

Targeted therapy for liver cancer: Current status and future directions

Qian Wang^{1*}

¹School of Medicine, Shandong Xiehe University, 250000, China

Article Info



Article Type:
Review

Article History:

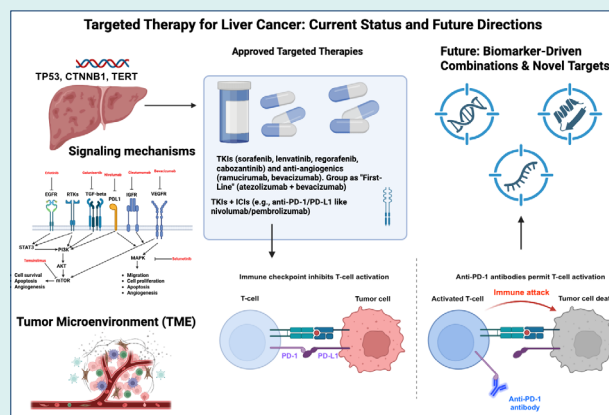
Received: 30 Jul. 2025
Revised: 9 Oct. 2025
Accepted: 11 Oct. 2025
ePublished: 14 Dec. 2025

Keywords:

Hepatocellular carcinoma
Tyrosine kinase inhibitors
Immune checkpoint inhibitors
Targeted therapy
Wnt/ β -catenin
FGFR4

Abstract

Because of its intrinsic tumor heterogeneity, poor response to traditional chemotherapy, and lack of viable molecular targets, liver cancer mostly hepatocellular carcinoma (HCC) continues to be a major worldwide health concern. With a focus on molecular processes, resistance routes, and combination therapy approaches, this review provides a thorough analysis of the status and new advancements in targeted therapeutics for liver cancer. By blocking the mechanisms that lead to angiogenesis and tumor growth, first-line systemic treatments, such the multi-tyrosine kinase inhibitors (TKIs) lenvatinib and sorafenib, have shown moderate improvements in survival. However, their long-term efficacy is significantly reduced by intrinsic and acquired resistance, which is why second-line medications like regorafenib, cabozantinib, and ramucirumab are being studied. When combined with anti-VEGF treatments, parallel developments in immunotherapy, in particular immune checkpoint inhibitors (ICIs) such as atezolizumab and nivolumab, have shown promising outcomes. The review highlights the role of the tumor microenvironment, epigenetic regulators including EZH2 and HDACs, and key oncogenic drivers and aberrant signaling cascades in HCC, such as the Wnt/ β -catenin, PI3K/AKT/mTOR, and RAS/RAF/MEK/ERK pathways. It also covers metabolic vulnerabilities, DNA damage response pathways, and new targets including FGFR4, AXL, and c-MET. To get around resistance mechanisms and improve therapeutic effectiveness, special attention is paid to logical combination treatments, which include combining targeted medicines with ICIs, irradiation, or synthetic lethality techniques. In the end, the review promotes the combination of dynamic molecular profiling and biomarker-driven precision medicine to enhance patient stratification, improve treatment decision-making, and provide long-lasting clinical effects. A strategic foundation for future advancements and individualized treatment of hepatocellular carcinoma is provided by this comprehensive synthesis.



Introduction

With an 18% 5-year survival rate, liver cancer is the fourth most prevalent cause of cancer-related mortality globally and the sixth most common kind of cancer overall.¹⁻³ Hepatocellular carcinoma (HCC) accounts for 90% of the cases.⁴ Hepatitis B virus (HBV) infection is the major risk factor, accounting for 50% of HCC cases.⁵ Hepatitis C virus (HCV) infection, long-term alcohol use, and non-

alcoholic fatty liver disorders (NAFLD) are other causes.⁶ Even if HBV vaccination and efficient HCV antivirals have reduced virus-related HCC, excessive alcohol use and the rising rates of obesity and diabetes in Western countries are the major causes of the overall increase in HCC incidence.⁷ A complicated multistep process, the pathophysiology of HCC has a diverse mutational landscape and histological characteristics.⁸⁻¹¹ 80% of



*Corresponding author: Qian Wang, Emails: 18663799731@163.com, Qian.wang1200@gmail.com



© 2025 The Author(s). This work is published by BioImpacts as an open access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>). Non-commercial uses of the work are permitted, provided the original work is properly cited.

