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Review Article

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# Review of Nanomedicine Applications in the Treatment of Hepatocellular Carcinoma

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## Abstract

Cancer constitutes a substantial global economic and health burden, prompting the use of conventional treatment approaches like chemotherapy, radiotherapy, and surgery. Unfortunately, many patients are diagnosed in the middle to advanced stages, limiting curative options. Liver cancer is particularly challenging due to its resistance to chemotherapy and radiotherapy. Conventional molecularly targeted drugs for liver cancer encounter issues such as brief effectiveness, inadequate drug concentration, and drug resistance. Therefore, it is imperative to urgently develop novel diagnostic and therapeutic strategies that can enhance the diagnosis, treatment, and long-term outlook for liver cancer. Nanomedicine, a facet of nanotechnology in medical research, offers promising avenues for advanced cancer treatment. This review aims to consolidate the shortcomings of conventional HCC treatments, underscore the significance, limitations, and future potential of nanomaterial research, and outline the evolution and implementation of nanotechnology for diagnosing and treating HCC. In addition, various strategies employed for drug delivery using nanosystems and various models used for experimentation were presented in detail.

## Keywords

Conventional treatments, Hepatocellular carcinoma, Mortality, Nanomedicine, Prevalence, Therapeutic approach

## Abbreviations

5-FU: 5-Fluorouracil; ASGPRs: Asialoglycoprotein receptors; ATO: Arsenic trioxide; AuNPs: Gold nanoparticles; CAR: Chimeric antigen receptor; CDT: Chemodynamic therapy; CSCs: Cancer stem cells; CT: Computerized tomography; CTCs: Circulating tumor cells; DNA: Deoxyribonucleic acid; DOX: Doxorubicin; DEX: Dexamethasone; ECM: Extracellular matrix; EPR: Enhanced permeability and retention; $H_2O_2$: Hydrogen peroxide; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; HSCs: Hepatic stellate cells; LEN: Lenvatinib; MOFs: Metal-organic frameworks; MRI: Magnetic resonance imaging; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; NPs: Nanoparticles; $OH^-$: Hydroxyl radicals; PBCA: Polybutylcyanoacrylate; PDT: Photodynamic therapy; PHH: Primary human hepatocytes; PS: Photosensitizers; PTT: Photothermal therapy; PTX: Paclitaxel; QDs: Quantum dots; RFA: Radiofrequency ablation; RNA: Ribonucleic acid; ROS: Reactive oxygen species; SDT: Sonodynamic therapy; SPIONs: Superparamagnetic iron oxide nanoparticles; SFN: Sorafenib; TACE: Transarterial chemoembolization; TARE: Transarterial radioembolization; TKIs:

Tyrosine kinase inhibitors; TME: Tumor microenvironment; VEGFR: Vascular endothelial growth factor receptor

## Introduction

Liver cancer is a malignancy affecting both the liver's structure and the intrahepatic bile ducts. It encompasses various subtypes such as fibrolamellar carcinoma, hepatoblastoma, hepatic angiosarcoma, cholangiocarcinoma, and hepatocellular carcinoma. Among these, HCC, also known as hepatoma, is the most prevalent primary liver tumor. HCC stands as a significant malignancy with considerable morbidity and mortality rates, posing a substantial threat to patients' overall well-being and life expectancy [1].

### Fibrosis, cirrhosis, and carcinoma

Fibrosis, cirrhosis, and HCC are three distinct medical conditions that primarily affect the liver (Figure 1) [2–6]. Fibrosis is a response to liver injury and inflammation. It is characterized by the excessive accumulation of fibrous scar tissue in the liver. When the liver is damaged, it attempts to repair itself by forming scar tissue. At the fibrosis stage, the liver may still be able to function relatively well, and if the cause is treated or eliminated, the liver may recover. In fibrosis, the immune system responds to ongoing liver injury and inflammation by promoting tissue repair, which involves the activation of certain immune cells like macrophages and fibroblasts. These cells play a role in the deposition of collagen and other ECM components, leading to the formation of scar tissue. Over time, if the underlying cause of liver injury persists (such as chronic alcohol abuse, viral hepatitis, or NAFLD), the fibrosis can progress and lead to cirrhosis [7].

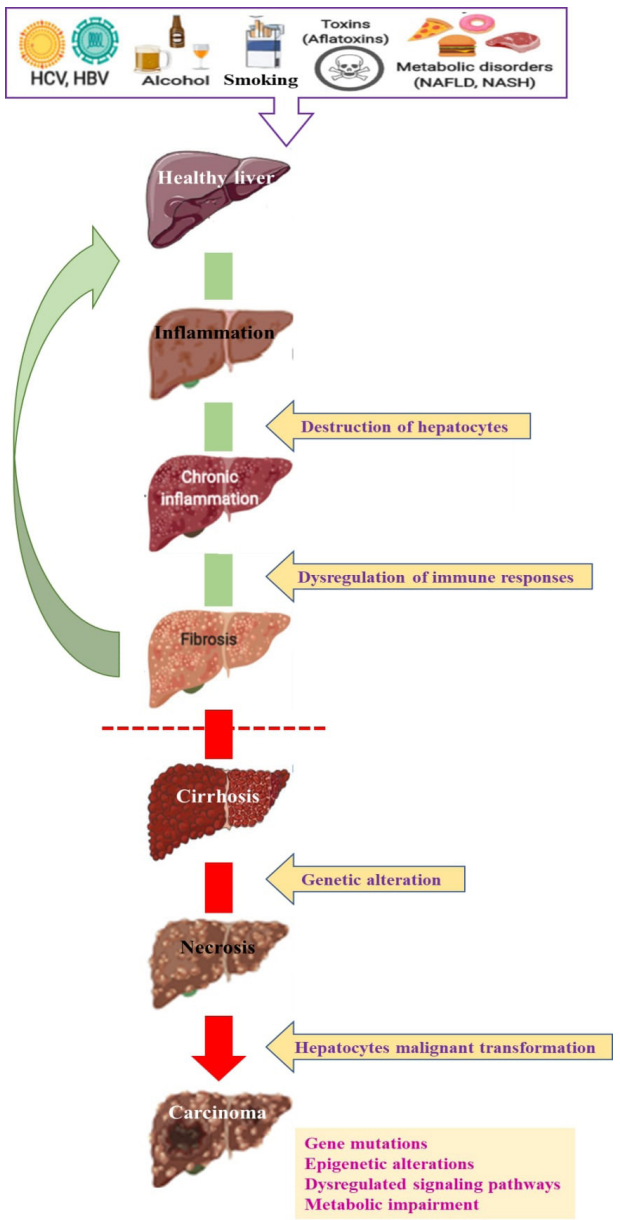


**Figure 1:** Progression of HCC [2–6].

Cirrhosis is an advanced stage of liver disease in which extensive scarring (fibrosis) has developed and has become widespread throughout the liver. The liver's normal structure is severely disrupted, and healthy liver tissue is replaced by non-functioning scar tissue. Cirrhosis can be the result of various chronic liver diseases and conditions, including long-term alcohol abuse, viral hepatitis (e.g., HBV or HCV), NAFLD, autoimmune hepatitis, and others. As cirrhosis progresses, liver function declines, and complications such as portal hypertension, ascites (fluid accumulation in the abdomen), hepatic encephalopathy (mental confusion due to liver failure), and jaundice can occur. Cirrhosis is considered irreversible, but early diagnosis and management can slow down its progression and improve the patient's quality of life. In cirrhosis, the chronic liver injury triggers a sustained immune response. The continuous inflammation and damage lead to ongoing activation of immune cells and the release of pro-inflammatory molecules. The immune response in cirrhosis can be dysregulated and contribute to the progression of fibrosis and the development of complications [7].

HCC accounts for about 90% of occurrences of primary liver cancer and is more common in those with cirrhosis and chronic liver disease [8]. The majority of HCC patients have a clinical history of chronic liver conditions. Inflammation, fibrosis, necrosis, and cirrhosis are pathological changes to the liver's structure and function that happen in response to shocks and injuries [9]. The main clinical risk factor for liver cancer in people is cirrhosis [10]. Cirrhosis frequently emerges in the context of NAFLD or NASH, as well as viral infections, alcohol-related damage, or autoimmune hepatitis. Metabolic syndrome, encompassing conditions like obesity, type 2 diabetes, and dyslipidemia, is strongly associated with NAFLD and NASH. Significantly, obesity stands as a distinct risk factor for the emergence of HCC [11]. Approximately 50% of HCC cases are linked to chronic HBV infection, and an additional 25% are connected to chronic HCV infection. Additional factors that elevate the risk of HCC include alcoholic liver disease and consumption of food contaminated with aflatoxin [12]. HCC has a multifaceted origin, with numerous complicated factors influencing both the progression of the disease and the outlook for patients [7].

### Associated key risk factors

Prevalence of HCC varies geographically and is influ-

enced by several factors, including the prevalence of risk factors associated with its development. HCC is more common in certain regions with a high burden of chronic liver diseases, such as cirrhosis, hepatitis B, and hepatitis C (Figure 1). Here are some key risk factors associated with the development of HCC:

**Chronic HBV and HCV infection**: Chronic infection with the HBV or HCV is one of the most significant risk factors for HCC. HBV and HCV can lead to chronic liver inflammation and cirrhosis, increasing the risk of HCC development over time.

**Cirrhosis:** Cirrhosis, regardless of the underlying cause (e.g., alcohol-related liver disease, NAFLD, chronic viral hepatitis), is strongly associated with an increased risk of HCC. The presence of cirrhosis represents a significant risk for HCC development.

**Chronic alcohol consumption:** Prolonged and excessive alcohol consumption can lead to liver damage and cirrhosis, increasing the risk of developing HCC.

**NAFLD and NASH:** NAFLD, a condition characterized by the accumulation of fat in the liver, can progress to NASH (severe form of NAFLD characterized by inflammation and liver cell damage) and eventually lead to cirrhosis, increasing the risk of HCC.

**Hemochromatosis:** Hemochromatosis is a hereditary disorder that causes excess iron to be stored in the liver and other organs. Over time, this iron overload can lead to cirrhosis and increase the risk of HCC.

**Diabetes:** Individuals with diabetes, especially if associated with obesity, have an increased risk of developing NASH, cirrhosis, and subsequently, HCC.

**Aflatoxin exposure:** Aflatoxins are toxic substances produced by certain molds that can contaminate certain foods, such as grains, nuts, and legumes. Chronic exposure to aflatoxins is associated with an increased risk of HCC.

## Incidence, Prevalence, and Mortality

Cancers, among non-communicable diseases, present a significant global public health concern, and their occurrence is increasing annually [1]. Cancer is responsible for one out of every six deaths globally, making it the second leading cause of death after cardiovascular diseases (Figure 2) [13]. The mortality attributed to cancer is rising rapidly worldwide across all age groups (Figure 3). These trends result from a complex interplay of factors including population aging, demographic growth, and shifts in the prevalence and distribution of major cancer risk factors. Several of these risk factors are tied to socioeconomic development [14]. A comparison of the incidence and mortality rates of the top 10 cancers globally and in India (Figure 4) [15].

Based on data sourced from the GLOBOCAN 2020 database [15], which encompasses 185 countries, an examination of primary liver cancer instances and fatalities reveals significant disparities across different geographical regions (Figure 5). Liver cancer imposes a noteworthy global burden. According to estimates from 2020, it ranks as the seventh most frequently diagnosed cancer and the third leading cause of

