantial demands on the large-scale production and economic feasibility of nanomedicines.

Future Directions

Personalized Nanomedicine: Customizing Nanomedicines Based on the Tumor Microenvironment of Patients

In the context of personalized nanomedicine, the precise modulation of therapeutic strategies according to the TME of liver cancer patients is critical. The development of dynamic, responsive nanomedicines—specifically nanoparticles sensitive to the unique features of HCC—can significantly enhance therapeutic efficacy. Such nanoparticles can be designed to exploit tumor-specific characteristics, including the acidic milieu and the overexpression of matrix metalloproteinase-14 (MMP-14).²⁸² This targeted approach enables the localized release of chemotherapeutic agents, such as sorafenib, thereby improving both efficacy and tolerability. Furthermore, incorporating liquid biopsy technologies facilitates the identification of tumor-specific biomarkers, allowing for the selection of appropriate targeting ligands and culminating in the development of truly personalized treatment regimens.

To address the heterogeneity observed in HCC, multi-drug co-loaded nanosystems can be engineered. These systems may incorporate a combination of contrast agents, chemotherapeutics, and immune adjuvants, with their compositions tailored based on the individual patient's genetic mutations (eg, c-Myc, TP53). This genetic customization is expected to mitigate drug resistance and enhance the precision of therapeutic interventions.

Two actionable strategies for personalization in HCC are outlined as follows:

Patient-Derived/Patient-Specific Nanovaccines: A Feasible Translational Workflow

This strategy offers a concrete implementation pathway that links patient sampling and antigen identification with the construction of nanovaccines, followed by immune monitoring and optimization. Specifically, tumor tissue or circulating tumor-derived materials (eg, ctDNA, exosomes) can be used to identify patient-specific antigen repertoires, which are then incorporated into nanoparticle- or virus-like particle-based vaccine formulations. These vaccines are combined with immunostimulatory adjuvants to enhance antigen-presenting cell uptake and T-cell priming, followed by iterative optimization based on immune readouts to refine therapeutic efficacy.²⁸³

Preclinical evidence has substantively supported this approach, particularly in the context of HCC, where neoantigen-based nanovaccines have demonstrated robust antitumor immunity and a reduction in postoperative recurrence. Specifically, a personalized vaccine constructed by conjugating patient-derived tumor neoantigen peptides to hepatitis B core protein virus-like particles (HBc-MWK) showed significant tumor inhibition and prevention of recurrence in xenograft models, while also inducing long-term immune memory.²⁸⁴ Given the high recurrence rate after HCC resection, this personalized nanovaccine strategy offers a promising, precision-based approach for post-surgical relapse prevention.²⁸⁵

Biomarker-Guided Nanodrug Design and Stratified Deployment: Engineering-Based Personalization

An “engineering-forward” approach expands the current paradigm by utilizing patient-specific biomarkers and TME features to inform the design of nanomedicines. This encompasses critical elements such as target selection, ligand functionalization, and the incorporation of stimuli-responsive release mechanisms. Utilizing liquid biopsy and tissue profiling can help identify tumor-specific biomarkers (eg, alpha-fetoprotein (AFP) and glypican-3 (GPC3)), which not only enable patient stratification but also inform ligand selection for enhanced tumor-targeted delivery and increased drug accumulation.

Furthermore, distinct TME signatures—such as elevated matrix metalloproteinase 2 (MMP2) activity, acidity, or hypoxia—can be used to match patients with stimuli-responsive nanoplateforms designed to release their payloads selectively under these conditions. For example, a stroma-immune co-targeting nanoplateform developed by Yin et al responds to MMP2-enriched HCC microenvironments, reprogramming cancer-associated fibroblasts (CAFs) and degrading PD-L1, thereby overcoming both fibrotic and immunosuppressive barriers. This platform demonstrated significant tumor suppression and immune activation in a humanized immune patient-derived xenograft (PDX) model. This case highlights how nanomedicine can be personalized based on individual stromal and immune TME profiles.²⁸⁶

Bionic Nanocarrier Design: Enhancing Targeting with Exosomes or Cell Membrane Coating

The design of bionic nanocarriers has gained considerable attention due to the homologous targeting between bionic materials and the cells they mimic, as well as the reduced susceptibility of hydrophobic substances to phagocytosis by Kupffer cells. This characteristic enhances the accumulation of drugs in tumor tissues. One promising development is the design of liver-derived bionic carriers, such as nanoscale vesicle carriers. For example, heat-sensitive liposomes coated with bionic liver cancer cell membranes (HepM-TSL) have been shown to precisely target areas of liver cancer recurrence by exploiting the homologous aggregation ability of liver tumor cell membranes.²⁸⁷ This approach not only reduces uptake by Kupffer cells but also enhances therapeutic efficacy while minimizing side effects on normal tissues. Additionally, tumor-derived exosomes, which have proven useful in liquid biopsy applications, exhibit considerable potential for drug delivery. Their homologous targeting ability allows them to penetrate tumor barriers easily and deliver therapeutic substances like siRNA. Moreover, the combination of dual-membrane coating technology and immune camouflage techniques not only extends the half-life of nanocarriers but also enhances their tumor accumulation, thereby improving therapeutic outcomes.

Artificial Intelligence-Assisted Optimization of Nanomaterials: Predicting Drug Release Kinetics and Toxicity

With advancements in artificial intelligence (AI) and data quantification, AI is becoming increasingly prevalent in the field of nanomedicine. In the future, AI and big data algorithms can be utilized to optimize nanomaterials for various applications. In liver cancer diagnosis and imaging, AI can enhance the accuracy of tumor detection, differentiate between benign and malignant lesions, and facilitate the early detection of tumors. In predictive modeling, machine learning algorithms can analyze multi-variable datasets to forecast disease progression, treatment responses, and patient survival, thus aiding in targeted treatment decision-making. For personalized treatment planning, AI algorithms can integrate diverse data sources, including multi-omics data from patients, to create individualized treatment strategies for specific HCC subtypes, optimize liver transplantation protocols, and assist in screening patients for clinical trials.²⁸⁸ Additionally, AI-driven nanomedicines can employ deep learning techniques to predict key factors such as liver accumulation rates, metabolic pathways, and potential liver toxicity risks of nanomaterials, thus enabling the identification of safer and more effective materials. Moreover, AI algorithms can be trained to design nanoparticles with optimized characteristics, such as ideal pore size, surface functionalization, and drug release profiles. This approach can improve the design of specific nanoparticles, optimize the structure of sustained-release formulations, and enable more precise and efficient drug delivery.

Outlook

Laboratory studies have demonstrated that nanotechnology offers innovative, highly targeted, and low-toxicity solutions for cancer treatment, effectively integrating diagnostic and therapeutic functions to achieve simultaneous diagnosis and treatment. Currently, clinical applications of nanotechnology in the diagnosis and treatment of HCC primarily focus on drug delivery systems. Future research should place greater emphasis on understanding the unique characteristics of HCC, the mechanisms underlying targeted drug action, and the metabolic pathways of therapeutic agents, while ensuring safety during clinical application. Moreover, conventional single diagnostic and therapeutic approaches often have limitations. Therefore, multi-modal synergistic strategies that combine chemotherapy, radiotherapy, magnetic hyperthermia, and other techniques offer significant potential. These strategies can exploit the strengths of each modality in the treatment of HCC, overcoming current challenges and contributing at various stages, including monitoring, treatment, and prognosis. Moving forward, it will be crucial to further integrate basic research with clinical translation, establish standardized physiological parameters for nanotechnology and targeted molecular biology, optimize the preparation processes and quality control standards for nanomaterials, and develop large-scale, standardized production protocols for nanomedicines. Clinical trials are essential to advancing the practical application of nanotechnology in HCC treatment, building a multi-modal treatment system, enabling personalized therapies, and ultimately improving the survival rates and quality of life for HCC patients.

Data Sharing Statement

No data was used for the research described in the article.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Moris D, Martinino A, Schiltz S, et al. Advances in the Treatment of Hepatocellular Carcinoma: an Overview of the Current and Evolving Therapeutic Landscape for Clinicians. *Ca a Cancer J Clinicians*. 2025;1–30. doi:10.3322/caac.70018
2. Kim DY. Changing Etiology and Epidemiology of Hepatocellular Carcinoma: Asia and Worldwide. *J Liver Cancer*. 2024;24(1):62–70. doi:10.17998/jlc.2024.03.13
3. Bray F, Laversanne M, Sung H, et al. Global Cancer Statistics 2022: globocan Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries, CA: a Cancer. *J Clin*. 2024;74:229–263.
4. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A Global View of Hepatocellular Carcinoma: trends, Risk, Prevention and Management. *Nat Rev Gastroenterol Hepatol*. 2019;16(10):589–604. doi:10.1038/s41575-019-0186-y
5. World Health Organization. *Global Cancer Observatory: Cancer Today*. Lyon FIAFRC. Ed.;2024.
6. Tran S, Zou B, Kam L, et al. Updates in Characteristics and Survival Rates of Hepatocellular Carcinoma in a Nationwide Cohort of Real-World Us Patients, 2003–2021. *J Hepatocell Carcinoma*. 2023;10:2147–2158. doi:10.2147/JHC.S420603
7. Yao X, Ling X, Zhu Z, Cao X, Tang S. Temporal Trends of Liver Cancer Burden, Comparative Analysis of Risk Factors and Trend Forecasts to 2024 in China, USA, the Republic of Korea, and Mongolia: an Analysis Based on Multiple Data Sources from Global Burden of Disease 2019, the Global Cancer Observatory, and Cancer Incidence in Five Continents. *J Glob Health*. 2025;15:04040. doi:10.7189/jogh.15.04040
8. Li Q, Ding C, Cao M, et al. Global Epidemiology of Liver Cancer 2022: an Emphasis on Geographic Disparities. *Chin Med J*. 2024;137(19):2334–2342. doi:10.1097/CM9.0000000000003264
9. Singal AG, Kanwal F, Llovet JM. Global Trends in Hepatocellular Carcinoma Epidemiology: implications for Screening, Prevention and Therapy. *Nat Rev Clin Oncol*. 2023;20(12):864–884. doi:10.1038/s41571-023-00825-3
10. Zhang CH, Cheng Y, Zhang S, Fan J, Gao Q. Changing Epidemiology of Hepatocellular Carcinoma in Asia. *Liver Int*. 2022;42(9):2029–2041. doi:10.1111/liv.15251
11. Dasgupta P, Henshaw C, Youlden DR, Clark PJ, Aitken JF, Baade PD. Global Trends in Incidence Rates of Primary Adult Liver Cancers: a Systematic Review and Meta-Analysis. *Front Oncol*. 2020;10:171. doi:10.3389/fonc.2020.00171
12. Shen C, Jiang X, Li M, Luo Y. Hepatitis Virus and Hepatocellular Carcinoma: recent Advances. *Cancers*. 2023;15(2):533. doi:10.3390/cancers15020533
13. Kulik L, El-Serag HB. Epidemiology and Management of Hepatocellular Carcinoma. *Gastroenterology*. 2019;156(2):477–491.e471. doi:10.1053/j.gastro.2018.08.065
14. Tabori NE, Sivananthan G. Treatment Options for Early-Stage Hepatocellular Carcinoma. *Semin Intervent Radiol*. 2020;37(05):448–455. doi:10.1055/s-0040-1720950
15. Numata K, Wang F. New Developments in Ablation Therapy for Hepatocellular Carcinoma: combination with Systemic Therapy and Radiotherapy. *Hepatobiliary Surg Nutr*. 2022;11(5):766–769. doi:10.21037/hbsn-22-363
16. Ban D, Ogura T, Akahoshi K, Tanabe M. Current Topics in the Surgical Treatments for Hepatocellular Carcinoma. *Ann Gastroenterol Surg*. 2018;2(2):137–146. doi:10.1002/ags3.12065
17. Heimbach JK, Kulik LM, Finn RS, et al. Aasld Guidelines for the Treatment of Hepatocellular Carcinoma. *Hepatology*. 2018;67(1):358–380. doi:10.1002/hep.29086
18. Vivarelli M, Mocchegiani F, Wakabayashi T, et al. Cacciaguerra, Prevention of Post-Hepatectomy Liver Failure in Cirrhotic Patients Undergoing Minimally Invasive Liver Surgery for Hcc: has the Round Ligament to Be Preserved? *Cancers*. 2024;16(2):364. doi:10.3390/cancers16020364
19. Farkas S, Hackl C, Schlitt HJ. Overview of the Indications and Contraindications for Liver Transplantation. *Cold Spring Harbor Perspectives Med*. 2014;4(5):a015602. doi:10.1101/cshperspect.a015602