HCC cases have telomerase activation brought on by mutations or rearrangements in the TERT promoter.^{9–12} The identification of important driver genes in HCC, such as TP53 (28–36%), CTNNB1 (17–37%), AXIN1 (4–14%), ARID1A (16.8%), and ARID2 (5.6%), has been made easier by next-generation sequencing. These genes are involved in controlling the cell cycle, Wnt/ β -catenin signaling, and epigenetic changes.^{13–15}

Nowadays, the primary cancer driver genes are still undetectable, and only 25% of individuals with HCC have at least one possible actionable mutation.¹⁶ Unfortunately, hepatic resection and liver transplantation are the primary curative treatments for HCC since it does not respond to traditional chemotherapy.¹⁷ Systemic treatments based on TKIs have improved treatment results for HCC since 2010. By blocking VEGFR2, sorafenib inhibits angiogenesis and the RAF-MEK-ERK signaling cascade.¹⁸ It serves as a first-line treatment, although provides a survival advantage of about 2.8 months compared to placebo.¹⁸ The phase 3 REFLECT study conducted in 2018 confirmed the effectiveness of lenvatinib, a TKI with enhanced inhibition of VEGF receptors and the FGFR family, revealing a small increase in median overall survival compared to sorafenib (13.6 vs. 12.3 months).¹⁹ Some TKIs (regorafenib) are second-line treatments for advanced HCC²⁰ and cabozantinib²¹) and ramucirumab, a monoclonal antibody specific for VEGFR2²², It has shown a particular advantage for individuals with elevated AFP serum levels after sorafenib's failure (REACH trial).²²

Since 2017, novel treatments that target the microenvironment of liver tumors have surfaced. The FDA approved pembrolizumab and nivolumab, two PD-1-targeting immune checkpoint inhibitors (ICIs), as second-line treatments for advanced HCC; nevertheless, their overall survival benefits were not as great as those of sorafenib.^{23,24} As a second-line treatment for advanced HCC, the FDA has authorized the combination of nivolumab and ipilimumab, a monoclonal antibody targeting CTLA-4.²⁵ Atezolizumab (anti-PD-L1) and bevacizumab (anti-VEGF) were approved by the FDA in 2020 as a first-line treatment for advanced HCC after the phase 3 IMBRAVE-150 trial (NCT03434379) demonstrated better efficacy than sorafenib, with a median progression-free survival of 6.8 months as opposed to 4.3 months.²⁶ Even with these encouraging developments, only 20–30% of HCC patients benefit with immunotherapy, and there are still no trustworthy biomarkers to detect responders with certainty.^{25,27} Ultimately, in order to create novel combination medicines that may inhibit tumor development and overcome immunotherapy resistance, a deeper understanding of the liver cancer microenvironment is needed. Finding response and resistance indicators is also crucial for improving patient selection and facilitating individualized treatment plans. An extensive examination

of both established and new targeted treatments for liver cancer, including HCC, is provided in this study. It looks at resistance routes, important molecular factors, and new treatment approaches, including as combination regimens. In order to direct future efforts to improve clinical outcomes in the care of liver cancer, emphasis is put on translational advancements and the potential of precision medicine. The comprehensive workflow of liver cancer therapy was showed in Fig. 1.

Molecular pathogenesis of liver cancer

Genetic and epigenetic drivers (e.g., TP53, CTNNB1, TERT)

The development and spread of liver cancer are influenced by a complex interaction of environmental, genetic, and epigenetic variables. Genetic changes such point mutations, gene amplifications, promoter region modifications, and copy number variations often brought on by viral infections or long-term hepatotoxic insults are what drive hepatocarcinogenesis. Hepatocytes undergo metamorphosis as a result of these alterations, and HCC is often associated with abnormalities in important oncogenes and tumor suppressor genes.^{9,28} Notably, frequent genetic changes in genes such CTNNB1, TERT, CDKN2A, SMARCA2, and HGF are linked to alcohol-related HCC. As one of the most frequent genetic events in liver cancer, TERT promoter mutations that result in telomerase reactivation are especially common among these, occurring in around 60% of cases.^{29,30} Mutations in CTNNB1, which encodes β -catenin, and TP53, a crucial tumor suppressor, are often seen in HCC and are essential in interfering with apoptosis, cell cycle regulation, and Wnt/ β -catenin signaling, which promotes unregulated proliferation and survival. These mutations establish different molecular subgroups of HCC and are usually mutually exclusive. Large, well-differentiated, cholestatic tumors with microtrabecular or pseudoglandular architecture and little inflammatory infiltrates are often seen in CTNNB1-mutant tumors. On the other hand, tumors with TP53 mutations are often poorly differentiated, compact, multinucleated, pleomorphic, and have a high rate of vascular invasion.²⁹ The development of liver cancer is also influenced by epigenetic changes, such as modifications to histones, DNA, and RNA.³¹ While worldwide DNA hypomethylation may activate oncogenes and aid in the development of tumors, abnormal methylation of tumor suppressor gene promoters silences their production. Furthermore, by altering the chromatin architecture, histone modifications including methylation, phosphorylation, acetylation, ubiquitylation, and sumoylation control the expression of genes. Acetylation and methylation are two of them that have a special impact on chromatin remodeling and gene transcription regulation.³² Gene expression in NAFLD and NASH is impacted by enzyme changes in histone