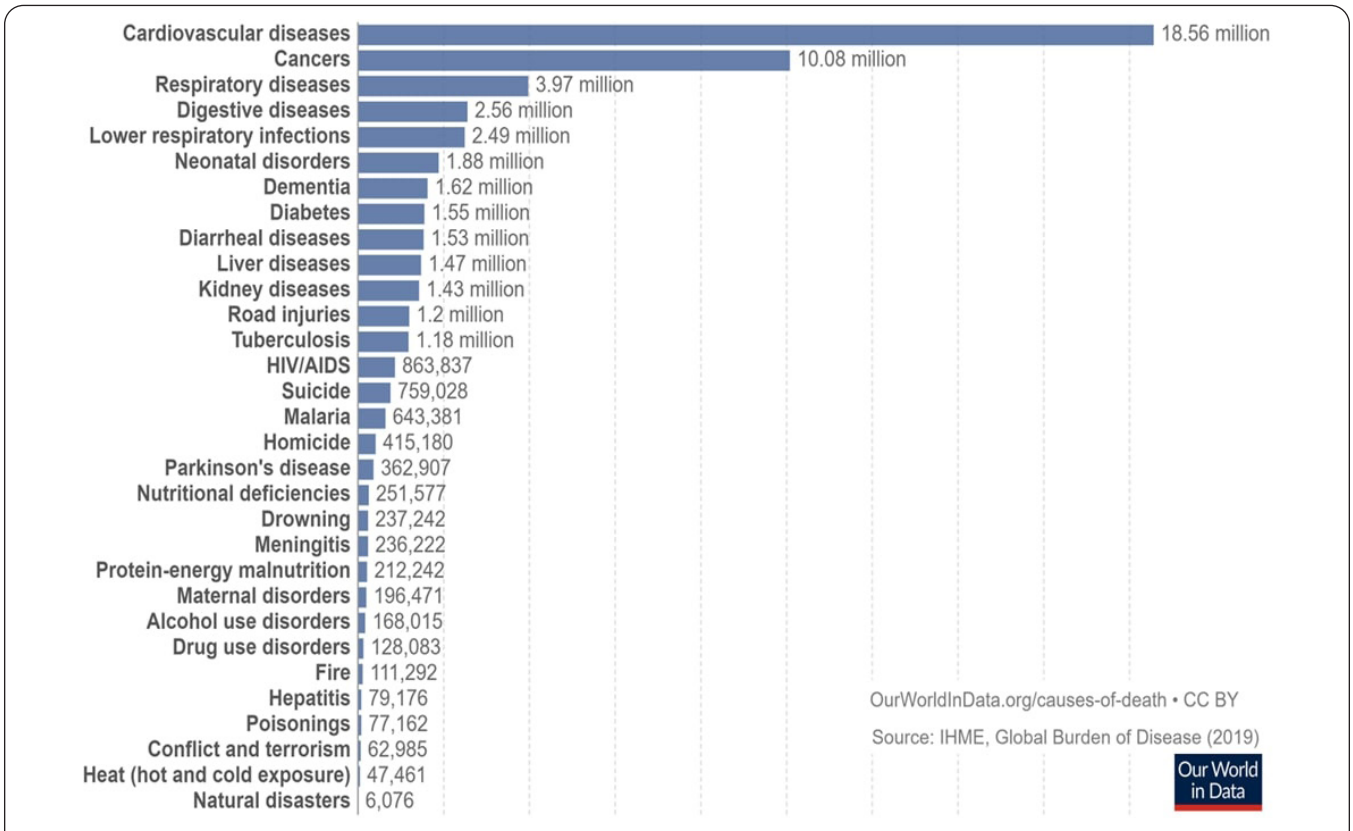


**Figure 2:** Number of deaths by cause, world, 2019, all ages [95].

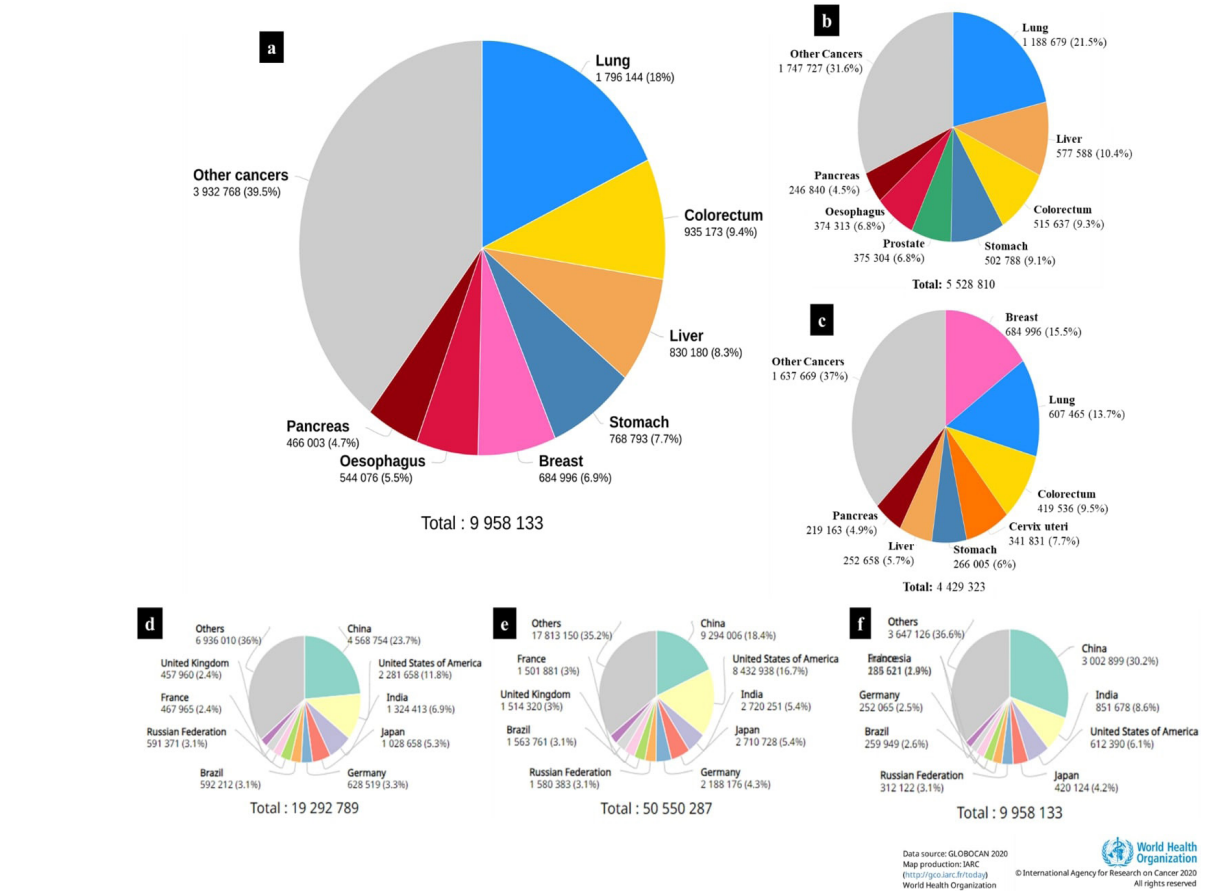


**Figure 3:** Estimated mortality due to various cancers worldwide, 2020, all ages, **(a)** both sexes, **(b)** males, and **(c)** females. Estimated number of **(d)** new cases (incidence), **(e)** prevalence (5 years), and **(f)** mortality of all cancers worldwide (as per countries), 2020, all ages, both sexes [15].

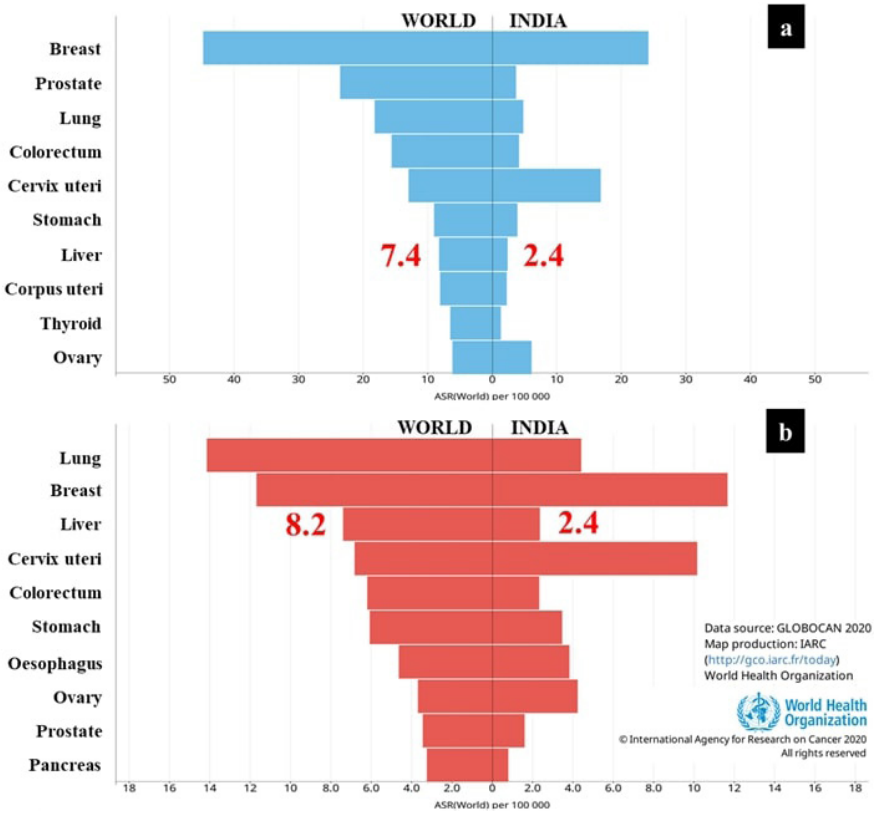


**Figure 4:** Estimated age-standardized **(a)** incidence and **(b)** mortality rates of top 10 cancer observed in India and worldwide, 2020, both sexes, all ages [15].

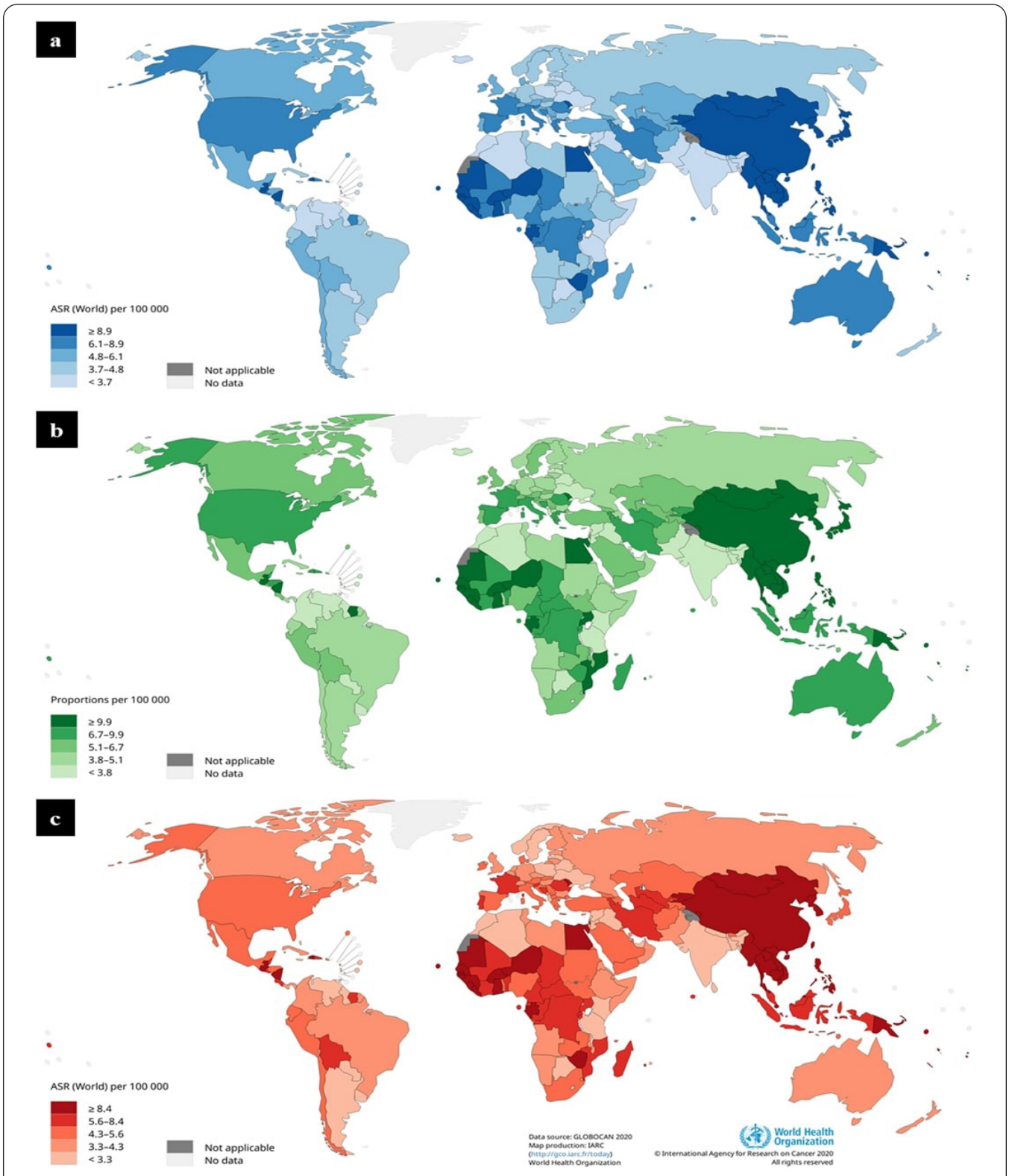


**Figure 5:** Estimated **(a)** age-standardized incidence, **(b)** prevalence (5 years), and **(c)** age-standardized mortality rates of liver cancer around the world, 2020, both sexes, all ages [15].

cancer-related mortality (Figure 4) [16]. Liver cancer is also positioned as the second highest contributor to premature cancer-related deaths [17]. The mortality pattern attributed to liver cancer, both on a global scale and within specific world regions (Figure 6), is constructed by GLOBOCAN using the most reliable sources of cancer fatality data available within each country 15. In the year 2020, an estimated 905,700 individuals received a liver cancer diagnosis, with 830,200 of them succumbing to the disease (Figure 6a) [17]. Over half of the worldwide liver cancer cases and deaths were concentrated in Eastern Asia, accounting for 54.3% and 54.1% respectively, despite this region constituting only 21.5% of the global population in 2020. Notably, China alone accounted for 45.3% of global liver cancer cases and 47.1% of liver cancer-related fatalities (Figure 6b) [15].