20. Koh JH, Tan DJH, Ong Y, et al. Liver Resection Versus Liver Transplantation for Hepatocellular Carcinoma within Milan Criteria: a Meta-Analysis of 18,421 Patients. *Hepatobiliary Surg Nutr.* **2022**;11(1):78–93. doi:10.21037/hbsn-21-350
21. Hong SK, Lee KW, Hong SY, et al. Efficacy of Liver Resection for Single Large Hepatocellular Carcinoma in Child-Pugh a Cirrhosis: analysis of a Nationwide Cancer Registry Database. *Front Oncol.* **2021**;11:674603. doi:10.3389/fonc.2021.674603
22. Tanaka T, Takata K, Miyayama T, et al. Long-Term Outcome and Eligibility of Radiofrequency Ablation for Hepatocellular Carcinoma over 3.0 cm in Diameter. *Sci Rep.* **2023**;13(1):16286. doi:10.1038/s41598-023-43516-w
23. Xu Q, Kobayashi S, Ye X, Meng X. Comparison of Hepatic Resection and Radiofrequency Ablation for Small Hepatocellular Carcinoma: a Meta-Analysis of 16,103 Patients. *Sci Rep.* **2014**;4(1):7252. doi:10.1038/srep07252
24. Schindler P, Kaldewey D, Rennebaum F, et al. Safety, Efficacy, and Survival of Different Transarterial Chemoembolization Techniques in the Management of Unresectable Hepatocellular Carcinoma: a Comparative Single-Center Analysis. *J Cancer Res Clin Oncol.* **2024**;150(5):235. doi:10.1007/s00432-024-05722-5
25. Hu WY, Wei HY, Li KM, Wang RB, Xu XQ, Feng R. Linc00511 as a Cerna Promotes Cell Malignant Behaviors and Correlates with Prognosis of Hepatocellular Carcinoma Patients by Modulating Mir-195/Eya1 Axis. *Biomed Pharmacother.* **2020**;121:109642. doi:10.1016/j.biopha.2019.109642
26. Inchingolo R, Posa A, Mariappan M, Spiliopoulos S. Locoregional Treatments for Hepatocellular Carcinoma: current Evidence and Future Directions. *World J Gastroenterol.* **2019**;25(32):4614–4628. doi:10.3748/wjg.v25.i32.4614
27. Podlasek A, Abdulla M, Broering D, Bzeizi K. Recent Advances in Locoregional Therapy of Hepatocellular Carcinoma. *Cancers.* **2023**;15(13):3347. doi:10.3390/cancers15133347
28. Jindal A, Thadi A, Shailubhai K. Hepatocellular Carcinoma: etiology and Current and Future Drugs. *J Clin Exp Hepatol.* **2019**;9(2):221–232. doi:10.1016/j.jceh.2019.01.004
29. Villanueva A. Hepatocellular Carcinoma. *N Engl J Med.* **2019**;380:1450–1462. doi:10.1056/NEJMr1713263
30. Yang C, Zhang H, Zhang L, et al. Evolving Therapeutic Landscape of Advanced Hepatocellular Carcinoma. *Nat Rev Gastroenterol Hepatol.* **2023**;20(4):203–222. doi:10.1038/s41575-022-00704-9
31. Wang G. Editorial: nanomaterials for Biology and Medicine. *Front Chem.* **2023**;11:1244175. doi:10.3389/fchem.2023.1244175
32. Wu H, Wang MD, Liang L, et al. Nanotechnology for Hepatocellular Carcinoma: from Surveillance, Diagnosis to Management. *Small.* **2021**;17(6):e2005236. doi:10.1002/sml.202005236
33. Gavass S, Quazi S, Karpiński TM. Nanoparticles for Cancer Therapy: current Progress and Challenges. *Nanoscale Res Lett.* **2021**;16(1):173. doi:10.1186/s11671-021-03628-6
34. Hoshyar N, Gray S, Han H, Bao G. The Effect of Nanoparticle Size on In Vivo Pharmacokinetics and Cellular Interaction. *Nanomedicine.* **2016**;11(6):673–692. doi:10.2217/nmm.16.5
35. Chen D, Tang Q, Zou J, et al. Ph-Responsive Peg-Doxorubicin-Encapsulated Aza-Bodipy Nanotheranostic Agent for Imaging-Guided Synergistic Cancer Therapy. *Adv Healthc Mater.* **2018**;7(7):e1701272. doi:10.1002/adhm.201701272
36. Gupta S, Murthy CN, Prabha CR. Recent Advances in Carbon Nanotube Based Electrochemical Biosensors. *Int J Biol Macromol.* **2018**;108:687–703. doi:10.1016/j.ijbiomac.2017.12.038
37. Kyriakides TR, Raj A, Tseng TH, et al. Biocompatibility of Nanomaterials and Their Immunological Properties. *Biomed Mater.* **2021**;16:042005.
38. Haghighi FH, Binaymotlagh R, Fratoddi I, Chronopoulou L, Palocci C. Peptide-Hydrogel Nanocomposites for Anti-Cancer Drug Delivery. *Gels.* **2023**;9(12):953. doi:10.3390/gels9120953
39. Yao Y, Zhou Y, Liu L, et al. Nanoparticle-Based Drug Delivery in Cancer Therapy and Its Role in Overcoming Drug Resistance. *Front Mol Biosci.* **2020**;7:193. doi:10.3389/fmolb.2020.00193
40. Liu M, Anderson RC, Lan X, Conti PS, Chen K. Recent Advances in the Development of Nanoparticles for Multimodality Imaging and Therapy of Cancer. *Med Res Rev.* **2020**;40(3):909–930. doi:10.1002/med.21642
41. Sengupta S, Parikh ND. Biomarker Development for Hepatocellular Carcinoma Early Detection: current and Future Perspectives. *Hepatol Oncol.* **2017**;4(4):111–122. doi:10.2217/hep-2017-0019
42. Yan S, Na J, Liu X, Wu P. Different Targeting Ligands-Mediated Drug Delivery Systems for Tumor Therapy. *Pharmaceutics.* **2024**;16(2):248. doi:10.3390/pharmaceutics16020248
43. Sabit H, Pawlik TM, Radwan F, et al. Precision Nanomedicine: navigating the Tumor Microenvironment for Enhanced Cancer Immunotherapy and Targeted Drug Delivery. *Mol Cancer.* **2025**;24(1):160. doi:10.1186/s12943-025-02357-z
44. Ye W, Wang J, Zheng J, Jiang M, Zhou Y, Wu Z. Association between Higher Expression of Vav1 in Hepatocellular Carcinoma and Unfavourable Clinicopathological Features and Prognosis. *Protein Pept Lett.* **2024**;31(9):706–713. doi:10.2174/0109298665330781240830042601
45. Mu R, Chang M, Feng C, et al. Analysis of the Expression of PRDX6 in Patients with Hepatocellular Carcinoma and its Effect on the Phenotype of Hepatocellular Carcinoma Cells. *Curr Genomics.* **2024**;25(1):2–11. doi:10.2174/0113892029273682240111052317
46. Qi L, Tan Y, Zhou Y, et al. Proteogenomic Identification and Analysis of KIF5B as a Prognostic Signature for Hepatocellular Carcinoma. *Curr Gene Ther.* **2025**;25(4):532–545. doi:10.2174/0115665232308821240826075513
47. Gudivada IP, Amajala KC. Integrative Bioinformatics Analysis for Targeting Hub Genes in Hepatocellular Carcinoma Treatment. *Curr Genomics.* **2025**;26(1):48–80. doi:10.2174/0113892029308243240709073945
48. Chen M, Guo Z, Chen Q, et al. Pd Nanosheets with Their Surface Coordinated By radioactive Iodide as a High-Performance Theranostic Nanoagent for Orthotopic Hepatocellular Carcinoma Imaging and Cancer Therapy. *Chem Sci.* **2018**;9(18):4268–4274. doi:10.1039/C8SC00104A
49. Li Z, Chen Y, Yang Y, et al. Recent Advances in Nanomaterials-Based Chemo-Photothermal Combination Therapy for Improving Cancer Treatment. *Front Bioeng Biotechnol.* **2019**;7:293. doi:10.3389/fbioe.2019.00293
50. Tang L, Yang X, He L, Zhu C, Chen Q. Preclinical Advance in Nanoliposome-Mediated Photothermal Therapy in Liver Cancer. *Lipids Health Dis.* **2025**;24(1):31. doi:10.1186/s12944-024-02429-x
51. Zhang X, Si S, Lieberwirth I, Landfester K, Mäiländer V. Engineered Protein Corona Sustains Stealth Functionality of Nanocarriers in Plasma. *J Nanobiotechnology.* **2025**;23(1):512. doi:10.1186/s12951-025-03565-x