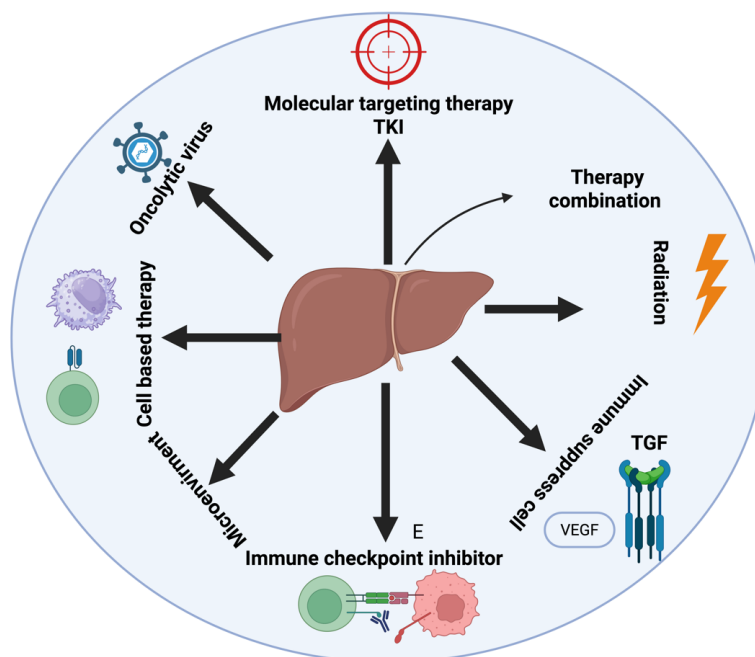


Fig. 1. Comprehensive therapeutic strategies for liver cancer. This figure illustrates a range of current and emerging therapeutic strategies for liver cancer, highlighting approaches that target both the tumor and its surrounding microenvironment. Key treatments include molecular targeting therapies such as TKIs, which block cancer-driving signaling pathways, and immune checkpoint inhibitors that restore T-cell function by blocking inhibitory pathways like PD-1/PD-L1. Additional strategies involve targeting immune suppressive cells to overcome tumor-induced immunosuppression, and radiation therapy to directly destroy cancer cells. Therapies aimed at modulating the tumor microenvironment, including inhibition of TGF- β and VEGF, are also shown to support anti-tumor immunity. Oncolytic virus therapy leverages viruses engineered to selectively infect and kill cancer cells, while cell-based therapies use immune cells like CAR-T or NK cells to directly target the tumor. The integration of these treatments through combination therapies is emphasized as a promising approach to enhance effectiveness and overcome therapeutic resistance in liver cancer.

acetylation and methylation, which may cause apoptosis, impaired hepatic metabolism, and an elevated risk of HCC. Genes are regulated by these epigenetic changes without changing the DNA sequence.

Signaling mechanisms that are aberrant

Wnt/ β -catenin

During HCC formation, the Wnt/ β -catenin cascade is crucial. Wnt ligands signal cell surface Frizzled receptors.^{33,34} Genetic mutations or Wnt pathway dysregulation may accelerate HCC development. A protein complex consisting of GSK-3 β , APC, and Axin phosphorylates β -catenin, causing proteasome degradation in the absence of Wnt ligands. GSK-3 β activity is inhibited by Wnt ligands binding to Frizzled receptors, preventing β -catenin degradation.^{34,35} This hinders β -catenin degradation and phosphorylation. Wnt activation causes it to enter the nucleus from the cytoplasm. This is made easier by APC and Axin destruction complex disruption. By binding to T-cell factor/lymphoid enhancer factor (TCF/LEF), stabilized β -catenin activates Wnt target gene transcription in the nucleus.³⁶ The β -catenin/TCF-LEF complex regulates gene expression crucial for cell survival, differentiation, and proliferation. In HCC, dysregulation of the Wnt/ β -catenin pathway leads to unregulated activation of genes that promote angiogenesis, prevent apoptosis, and

promote proliferation.