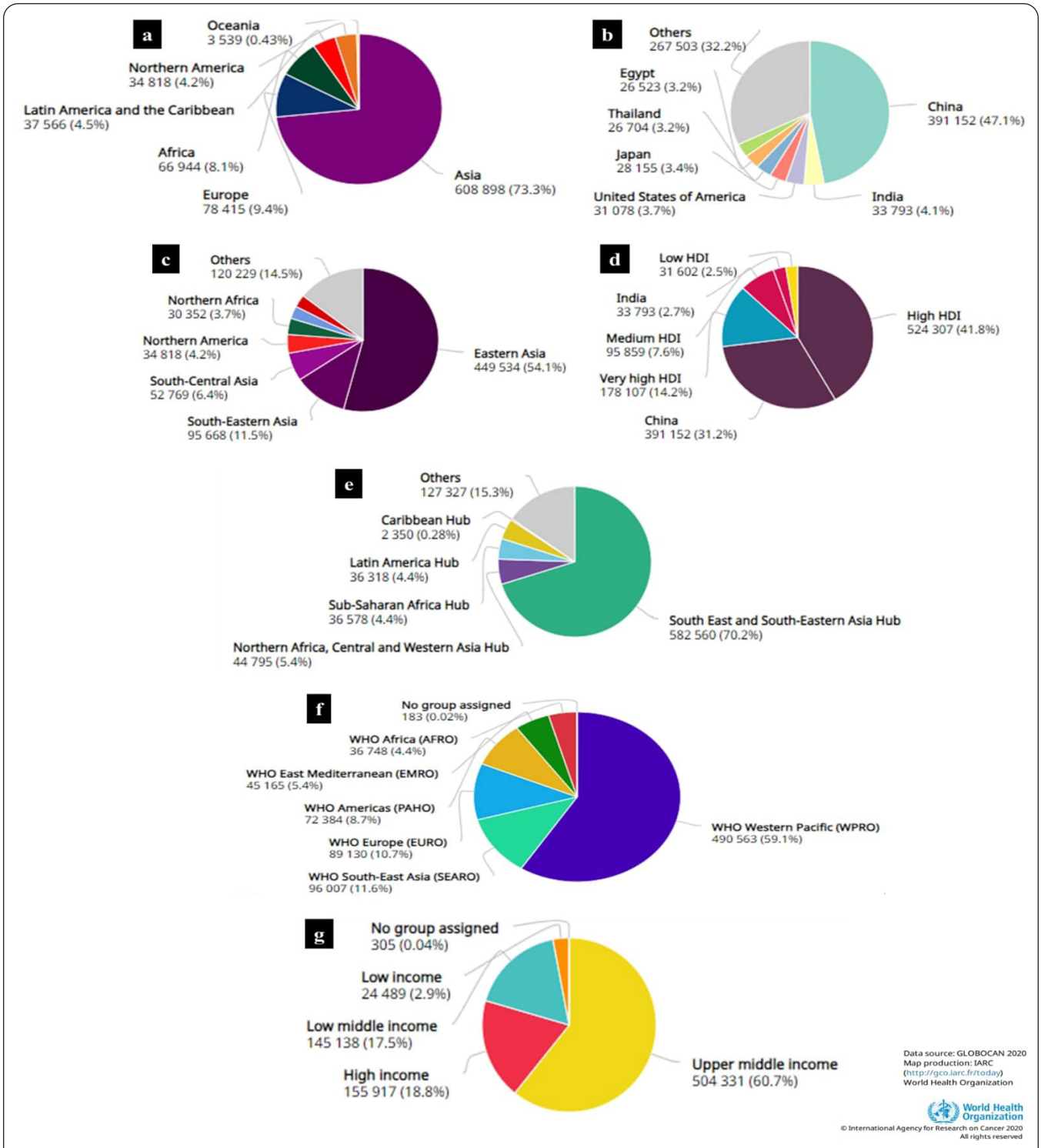


**Figure 6:** Estimated mortality (Number of deaths worldwide, 2020, both sexes, all ages = 830,180) of liver cacner across **(a)** continents, **(b)** countries, **(c)** world areas, **(d)** HDI, **(e)** GICR hubs, **(f)** WHO regions, and **(g)** income levels [15].

Among the countries examined, liver cancer was identified as the third most prevalent cause of cancer-related mortality in 46 nations, while it held the position of the fifth most common cause of death in an additional 90 countries [17]. Asia has highest mortality with 73.3% among which 47.1% is from China and India stands distant second with 4.1% (Figure 6a and 6b). Table 1 presents the number of new cases (incidence), prevalence (5 years) and deaths (mortality) observed in India for liver cancer across age groups based on gender in 2020 15. The incidence of new cases of liver cancer is projected to increase by 55% between 2020 and 2040, and ~1.3 million people could die by 2040 from this devastating disease (Figure 7) [15]. Significant diversity exists in the occurrence and underlying factors of HCC across different countries, underscoring the influence of regional variations [18]. The occurrence and frequency of the condition differ among various global regions, illustrating distinctions in economic, cultural, demographic, biological factors, and governmental health priorities [1].

**Table 1:** Incidence, prevalence (5 years), and mortality of liver cancer in India across all ages, both sexes, 2020 [15].

| Age (Years) | Gender | Incidence | Prevalence (5 years) | Mortality |
|---|---|---|---|---|
| 0 to 9 | Male | 285 (12.8%; N = 2226) | 1256 (9.9%; N = 12740) | 138 (13%; N = 1059) |
| | Female | 141 (9.2%; N = 1535) | 421 (8.2%; N = 5112) | 68 (9.4%; N = 724) |
| 10 to 19 | Male | 41 (3.2%; N = 1285) | 126 (3.1%; N = 4049) | 32 (3.8%; N = 847) |
| | Female | 46 (6.9%; N = 670) | 171 (7.3%; N = 2327) | 42 (9%; N = 468) |
| 20 to 29 | Male | 271 (6.2%; N = 4405) | 478 (5.8%; N = 8180) | 276 (8.6%; N = 3203) |
| | Female | 151 (7.8%; N = 1946) | 318 (7.7%; N = 4108) | 154 (10.6%; N = 1459) |
| 30 to 39 | Male | 784 (3.5%; N = 22545) | 1213 (3.6%; N = 33410) | 801 (4.2%; N = 19009) |
| | Female | 649 (10.5%; N = 6177) | 1259 (10.4%; N = 12055) | 662 (13.2%; N = 5019) |
| 40 to 49 | Male | 2423 (3.2%; N = 74969) | 2666 (3.1%; N = 84704) | 2454 (3.7%; N = 67156) |
| | Female | 1529 (7.8%; N = 19670) | 2082 (7.7%; N = 27169) | 1570 (8.9%; N = 17692) |
| 50 to 59 | Male | 6110 (3.9%; N = 157769) | 6546 (3.7%; N = 175776) | 6557 (4.6%; N = 142820) |
| | Female | 2626 (5.5%; N = 47726) | 3223 (5.3%; N = 60589) | 2732 (6.3%; N = 43407) |
| 60 to 69 | Male | 9102 (4.9%; N = 185194) | 8996 (4.6%; N = 196338) | 8746 (5.2%; N = 168079) |
| | Female | 3208 (4.4%; N = 73520) | 3442 (4.2%; N = 82538) | 3149 (4.7%; N = 67422) |
| 70+ | Male | 5216 (2.8%; N = 183927) | 4535 (2.5%; N = 178720) | 4561 (2.6%; N = 175349) |
| | Female | NA | NA | NA |

**Note:** "N' represents the total number of cases reported worldwide.

## Present Treatment Approaches

The treatment of HCC depends on several factors, including the stage of the cancer, the overall health of the patient, the underlying liver function, presence of vascular invasion, presence of ascites or encephalopathy, the availability of different treatment options, and patient preferences.

### Surgical resection

Surgical resection involves the surgical removal of the tumor and surrounding healthy liver tissue. It is most effective when HCC is detected early and is localized to a specific area of the liver. Surgical resection aims to achieve complete removal of the tumor and offers the best chance for a potential cure. However, not all patients are eligible for surgery due to factors such as tumor size, location, hepatic reserve, the expected volume of resection, and liver function [19].

### Liver transplantation

Liver transplantation is considered the definitive treatment for selected patients with early-stage HCC and cirrhosis. It involves replacing the diseased liver with a healthy liver from a deceased or living donor. Liver transplantation has the potential to eliminate both detectable and undetectable nodules, as well as pre-neoplastic lesions, while also addressing the underlying cirrhosis.

### Local ablation therapy

Local ablation therapies are minimally invasive procedures that target and destroy the tumor within the liver, which is tumor necrosis by thermal and chemical methods [20]. They are suitable for small HCCs (especially <2 cm, single tumor) and can be used in patients who are not candidates for surgery or transplantation 20. Localized ablation techniques offer benefits such as minimal impact on the body by precisely targeting the tumor and its neighboring tissue, high efficacy, and brief procedure duration [21].

#### Types

Radiofrequency ablation: RFA uses high-frequency electrical currents to generate heat and destroy the tumor [21].

**Microwave ablation**: Similar to RFA but uses microwaves to heat and destroy the tumor [21].

Laser photocoagulation: Uses focused laser energy to heat and vaporize or coagulate tumor tissue [21].

**Percutaneous ethanol injection:** Involves the injection of alcohol directly into the tumor to kill cancer cells [21].

**Cryoablation:** Uses extreme cold to freeze and destroy the tumor [21].

**High-intensity focused ultrasound:** Employs focused ultrasound waves to generate heat at the target site, leading to tissue destruction [21].

**Irreversible electroporation:** Involves the application of short electrical pulses to create temporary pores in the cell membrane, allowing therapeutic agents to enter the cells more effectively [21].

### Transarterial therapy

Transarterial therapy is a type of locoregional treatment. These therapies are designed to target the tumor directly within the liver while minimizing damage to healthy surrounding

**Figure 7**: Projected increase in estimated number of **(a)** new cases (incidence) and **(b)** deaths (mortality) due to liver cancer across World, Asia, South-Eastern Asia, and in India, 2020 to 2040, all ages, both sexes [15].

tissues. Transarterial therapies are usually performed by interventional radiologists. These are not curative treatments but can be valuable options for patients who are not candidates for surgery or transplantation.

## Types

Transarterial embolization: Transarterial embolization is a method that relies on obstructing the primary artery that supplies essential nutrients to the tumor tissue by introducing an embolic substance. This leads to the tumor's intended necrosis due to inadequate blood supply [21].

Transarterial chemoembolization: TACE is a locoregional therapy that delivers chemotherapy drugs directly into the blood vessels supplying the tumor. It also involves the use of embolic agents to block the blood flow to the tumor, starving it of nutrients [21].

Transarterial radioembolization: TARE involves the introduction of microspheres containing a radioactive isotope through injection (yttrium-90) into the arteries supplying the HCC [19, 21].

### Systemic therapy

Systemic therapies are drugs that circulate throughout the body to treat HCC. These therapies are particularly relevant when the cancer has spread beyond the liver or when local treatments like surgery or ablation are not feasible due to the advanced stage of the disease. These therapies may include:

#### Types

**Chemotherapy:** Traditional chemotherapy is rarely used as a first-line treatment for HCC due to limited effectiveness and significant side effects.

**Targeted therapies:** These drugs specifically target molecules or molecular pathways involved in cancer growth and survival, such as TKIs (e.g., SFN, LEN, regorafenib, and cabozantinib). They are used in advanced HCC.

**Immunotherapy:** Immune checkpoint inhibitors, such as nivolumab, atezolizumab, and pembrolizumab, are used for some patients with advanced HCC to boost the immune system's response against cancer cells.

**Radiation therapy:** Uses radioactive substances that target and destroy cancer cells throughout the body. It is used in select cases of advanced HCC.

### Supportive care

In advanced HCC or cases where curative treatments are not feasible, supportive care aims to manage symptoms, improve quality of life, and provide palliative relief. This may include pain management, nutritional support, and management of complications related to liver disease.

Each treatment approach has its advantages and disadvantages (Table 2), and the choice of treatment can only be made in consultation with a multidisciplinary team of specialists, including hepatologists, oncologists, surgeons, and interventional radiologists. The treatment plan should be tailored to the individual patient's specific circumstances and preferences to optimize outcomes and quality of life. Additionally, ongoing research is exploring new treatment strategies and combinations to improve outcomes for patients with HCC.