52. Wang YQ, Huang C, Ye PJ, et al. Prolonged Blood Circulation Outperforms Active Targeting for Nanocarriers-Mediated Enhanced Hepatocellular Carcinoma Therapy in Vivo. *J Control Release*. 2022;347:400–413. doi:10.1016/j.jconrel.2022.05.024
53. Serrano A, Casares N, Trocóniz IF, et al. Foxp3 Inhibitory Peptide Encapsulated in a Novel Cd25-Targeted Nanoliposome Promotes Efficient Tumor Regression in Mice. *Acta Pharmacol Sin*. 2025;46(1):171–183. doi:10.1038/s41401-024-01338-0
54. Xu H, Li S, Liu Y, Sung YY, Zhou Y, Wu H. A Novel Ph-Sensitive Nanoparticles Encapsulating Anti-Pd-1 Antibody and Mdk-Sirna Overcome Immune Checkpoint Blockade Resistance in Hcc Via Reshaping Immunosuppressive Tme. *J Exp Clin Cancer Res*. 2025;44(1):148. doi:10.1186/s13046-025-03396-6
55. Li H, Wang S, Yang Z, Meng X, Niu M. Nanomaterials Modulate Tumor-Associated Macrophages for the Treatment of Digestive System Tumors. *Bioact Mater*. 2024;36:376–412. doi:10.1016/j.bioactmat.2024.03.003
56. Kusi D, Sun Y, Liu C. Advances in Manganese-Based Nanomaterials for Cancer Therapy Via Regulating Non-Ferrous Ferroptosis. *Int J Pharm*. 2025;669:125101. doi:10.1016/j.ijpharm.2024.125101
57. Xia C, Li M, Ran G, et al. Redox-Responsive Nanoassembly Restrained Myeloid-Derived Suppressor Cells Recruitment through Autophagy-Involving Lactate Dehydrogenase a Silencing for Enhanced Cancer Cancer Immunotherapy. *J Control Release*. 2021;335:557–574. doi:10.1016/j.jconrel.2021.05.034
58. Liu Z, Yao Z, Yang H, Wu S, Tang Z. Leveraging the Dual Role of Ros in Liver Diseases with Nanomaterials: clearing and Amplifying for Therapy. *Nanoscale*. 2025;17(7):3688–3697. doi:10.1039/D4NR04469J
59. Johansson JM, Rietz HD, Hedlund H, et al. Cellular and Biophysical Barriers to Lipid Nanoparticle Mediated Delivery of Rna to the Cytosol. *Nat Commun*. 2025;16(1):5354. doi:10.1038/s41467-025-60959-z
60. Wei X, Cao W, Wang S, et al. Progress in the Application of Novel Nanomaterials in Targeted Therapy for Liver Cancer. *Int J Nanomed*. 2025;20:2623–2643. doi:10.2147/IJN.S509409
61. Nadarevic T, Colli A, Giljaca V, et al. Magnetic Resonance Imaging for the Diagnosis of Hepatocellular Carcinoma in Adults with Chronic Liver Disease. *Cochrane Database Syst Rev*. 2022;5(5):Cd014798. doi:10.1002/14651858.CD014798.pub2
62. Hennedige T, Venkatesh SK. Imaging of Hepatocellular Carcinoma: diagnosis, Staging and Treatment Monitoring. *Cancer Imaging*. 2013;12(3):530–547. doi:10.1102/1470-7330.2012.0044
63. Usman S, Smith L, Brown N, Major V. Diagnostic Accuracy of Magnetic Resonance Imaging Using Liver Tissue Specific Contrast Agents and Contrast Enhanced Multi Detector Computed Tomography: a Systematic Review of Diagnostic Test in Hepatocellular Carcinoma (Hcc). *Radiography*. 2018;24(4):e109–e114. doi:10.1016/j.radi.2018.05.002
64. Patil-Sen Y, Torino E, De Sarno F, et al. Biocompatible Superparamagnetic Core-Shell Nanoparticles for Potential Use in Hyperthermia-Enabled Drug Release and as an Enhanced Contrast Agent. *Nanotechnology*. 2020;31(37):375102. doi:10.1088/1361-6528/ab91f6
65. Bu L, Xie J, Chen K, et al. Assessment and Comparison of Magnetic Nanoparticles as Mri Contrast Agents in a Rodent Model of Human Hepatocellular Carcinoma. *Contrast Media Mol Imaging*. 2012;7(4):363–372. doi:10.1002/cmmi.494
66. Zhang Y, Numata K, Du Y, Maeda S. Contrast Agents for Hepatocellular Carcinoma Imaging: value and Progression. *Front Oncol*. 2022;12:921667. doi:10.3389/fonc.2022.921667
67. Lu J, Sun J, Li F, et al. Highly Sensitive Diagnosis of Small Hepatocellular Carcinoma Using Ph-Responsive Iron Oxide Nanocluster Assemblies. *J Am Chem Soc*. 2018;140(32):10071–10074. doi:10.1021/jacs.8b04169
68. Xu YH, Yang J, Meng J, Wang H. Targeted Mr Imaging Adopting T1-Weighted Ultra-Small Iron Oxide Nanoparticles for Early Hepatocellular Carcinoma: an in Vitro and in Vivo Study. *Chin Med Sci J*. 2020;35(2):142–150. doi:10.24920/003643
69. Liu H, Teng X, Yu S, Yang W, Kong T, Liu T. Recent Advances in Photoacoustic Imaging: current Status and Future Perspectives. *Micromachines*. 2024;15(8):1007. doi:10.3390/mi15081007
70. Hui X, Malik MOA, Pramanik M. Looking Deep inside Tissue with Photoacoustic Molecular Probes: a Review. *J Biomed Opt*. 2022;27:070901. doi:10.1117/1.JBO.27.7.070901
71. Upputuri PK, Pramanik M. Recent Advances in Photoacoustic Contrast Agents for in Vivo Imaging. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2020;12:e1618. doi:10.1002/wnan.1618
72. Liu T, Wei Q, Wang J, Jiao S, Zhang HF. Combined Photoacoustic Microscopy and Optical Coherence Tomography Can Measure Metabolic Rate of Oxygen. *Biomed Opt Express*. 2011;2:1359–1365. doi:10.1364/BOE.2.001359
73. Sridharan B, Lim HG. Advances in Photoacoustic Imaging Aided by Nano Contrast Agents: special Focus on Role of Lymphatic System Imaging for Cancer Theranostics. *J Nanobiotechnology*. 2023;21:437. doi:10.1186/s12951-023-02192-8
74. Deng H, Shang W, Lu G, et al. Targeted and Multifunctional Technology for Identification between Hepatocellular Carcinoma and Liver Cirrhosis. *ACS Appl Mater Interfaces*. 2019;11(16):14526–14537. doi:10.1021/acsami.8b20600
75. Mundada K, Pellerito JS, Srivastava B, Revzin MV. Ultrasound Contrast Agents: current Role in Adults and Children for Various Indications. *Radiol Clin North Am*. 2024;62(6):1035–1062. doi:10.1016/j.rcl.2024.07.010
76. Hawley J, Tang Y, Sjöström A, Fuentes-Albuero A, Tranquart F. The Clinical Utility of Liver-Specific Ultrasound Contrast Agents During Hepatocellular Carcinoma Imaging. *Ultrasound Med Biol*. 2025;51(3):415–427. doi:10.1016/j.ultrasmedbio.2024.10.011
77. Ying X, Dong S, Zhao Y, Chen Z, Jiang J, Shi H. Research Progress on Contrast-Enhanced Ultrasound (Ceus) Assisted Diagnosis and Treatment in Liver-Related Diseases. *Int J Med Sci*. 2025;22(5):1092–1108. doi:10.7150/ijms.101789
78. Wegierak D, Nittayacharn P, Cooley MB, et al. Nanobubble Contrast Enhanced Ultrasound Imaging: a review. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2024;16:e2007. doi:10.1002/wnan.2007
79. Qiu Y, Wu Z, Chen Y, et al. Nano Ultrasound Contrast Agent for Synergistic Chemo-Photothermal Therapy and Enhanced Immunotherapy against Liver Cancer and Metastasis. *Adv Sci*. 2023;10(21):e2300878. doi:10.1002/adv.202300878
80. Zeng F, Du M, Chen Z. Nanosized Contrast Agents in Ultrasound Molecular Imaging. *Front Bioeng Biotechnol*. 2021;9:758084. doi:10.3389/fbioe.2021.758084
81. Pelizzaro F, Cardin R, Penzo B, et al. Liquid Biopsy in Hepatocellular Carcinoma: where Are We Now? *Cancers*. 2021;13(9):2274. doi:10.3390/cancers13092274
82. Pandey S, Yadav P. Liquid Biopsy in Cancer Management: integrating Diagnostics and Clinical Applications. *Pract Lab Med*. 2025;43:e00446. doi:10.1016/j.plabm.2024.e00446

83. Tan P, Grundy L, Makary P, Eng KH, Ramsay G, Bekheit M. The Value of Liquid Biopsy in the Diagnosis and Staging of Hepatocellular Carcinoma: a Systematic Review. *Transl Gastroenterol Hepatol*. 2021;6:54. doi:10.21037/tgh.2020.01.11
84. Liu M, Wen Y. Point-of-Care Testing for Early-Stage Liver Cancer Diagnosis and Personalized Medicine: biomarkers, Current Technologies and Perspectives. *Heliyon*. 2024;10(19):e38444. doi:10.1016/j.heliyon.2024.e38444
85. Chen K, Du Z, Zhang Y, Bai R, Zhu L, Xu W. Exploring Nucleic Acid Nanozymes: a New Frontier in Biosensor Development. *Biosensors*. 2025;15(3):142. doi:10.3390/bios15030142
86. Aydin S, Emre E, Ugur K, et al. An Overview of Elisa: a Review and Update on Best Laboratory Practices for Quantifying Peptides and Proteins in Biological Fluids. *J Int Med Res*. 2025;53(2):3000605251315913. doi:10.1177/03000605251315913
87. Hariri AA, Newman SS, Tan S, et al. Improved Immunoassay Sensitivity and Specificity Using Single-Molecule Colocalization. *Nat Commun*. 2022;13(1):5359. doi:10.1038/s41467-022-32796-x
88. Zhao D, Cao J, Zhang L, Zhang S, Wu S. Targeted Molecular Imaging Probes Based on Magnetic Resonance Imaging for Hepatocellular Carcinoma Diagnosis and Treatment. *Biosensors*. 2022;12(5):342. doi:10.3390/bios12050342
89. Chen P, Jiang P, Lin Q, et al. Simultaneous Homogeneous Fluorescence Detection of Afp and Gpc3 in Hepatocellular Carcinoma Clinical Samples Assisted by Enzyme-Free Catalytic Hairpin Assembly. *ACS Appl Mater Interfaces*. 2022;14(25):28697–28705. doi:10.1021/acsami.2c09135
90. Ridder DA, Weinmann A, Schindeldecker M, et al. Comprehensive Clinicopathologic Study of Alpha Fetoprotein-Expression in a Large Cohort of Patients with Hepatocellular Carcinoma. *Int J Cancer*. 2022;150(6):1053–1066. doi:10.1002/ijc.33898
91. Li R, Fan H, Zhou H, et al. Nanozyme-Catalyzed Metasurface Plasmon Sensor-Based Portable Ultrasensitive Optical Quantification Platform for Cancer Biomarker Screening. *Adv Sci*. 2023;10(24):e2301658. doi:10.1002/adv.202301658
92. Olorundare FOG, Sipuka DS, Sebokolodi TI, et al. An Electrochemical Immunosensor on a Carbon Nanofiber/Gold Nanoparticles Platform for the Detection of Alpha-Feto Protein Cancer Biomarker. *Sens Bio-Sens Res*. 2023;41:100574. doi:10.1016/j.sbsr.2023.100574
93. Haruyama Y, Kataoka H. Glypican-3 Is a Prognostic Factor and an Immunotherapeutic Target in Hepatocellular Carcinoma. *World J Gastroenterol*. 2016;22(1):275–283. doi:10.3748/wjg.v22.i1.275
94. Li G, Wang B, Li L, et al. H-Rgo-Pd Nps Nanozyme Enhanced Silver Deposition Strategy for Electrochemical Detection of Glypican-3. *Molecules*. 2023;28(5):2271. doi:10.3390/molecules28052271
95. Shegekar T, Vodithala S, Juganavar A. The Emerging Role of Liquid Biopsies in Revolutionising Cancer Diagnosis and Therapy. *Cureus*. 2023;15(8):e43650. doi:10.7759/cureus.43650
96. Plaks V, Koopman CD, Werb Z. Cancer. Circulating Tumor Cells. *Science*. 2013;341(6151):1186–1188. doi:10.1126/science.1235226
97. Zhao L, Song J, Sun Y, et al. Tumor-derived proliferative CTCs and CTC clusters predict aggressiveness and early recurrence in hepatocellular carcinoma patients. *Cancer Med*. 2023;12(13):13912–13927. doi:10.1002/cam4.5946
98. Edd JF, Mishra A, Smith KC, et al. Isolation of Circulating Tumor Cells. *Iscience*. 2022;25(8):104696. doi:10.1016/j.isci.2022.104696
99. Lu X, Tan S, Wu M, Ju H, Liang X, Li P. Evaluation of a New Magnetic Bead as an Integrated Platform for Systematic Ctc Recognition, Capture and Clinical Analysis. *Colloids Surf B Biointerfaces*. 2021;199:111542. doi:10.1016/j.colsurfb.2020.111542
100. Mao Y, Zhang Y, Yu Y, et al. Self-Assembled Supramolecular Immunomagnetic Nanoparticles through π - π Stacking Strategy for the Enrichment of Circulating Tumor Cells. *Regen Biomater*. 2023;10:rbad016. doi:10.1093/rb/rbad016
101. Tkach M, Théry C. Communication by Extracellular Vesicles: where We Are and Where We Need to Go. *Cell*. 2016;164(6):1226–1232. doi:10.1016/j.cell.2016.01.043
102. Chen J, Li P, Zhang T, et al. Review on Strategies and Technologies for Exosome Isolation and Purification. *Front Bioeng Biotechnol*. 2021;9:811971. doi:10.3389/fbioe.2021.811971
103. Zhang X, Wei X, Qi J, et al. Simultaneous Detection of Bladder Cancer Exosomal Micrnas Based on Inorganic Nanoflare and Dnazyme Walker. *Anal Chem*. 2022;94(11):4787–4793. doi:10.1021/acs.analchem.1c05588
104. Yasamineh S, Nikben N, Ahmed MH, Kareem RA, Al-Aridhy AK, Hooshair MH. Increasing the Sensitivity and Accuracy of Detecting Exosomes as Biomarkers for Cancer Monitoring Using Optical Nanobiosensors. *Cancer Cell Int*. 2024;24(1):189. doi:10.1186/s12935-024-03379-1
105. Bashir S, Zia MA, Shoukat M, Kaleem I, Bashir S. Nanoparticles as a Novel Key Driver for the Isolation and Detection of Circulating Tumour Cells. *Sci Rep*. 2024;14(1):22580. doi:10.1038/s41598-024-67221-4
106. Kalogianni DP. Nanotechnology in Emerging Liquid Biopsy Applications. *Nano Converg*. 2021;8(1):13. doi:10.1186/s40580-021-00263-w
107. Xiong Q, Wang J, Bai Y, Song T. Recent Progress in Nanomedicine for Hepatocellular Carcinoma Therapy. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi*. 2018;35(2):314–319. doi:10.7507/1001-5515.201705008
108. Ejigah V, Owoseni O, Bataille-Backer P, Ogundipe OD, Fisus FA, Adesina SK. Approaches to Improve Macromolecule and Nanoparticle Accumulation in the Tumor Microenvironment by the Enhanced Permeability and Retention Effect. *Polymers*. 2022;14(13):2601. doi:10.3390/polym14132601
109. Lo A, Lin CT, Wu HC. Hepatocellular Carcinoma Cell-Specific Peptide Ligand for Targeted Drug Delivery. *Mol Cancer Ther*. 2008;7:579–589. doi:10.1158/1535-7163.MCT-07-2359
110. Gressler S, Hipfinger C, Part F, Pavlicek A, Zafiu C, Giese B. A Systematic Review of Nanocarriers Used in Medicine and Beyond — definition and Categorization Framework. *J Nanobiotechnol*. 2025;23(1):90. doi:10.1186/s12951-025-03113-7
111. Lu H, Zhang S, Wang J, Chen Q. A Review on Polymer and Lipid-Based Nanocarriers and Its Application to Nano-Pharmaceutical and Food-Based Systems. *Front Nutr*. 2021;8:783831. doi:10.3389/fnut.2021.783831
112. Cho K, Wang X, Nie S, Chen ZG, Shin DM. Therapeutic Nanoparticles for Drug Delivery in Cancer. *Clin Cancer Res*. 2008;14(5):1310–1316. doi:10.1158/1078-0432.CCR-07-1441
113. Jaradat E, Meziane A, Lamprou DA. Conventional Vs Pegylated Loaded Liposomal Formulations by Microfluidics for Delivering Hydrophilic Chemotherapy. *Int J Pharm*. 2024;655:124077. doi:10.1016/j.ijpharm.2024.124077
114. Wilhelm S, Tavares AJ, Dai Q, et al. Analysis of Nanoparticle Delivery to Tumours. *Nature Rev Mater*. 2016;1(5):1–12. doi:10.1038/natrevmats.2016.14
115. Szyk P, Czarczynska-Goslinska B, Ziegler-Borowska M, Larrosa I, Goslinski T. Sorafenib-Drug Delivery Strategies in Primary Liver Cancer. *J Funct Biomater*. 2025;16(4):148. doi:10.3390/jfb16040148

116. Bazak R, Hourri M, Achy SE, Hussein W, Refaat T. Passive Targeting of Nanoparticles to Cancer: a Comprehensive Review of the Literature. *Mol Clin Oncol*. 2014;2(6):904–908. doi:10.3892/mco.2014.356
117. Yu Z, Huang L, Guo J. Anti-Stromal Nanotherapeutics for Hepatocellular Carcinoma. *J Control Release*. 2024;367:500–514. doi:10.1016/j.jconrel.2024.01.050
118. Yoo J, Park C, Yi G, Lee D, Koo H. Active Targeting Strategies Using Biological Ligands for Nanoparticle Drug Delivery Systems. *Cancers*. 2019;11(5):640. doi:10.3390/cancers11050640
119. Liu Y, Wu Y, Li Z, Wan D, Pan J. Targeted Drug Delivery Strategies for the Treatment of Hepatocellular Carcinoma. *Molecules*. 2024;29(18):4405. doi:10.3390/molecules29184405
120. Li M, Zhang W, Wang B, Gao Y, Song Z, Zheng QC. Ligand-Based Targeted Therapy: a Novel Strategy for Hepatocellular Carcinoma. *Int J Nanomed*. 2016;11:5645–5669. doi:10.2147/IJN.S115727
121. van der Meel R, Vehmeijer LJ, Kok RJ, Storm G, van Gaal EV. Ligand-Targeted Particulate Nanomedicines Undergoing Clinical Evaluation: current Status. *Adv Drug Deliv Rev*. 2013;65:1284–1298. doi:10.1016/j.addr.2013.08.012
122. Zhou XQ, Li YP, Dang SS. Precision Targeting in Hepatocellular Carcinoma: exploring Ligand-Receptor Mediated Nanotherapy. *World J Hepatol*. 2024;16:164–176. doi:10.4254/wjh.v16.i2.164
123. Luo XY, Wu KM, He XX. Advances in Drug Development for Hepatocellular Carcinoma: clinical Trials and Potential Therapeutic Targets. *J Exp Clin Cancer Res*. 2021;40:172. doi:10.1186/s13046-021-01968-w
124. Zhang X, Li J, Yan M. Targeted Hepatocellular Carcinoma Therapy: transferrin Modified, Self-Assembled Polymeric Nanomedicine for Co-Delivery of Cisplatin and Doxorubicin. *Drug Dev Ind Pharm*. 2016;42(10):1590–1599. doi:10.3109/03639045.2016.1160103
125. Zhang X, Chen Y, Li X, et al. Carrier-Free Self-Assembled Nanomedicine Based on Celastrol and Galactose for Targeting Therapy of Hepatocellular Carcinoma Via Inducing Ferroptosis. *Eur J Med Chem*. 2024;267:116183. doi:10.1016/j.ejmech.2024.116183
126. He C, Zhou J, Wu X, et al. Hyaluronic Acid-Zein Shell-Core Biopolymer Nanoparticles Enhance Hepatocellular Carcinoma Therapy of Celastrol Via Cd44-Mediated Cellular Uptake. *Int J Biol Macromol*. 2024;281:136096. doi:10.1016/j.ijbiomac.2024.136096
127. Gao Y, Wang JP, Hong DF, Yang C, Naranmandura H. Mesenchymal Stem Cell-Mediated Targeted Drug Delivery Systems for Hepatocellular Carcinoma: current Advances and Future Directions. *Bioengineering*. 2025;12(11):1206. doi:10.3390/bioengineering12111206
128. Shao X, Zhao X, Wang B, Fan J, Wang J, An H. Tumor Microenvironment Targeted Nano-Drug Delivery Systems for Multidrug Resistant Tumor Therapy. *Theranostics*. 2025;15(5):1689–1714. doi:10.7150/thno.103636
129. Sung YC, Jin PR, Chu LA, et al. Delivery of Nitric Oxide with a Nanocarrier Promotes Tumour Vessel Normalization and Potentiates Anti-Cancer Therapies. *Nat Nanotechnol*. 2019;14(12):1160–1169. doi:10.1038/s41565-019-0570-3
130. Zhou W, Jia Y, Liu Y, Chen Y, Zhao P. Tumor Microenvironment-Based Stimuli-Responsive Nanoparticles for Controlled Release of Drugs in Cancer Therapy. *Pharmaceutics*. 2022;14(11):2346. doi:10.3390/pharmaceutics14112346
131. Duan S, Xia Y, Tian X, et al. A Multi-Bioresponsive Self-Assembled Nano Drug Delivery System Based on Hyaluronic Acid and Geraniol against Liver Cancer. *Carbohydr Polym*. 2023;310:120695. doi:10.1016/j.carbpol.2023.120695
132. Sun H, Nai J, Deng B, et al. Angelica Sinensis Polysaccharide-Based Nanoparticles for Liver-Targeted Delivery of Oridonin. *Molecules*. 2024;29(3):731. doi:10.3390/molecules29030731
133. Cabral H, Li J, Miyata K, Kataoka K. Controlling the Biodistribution and Clearance of Nanomedicines. *Nat Rev Bioeng*. 2024;2:214–232.
134. van Eerden RAG, Mathijssen RHJ, Koolen SLW. Recent Clinical Developments of Nanomediated Drug Delivery Systems of Taxanes for the Treatment of Cancer. *Int J Nanomed*. 2020;15:8151–8166. doi:10.2147/IJN.S272529
135. Abdifetah O, Na-Bangchang K. Pharmacokinetic Studies of Nanoparticles as a Delivery System for Conventional Drugs and Herb-Derived Compounds for Cancer Therapy: a Systematic Review. *Int J Nanomed*. 2019;14:5659–5677. doi:10.2147/IJN.S213229
136. Jia Y, Yao D, Bi H, et al. Based Nano-Delivery Systems for Anticancer Therapeutics. *Phytomedicine*. 2024;128:155521. doi:10.1016/j.phymed.2024.155521
137. Kasivisvanathan V, Thapar A, Oskrochi Y, Picard J, Leen EL. Irreversible Electroporation for Focal Ablation at the Porta Hepatis. *Cardiovasc Intervent Radiol*. 2012;35:1531–1534. doi:10.1007/s00270-012-0363-7
138. Zhang X, Zhang X, Ding X, et al. Novel Irreversible Electroporation Ablation (Nano-Knife) Versus Radiofrequency Ablation for the Treatment of Solid Liver Tumors: a Comparative, Randomized, Multicenter Clinical Study. *Front Oncol*. 2022;12:945123. doi:10.3389/fonc.2022.945123
139. Narayanan G, Froud T, Suthar R, Barbary K. Irreversible Electroporation of Hepatic Malignancy. *Semin Intervent Radiol*. 2013;30(01):67–73. doi:10.1055/s-0033-1333655
140. Aycock KN, Davalos RV. Irreversible Electroporation: background, Theory, and Review of Recent Developments in Clinical Oncology. *Bioelectricity*. 2019;1(4):214–234. doi:10.1089/bioe.2019.0029
141. Yun JH, Fang A, Khorshidi F, et al. New Developments in Image-Guided Percutaneous Irreversible Electroporation of Solid Tumors. *Curr Oncol Rep*. 2023;25(11):1213–1226. doi:10.1007/s11912-023-01452-y
142. Chen X, Ren Z, Yin S, et al. The Local Liver Ablation with Pulsed Electric Field Stimulate Systemic Immune Reaction against Hepatocellular Carcinoma (Hcc) with Time-Dependent Cytokine Profile. *Cytokine*. 2017;93:44–50. doi:10.1016/j.cyto.2017.05.003
143. Shi X, O'Neill C, Wang X, et al. Irreversible Electroporation Enhances Immunotherapeutic Effect in the Off-Target Tumor in a Murine Model of Orthotopic Hcc. *Am J Cancer Res*. 2021;11(6):3304–3319.
144. Niessen C, Igl J, Pregler B, et al. Factors Associated with Short-Term Local Recurrence of Liver Cancer after Percutaneous Ablation Using Irreversible Electroporation: a Prospective Single-Center Study. *J Vasc Interv Radiol*. 2015;26(5):694–702. doi:10.1016/j.jvir.2015.02.001
145. Aycock KN, Zhao Y, Lorenzo MF, Davalos RV. A Theoretical Argument for Extended Interpulse Delays in Therapeutic High-Frequency Irreversible Electroporation Treatments. *IEEE Trans Biomed Eng*. 2021;68(6):1999–2010. doi:10.1109/TBME.2021.3049221
146. Peeken JC, Vaupel P, Combs SE. Integrating Hyperthermia into Modern Radiation Oncology: what Evidence Is Necessary? *Front Oncol*. 2017;7:132. doi:10.3389/fonc.2017.00132
147. Dennis CL, Ivkov R. Physics of Heat Generation Using Magnetic Nanoparticles for Hyperthermia. *Int J Hyperthermia*. 2013;29(8):715–729. doi:10.3109/02656736.2013.836758
148. Attaluri A, Jackowski J, Sharma A, et al. Design and Construction of a Maxwell-Type Induction Coil for Magnetic Nanoparticle Hyperthermia. *Int J Hyperthermia*. 2020;37(1):1–14. doi:10.1080/02656736.2019.1704448