## Role of Nanomedicine

Genetic mutations occurring in the context of cancer lead to the disruption of tyrosine kinases [22], a group of essential proteins in HCC, including VEGFR [23], which play crucial roles in HCC's development. SFN, the first approved TKI,

**Table 2:** Advantages and disadvantages of various treatment approaches associated with HCC [19, 21].

| Method(s) | Advantage(s) | Disadvantage(s) |
|---|---|---|
| Surgical resection | Can potentially cure HCC if the tumor is small and localized. | Not all HCC cases are suitable for surgical resection, especially in advanced stages. |
| | Offers the chance for complete removal of the tumor. | Surgical resection can be associated with postoperative complications, such as bleeding, infection, and liver failure. |
| | Long-term survival can be achieved in some cases. | Poor long-term benefits due to high risk of recurrence and low disease-free survival rates. |
| Liver transplantation | Liver transplantation can potentially cure both the cancer and the underlying liver condition. | Limited availability of donor organs. |
| | Offers a new liver without underlying liver disease for selected patients. | Strict criteria for patient eligibility for transplantation. |
| | Holds high surgical morbidity and mortality rates. | Long waiting times for suitable donor livers. |
| | Long-term survival can be achieved in appropriately selected candidates. | The need for lifelong immunosuppression after transplantation. |
| Local ablation therapy | Minimally invasive procedures with shorter hospital stays and quicker recovery. | May not be suitable for larger tumors or tumors located near critical structures. |
| | Effective for small HCCs when complete tumor removal is feasible. | There is a risk of local tumor recurrence. |
| | Can be repeated if necessary. | |
| Transarterial therapy | Effective in controlling tumor growth and reducing symptoms in intermediate-stage HCC. | TACE is a palliative treatment and not curative. |
| | Minimizes the systemic side effects of chemotherapy. | May require multiple sessions for optimal results. |
| | | Post-embolization syndrome, with pain and fever, can occur. |
| Systemic therapy | Can be used in advanced HCC when curative treatments are not feasible. | Not all patients respond to systemic therapies, might require careful patient selection. |
| | Some targeted therapies and immunotherapies have shown promising results in improving survival in certain patients. | High costs of some targeted and immunotherapies can be a barrier to access. |
| | Can target cancer cells throughout the body, including those not visible in imaging. | Systemic therapies can cause side effects that may impact quality of life. |
| | Can provide palliative relief in advanced HCC. | |

significantly extended both the time of progression and overall survival [24]. A total of six TKIs have received approval for HCC treatment. LEN and donafenib, along with SFN, constitute the primary treatment line. Apatinib, cabozantinib, and regorafenib are categorized as secondary treatment options. In addition to TKIs, other treatments encompass ramucirumab, nivolumab, and bevacizumab [25].

Despite the availability of numerous approved medications, the drug treatment for unresectable and advanced HCC still falls short of achieving the desired outcomes. These suboptimal results can be attributed to various factors, including limited drug availability within the body and the non-targeted delivery of drugs [26]. These factors contribute to an increased risk of side effects while maintaining low drug concentrations in the intended tissue [26]. An important strategy to enhance treatment effectiveness involves the utilization of NPs [27]. Other strategies involve the exploration of novel systemic therapies [28]. Nonetheless, the development of new systemic drugs is a gradual process, evident from the nearly decade-long timeline it took to diversify the range of medications beyond SFN [29].

Nanomedicine plays a significant role in the management of HCC. Hepatocytes and hepatic stellate cells are only accessible by small particles (preferably <100 nm) that can penetrate the liver fenestrae [2]. NPs and nanotechnology-based approaches offer unique advantages in the diagnosis, treatment, and monitoring of HCC. NPs offer a range of benefits including stable payload retention, specific delivery to tumors, efficient cellular uptake, a favorable surface-to-volume ratio, and the capacity to encapsulate multiple therapeutic agents simultaneously [30]. Moreover, they can enhance the availability of drugs [31]. Various types of NPs are available, encompassing polymeric, lipid-based, metallic, and silica-based formulations (Table 3). Yet, most NPs approved for drug delivery in clinical

**Table 3:** Various types of NPs used in nanomedicine [6, 51, 95, 96].

| Type(s) | Advantage(s) | Disadvantage(s) |
|---|---|---|
| Dendrimers | Monodispersity. | Encapsulate low molecular weight compounds. |
| | Controlled modification process. | - |
| | Properties can be tunable. | - |
| | Facile synthesis. | - |
| | Superior biocompatibility and accumulation. | - |
| Liposomes and their derivatives | Encapsulation of broad range of molecules. | Encapsulation efficiency is lower. |
| | Ease in nano-formulation. | - |
| | Superior bioavailability. | - |
| Solid lipid NPs | Physical stability is higher. | Encapsulation efficiency of hydrophilic drugs is lower. |
| | Toxicity towards mammalian cells is minimal. | - |
| | Good permeability in tumor tissues. | - |
| Polymeric NPs | Superior control of encapsulated drug molecules. | Chance of particle aggregation/agglomeration. |
| | Easily achievable surface modifications. | - |
| | Particle characteristics can be tunable. | - |
| Metal NPs | Better suited for applications in theranostics. | Shows minimal to moderate toxicity in mammalian cells. |
| | Can be synthesized in a variety of sizes, shapes, and geometries. | - |
| | Shows superior magnetic and optical properties. | - |
| QDs | Minimal cytotoxicity. | Colloidal instability. |
| | Good biocompatibility. | Pharmacological and toxicological effects due to heavy metals. |
| | Resistant to chemical degradation. | |
| Carbon based nanostructures | High loading capacity. | Requires surface modifications to reduce unwanted toxicity towards normal cells. |
| | Efficient surface modifications. | - |
| | Easily diffuse and interact with intracellular organelles and macromolecules. | - |
| Mesoporous silica NPs | Enhanced solubility of hydrophobic drugs. | Burst release of drugs. |
| | Tunable NPs size and porosity. | Not stable for longer durations. |
| | Superior biocompatibility and biodegradability. | - |

**Table 4:** Various liver cell types, associated receptors, and corresponding ligands used [2, 69, 82].

| Cell type(s) | Receptor(s) | Ligand(s) |
|---|---|---|
| Hepatic stellate cells | Retinol binding protein | Retinol |
| | Platelet-derived growth factor receptor-β | Cyclic C*SRNLIDC* peptide |
| | Mannose 6-phosphate receptor | Mannose 6-phosphate |
| | Collagen type VI Receptor | C*GRGDSPC* peptide |
| | | cRGD* peptide |
| Kupffer cells | Fc Receptors | CC52 antibody |
| | Scavenger Receptors | Immunoglobulin G and calcitriol |
| | Galactose receptor | Galactose |
| | Fucose receptor | 4-aminophenyl-β-l-fucopyranoside |
| | | Fucose |
| | Mannose receptor | 4-aminophenyl-α-d-mannopyranoside |
| | | Mannose |
| Sinusoidal endothelial cells | Hyaluronan receptor | Hyarluronic acid stearylamine |
| | Scavenger receptors | Aconitylated human serum albumin |
| | Low-density lipoprotein receptor | KLGR peptide |
| Hepatocyte | C-X-C chemokine receptor type 4 | Plerixafor |
| | Folate receptor | Folate |
| | Low-density lipoprotein receptor | Apo E |
| | Scavenger receptor B1 | Apo A-I |
| | Heparan sulfate proteoglycans | CKNEKKNKIERNNKLKQPP peptide |
| | Transferrin receptors | DT7 and LT7 peptides, transferrin |
| | Asialoglycoprotein receptor | Pullulan |
| | | Soybean sterylglucoside |
| | | Lactosyl-norcantharidin |
| | | Lactose |
| | | PreS1 peptide |
| | | Asialofetuin |
| | | N-acetyl galactosamine |
| | | Galactose |

settings are either polymeric or lipid-based [32]. Lipid-based NPs exhibit significant potential for addressing liver-related illnesses. This potential is exemplified by the clinical approval of the liver-targeting nanomedicine ONPATTRO®, marking a noteworthy advancement in liver disease treatment [2].

Research focused on cancer nanomedicines has demonstrated that NPs within the size range of 40 to 400 nm can lead to extended circulation times and enhanced accumulation within tumors [33]. However, liver architecture introduces leaky vessels and anomalous vasculature due to chronic liver conditions. Therefore, the prudent selection of NPs sizes, charges, and surface characteristics becomes imperative to engineer NPs capable of passively infiltrating HCC tumors [6]. Numerous receptors and proteins are present across HCC tumors and normal liver cells, encompassing sinusoidal endothelial cells, HSCs, Kupffer cells, and hepatocytes. Hepatocyte receptors, such as heparan sulfates, folate receptors, transferrin receptors, and ASGPRs, could function as distinct binding sites for NPs. Additional receptors, like scavenger receptors, low-density lipoprotein, and C-X-C type 4, have also been observed (Table 4) [2, 6]. The subsequent section outlines key roles and instances (Table 5) illustrating the application of nanomedicine in HCC.

## Early detection, diagnosis, and monitoring

NPs can serve as contrast agents in imaging modalities like MRI, CT, and ultrasound, enhancing the detection and characterization of HCC lesions at an early stage. NPs can be functionalized with tumor-specific ligands to improve the sensitivity and specificity of imaging, facilitating early diagnosis.

### Image-guided

NPs can be used as contrast agents in various imaging modalities, such as MRI, CT, and ultrasound. These NPs can help improve the detection and characterization of HCC lesions, allowing for early diagnosis and monitoring of treatment response. Iron oxide NPs have been used as MRI contrast agents for improved imaging and early detection of HCC lesions.

**Table 5:** Application of NPs in HCC.

| NPs type | Drug used | Properties | Ligand used | Target | EE/DLC/ DLE (%) | *In vitro* | *In vivo* | Outcome(s) |
|---|---|---|---|---|---|---|---|---|
| Liposomes [97] | DOX | 108 nm; -23.8 mV | PNA-GA | GA receptor | 94.1; 9.3; - | HepG2; SMMC-7721 | Xenograft tumour BALB/C-nu mice | Excellent liver and tumour-targeting effects |
| Polymeric (PL-GA-PEG) [98] | SFN; MPT | 145 and 147 nm; -15.47 and -17.80 mV | LFC131 | CXCR4 | 52.17 and 1.03; 61.34 and 1.23; - | HepG2; HuH7; SMMC-7721 | Female BALB/c nude mice | Co-delivery showed prolonged circulation and tumor growth suppression |
| LF shell-oily core NCap [99] | SFN; QRC | 169 and 230 nm; +8.81 and +14.4 mV | LA/GA | ASGP and GA receptors | 85.6 and 86.5; -; - | HepG2 | DEN induced HCC-mice | Combined delivery with LF-NCap showed potential therapeutic effect |
| TPGS-b-PCL/ Pluronic P123 [100] | SFN | 99.1 nm; -16.6 mV | Anti-GPC3 antibody | GPC3 receptor | 76.3; 9.5; - | HepG2 | HepG2 xenograft-bearing nude mice | Inhibited tumor growth without obvious side effects |
| Polymeric (PL-GA-PVA) [101] | PTX; GAL; ATF | 181.5, 240.9 and 242 nm; -10.7, -13 mV | Aptamer TLS 9a (L5) | TAG-72 and HSP70 | 67.31; 5.98; - | HepG2; HuH-7 | Sprague-Dawley male rats | Significantly prolonged survival |
| Chimeric (PEG-P(TMC-DTC)-PAsp) [102] | Saporin | 36 nm; -22.5 mV | GE11 | EGFR receptor | 66.1; 3.2; - | SMMC-7721 | Orthotopic SMMC-7721 HCC-bearing mice | Low toxicity, effective tumor inhibition, and significant improvement in survival |
| Polymeric (PLGA-DOPC-TPGS-peptide modified PEG) [103] | SFN; Peptide | 116.8 nm; -24.4 mV | GPC3 | GPC3 receptor | 83.5; -; - | Hep3B; SK-Hep1 | Subcutaneous Hep3B HCC-bearing female nude athymic mice | High entrapment efficiency and tumor regression in HCC xenograft tumors |
| Liposomes [104] | DTX | ~100 nm | DT7 peptide | Tf receptor | - | HepG2 | Subcutaneous HepG2 HCC-bearing BALB/c nude mice | High tumor accumulation and antitumor effect |
| Bimimetic (CuS) [105] | SFN | ~210 nm; -15.2 mV | Anti-VEGFR receptor | VEGFR receptor | 94.7; 88 (by weight); - | HepG2 | Intraperitoneal H22 Hepatoma xenograft female BALB/c mice | Showed synergistic photothermal and chemotherapy effect |
| Liposomes [106] | GALARV | 93.83 nm; -27.3 mV | Galactosyl ceramide (ASGPR) | Galactose | >99; -; - | HrpG2; Hep3B | - | Novel active nanotherapeutic approach |
| Ionizable Lipid NPs [107] | mFLuc | - | Hepatocytes and LSECs | Mannose | - | HepG2 | C57BL/6 mice | Shown great potential for cell type-specific delivery of mRNA |
| BSA NPs [108] | SFN | 158 nm; -16.27 mV | Folate | Folic acid receptor | 77.25; 7.73; - | LO2; SMMC-7721 | Female Sprague-Dawley rats | Improved therapeutic effect and reduced side effects |
| Redox-responsive self-assembled NPs [109] | CPT | 114.40 nm; -23.67 mV | Lactose conjugate with CPT | ASGPR | 60; -; - | HepG2; HUVES | Subcutaneous HepG2 Female BALB/c nude mice | Enhanced antitumor ability and reduced side effects |
| Redox-sensitive pullulan NPs [110] | PTX | 170 nm; -15 mV | Folic acid and Lipoic acid | ASGPR and Folate receptors | -; -; 75 | SMMC-7721 | Sprague-Dawley rats | Dual-targeting capacity |
| Liposomes (DSPE-PEG2000-Mal) [111] | RSV; CUR | ~140 nm; ~-10 mV | SP94 | Membrane receptors | 85.9 and 92.1; -; 62.05 and 72.23 | HepG2 | Subcutaneous HepG2 Xenograft BALB/c nude mice | Enhanced therapeutic potential with good safety profile |
| Metal NPs (Fe@ Fe3O4) [112] | Ginsenoside Rg3 | ~600 nm; ~-20 mV | - | - | ~80; -; - | - | DEN induced orthotopic HCC-mice | Prolonged the survival of HCC mice |
| MSNPs with PM [113] | SFN | ~100 nm; -20 mV | Anti-PD-L1 | Collagen IV | -; -; ~75 | - | Orthotopic HCC-mice | Efficiently inhibit post-surgical HCC relapse without obvious side effects |
| LCC NPs [114] | SFN; miR-375 | ~100.7 nm; +40.37 mV | - | ATG7 gene | -; -; 35.2 and 55 | HepG2 | Xenograft HCC BALB/c nude mice | pH dependent drug release and enhanced therapeutic efficiency |
| Polymeric NPs (PL-GA-PEG-AEAA) [115] | DOX; Icaritin | 138 nm; +3 mV | - | To induce autophagy | 68.5 and 96.7; 10.8 and 5.1; - | HuH7; Hepa1-6 | Hapa1-6 hemisplenic HCC-C57BL/g mice | Low dose synergy therapeutic effect |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| CUR@Hb NPs [116] | CUR | 6.5 nm | - | - | -; 18.5; 60 | SMMC7721 | Female SPF grade nude mic | Inhibited tumor cell migration and enhanced radiosensitivity of HCC |
| Liposomes [117] | Tumor-derived RNA | 209.68 nm; +42.03 mV | - | Dendritic cells | - | Hepa1-6 | C57BL/6 male mice | Potent anti-tumor RNA vaccines |
| Core-shell NPs (Albumin-Albumin/lactosylated) [118] | DOX | 254 nm; -26 mV | - | ASGPR | 91; -; - | HepG2 | - | Efficiently decreased cell viability than free DOX |
| Polymer NPs (PLGA) [119] | YSL | 222.6 nm; +18.23 mV | R6LRVG | Intestinal epithelial cells | 70.27; 19.69; - | BEL-7402 | Female BALB/c mice | Potential oral delivery system |