149. Chang P, Purushotham S, Rumpel H, et al. Novel Dual Magnetic Drug Targeting and Hyperthermia Therapy in Hepatocellular Carcinoma with Thermosensitive Polymer-Coated Nanoparticles. *J Gastroint Dig Syst.* 2014;4:198.
150. Gharibkandi NA, Žuk M, Muftuler FZB, Wawrowicz K, Żelechowska-Matysiak K, Bilewicz A. 198au-Coated Superparamagnetic Iron Oxide Nanoparticles for Dual Magnetic Hyperthermia and Radionuclide Therapy of Hepatocellular Carcinoma. *Int J Mol Sci.* 2023;24(6):5282. doi:10.3390/ijms24065282
151. Kraus S, Arbib S, Ventura-Bixenspaner H, et al. Case Report: treatment with Magnetic Nanoparticle-Based Hyperthermia Stabilizes Metastatic Disease in Stage Iv Primary Liver Cancer Patients. *Front Oncol.* 2025;15:1742249. doi:10.3389/fonc.2025.1742249
152. Beković M, Trbušić M, Gyergyek S, et al. Numerical Model for Determining the Magnetic Loss of Magnetic Fluids. *Materials.* 2019;12(4):591. doi:10.3390/ma12040591
153. Iglesias CAM, de Araújo JCR, Xavier J, et al. Magnetic Nanoparticles Hyperthermia in a Non-Adiabatic and Radiating Process. *Sci Rep.* 2021;11(1):11867. doi:10.1038/s41598-021-91334-9
154. Zapata J, Restrepo J. Dynamic Hysteretic Properties and Specific Loss Power of Magnetic Nanoparticles in a Viscous Medium at Different Thermal Baths. *AIP Adv.* 2023;13(10). doi:10.1063/5.0164259
155. Auerbach M, Ballard H. Clinical Use of Intravenous Iron: administration, Efficacy, and Safety. *Hematology Am Soc Hematol Educ Program.* 2010;2010 (1):338–347. doi:10.1182/asheducation-2010.1.338
156. Zhang J, Zhang M, Ji J, et al. Glycyrrhetic Acid-Mediated Polymeric Drug Delivery Targeting the Acidic Microenvironment of Hepatocellular Carcinoma. *Pharm Res.* 2015;32(10):3376–3390. doi:10.1007/s11095-015-1714-2
157. Kim CK, Choi EJ, Choi SH, Park JS, Haider KH, Ahn WS. Enhanced P53 Gene Transfer to Human Ovarian Cancer Cells Using the Cationic Nonviral Vector, Ddc. *Gynecol Oncol.* 2003;90:265–272. doi:10.1016/S0090-8258(03)00248-8
158. Zhang WW, Li L, Li D, et al. The First Approved Gene Therapy Product for Cancer Ad- p53 (Gendicine): 12 Years in the Clinic. *Hum Gene Ther.* 2018;29(2):160–179. doi:10.1089/hum.2017.218
159. Chakraborty C. Potentiality of Small Interfering Rnas (Sirna) as Recent Therapeutic Targets for Gene-Silencing. *Curr Drug Targets.* 2007;8 (3):469–482. doi:10.2174/138945007780058988
160. Oh HR, Jo H-Y, Park JS, et al. Galactosylated Liposomes for Targeted Co-Delivery of Doxorubicin/Vimentin Sirna to Hepatocellular Carcinoma. *Nanomaterials.* 2016;6(8):141. doi:10.3390/nano6080141
161. Li LC, Okino ST, Zhao H, et al. Small Dsrnas Induce Transcriptional Activation in Human Cells. *Proc Natl Acad Sci U S A.* 2006;103 (46):17337–17342. doi:10.1073/pnas.0607015103
162. Setten RL, Lightfoot HL, Habib NA, Rossi JJ. Development of Mtl-Cebpa: small Activating Rna Drug for Hepatocellular Carcinoma. *Curr Pharm Biotechnol.* 2018;19(8):611–621. doi:10.2174/1389201019666180611093428
163. Li HL, Gee P, Ishida K, Hotta A. Efficient Genomic Correction Methods in Human Ips Cells Using Crispr-Cas9 System. *Methods.* 2016;101:27–35. doi:10.1016/j.ymeth.2015.10.015
164. Ashok B, Peppas NA, Wechsler ME. Lipid and Polymer-Based Nanoparticle Systems for the Delivery of Crispr/Cas9. *J Drug Delivery Sci Technol.* 2021;65:102728. doi:10.1016/j.jddst.2021.102728
165. Liu Q, Zhao K, Wang C, et al. Multistage Delivery Nanoparticle Facilitates Efficient Crispr/Dcas9 Activation and Tumor Growth Suppression in Vivo. *Adv Sci.* 2019;6:1801423. doi:10.1002/advs.201801423
166. Kearney CJ, Lucas CR, O'Brien FJ, Castro CE. DNA Origami: folded DNA-Nanodevices That Can Direct and Interpret Cell Behavior. *Adv Mater.* 2016;28(27):5509–5524. doi:10.1002/adma.201504733
167. Hannewald N, Winterwerber P, Zechel S, et al. DNA Origami Meets Polymers: a Powerful Tool for the Design of Defined Nanostructures. *Angew Chem Int Ed Engl.* 2021;60(12):6218–6229. doi:10.1002/anie.202005907
168. Ghosal S, Bag S, Bhowmik S. Unravelling the Drug Encapsulation Ability of Functional DNA Origami Nanostructures: current Understanding and Future Prospects on Targeted Drug Delivery. *Polymers.* 2023;15(8):1850. doi:10.3390/polym15081850
169. Zhang Y, Tian X, Wang Z, et al. Advanced Applications of DNA Nanostructures Dominated by DNA Origami in Antitumor Drug Delivery. *Front Mol Biosci.* 2023;10:1239952. doi:10.3389/fmolb.2023.1239952
170. Zhang H, Qu X, Chen H, et al. Fabrication of Calcium Phosphate-Based Nanocomposites Incorporating DNA Origami, Gold Nanorods, and Anticancer Drugs for Biomedical Applications. *Adv Health Mater.* 2017;6(20). doi:10.1002/adhm.201700664
171. Riley RS, June CH, Langer R, Mitchell MJ. Delivery Technologies for Cancer Immunotherapy. *Nat Rev Drug Discov.* 2019;18(3):175–196. doi:10.1038/s41573-018-0006-z
172. Zhang L, Huang J, Chen X, et al. Self-Assembly Nanovaccine Containing Tlr7/8 Agonist and Stat3 Inhibitor Enhances Tumor Immunotherapy by Augmenting Tumor-Specific Immune Response. *J Immunother Cancer.* 2021;9(8):e003132. doi:10.1136/jitc-2021-003132
173. Ren X, Su D, Shi D, Xiang X. The Improving Strategies and Applications of Nanotechnology-Based Drugs in Hepatocellular Carcinoma Treatment. *Front Bioeng Biotechnol.* 2023;11:1272850. doi:10.3389/fbioe.2023.1272850
174. Lin YX, Wang Y, Blake S, et al. Rna Nanotechnology-Mediated Cancer Immunotherapy. *Theranostics.* 2020;10(1):281–299. doi:10.7150/thno.35568
175. Chen Y, Zhou Q, Jia Z, et al. Enhancing Cancer Immunotherapy: nanotechnology-Mediated Immunotherapy Overcoming Immunosuppression. *Acta Pharm Sin B.* 2024;14(9):3834–3854. doi:10.1016/j.apsb.2024.05.032
176. Xiang S, Zhan H, Zhan J, Li X, Lin X, Sun W. Breaking Hypoxic Barrier: oxygen-Supplied Nanomaterials for Enhanced T Cell-Mediated Tumor Immunotherapy. *Int J Pharm X.* 2025;10:100400. doi:10.1016/j.ijpx.2025.100400
177. Hu Y, He K, Zhang M, et al. Multi-Faceted Nanodrug Reinforcing Metalloimmunotherapy for Hepatocellular Carcinoma. *Biomaterials.* 2026;329:123899. doi:10.1016/j.biomaterials.2025.123899
178. Abdullah KA, Tahir TF, Qader AF, Omer RA, Othman KA. Nanozymes: classification and Analytical Applications—a Review. *J Fluoresc.* 2024;34(1):1–15. doi:10.1007/s10895-023-03262-8
179. Yang W, Yang X, Zhu L, Chu H, Li X, Xu W. Nanozymes: activity Origin, Catalytic Mechanism, and Biological Application. *Coord Chem Rev.* 2021;448:214170. doi:10.1016/j.ccr.2021.214170
180. Li X, Hu J, Zhao Q, Yao W, Jing Z, Jin Z. Towards Precision Medicine: design Considerations for Nanozymes in Tumor Treatment. *J Transl Med.* 2024;22(1):1033. doi:10.1186/s12967-024-05845-w

181. Gao S, Lin H, Zhang H, Yao H, Chen Y, Shi J. Nanocatalytic Tumor Therapy by Biomimetic Dual Inorganic Nanozyme-Catalyzed Cascade Reaction. *Adv Sci*. 2019;6(3):1801733. doi:10.1002/adv.201801733
182. Wang X, Xiong T, Cui M, et al. Targeted Self-Activating Au-Fe₃O₄ Composite Nanocatalyst for Enhanced Precise Hepatocellular Carcinoma Therapy Via Dual Nanozyme-Catalyzed Cascade Reactions. *Appl Mater Today*. 2020;21:100827. doi:10.1016/j.apmt.2020.100827
183. Fan K, Xi J, Fan L, et al. In Vivo Guiding Nitrogen-Doped Carbon Nanozyme for Tumor Catalytic Therapy. *Nat Commun*. 2018;9(1):1440. doi:10.1038/s41467-018-03903-8
184. Chen K, Li Q, Zhao X, et al. Biocompatible melanin based theranostic agent for in vivo detection and ablation of orthotopic micro-hepatocellular carcinoma. *Biomater Sci*. 2020;8(15):4322–4333. doi:10.1039/D0BM00825G
185. Sun J, Jin Z, Jin Y, Yuan H, Jin G, Quan J. MRI-Based Multifunctional Nanoliposomes for Enhanced Hcc Therapy and Diagnosis. *Mol Pharm*. 2025;22(2):787–807. doi:10.1021/acs.molpharmaceut.4c00917
186. Lai X, Lu T, Zhang F, et al. Lysosome-Targeted Theranostics: integration of Real-Time Fluorescence Imaging and Controlled Drug Delivery Via Zn(II)-Schiff Base Complexes. *J Inorg Biochem*. 2025;272:113015. doi:10.1016/j.jinorgbio.2025.113015
187. Algar WR, Massey M, Rees K, et al. Photoluminescent Nanoparticles for Chemical and Biological Analysis and Imaging. *Chem Rev*. 2021;121(15):9243–9358. doi:10.1021/acs.chemrev.0c01176
188. Zhang W, Hu Z, Tian J, Fang C. A Narrative Review of near-Infrared Fluorescence Imaging in Hepatectomy for Hepatocellular Carcinoma. *Ann Transl Med*. 2021;9(2):171. doi:10.21037/atm-20-5341
189. He P, Su S, Fang C, et al. Residual Cancerous Lesion and Vein Tumour Thrombus Identified Intraoperatively Using a Fluorescence Navigation System in Liver Surgery. *ANZ J Surg*. 2019;89(7–8):E308–e314. doi:10.1111/ans.15282
190. Fang CH, Zhang P, Luo HL, et al. Application of Augmented-Reality Surgical Navigation Technology Combined with Icg Molecular Fluorescence Imaging in Laparoscopic Hepatectomy. *Zhonghua Wai Ke Za Zhi*. 2019;57(8):578–584. doi:10.3760/cma.j.issn.0529-5815.2019.08.004
191. Gao F, Xie Q, Ran X, et al. Use of Indocyanine Green-Human Serum Albumin Complexes in Fluorescence Image-Guided Laparoscopic Anatomical Liver Resection: a Case Series Study (with Video). *Surg Endosc*. 2024;38(11):6938–6947. doi:10.1007/s00464-024-11295-8
192. Zhu J, Chu C, Li D, et al. Superior Fluorescent Nanoemulsion Illuminates Hepatocellular Carcinoma for Surgical Navigation. *Front Bioeng Biotechnol*. 2022;10:890668. doi:10.3389/fbioe.2022.890668
193. Gong J, Hu J, Yan X, et al. Injectable Hydrogels Including Magnetic Nanosheets for Multidisciplinary Treatment of Hepatocellular Carcinoma Via Magnetic Hyperthermia. *Small*. 2024;20(3):e2300733. doi:10.1002/smll.202300733
194. Pillai K, Ke K, Mekawy A, Akhter J, Morris DL. Enhancement of Treatment Efficacy of Hepatic Tumours Using Trans-Arterial-Chemoembolization. *Am J Cancer Res*. 2023;13(5):1623–1639.
195. Ashour R, Rewisha E, Rady MA, Elkhadry SW, Abdelhalim H, Atef M. Effectiveness of Sorafenib in Treating Intermediate-Stage Hepatocellular Carcinoma Patients Refractory to Transarterial Chemoembolization. *BMC Cancer*. 2024;24(1):1466. doi:10.1186/s12885-024-13199-1
196. Cheng AL, Kang YK, Chen Z, et al. Efficacy and Safety of Sorafenib in Patients in the Asia-Pacific Region with Advanced Hepatocellular Carcinoma: a Phase III Randomised, Double-Blind, Placebo-Controlled Trial. *Lancet Oncol*. 2009;10(1):25–34. doi:10.1016/S1470-2045(08)70285-7
197. Kong FH, Ye QF, Miao XY, et al. Current Status of Sorafenib Nanoparticle Delivery Systems in the Treatment of Hepatocellular Carcinoma. *Theranostics*. 2021;11(11):5464–5490. doi:10.7150/thno.54822
198. Zheng L, Li C, Huang X, et al. Thermosensitive Hydrogels for Sustained-Release of Sorafenib and Selenium Nanoparticles for Localized Synergistic Chemoradiotherapy. *Biomaterials*. 2019;216:119220. doi:10.1016/j.biomaterials.2019.05.031
199. Wu J, Zhao X, Ren B, Duan X, Sun J. Radioresistance in Hepatocellular Carcinoma: biological Bases and Therapeutic Implications. *Int J Mol Sci*. 2025;26(5):1839. doi:10.3390/ijms26051839
200. Arif M, Nawaz AF, Khan SU, et al. Nanotechnology-Based Radiation Therapy to Cure Cancer and the Challenges in Its Clinical Applications. *Heliyon*. 2023;9(6):e17252. doi:10.1016/j.heliyon.2023.e17252
201. Baskar R, Lee KA, Yeo R, Yeoh KW. Cancer and Radiation Therapy: current Advances and Future Directions. *Int J Med Sci*. 2012;9(3):193–199. doi:10.7150/ijms.3635
202. Nguyen V-K, Tsai S-W, Cho I-C, et al. Gold Nanoparticle-Enhanced Production of Reactive Oxygen Species for Radiotherapy and Phototherapy. *Nanomaterials*. 2025;15(4):317. doi:10.3390/nano15040317
203. Gerken LRH, Gerdes ME, Pruschy M, Herrmann IK. Prospects of Nanoparticle-Based Radioenhancement for Radiotherapy. *Mater Horiz*. 2023;10(10):4059–4082. doi:10.1039/D3MH00265A
204. Taghizadeh S, Alimardani V, Roudbali PL, Ghasemi Y, Kaviani E. Gold Nanoparticles Application in Liver Cancer. *Photodiagnosis Photodyn Ther*. 2019;25:389–400. doi:10.1016/j.pdpdt.2019.01.027
205. Malone CD, Egbulefu C, Zheleznyak A, et al. Activation of Nano-Photosensitizers by Y-90 Microspheres to Enhance Oxidative Stress and Cell Death in Hepatocellular Carcinoma. *Sci Rep*. 2022;12(1):12748. doi:10.1038/s41598-022-17185-0
206. Kciuk M, Yahya EB, Mohamed MMI, et al. Recent Advances in Molecular Mechanisms of Cancer Immunotherapy. *Cancers*. 2023;15(10):2721. doi:10.3390/cancers15102721
207. Dobosz P, Dzieciatkowski T. The Intriguing History of Cancer Immunotherapy. *Front Immunol*. 2019;10:2965. doi:10.3389/fimmu.2019.02965
208. Lu Q, Kou D, Lou S, et al. Nanoparticles in Tumor Microenvironment Remodeling and Cancer Immunotherapy. *J Hematol Oncol*. 2024;17(1):16. doi:10.1186/s13045-024-01535-8
209. Yu Z, Guo J, Liu Y, et al. Nano Delivery of Simvastatin Targets Liver Sinusoidal Endothelial Cells to Remodel Tumor Microenvironment for Hepatocellular Carcinoma. *J Nanobiotechnology*. 2022;20(1):9. doi:10.1186/s12951-021-01205-8
210. Zhou Z, Zhang B, Zai W, et al. Perfluorocarbon Nanoparticle-Mediated Platelet Inhibition Promotes Intratumoral Infiltration of T Cells and Boosts Immunotherapy. *Proc Natl Acad Sci U S A*. 2019;116(24):11972–11977. doi:10.1073/pnas.1901987116
211. Whiteside TL. Foxp3+ Treg as a Therapeutic Target for Promoting Anti-Tumor Immunity. *Expert Opin Ther Targets*. 2018;22(4):353–363. doi:10.1080/14728222.2018.1451514
212. Cabral H, Kinoh H, Kataoka K. Tumor-Targeted Nanomedicine for Immunotherapy. *Acc Chem Res*. 2020;53(12):2765–2776. doi:10.1021/acs.accounts.0c00518