**Note:** Properties: Size values presented were results of dynamic light scattering measurement.

**Abbreviations:** AEAA: Aminoethyl anisamide; Anti-PD-L1: Rat-derived anti-mouse PD-L1 antibody, an immune checkpoint inhibitor; ASGP: Asialoglycoprotein; ASGPR: Asialoglycoprotein receptor; ATF: Apotransferrin; ATG7 gene: Autophagy-related gene; BSA: Bovine serum albumin; CPT: Camptpthecin; CUR: Curcumin; CuS: Copper sulfate; CXCR4: C-X-C chemokine receptor type 4; DEN: Diethylnitrosamine; DLC: Drug loading capacity; DLE: Drug loading efficiency; DOPC: 1,2-dioleoyl-sn-glycero-3-phosphocholine; DOX: Doxorubicin; DSPE-PEG2000-Mal: 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide (polyethylene glycol)-2000]; DTX: Docetaxel; EE: Entrapment/encapsulation efficiency; EGFR: Epidermal growth factor receptor; GA: Glycyrrhetinic acid; GAL: Galactosamine; GALARV: Galactosylated nanoliposomes incorporating ARV (BRD4 protein); GPC-3: Glypican-3; Hb: Hemoglobin; HSP70: heat shock protein 70; LA: Lactobionic acid; LCC: Lipid coated calcium carbonate; LF: Lactoferrin; LFC131: peptide inhibitor of CXCR4; LSECs: Liver sinusoidal endothelial cells; mFLuc: Messenger RNA firefly luciferase; MSNPs: Mesoporous silica nanoparticles; MTP: Metapristone; NCap: Nanocapsules; PEG: Polyethylene glycol; PEG-P(TMC-DTC)-PAsp: poly(ethylene glycol)-b-poly(trimethylene carbonate-co-dithiolane trimethylene carbonate)-b-poly(aspartic acid); PLGA: poly (lactic-co-glycolic acid); PM: Platelet membrane; PNA: Peanut agglutinin; PTX: Paclitaxel; PVA: Polyvinyl alcohol; QRC: Quercetin; R6LRVG: Cell penetrating peptide (R6) with the polypeptide of LRVG (targeting intestinal epithelial cells); RSV: Resveratrol; SFN: Sorafenib; SP94: Short peptide ligand SFSIIHTPILPL; TAG-72: Tumor-associated glycoprotein 72; Tf: Transferrin receptor; TPGS: D-α-Tocopherol polyethylene glycol 1000 succinate; TPGS-b-PCL: D-α-tocopheryl polyethylene glycol 1000 succinate-poly(caprolactone); VEGFR: Vascular endothelial growth factor receptor; and YSL: Tyroserleutide.

These NPs provide high-resolution images to aid in diagnosis and treatment planning [20]. NPs can enhance the accuracy of image-guided biopsies by improving the visualization of the tumor and guiding the biopsy needle to the precise location for tissue sampling. This reduces the need for repeated biopsies and enhances diagnostic accuracy. NPs with imaging capabilities can help monitor the response to treatment. Changes in NPs accumulation and distribution within the tumor can provide real-time feedback on treatment effectiveness.

### Biosensors

Biosensors serve as devices employed to identify and analyze cancer biomarkers by converting biological elements like proteins, RNA, or DNA into electrical signals [34]. These biosensors are recognized for their ability to offer satisfactory imaging of cancer cells, monitoring metastasis and cancer cell angiogenesis, swift and precise detection, and the capability to forecast the effectiveness of anti-cancer chemotherapy agents [34]. Biosensors are adept at identifying the presence of tumors, whether benign or malignant, and gauging the treatment's success in eradicating cancerous cells [35]. The use of nanomaterials as biosensors has emerged as a promising strategy for cancer detection due to their sensitivity and precise measurement [34]. Nanomaterials, valued for their remarkable optical, magnetic, and physicochemical, are commonly harnessed as biosensors in the forms of carbon nanotubes, quantum dots, AuNPs, and magnetic NPs [36]. HCC can be detected through various aberrant alterations in nucleic acid levels that activate pathways linked to carcinogenesis processes. Detection of cell-free nucleic acids in blood from tumors constitutes another detection method. Alpha-fetoprotein (AFP), a fetal-specific glycoprotein, assumes significance as a diagnostic biomarker due to its anomalous expression in HCC. Additional biomarkers for HCC encompass osteopontin (OPN), AFP-L3, glyoxylate reductase homolog, Golgi protein-73 (GP73), alpha-L-fucosidase (AFU), des-γ-carboxyprothrombin (DCP), and glypican-3 (GPC3) [37]. An electrochemical approach represents another pivotal method for biosensing HCC biomarkers, boasting merits such as affordability, ease of miniaturization, sensitivity, and integration. Various research endeavors have been undertaken to devise biosensors for the detection of HCC biomarkers.

## Targeted drug delivery

NPs can be engineered to deliver anticancer drugs specifically to the tumor site. By functionalizing NPs with targeting ligands, such as antibodies or peptides, they can selectively accumulate in HCC cells while sparing healthy liver tissue. This targeted drug delivery approach enhances the efficacy of chemotherapy and reduces systemic toxicity, improving the overall therapeutic index. Liposomes, which are lipid-based NPs, have been used to deliver the chemotherapy drug DOX specifically to HCC cells. Efficient drug delivery to tumor sites is a prerequisite for the high efficacy of nanodrugs and reduces systemic side effects [38]. SFN and 5-FU, a targeted therapy used in advanced HCC, has been encapsulated in NPs to enhance its delivery and improve its effectiveness against HCC cells [39].

### Chemotherapeutic drugs

Chemotherapy, undergoing certain modifications, has surfaced as a swiftly evolving field with promising innovations aimed at surmounting existing challenges in HCC treatment [34]. Furthermore, the drugs (such as DOX, 5-FU, PTX, and ATO) utilized in this therapeutic approach prioritize specific carcinogenic pathways to mitigate systemic toxicity [34].

### Molecularly targeted drugs

Chemotherapy agents exhibit diminished efficacy and more prominent detrimental side effects in HCC, particularly in advanced stages of mid-to-late progression [40]. In contemporary clinical practice, molecularly targeted drug therapy constitutes the primary and secondary treatment approaches. First-line medications like SFN and LEN fall within the category of multiple receptor TKIs, which have the potential to enhance overall survival [41]. Nonetheless, their impact is constrained by adverse effects and limited bioavailability [42].

### Natural plant medicines

Natural plant-derived medications are employed in anti-HCC treatment due to their minimal toxicity. Nevertheless, a notable constraint in their broad utilization stems from their inadequate water solubility [20]. Because many bioactive compounds achieve their anticancer effects through a combination of overlapping pathways, examining their individual actions can be intricate. Pinpointing key molecular targets and understanding the mechanisms by which plant-derived bioactives combat cancer would increase the potential for applying them in translational approaches to HCC treatment. For example, fucoidan [43] is a naturally occurring sulfated polysaccharide known for its diverse biological effects, which encompass anticancer and antioxidant activities. Fucoidan has been shown to interfere with cancer cell proliferation, induce apoptosis (programmed cell death), and possess antioxidant capabilities that help protect cells from oxidative stress.

## Radiotherapy

Among the various treatments discussed for liver cancer, radiotherapy stands out as a clinically effective method for eliminating tumor cells using high-energy photon radiation, specifically gamma or X-rays radiation [34]. Radiotherapy operates through both direct and indirect ionization mechanisms. In the direct mechanism, DNA strands are promptly broken, leading to cell demise. The indirect process generates ROS, inducing cellular stress and DNA fragmentation that ultimately causes cell death [44, 45]. However, ionizing radiation could potentially harm not only the targeted tumor tissue but also the surrounding healthy tissue [34]. To address this, the concept of radiosensitizers emerged to enhance the precision and effectiveness of radiation. Radiosensitizers encompass substances that, when combined with radiation, expedite tumor eradication [46]. These substances span small molecules like oxygen [47], macromolecules including oligonucleotides [48], and nanomaterials. Noble and heavy metal NPs like bismuth, tungsten, tantalum, gadolimium, platinum, silver, and gold possess the capability to absorb X-ray energy and interact with tumor cells, releasing photoelectrons and secondary electrons. These secondary electrons directly interact with DNA and water, heightening ROS production and intensifying the tumor cells' susceptibility to radiation. These specialized noble metal NPs stimulate ROS generation while simultaneously suppressing the p53 signaling pathway, leading to cellular autophagy [49].

## Photothermal therapy

PTT has garnered significant attention due to its remarkable ability to eliminate tumor tissues while sparing normal tissues from harm [50]. PTT relies on a photosensitizer, which absorbs light and converts it into heat energy, inducing localized hyperthermia and subsequent tumor ablation [50]. Notably, the PTT mechanism operates at lower energy levels and longer wavelengths of light, thereby minimizing the risk of collateral damage to neighboring cells and tissues [50]. Although PTT effectively tackles primary tumors through thermal ablation, it often falls short in completely eradicating tumors due to limitations in depth of penetration and an inability to prevent metastasis and cancer recurrence when used as a sole therapy [51]. The primary challenges associated with PTT agents revolve around limited targeting precision and suboptimal drug delivery capabilities [52]. Thus, the design of functional drug delivery nanosystems becomes crucial in enhancing therapeutic outcomes by precisely guiding antitumor drugs to their intended sites of action. To address the limitations of PTT when employed in isolation, combined therapies have gained substantial prominence in recent times.

## Photodynamic therapy

PDT, also known as photochemotherapy, involves the utilization of photosensitizing drugs in conjunction with specific light wavelengths to initiate reactions that generate cytotoxic ROS, including singlet oxygen and free radicals, for the treatment of diverse tumors [34, 53, 54]. Its non-invasive nature has garnered substantial interest for treating various malignancies [34]. PDT is considered a viable palliative approach for advanced liver cancer and has proven effective in managing hepatoblastoma, cholangiocarcinoma, liver metastases and HCC [53]. Nonetheless, PDT's clinical application in liver cancer is constrained due to factors like inadequate sensitivity and specificity for malignant liver tissue and challenges in delivering light to the liver's dense composition [55]. The pivotal role of PS in PDT has prompted technological enhancements to address these limitations [34]. An optimal PS molecule should be pure, with selective tumor tissue accumulation to minimize toxicity and side effects, while swiftly clearing normal tissues. Nanotechnology integration, particularly nanoscale PS delivery systems, has bolstered PDT's efficacy while mitigating its adverse effects [51]. Noteworthy PSs used include metal phthalocyanines, AuNPs, polymeric micelles, Radachlorin (a second-generation PS), pH-responsive PS, and MOFs [34]. While a few PSs like verteporfin, photofrin, and foscan have gained authorization, others such as silicon phthalocyanine are presently undergoing clinical trials [34].

Despite strides in this technique, significant hurdles remain in deploying PDT for liver cancers. Consequently, combined therapies involving PDT and other modalities like immunotherapy, chemotherapy, and PTT, are gaining traction due to their superior outcomes compared to standalone PDT.

## Sonodynamic therapy

Given the fleeting lifespan of ROS formed from light energy, their diffusion is limited to a depth ranging from 10 to

55 nm [56]. Consequently, only cells in close proximity to the photosensitizer experience apoptosis, while others remain unaffected [34]. To address the limitations of PDT, an alternative approach called SDT has emerged. SDT constitutes a novel non-invasive strategy for treating cancer, employing a combination of low-intensity ultrasound and sonosensitizer drugs to generate ROS capable of prompting apoptosis in cancer cells [51]. In SDT, a sonosensitizer is combined with ultrasound to enable enhanced penetration into deep tissues [57]. The mechanism underlying SDT closely mirrors PDT, involving ROS generation. Additionally, various mechanisms have been proposed to explain the energy transfer between ultrasound and sonosensitizer, encompassing and pyrolysis, sonoluminescence, and acoustic cavitation [34].

## Chemodynamic therapy

Cancer cells exhibit heightened sensitivity to ROS-mediated cancer therapies [58], including chemotherapy, radiotherapy, SDT, and PDT [34]. CDT employs chemodynamic agents (Fenton agents) to trigger Fenton or Fenton-like reactions, generating ROS by converting $H_2O_2$ into toxic OH- radicals. These radicals subsequently impair the cell's genetic components, culminating in cell death [34]. CDT possesses distinct advantages over other ROS-mediated cancer therapies such as chemotherapy, PTT, PDT, and SDT. It offers specific responsiveness to $H_2O_2$, targets tumor cells specifically, avoids drug resistance, and doesn't require external stimulation [34]. As a result, CDT presents a more dependable approach with potential for clinical implementation. Electron-rich hybrid nanomaterials [59], nanozymes [60], transition-based metal inorganic NPs [61], MOFs [62], covalent organic frameworks [63], and macromolecular nanomaterials [64] are among the agents employed in CDT. Recent advancements in nanotechnology have significantly propelled CDT, particularly through the generation of OH- radicals via Fenton and Fenton-like reactions [65, 66].

## Immunotherapy

Harnessing the body's immune system as a means to combat infections and diseases has emerged as a highly promising approach in the treatment of liver cancer [34]. Notable recent strategies encompass immune checkpoint inhibitors like Nivolumab (anti-PD1) and Tremelimumab (anti-CTLA-4), as well as adoptive immune cell transfer therapies involving gamma delta (γδ) T-cells, αβ T-cells (including CAR-T cells and CD8+ T cells), and natural killer (NK) cells [35]. Additionally, the utilization of T-cell vaccinations or cytokines represents another avenue to impede cancer progression and induce cancer cell death [35]. Exploring innovative treatment methods has led to the recognition of NPs as holding significant promise. Nanomedicine-based immunotherapy is a novel approach to tumor treatment that has recently garnered substantial attention [51].

## Theranostics

Theranostics represents a consolidated approach involving both therapy and diagnosis. This innovative technology facilitates diagnosis, precise delivery of treatment, and monitoring of therapeutic responses through a single intelligent nanostructured carrier [67]. Additionally, nano-sized materials can selectively accumulate at tumor sites, enabling targeted drug delivery and minimizing the impact on healthy tissues, thereby reducing side effects. As a result, nano-theranostics emerges as a compelling option for concurrent cancer diagnosis and treatment, holding immense potential for clinical applications [68, 69]. Presently, theranostic delivery-based strategies are being acknowledged as effective treatments for liver cancer. Notable examples of nanocarriers include polymeric NPs, micelles, and SPIONs, all contributing to the development of theranostic drug delivery for liver cancer [51]. In a similar vein, QDs, which are semiconductor NPs, have been leveraged as fluorescent imaging agents for *in vivo* detection and tracking of HCC cells [51].

## CSCs

CSCs are currently recognized as the primary culprits behind the recurrence of tumors. Once chemotherapy has concluded, CSCs undergo a phase of rapid proliferation and subsequently transform, giving rise to fresh tumor tissues. Much like their normal stem cell counterparts, this particular group of cells within the tumor tissue, unevenly dispersed, possesses the ability to self-renew. This group plays a crucial role in the tumor's sustenance and contributes to variations in its genetic and metabolic attributes. The genetic instability within CSCs subsequently leads to the emergence of new phenotypic markers in this subset of cells. Consequently, several surface markers and metabolic pathways, which experience heightened activation in CSCs, have the potential to serve as distinct targets for the development of diagnostic and therapeutic strategies [70].

### Nanotherapy

Nanotherapy can enable localized treatment by delivering therapeutic agents directly to the tumor site. This can be achieved through intra-arterial administration or by designing NPs that respond to specific conditions in the TME, releasing their cargo only when they reach the target site.

### Gene therapy

Recent investigations have delved into various genetic mutations and pathways associated with different liver cancers. This exploration has paved the way for innovative drug formulations that can effectively target these overly active pathways. Among emerging technologies for cancer treatment, the utilization of NPs as carriers in gene therapy has gained prominence. This is owing to their facile synthesis, compatibility with diverse organs, non-toxic nature, and low potential for causing immune responses [71]. However, for successful implementation, it is imperative that this approach is both efficient and safe, while also demonstrating specificity [20]. Within this context, RNA interference (RNAi) stands out as a promising technique for gene silencing in HCC. The interaction between NPs and cellular components presents a viable avenue for delivering RNAi. Consequently, integrating nanotechnology into gene therapy emerges as a potent strategy for targeted treatment of HCC.

### Drug resistance reversal

Reversing drug resistance in HCC is a critical challenge in the treatment of this type of liver cancer. Drug resistance occurs when cancer cells become less responsive to the effects of therapeutic agents, leading to treatment failure. In HCC, various mechanisms can contribute to drug resistance, including genetic mutations, alterations in drug metabolism, and the presence of CSCs. Overcoming drug resistance involves strategies aimed at restoring the sensitivity of cancer cells to treatment. Nanomedicine has offered effective approaches to address significant challenges, including drug resistance and the recurrence of HCC [70]. NPs can be engineered to deliver drugs that reverse drug resistance mechanisms in HCC cells, enhancing the effectiveness of chemotherapy and targeted therapies.

### Personalized medicine

Nanomedicine allows for the development of personalized treatment approaches based on the unique characteristics of a patient's HCC. This includes tailoring the choice of NPs, drug payload, and targeting ligands to match the individual's tumor profile.

### Monitoring of circulating tumor cells and biomarkers

Monitoring CTCs and biomarkers in HCC plays a crucial role in assessing disease progression, treatment response, and prognosis. CTCs are cancer cells that have detached from the primary tumor and entered the bloodstream. Biomarkers are measurable substances, such as proteins or genetic material, which provide information about the presence and characteristics of the disease. Nanotechnology-based platforms can be used to detect and analyze CTCs and biomarkers in the bloodstream. This information can aid in early detection of HCC recurrence and treatment response assessment.

### Radiofrequency ablation guidance

NPs can be used to improve the guidance and visualization of radiofrequency ablation procedures, increasing the precision and effectiveness of tumor ablation.

### Liver regeneration

Utilizing NPs to enhance liver regeneration in HCC requires a comprehensive understanding of the disease's underlying mechanisms and the interaction between NPs and liver tissue. NPs can be designed to carry growth factors, cytokines, or other therapeutic agents that promote liver regeneration. These NPs can deliver these factors directly to the damaged liver tissue, stimulating cell proliferation and tissue repair. NPs can also be incorporated into scaffolds or matrices that mimic the ECM environment of the liver. These structures provide mechanical support and cues for cell attachment, proliferation, and differentiation, facilitating liver tissue regeneration. NPs can be designed to release pro-angiogenic factors that stimulate the formation of new blood vessels. Enhanced blood supply supports tissue regeneration by ensuring an adequate oxygen and nutrient supply to the regenerating tissue. NPs with antioxidant properties can help reduce oxidative stress and create a more favorable environment for tissue repair.

### Minimally invasive procedures

Minimally invasive procedures using nanomedicine in HCC offer innovative ways to diagnose, treat, and manage the disease with reduced invasiveness and improved precision. Nanomedicine utilizes NPs and nanoscale materials to enhance the effectiveness and safety of medical interventions. NPs enable minimally invasive procedures, requiring smaller incisions or catheter insertions compared to traditional surgical methods such as percutaneous injection or intravenous administration, reducing the need for invasive surgeries, hospitalization time, and improving patient comfort.

### Combination therapy

Nanomedicine allows for the integration of multiple therapeutic agents within a single NP, enabling combination therapy to target multiple pathways involved in HCC growth and progression. Polymeric NPs have been utilized to co-deliver multiple therapeutic agents, such as chemotherapeutic drugs and targeted therapies, to target different pathways involved in HCC growth and progression. Chen et al. [72] reported a drug delivery system for photothermal-chemotherapy of HCC by utilizing aptamer-modified graphene quantum dots and magnetic chitosan NPs. The findings of this research demonstrate that drug delivery nanosystems have a notable capacity to suppress tumor growth and prolong the lifespan of mice with tumors. The drug delivery nanosystems, identified as DOX-$Fe_3O_4$@CGA, exhibit robust potential for synergistic tumor treatment, opening avenues for the innovation and advancement of new therapies for HCC.

Overall, nanomedicine offers promising opportunities for more effective and targeted therapies in the management of HCC. However, it is important to continue research and clinical trials to fully understand the safety and efficacy of these nanotechnology-based approaches in HCC treatment. As the field of nanomedicine continues to evolve, it holds great potential to revolutionize the way we diagnose and treat HCC.

## Targeting Strategies

Specialized therapeutic NPs offer a solution to the issues associated with conventional NPs, resulting in reduced dosing frequency and decreased toxicity to non-target organs [34]. The tumor's pathophysiological characteristics lend themselves to passive targeting benefits. Conversely, active targeting relies on ligands that attach to specific cells within the tumor site [73]. This approach simultaneously diminishes accumulation in unintended tissues, collectively enhancing the therapeutic index [74].

When formulating nanocarriers for targeted tumor delivery, it's crucial to consider their physical and chemical attributes, including size and surface characteristics, to achieve optimal accumulation at the diseased site. For instance, strong interactions between serum proteins and NPs could lead to rapid elimination from the bloodstream. The surface features of nanocarriers, such as charge and hydrophobicity, influence how proteins adhere to them, triggering complement system activation and swift uptake by phagocytes. In comparison to positively charged and hydrophobic particles, neutral and hy-

drophilic ones generally experience less susceptibility to opsonization [75]. Alongside surface properties, particle size also significantly impacts the fate of nanomedicines *in vivo*. Hydrophilic NPs smaller than approximately 5 nm are efficiently cleared through renal filtration, while larger nanoparticles (> ~200 nm) have a tendency to accumulate rapidly in healthy organs like the lungs, spleen, and liver [76, 77]. Intriguingly, nanocarriers sized between ~5 and ~250 nm can pass through leaky tumor vessels, allowing for gradual and efficient accumulation over time. This phenomenon is partly due to the inadequate lymphatic drainage often observed in tumors [74].

Even when achieving satisfactory tumor accumulation, the effectiveness of nanomedicines heavily relies on how deeply they can penetrate into the tumor interstitium [78, 79]. This penetration is significantly influenced by both the TME and the physical and chemical attributes of the nanocarriers. The TME is typically characterized by an abundance of stromal elements like collagen, hyaluronan, and proteoglycan networks, along with elevated interstitial fluid pressure, collectively creating a formidable biological obstacle for efficient tumor penetration [74, 80]. Among the various strategies employed to facilitate such penetration, the simplest approach involves reducing the size of the nanocarriers [81].

## Active targeting

Actively targeted nanomedicines are endowed with surface modifications containing recognition elements that selectively adhere to receptors that are either expressed or overexpressed at the intended location, such as tumor or endothelial cells [74]. Frequently utilized targeting ligands encompass small molecules (like proteins, peptides, and folic acid), aptamers, and nanobodies (Table 6) [38, 82]. Additionally, beyond nanocarriers, both radioisotopes and drugs have been altered with targeting ligands to facilitate molecular imaging and radio-immunotherapy. While numerous studies have consistently demonstrated considerably heightened cellular uptake of actively targeted nanoparticles *in vitro*, varying outcomes regarding the efficacy of tumor targeting using NPs with or without active targeting ligands have been documented.

## Passive targeting

Passive targeting refers to the specific buildup of NPs at particular sites due to distinct anatomical or pathophysiological attributes [83]. The effectiveness of passive targeting is influenced by factors such as physicochemical attributes, the mode of administration, and notably, the EPR effect 40. Among these factors, the EPR effect holds the most significant influence [38]. In passive targeting, inherent tumor characteristics like heightened vascular permeability and impaired lymphatic drainage significantly facilitate the EPR effect, leading to increased accumulation of NPs within tumor tissues [84].

## Triggered drug release

Both passive and active targeting strategies prominently rely on the EPR effect to facilitate effective accumulation within tumors [74]. Nonetheless, the EPR effect exhibits notable variability as a biological phenomenon, displaying considerable differences among various tumor types and metastases both within and between individuals [74]. This variability can result in instances where the EPR effect falls short. To tackle this issue, a recent solution has emerged in the form of intravascularly triggered drug release. This method hinges on the precise control of drug liberation from NPs at the tumor site through the manipulation of internal or external triggers 40. Distinct alterations in factors like protein and enzyme expression, hyperthermia, hypoxia, redox potential, pH, as well as the stromal cell composition within the TME, set it apart from other tissue environments [85, 86]. Additionally, external stimuli such as magnetic fields, light, ultrasound, and heat can also be harnessed to target the tumor effectively [87].