213. Hao L, Li S, Ye F, et al. The Current Status and Future of Targeted-Immune Combination for Hepatocellular Carcinoma. *Front Immunol.* **2024**;15:1418965. doi:10.3389/fimmu.2024.1418965
214. Wang X, Fan R, Mu M, et al. Harnessing Nanoengineered Car-T Cell Strategies to Advance Solid Tumor Immunotherapy. *Trends Cell Biol.* **2025**;35(9):782–798. doi:10.1016/j.tcb.2024.11.010
215. Chada NC, Wilson JT. Jump-Starting Chimeric Antigen Receptor-T Cells to Go the Extra Mile with Nanotechnology. *Curr Opin Biotechnol.* **2024**;89:103179. doi:10.1016/j.copbio.2024.103179
216. Kolluri A, Li D, Li N, Duan Z, Roberts LR, Ho M. Human Vh-Based Chimeric Antigen Receptor T Cells Targeting Glypican 3 Eliminate Tumors in Preclinical Models of Hcc. *Hepatol Commun.* **2023**;7(2):e0022. doi:10.1097/HC9.0000000000000022
217. Yang Z, Cheng C, Li Z, et al. Advancing Liver Cancer Treatment with Dual-Targeting Car-T Therapy. *J Nanobiotechnology.* **2025**;23(1):462. doi:10.1186/s12951-025-03512-w
218. Habibollahi P, Gurevich A, Hui JZ, et al. Integrated Imaging Probe and Bispecific Antibody Development Enables in Vivo Targeting of Glypican-3-Expressing Hepatocellular Carcinoma. *Mol Cancer Ther.* **2024**;23:1815–1826. doi:10.1158/1535-7163.MCT-23-0470
219. Chen L, Zhu Y, Feng M, Zuo D, Chen G, Ji K. Targeting Cd16a on Nk Cells and Gpc3 in Hepatocellular Carcinoma: development and Functional Validation of a Therapeutic Bispecific Antibody. *Front Immunol.* **2025**;16:1599764. doi:10.3389/fimmu.2025.1599764
220. Chen X, Chen Y, Liang R, et al. Combination Therapy of Hepatocellular Carcinoma by Gpc3-Targeted Bispecific Antibody and Irinotecan Is Potent in Suppressing Tumor Growth in Mice. *Mol Cancer Ther.* **2022**;21:149–158. doi:10.1158/1535-7163.MCT-20-1025
221. Lu Y, Liu Y, Zuo X, et al. Cxcl12(+) Tumor-Associated Endothelial Cells Promote Immune Resistance in Hepatocellular Carcinoma. *J Hepatol.* **2025**;82:634–648. doi:10.1016/j.jhep.2024.09.044
222. Zhu M, Wu Y, Zhu T, et al. Multifunctional Bispecific Nanovesicles Targeting Slamf7 Trigger Potent Antitumor Immunity. *Cancer Immunol Res.* **2024**;12(8):1007–1021. doi:10.1158/2326-6066.CIR-23-1102
223. Ning Y, Yang A, Zhou S, et al. B-Glucan Nanotubes Loaded with Iron Diselenide for Enhanced Photothermal and Photodynamic Therapy in Hepatocellular Carcinoma. *Acta Biomater.* **2025**;207:577–590. doi:10.1016/j.actbio.2025.10.005
224. Wang J, Qiu J, Tsang KC, et al. Enhanced Functionality of Anti-Gpc3 Car-T Cells against Hepatocellular Carcinoma through Locoregional Administration. *Liver Int.* **2025**;45:e70450. doi:10.1111/liv.70450
225. Liu X, Liu Y, Zhao D, Shan D, Guo C, Jia L. Nanomaterials Mediated Enhancement of Car-T for Hcc: revolutionizing Immunotherapy Strategies. *Int J Nanomed.* **2025**;20:7489–7500. doi:10.2147/IJN.S527315
226. Sui M, Liu T, Song X, et al. The Molecular Receptor Nkbb Enhances the Persistence and Anti-Hepatocellular Carcinoma Activity of Gpc3 Car-T Cells. *Pharmacol Res.* **2025**;212:107619. doi:10.1016/j.phrs.2025.107619
227. Guan X, Sun L, Shen Y, et al. Nanoparticle-Enhanced Radiotherapy Synergizes with Pd-L1 Blockade to Limit Post-Surgical Cancer Recurrence and Metastasis. *Nat Commun.* **2022**;13(1):2834. doi:10.1038/s41467-022-30543-w
228. Wang J, Guo Q, He L, et al. A Nanoradiosensitizer Potentiates Tumor Radiotherapy through Jfk Inhibition and Hypoxia Alleviation. *Nano Lett.* **2025**;25:5435–5443. doi:10.1021/acs.nanolett.5c00677
229. Yalikun K, Li Z, Zhang J, et al. Hepatic Artery Infusion Chemotherapy Combined with Camrelizumab and Apatinib as Conversion Therapy for Patients with Unresectable Hepatocellular Carcinoma: a Single-Arm Exploratory Trial. *BMC Cancer.* **2025**;25(1):838. doi:10.1186/s12885-025-14250-5
230. Yu S, Wang Y, He P, et al. Effective Combinations of Immunotherapy and Radiotherapy for Cancer Treatment. *Front Oncol.* **2022**;12:809304. doi:10.3389/fonc.2022.809304
231. Mokhtari RB, Homayouni TS, Baluch N, et al. Combination Therapy in Combating Cancer. *Oncotarget.* **2017**;8(23):38022–38043. doi:10.18632/oncotarget.16723
232. Li Q, Lei X, Zhu J, et al. Radiotherapy/Chemotherapy-Immunotherapy for Cancer Management: from Mechanisms to Clinical Implications. *Oxid Med Cell Longev.* **2023**;2023:7530794. doi:10.1155/2023/7530794
233. Farinha D, Sarmiento-Ribeiro AB, Faneca H. Combination of Gene Therapy and Chemotherapy in a New Targeted Hybrid Nanosystem to Hepatocellular Carcinoma. *Int J Nanomed.* **2024**;19:12505–12527. doi:10.2147/IJN.S474665
234. Zhu F, Wang BR, Zhu ZF, et al. Photodynamic Therapy: a Next Alternative Treatment Strategy for Hepatocellular Carcinoma? *World J Gastrointest Surg.* **2021**;13(12):1523–1535. doi:10.4240/wjgs.v13.i12.1523
235. Ismail AF, Ali MM, Ismail LF. Photodynamic Therapy Mediated Antiproliferative Activity of Some Metal-Doped ZnO Nanoparticles in Human Liver Adenocarcinoma Hepg2 Cells under Uv Irradiation. *J Photochem Photobiol B.* **2014**;138:99–108. doi:10.1016/j.jphotobiol.2014.04.006
236. Zhang D, Zheng A, Li J, et al. Smart Cu(Ii)-Aptamer Complexes Based Gold Nanoplatfor for Tumor Micro-Environment Triggered Programmable Intracellular Prodrug Release, Photodynamic Treatment and Aggregation Induced Photothermal Therapy of Hepatocellular Carcinoma. *Theranostics.* **2017**;7(1):164–179. doi:10.7150/thno.17099
237. Li Z, He J, Li B, et al. Titanium Dioxide Nanoparticles Induce Endoplasmic Reticulum Stress-Mediated Apoptotic Cell Death in Liver Cancer Cells. *J Int Med Res.* **2020**;48:300060520903652. doi:10.1177/0300060520903652
238. Pandey PR, Young KH, Kumar D, Jain N. Rna-Mediated Immunotherapy Regulating Tumor Immune Microenvironment: next Wave of Cancer Therapeutics. *Mol Cancer.* **2022**;21:58. doi:10.1186/s12943-022-01528-6
239. Zhang X, Su K, Wu S, et al. One-Component Cationic Lipids for Systemic mRNA Delivery to Splenic T Cells. *Angew Chem Int Ed Engl.* **2024**;63(26):e202405444. doi:10.1002/anie.202405444
240. Cheng Q, Wei T, Farbiak L, Johnson LT, Dilliard SA, Siegwart DJ. Selective Organ Targeting (Sort) Nanoparticles for Tissue-Specific mRNA Delivery and Crispr-Cas Gene Editing. *Nat Nanotechnol.* **2020**;15(4):313–320. doi:10.1038/s41565-020-0669-6
241. Zhao C, Wang B, Liu E, Zhang Z. Loss of PTEN Expression Is Associated with P13k Pathway-Dependent Metabolic Reprogramming in Hepatocellular Carcinoma. *Cell Commun Signal.* **2020**;18(1):131. doi:10.1186/s12964-020-00622-w
242. Zhang Y, Xie F, Yin Y, et al. Immunotherapy of Tumor Rna-Loaded Lipid Nanoparticles against Hepatocellular Carcinoma. *Int J Nanomed.* **2021**;16:1553–1564. doi:10.2147/IJN.S291421
243. Ma Y, Xiao CL, Han JX, et al. Synergistic Nanobubble-Mediated Sonodynamic Therapy and Microrna Immunotherapy Suppresses Hepatocellular Carcinoma. *Mat Today Nano.* **2026**;33:100742. doi:10.1016/j.mtnano.2025.100742
244. Qi S, Zhou L, Liu W, et al. Pa/Mr Imaging-Guided Precision Phototherapy and Efficacy Evaluation of Hepatocellular Carcinoma Utilizing a Targeted Multifunctional Nanoprobe. *Front Immunol.* **2025**;16:1605048. doi:10.3389/fimmu.2025.1605048