**Table 6:** Advantages and disadvantages of ligands used in HCC active targeting [83].

| Ligand(s) | Advantage(s) | Disadvantage(s) |
|---|---|---|
| Di- and polysaccharides | Economical | Relatively inferior specificity. |
| | Superior permeability | |
| | Relatively smaller size | |
| Antibodies | Superior specificity | Possible immunogenicity. |
| | High affinity | Not economical. |
| | | Low permeability. |
| | | Relatively bigger size. |
| Peptides | Tunable properties. | Susceptible to proteolysis. |
| | Ease in synthesis. | |
| | Superior specificity. | |
| | High affinity. | |
| | Moderate size. | |
| Aptamer | Non-immunogenic. | Fast degradation and clearance. |
| | Superior permeability. | |
| | High solubility. | |

**Table 7:** Various cell lines (*in vitro*) used in therapeutic application of NPs against HCC [5, 88, 120, 122].

| Cell Line(s) | ATCC | Origin (Species/Race/Gender/Age) | HBV/HCV | Condition | Sensitivity to SFN (IC50; µM) | Mutant Gene(s) | Application(s) |
|---|---|---|---|---|---|---|---|
| HepG2 | HB-8065 | Human/African/M/15 | -/- | HCC | 6/7.42 | NRAS; TERT | A1; A2; A5; A6 |
| HepG2 (C3A) | CRL-10741 | Human/European/M/15 | -/- | Differentiated HCC | - | CTNNB1; NRAS | A1; A2; A5 |
| Hep3B2.1-7 | HB-8064 | Human/African American/M/8 | +/- | HCC | 3.31 | AXIN1; RB1 | A1; A2; A3; A4; A5; A6 |
| Hepa1-6 | CRL-1830 | Mouse/C57L strain | NA | Hepatoma | 10 to 50 | - | A1; A2 |
| HepaRG | - | Human/European/F/- | -/+ | HCC | - | - | A1; A2 |
| HuH-7 | JCRB0403* | Human/Japanese/M/57 | -/- | Well-differentiated HCC | 5.97 | KDR; POLD3; TERT; TP53 | A2; A5 |
| MHCC97 | - | Human/Asian/M/39 | +/Unknown | Metastasis HCC | - | BRD7; KEAP1; TP53 | A2; A5 |
| PLC/PRF/5 | CRL-8024 | Human/African/M/24 | +/- | Hepatoma | - | CDKN2A; NRAS; TP53 | A1; A2; A3; A4 |
| SK-HEP-1 | HTB-52 | Human/European/M/52 | -/Unknown | Adenocarcinoma | 5.3 ± 0.5 | BRAF; CDHN2A | A1; A2; A5; A6; |
| SNU-387 | CRL-2237 | Human/Asian/F/41 | +/- | Grade IV/V pleomorphic HCC | 10 to 50 | CDKN2A; NRAS; TP53 | A1; A2; A3; A4 |
| SNU-423 | CRL-2238 | Human/Asian/M/40 | +/- | Pleomorphic HCC | - | ND | A1; A2; A3; A4 |
| SNU-449 | CRL-2234 | Human/Asian/M/52 | +/- | Grade II-III/IV HCC | - | CDKN2A; TP53 | A1; A2 |
| SNU-475 | CRL-2236 | Human/Asian/M/43 | +/- | Grade II-IV/V HCC | 10 to 50 | TP53 | A1; A2; A3; A4 |

**Note:** A1: 3D cell culture; A2: Cancer research; A3: Infections disease research; A4: Sexually transmitted disease research; A5: Toxicology; A6: High-throughput screening; *JCRB: Japanese Collection of Research Bioresources; NA: Not available; ND; and Not determined.

## Cancer Models Used for Nanomedicine Evaluation

Significant advancements have been observed in the field of HCC, particularly in understanding its intricate epigenetic, genetic, biological, and molecular mechanisms, as well as its pathogenesis and therapeutic innovations. This intricate process has been elucidated through the utilization of preclinical experimental models, which will be briefly outlined below.

### Cell-based (*In vitro*) models

*In vitro* investigations involve cultivating isolated cells within specific culture media. Cancer cell lines are widely employed in routine research due to their ability to provide insights into molecular mechanisms, response to radiotherapy or chemotherapy, drug resistance, invasion, proliferation, cellular signaling pathways, and metabolism underlying tumor growth and metastasis. These cell lines encompass a majority of the genetic and epigenetic alterations found within tumors. They serve as indispensable research tools for drug screening, uncovering molecular targets, and facilitating the development of *in vivo* xenograft models, allowing for swift and cost-effective evaluation of potential therapeutic approaches. Approximately 30 cell lines have been examined in studies focusing on HCC. Among the commonly utilized lines are HuH-7, C3A, Hep3B, HepaRG, and HepG2 (Table 7). HepG2 stands out as the frequently employed HCC cell line due to its ready availability and characterization that aids in pharmacological and toxicological inquiries. In addition, PHH (Table 8) are considered the benchmark for *in vitro* investigations concerning drug-induced liver injury, toxicity, and biotransformation [88]. About 70 to 80% of the human liver consists of hepatocytes, with non-parenchymal cells like sinusoidal endothelial cells, HSCs, and Kupffer cells, accounting for 5 to 6% of the composition [89]. To replicate this *in vivo* cellular diversity, co-cultures can be devised wherein PHH or liver cell lines are combined with non-parenchymal liver cells such as immune cells, stem cells, endothelial cells, and HSCs [88]. A number of studies have suggested that HCC originates from CSCs. These CSCs possess self-renewal capabilities and give rise to the various cell lineages within HCCs [88]. Although 2D models offer valuable insights in liver studies, they lack the cell density and intricate microenvironment characteristic of *in vivo* settings [88]. To address these limitations, 3D models (Table 8) like spheroid cultures and organoid cultures have been developed. Spheroids can be derived from PHH as well as hepatic cell lines [90]. Most liver cell lines exhibit lesser differentiation compared to PHH, notably in terms of biotransformation capacity [91]. Over the years, 3D spheroid models have been introduced to mitigate this shortfall [88]. *Ex vivo* precision-cut liver slices obtained from rat, mouse, or human sources stand as tools that retain the complex native liver environment, including interactions between all liver cell types and the ECM [92,93].

### Animal-based (*In vivo*) models

*In vitro* studies are pivotal in identifying novel treatments and refining existing treatment approaches. However, the transition from these findings to human clinical trials has posed challenges [94]. Animal models hold a crucial role in com-

**Table 8:** Various *in vitro* (2D and 3D) models used in therapeutic application of NPs against HCC [5, 88].

| Model | Advantage(s) | Disadvantage(s) | Application(s) |
|---|---|---|---|
| PHH monolayer | Superior biotransformation efficiency. | Cannot replicate intricate *in vivo* TME. | Liver disease studies. |
| | Similar to *in vivo* phenotype. | Progressive dedifferentiation. | HCC initiation and progression studies. |
| | - | More donor-to-donor variability. | Drug induced injury and toxicity studies. |
| PHH sandwich culture | Retained morphology. | More donor-to-donor variability. | Drug induced liver injury studies. |
| | Prolonged viability. | Alteration in protein expression over time. | Carcinogens initiated HCC studies. |
| Liver cell lines mono-layer | Economical. | Cannot replicate intricate *in vivo* TME. | Silencing and overexpression studies. |
| | Allows genetic manipulation. | Low biotransformation capacity. | HCC epigenetic and molecular assessing studies. |
| | High-throughput analysis. | Compared to PHH, less differentiated. | Genotoxicity studies. |
| | Can be reproducible. | Cannot replicate intra- and inter-tumor diversity. | Drug safety and screening studies. |
| | Phenotype stability. | - | - |
| | Ease in use. | - | - |
| Co-culture | Cell-cell interactions are possible. | Higher interlaboratory variability. | Impact of TME and non-parenchymal cells on HCC can be studied. |
| | Resemblance to *in vivo* TME and heterogeneity of cell. | No standard protocols in place. | - |
| Stem-cell derived | Resemblance in PHH metabolism. | Absence of standard laboratory protocols for primary CSCs isolation. | HCC associated initiation and progression studies. |
| | Reproducibility. | - | Drug resistance studies. |
| | Expandable. | - | HCC therapy studies. |
| | Superior stability of culture. | - | - |
| Spheroid | Resemblance to *in vivo* cell heterogeneity and TME when combined with co-culture. | Difficult to achieve long-term culture. | Immunotherapy studies. |
| | Can study nutrient and oxygen gradients. | Lower throughput. | Angiogenesis studies. |
| | Superior biotransformation efficiency than 2D culture. | Lower uniformity. | Tumor progression studies. |
| | Can retain phenotypic and morphological functions. | Comparatively more expensive and time consuming than 2D culture. | Stem cells' role can be studied. |
| | - | More donor-to-donor variability. | Efficacy and delivery of drugs can be studied. |
| | - | - | Liver function and disease studied. |
| | - | - | Toxicity (cyto- and geno-) studies. |
| Organoid | Minimal requirement of starting material. | Moderate success rate. | Inter- and intra- tumor diversity studies of HCC. |
| | Tumor heterogeneity. | Time consuming. | Cellular and molecular characterization studies of HCC. |
| | Allows genetic manipulation. | Not economical. | HBV-associated HCC progression. |
| | Can achieve cryopreservation. | - | Gene therapy and toxicity studies. |
| | Expandable. | - | Personalized drug discovery and regenerative medicine studies. |
| | High throughput analysis can be done. | - | - |
| | Mimics architecture and functionality of native tissues. | - | - |
| Precision-cut liver slices | Resemblance to *in vivo* gene expression. | Availability is limited. | Liver disease studies. |
| | Possibility of automation. | Technically not easy. | Drug screening and toxicity studies. |
| | Economical. | Reduced cytochrome p450 and albumin expression over time. | Immunological studies. |
| | Polarized morphology can be achieved. | Lack of reproducibility due to variance in culture conditions. | - |
| | Attain *in vivo* TME. | Prolonged incubation periods. | - |
| | - | Labor intensive preparation. | - |
| | - | Not suitable for high-throughput analysis. | - |

**Table 9:** Various animal (*in vivo*) models used in therapeutic application of NPs against HCC [34 ,94 ,122].

| Animal(s) | Cancer induction via | Economics and maintenance | Specification(s) |
|---|---|---|---|
| Mouse | Genetic engineering | Economical. | Defined genetics with existence of "humanized" models; some differences still exist compared with human HCC, because these models are very homogeneous compared with heterogeneous human HCC. |
| | Syngeneic | Ease in maintenance. | |
| | Xenograft | Well established protocols. | |
| | Chemical induction | - | |
| Rat | Syngeneic | Comparatively lower cost. | DEN Wistar cirrhotic model is closer in human TME. |
| | Xenograft | Well established protocols. | |
| | - | DEN-induced Wistar model requires 3 months. | |
| Zebrafish | Genetic engineering | Lower testing and maintenance costs. | High molecular and genetic similarity with human; development of HCC models to study molecular and pathological factors. |
| | Chemical induction | Rapid development and feasibility in drug administration. | |
| | - | Toxicity and efficacy profiling. | |
| Rabbit | Allograft (Orthotopic) | Expensive. | VX2 tumor is of squamous cell origin, whereas human HCC is an adenocarcinoma; microRNA profiles are also different. |
| | - | Tumor growth is rapid (2 to 3 weeks). | |
| | - | Unknown genome organization and tumor biology. | |
| Woodchuck | Viral exposure (*In situ*) | Highly expensive. | Hepatic derived, but typically no fibrosis or scarring of woodchuck livers is noted. |
| | - | Requires few years to develop HCC model. | |
| | - | Requires special protocols. | |
| Swine | Genetic engineering | Highly expensive. | Most identical to human tumor biology; "Oncopig" is immunocompetent and thus amenable to experiments involving immunotherapies. |
| | Chemical induction | Requires few years to develop DEN-induced HCC model. | |
| | - | "Oncopig" is too expensive. | |

prehending the pathophysiology of HCC and its progression [94]. They serve as essential preclinical platforms for assessing treatment efficacy before clinical implementation [94]. The selection of an appropriate animal model is a critical aspect of designing translational experiments that ultimately inform new clinical protocols. Presently, various animal models for HCC exist, encompassing a range of species including mice, rats, zebrafish, rabbits, woodchucks, and swine (Table 9). The diverse methods employed to induce HCC in these animal models are detailed in Table 10. A crucial consideration is to ensure that the chosen animal model closely mimics the molecular, cellular, and tissue aspects of human HCC pathophysiology (Figure 8). Some animal tumor models may differ in origin or involve mutations stemming from DNA-damaging agents that may not reflect the hepatic context of human HCC. Additionally, if cirrhosis is absent in a model, the interpretation of treatment outcomes should be tempered by the fact that human HCC often arises in the context of cirrhosis. Moreover, an ideal animal model should replicate the TME akin to that of human HCC. This becomes particularly significant when studying interventions like embolization and immunotherapy, as the TME can significantly influence therapeutic responses. Distinctions in the TME between naturally occurring in situ tumor development and implanted tumors can lead to variations in therapeutic responses. The selection of an appropriate model for testing novel therapeutic agents involves a balance between advantages and disadvantages, taking into account factors such as tumor cell type, mutational status, cirrhosis presence, and the immune microenvironment of the tumor.