245. Chen Y, Jiang Z, Yan Z, et al. A Codelivery System Loaded with Pd11-Sirna and Sorafenib Enhances the Therapeutic Effect of Sorafenib on Hepatocellular Carcinoma Via Tat-Poly-Ss-Lysine Modified Chitosan. *Int J Biol Macromol.* **2024**;263:130162. doi:10.1016/j.ijbiomac.2024.130162
246. Bai B, Zheng Z, Zhu B, et al. An Fe(III)-Covalent Organic Framework (Cof)-Sorafenib Nanoplatfrom Induces Chemo-Ferroptosis for Enhanced Hepatocellular Carcinoma Immunotherapy. *Mater Today Bio.* **2025**;34:102135. doi:10.1016/j.mtbio.2025.102135
247. Chen Y, Lin G, Wang Z, et al. Predicting Anti-Tumor Efficacy of Multi-Functional Nanomedicine on Decellularized Hepatocellular Carcinoma-on-a-Chip. *Biosens Bioelectron.* **2024**;264:116668. doi:10.1016/j.bios.2024.116668
248. Guo C, Dou R, Wang L, et al. A Metal-Drug Self-Delivery Nanomedicine Alleviates Tumor Immunosuppression to Potentiate Synergistic Chemo/Chemodynamic Therapy against Hepatocellular Carcinoma. *Fundam Res.* **2025**;5:1440–1450. doi:10.1016/j.fmre.2024.12.014
249. Dong M, Liu Y, Xiao Y, et al. Tumor-Targeted PLGA Nanospheres Enhance Therapeutic Effect of Lenvatinib in Hepatocellular Carcinoma Via Photothermal and Photodynamic Therapy. *ACS Appl Mater Interfaces.* **2025**;17:44922–44938. doi:10.1021/acsami.5c09045
250. Chen T, Tam N, Mao Y, et al. A Multi-Hit Therapeutic Nanoplatfrom for Hepatocellular Carcinoma: dual Stimuli-Responsive Drug Release, Dual-Modal Imaging, and in Situ Oxygen Supply to Enhance Synergistic Therapy. *Mater Today Bio.* **2022**;16:100338. doi:10.1016/j.mtbio.2022.100338
251. Yang X, Zhang Q, Li D, et al. A Multifunctional Nanodrug Increases the Therapeutic Sensitivity of Lenvatinib to Hepatocellular Carcinoma by Inhibiting the Stemness of Hepatic Cancer Stem Cells. *Adv Healthc Mater.* **2024**;13(29):e2401398. doi:10.1002/adhm.202401398
252. Jianghong L, Tingting M, Yingping Z, et al. Aptamer and Peptide-Modified Lipid-Based Drug Delivery Systems in Application of Combined Sequential Therapy of Hepatocellular Carcinoma. *ACS Biomater Sci Eng.* **2021**;7(6):2558–2568. doi:10.1021/acsbomaterials.1c00357
253. Jia G, Wang T, Li R, et al. Radioiodine-131-Labeled Theranostic Nanoparticles for Transarterial Radioembolization and Chemoembolization Combination Therapy of Vx2 Liver Tumor. *Adv Healthc Mater.* **2023**;12(32):e2301559. doi:10.1002/adhm.202301559
254. Wu LP, Wang D, Li Z. Grand Challenges in Nanomedicine. *Mater Sci Eng C Mater Biol Appl.* **2020**;106:110302. doi:10.1016/j.msec.2019.110302
255. Grant CR, Liberal R. Liver Immunology: how to Reconcile Tolerance with Autoimmunity. *Clin Res Hepatol Gastroenterol.* **2017**;41:6–16. doi:10.1016/j.clinre.2016.06.003
256. Feng H, Zhuo Y, Zhang X, et al. Tumor Microenvironment in Hepatocellular Carcinoma: key Players for Immunotherapy. *J Hepatocell Carcinoma.* **2022**;9:1109–1125. doi:10.2147/JHC.S381764
257. Doherty DG. Immunity, Tolerance and Autoimmunity in the Liver: a Comprehensive Review. *J Autoimmun.* **2016**;66:60–75. doi:10.1016/j.jaut.2015.08.020
258. Cormode DP, Skajaa GO, Delshad A, et al. A Versatile and Tunable Coating Strategy Allows Control of Nanocrystal Delivery to Cell Types in the Liver. *Bioconjug Chem.* **2011**;22(3):353–361. doi:10.1021/bc1003179
259. Sahay G, Alakhova DY, Kabanov AV. Endocytosis of Nanomedicines. *J Control Release.* **2010**;145(3):182–195. doi:10.1016/j.jconrel.2010.01.036
260. Poisson J, Lemoine S, Boulanger C, et al. Liver Sinusoidal Endothelial Cells: physiology and Role in Liver Diseases. *J Hepatol.* **2017**;66(1):212–227. doi:10.1016/j.jhep.2016.07.009
261. Liu Y, Cong K, Li Q, Zhang D, Sheng J. New Insights into Combined Immunotherapy for Hepatocellular Carcinoma Associated with Liver Cirrhosis. *Front Immunol.* **2025**;16:1741398. doi:10.3389/fimmu.2025.1741398
262. Wu S, Anand N, Guo Z, et al. Bridging Immune Evasion and Vascular Dynamics for Novel Therapeutic Frontiers in Hepatocellular Carcinoma. *Cancers.* **2025**;17(11):1860. doi:10.3390/cancers17111860
263. Yuan G, Luo X, He K, et al. Intratumoral Self-Assembly of Renal-Clearable Gold Nanoparticles as Precise Photothermal Nanomedicine for Liver Tumor Therapy. *Sci Adv.* **2025**;11(17):eadw7032. doi:10.1126/sciadv.adw7032
264. Jiang K, Tian K, Yu Y, et al. Kupffer Cells Determine Intrahepatic Traffic of Pegylated Liposomal Doxorubicin. *Nat Commun.* **2024**;15(1):6136. doi:10.1038/s41467-024-50568-7
265. Zenze M, Singh M. Receptor Targeting Using Copolymer-Modified Gold Nanoparticles for Pcmv-Luc Gene Delivery to Liver Cancer Cells in Vitro. *Int J Mol Sci.* **2024**;25(9):5016. doi:10.3390/ijms25095016
266. Aobulaisan K, Li Q, Deng C, et al. Asgpr-Targeted Micelles Co-Delivering Lenvatinib and Cop1 Sirna for Hepatocellular Carcinoma Via Dual-Targeting. *Eur J Pharm Sci.* **2026**;216:107308. doi:10.1016/j.ejps.2025.107308
267. Li YQ, Liao Y, Zhang W, Rao GW, Zheng Q. Research Progress of Drugs Targeting Gpc3 in Cancer Therapy. *Bioorg Chem.* **2026**;169:109383. doi:10.1016/j.bioorg.2025.109383
268. Hosseini A, Mohammadnejad J, Narmani A, Jafari H. Folic Acid-Chitosan-PLGA Nano Delivery System against Liver Cancer Cells: in Vitro Studies. *J Biochem Mol Toxicol.* **2025**;39(9):e70478. doi:10.1002/jbt.70478
269. Fawaz W, Hanano A, Murad H, Yousfan A, Alghoraibi I, Hasian J. Polymeric Nanoparticles Loaded with Vincristine and Carbon Dots for Hepatocellular Carcinoma Therapy and Imaging. *Sci Rep.* **2024**;14(1):24520. doi:10.1038/s41598-024-75332-1
270. Jakic K, Selc M, Razga F, et al. Long-Term Accumulation, Biological Effects and Toxicity of Bsa-Coated Gold Nanoparticles in the Mouse Liver, Spleen, and Kidneys. *Int J Nanomed.* **2024**;19:4103–4120. doi:10.2147/IJN.S443168
271. Guo Z, Liu D, Zhang H, Yu W, Li Y, Gu N. Superparamagnetic Iron Oxide Nanoparticles in Clinical Applications: current Status and Future Perspectives. *EBioMedicine.* **2025**;122:106054. doi:10.1016/j.ebiom.2025.106054
272. Sun T, Kang Y, Liu J, et al. Nanomaterials and Hepatic Disease: toxicokinetics, Disease Types, Intrinsic Mechanisms, Liver Susceptibility, and Influencing Factors. *J Nanobiotechnology.* **2021**;19(1):108. doi:10.1186/s12951-021-00843-2
273. Pei Z, Lei H, Cheng L. Bioactive Inorganic Nanomaterials for Cancer Theranostics. *Chem Soc Rev.* **2023**;52(6):2031–2081. doi:10.1039/D2CS00352J
274. Lehrich BM, Delgado ER. Lipid Nanovesicle Platforms for Hepatocellular Carcinoma Precision Medicine Therapeutics: progress and Perspectives. *Organogenesis.* **2024**;20(1):2313696. doi:10.1080/15476278.2024.2313696
275. Gao F, Feng X, Li X. Recent Advances in Polymeric Nanoparticles for the Treatment of Hepatic Diseases. *Front Pharmacol.* **2025**;16:1528752. doi:10.3389/fphar.2025.1528752
276. He Y, Wang Y, Wang L, Jiang W, Wilhelm S. Understanding Nanoparticle-Liver Interactions in Nanomedicine. *Expert Opin Drug Deliv.* **2024**;21(6):829–843. doi:10.1080/17425247.2024.2375400

277. Pan J, Zhang C, Lu H, et al. Activation of Pregnane X Receptor-Organic Anion Transporting Polypeptide 1a/B /P-Glycoprotein/Multidrug Resistance Protein 2 Axis Mediates the Accelerated Blood and Liver Clearance of Pegylated Liposomes. *Drug Metab Dispos.* **2025**;53(11):100172. doi:10.1016/j.dmd.2025.100172
278. Roszkowski S, Durczynska Z. Advantages and Limitations of Nanostructures for Biomedical Applications. *Adv Clin Exp Med.* **2025**;34(3):447–456. doi:10.17219/acem/186846
279. Jarrar B, Almansour M, Al-Doaiss A, et al. Metallic and Metallic Oxide Nanoparticles Toxicity Primarily Targets the Mitochondria of Hepatocytes and Renal Cells. *Toxicol Ind Health.* **2024**;40(12):667–678. doi:10.1177/07482337241282860
280. Sun H, Jiang C, Wu L, Bai X, Zhai S. Cytotoxicity-Related Bioeffects Induced by Nanoparticles: the Role of Surface Chemistry. *Front Bioeng Biotechnol.* **2019**;7:414. doi:10.3389/fbioe.2019.00414
281. Singh N, Kim J, Kim J, et al. Covalent Organic Framework Nanomedicines: biocompatibility for Advanced Nanocarriers and Cancer Theranostics Applications. *Bioact Mater.* **2023**;21:358–380. doi:10.1016/j.bioactmat.2022.08.016
282. Zhang L, Jin S, Wei Y, et al. Prognostic Significance of Matrix Metalloproteinase 14 in Patients with Cancer: a Systematic Review and Meta-Analysis. *Clin Lab.* **2020**;86:1.
283. Desai N, Chavda V, Singh TRR, Thorat ND, Vora LK. Cancer Nanovaccines: nanomaterials and Clinical Perspectives. *Small.* **2024**;20:e2401631. doi:10.1002/sml.202401631
284. Zhou S, Wang C, Ning Y, et al. Virus-Like Particle-Based Personalized Neoantigen Nano-Vaccine for Tumor Immunotherapy and Recurrence Prevention. *ACS Nano.* **2025**;19(40):35385–35400. doi:10.1021/acsnano.5c06278
285. Gurunathan S, Thangaraj P, Wang L, Cao Q, Kim JH. Nanovaccines: an Effective Therapeutic Approach for Cancer Therapy. *Biomed Pharmacother.* **2024**;170:115992. doi:10.1016/j.biopha.2023.115992
286. Yin X, Zhao X, Shen Y, et al. Nanoparticle-Mediated Dual Targeting of Stromal and Immune Components to Overcome Fibrotic and Immunosuppressive Barriers in Hepatocellular Carcinoma. *J Control Release.* **2025**;383:113783. doi:10.1016/j.jconrel.2025.113783
287. Sun Y, Zhai W, Liu X, et al. Homotypic Cell Membrane-Cloaked Biomimetic Nanocarrier for the Accurate Photothermal-Chemotherapy Treatment of Recurrent Hepatocellular Carcinoma. *J Nanobiotechnology.* **2020**;18(1):60. doi:10.1186/s12951-020-00617-2
288. Bhange M, Telange D. Convergence of Nanotechnology and Artificial Intelligence in the Fight against Liver Cancer: a Comprehensive Review. *Discov Oncol.* **2025**;16(1):77. doi:10.1007/s12672-025-01821-y

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