## Clinical Trials Involving Nanomedicine

Clinical trials involving nanomedicine in HCC are exploring the potential of nanotechnology-based approaches to enhance diagnosis, treatment, and management of HCC. These trials aim to leverage the unique properties of NPs and nanoscale materials to improve the precision, efficacy, and safety of interventions. The landscape of clinical trials is continually evolving. Table 11 presents a list of clinical trials involving nanomedicine in HCC.

## Nanomedicine Associated Toxicity or Adverse Effects

While nanomedicine offers promising opportunities for targeted and efficient therapeutic interventions in HCC, there are potential concerns regarding its safety, including toxicity and adverse effects. These concerns arise from the unique properties of NPs or nanoscale materials and their interactions with biological systems. It's important to carefully evaluate and address these issues to ensure the safe clinical translation of nanomedicine-based approaches. Here are some considerations regarding nanomedicine-associated toxicity and adverse effects:

**Table 10**: Methods associated with induction of HCC in animal models [8, 34, 122, 123].

| Method(s) | Example(s) | Advantage(s) | Disadvantage(s) | Immune system origin |
|---|---|---|---|---|
| Chemical induction | DEN; CCl4; PB; NMOR; TAA | Occurrence of cirrhosis/fibrosis. | Tumor genetic background is undefined. | Animal |
| | | Chronic inflammation. | Takes long time for tumor induction. | |
| | | Functional tumor-immune system interface. | - | |
| Genetic engineering | HBx; GMNT knockout; Mdr2 (Abcb4) knockout; c-Myc + p53 | Functional tumor-immune system interface. | Requires stimuli to develop cirrhosis/fibrosis. | Animal |
| | | Gene mutations are specific. | Minimal tumor mutation burden, contradicting HCC microenvironment. | |
| | | Role of signaling pathways and specific proteins. | - | |
| Syngeneic | - | Occurrence of metastasis. | Requires stimuli to develop cirrhosis/fibrosis. | Animal |
| | | Functional tumor-immune system interface. | Minimal similarity with human HCC. | |
| | | Can examine complex cancer and non-cancer cells interactions. | - | |
| Xenograft | MH-134 cells; HCa-1 cells; Hepa1-6 cells; N1-S1 cells | Predictable tumor growth. | Absence of cirrhosis/fibrosis. | Deficient |
| | | Rapid and economical. | Not ideal to study tumor-immune system interface. | |
| | | - | Overlooks entire hepatocarcinogenesis process. | |
| Humanized | - | Most suitable to study immunotherapies. | Absence of cirrhosis/fibrosis. | Human |
| | | Suitable to study tumor-immune system interface of human origin. | Technical difficulties and not economical. | |
| | | - | Lack of complete human immune system reconstitution. | |

**Note:** CCl4: Carbon tetrachloride; DEN: Diethylnitrosamine; GNMT: Glycine N-methyltransferase; HBx: Hepatitis B virus X protein; Mdr2(ABCB4): Multidrug resistance 2(ATP-binding cassette, sub-family B, member 4); NMOR: N-nitrosomorpholine; PB: Phenobarbital; TAA: Thioacetamide

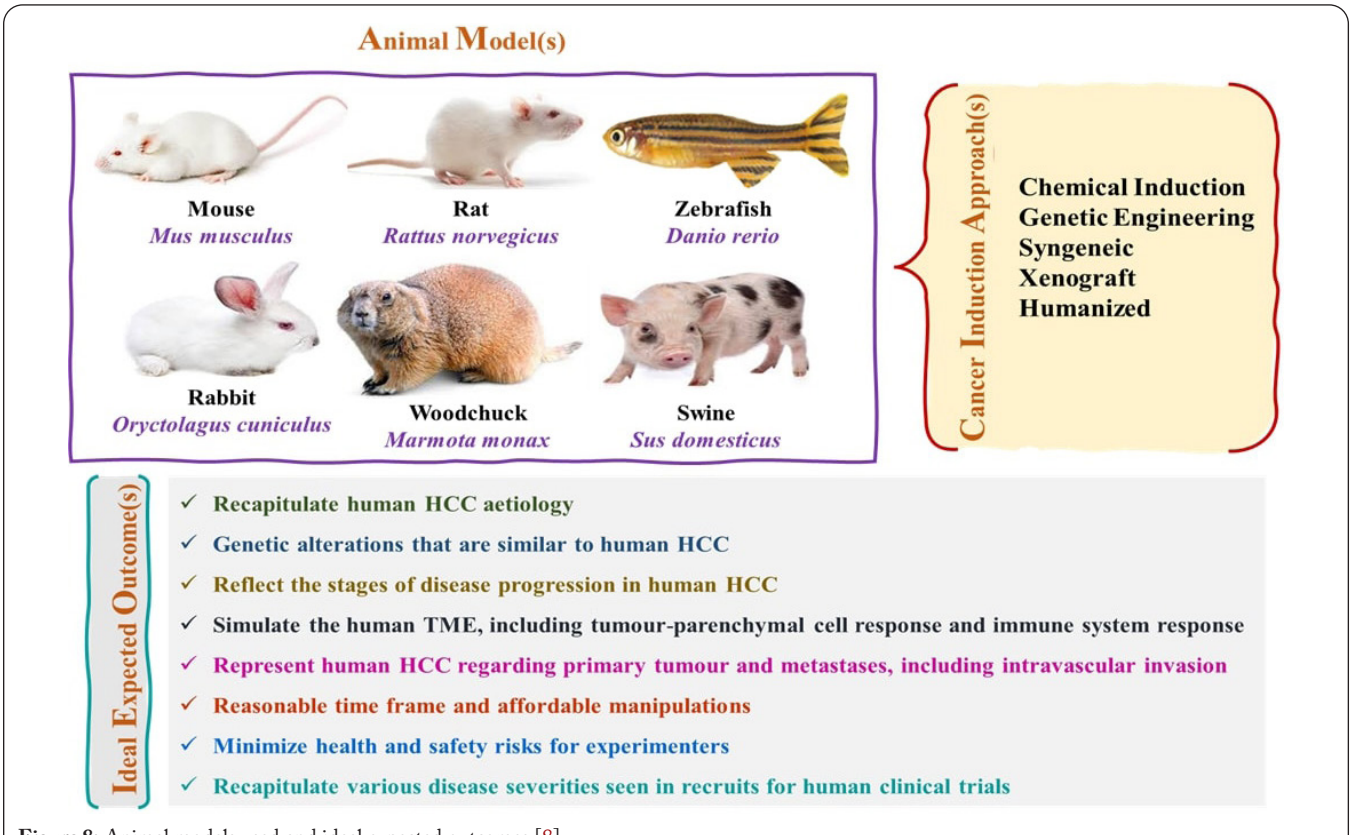


**Figure 8:** Animal models used and ideal expected outcomes [8].

**Table 11:** Clinical trials associated with HCC (https://www.clinicaltrials.gov).

| Trail number | Trail phase | Purpose | NPs Type | Drug used | Combination | Mechanism | Endpoint | Sponsor |
|---|---|---|---|---|---|---|---|---|
| NCT00617981 | III | Efficacy and safety of ThermoDox | Liposome | DOX | RFA | Response to temperature change | Non-resectable HCC | IMUNON, USA |
| NCT02314052 | I and II | Safety and tolerability of DCR-MYC | Lipid | Novel synthetic siRNA oligonucleotide | - | Inhibition of MYC oncogene | Inhibition of cancer cell growth | Dicerna Pharmaceuticals, USA |
| NCT02449109 | I and II | Safety and efficacy of nanodrug | - | Gemzar mix with Glycyrrhizin | Digital subtraction angiography | Active targeting | Interventional therapy | Fuda Cancer Hospital, China |
| NCT04331743 | Ib | Safety and efficacy | Liposome | Mitoxantrone hydrochloride | - | Passive targeting | Advanced HCC | CSPC ZhongQi Pharmaceutical Technology Co., Ltd., China |
| NCT00057395 | I and II | Safety and effectiveness | Liposome | Aroplatin | - | Passive targeting | Advanced solid malignancies | Aronex Pharmaceuticals, US |
| NCT01829971 | I | Safety of MRX34 | Liposome | miR-34a | DEX | Gene therapy | Advanced solid tumours | Mirna Therapeutics, Inc., US |
| NCT04050462 | II | Effect of combination therapy over monotherapy | Polymeric (PBCA) | BMS-986253 or Cabiralizumab | Nivolumab | Drug delivery | Interventional combination therapy | NYU Langone Health, US |
| NCT02716012 | Ia and Ib | Safety, tolerability, and anti-tumour activity study | Liposome | MTL-CEBPA (dsRNA) | SFN | Gene therapy (CEBPA gene) | Advanced HCC | Mina Alpha Limited, UK |
| NCT02191878 | I and II | Safety, pharmacokinetics, and preliminary anti-tumor activity | Lipid | TKM-080301 | - | Drug delivery | Advanced HCC - Interventional therapy | Arbutus Biopharma, US |

**Nanoparticle characteristics:** The size, shape, surface charge, and composition of NPs can influence their behavior within the body. Some NPs may have unexpected interactions with biological molecules, potentially leading to adverse effects.

**Accumulation and distribution:** NPs can accumulate in various organs and tissues, including the liver and spleen. Prolonged or excessive accumulation may affect the normal functions of these organs and potentially cause toxicity.

**Immune response:** NPs can trigger immune responses, leading to inflammation and potential damage to healthy tissues. The immune response can also influence the clearance and distribution of NPs.

**Long-term effects:** The long-term effects of NPs exposure are still being studied. Some NPs may have slow clearance rates, raising concerns about chronic exposure and potential cumulative effects.

**Bio-distribution and clearance:** Understanding how NPs are metabolized, excreted, and eliminated from the body is crucial. Impaired clearance mechanisms could lead to prolonged exposure and adverse effects.

**Organ-specific effects:** Different organs may respond differently to NPs exposure. For example, NPs that accumulate in the lungs could cause respiratory issues, while those that accumulate in the liver might affect liver function.

**Cellular effects:** NPs can interact with cell membranes and cellular organelles, potentially interfering with cellular processes and leading to cytotoxicity or other adverse effects.

**Genotoxicity:** Some NPs may have genotoxic effects, potentially leading to DNA damage and mutations.

**Allergic reactions:** Certain NPs or their surface modifications could trigger allergic reactions in sensitive individuals.

**Environmental impact:** The potential release of NPs into the environment raises concerns about their impact on ecosystems and potential bioaccumulation in the food chain.

## Limitations of Nanomedicine

The following are the few limitations restricting the applicability of nanoscale materials in the overall well-being of humans.

While numerous NPs have gained FDA approval for clinical use, concerns surrounding their safety persist when applied in human contexts. It remains essential to delve deeper into their potential toxicity during their presence in the body, their metabolic pathways, and the speed of their elimination. Further investigation and substantiation are necessary in these areas [20].

Many research endeavors have not comprehensively examined the toxicological characteristics, relying mainly on short-term animal trials to establish relative safety. Long-term toxicity of these nanomaterials has remained largely unexplored [20].

According to the available literature, nanomaterials are stored at room temperature for a duration ranging from one week to one month, and during this time, no precipitation,

dissolution, or other significant changes occur, indicating a relatively stable formation of NPs, however effects of long term storage is unavailable [20].

During preclinical testing of a novel therapeutic, the interventional radiologist must carefully evaluate the pros and cons of each model, taking into account the molecular mechanism of the treatment and the challenges associated with replicating that mechanism in humans [94].

While incorporating targeting ligands enhances the internalization of nanomedicines by cells *in vitro*, this enhancement may not always translate to increased accumulation within tumors *in vivo* [74].

The effectiveness of nanomedicines in targeting tumors is influenced by the physical and chemical characteristics of nanocarriers (such as size and surface properties), as well as the specific pathophysiological features of the targeted tissue [74].

## Conclusion

In conclusion, fibrosis is the initial stage of liver scarring, while cirrhosis represents the advanced and irreversible stage with severe liver damage. HCC, on the other hand, is a primary liver cancer that can develop in the context of cirrhosis or other chronic liver diseases. Nanomedicine offers a promising avenue for enhancing the diagnosis and management of HCC, holding significant potential for further advancements. Achieving early HCC diagnosis necessitates a multifaceted approach, incorporating diverse methods such as imaging and continuous monitoring, to enhance diagnostic precision and establish the groundwork for early interventions. In the realm of treatment, amalgamating various therapeutic strategies can mitigate harm to healthy liver cells, counter drug resistance, and enhance overall survival rates. Presently, the focus is on developing integrated nanoparticles that facilitate both diagnosis and treatment with heightened efficiency. Investigating the molecular mechanisms underlying the role of nanoparticles in HCC therapy could potentially be the next progressive stride.

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## Conflict of Interest

None.

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