PI3K/AKT/mTOR

HCC development requires the signaling cascade of PI3K, Akt, and mTOR.³⁷ When IGF and EGF bind to their cell surface receptors, PI3K is activated. Activated PI3K phosphorylates PIP2 into PIP3.³⁸ PIP3 recruits and activates Akt as a second messenger. Akt is phosphorylated by PDK1 and mTORC2 after binding PIP3. Akt-induced TSC2 phosphorylation and inhibition activates mTORC1. Through phosphorylation of downstream effectors including p70S6K and 4E-BP1, mTORC1 regulates protein synthesis, metabolism, and cell proliferation.^{39,40} These downstream effectors boost protein synthesis and cell division. PI3K/Akt/mTOR pathway dysregulation in HCC causes angiogenesis, metabolic reprogramming, uncontrolled cell proliferation, apoptosis resistance, and extended mTORC1 activation (Fig. 2).⁴¹

RAS/RAF/MEK/ERK

The RAS/RAF/MEK/ERK pathway affects cellular activities include differentiation, apoptosis, proliferation, and stress response. Its aberrant activation helps colon cancer, HCC, leukemia, and melanoma develop and advance.^{42–47} External stimuli activate RAS proteins, small GTPases. Activated RAS causes RAF dimerization and activation in the RAS/RAF/MEK/ERK cascade. RAF phosphorylates MEK, which activates ERK, propagating the signal downstream.⁴⁸ P-ERK, the active form of

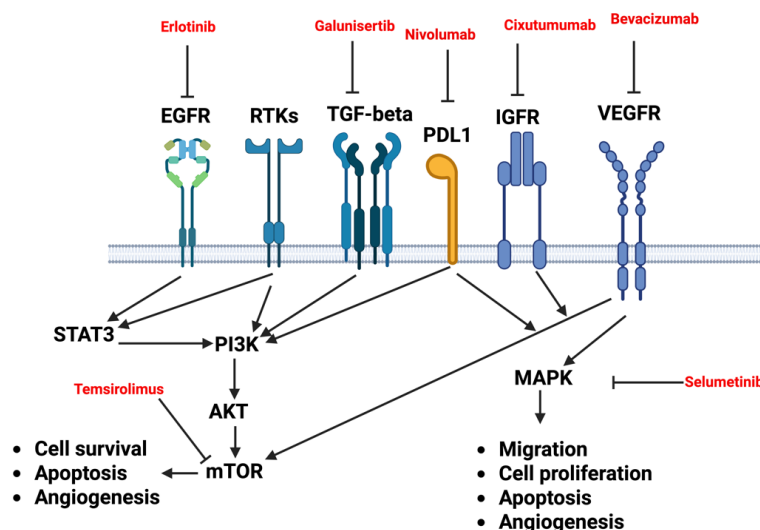


Fig. 2. In the management of unresectable hepatocellular carcinoma (HCC), several molecular targets have been identified for therapeutic intervention. Erlotinib exerts its antitumor effects by blocking epidermal growth factor receptor (EGFR) activity, which subsequently disrupts downstream signaling pathways, notably the STAT, PI3K/Akt, and mTOR axes, ultimately suppressing key oncogenic processes. Galunisertib, an inhibitor of the transforming growth factor-beta receptor (TGF β R), similarly impedes tumor progression by attenuating the PI3K/Akt/mTOR signaling cascade. Immune checkpoint modulation is achieved through agents such as nivolumab, which targets programmed death-ligand 1 (PD-L1), while cixutumumab and bevacizumab selectively inhibit the insulin-like growth factor receptor (IGFR) and vascular endothelial growth factor receptor (VEGFR), respectively. These agents disrupt tumor-promoting signals predominantly via the MAPK pathway. Additionally, temsirolimus and selumetinib act as direct inhibitors of mTOR and MAPK, respectively, offering targeted suppression of proliferative and survival mechanisms in recurrent HCC.

ERK, is a critical indicator of RAS/RAF/MEK/ERK signaling.⁴⁹ Around 30% of cancers are caused by RAS gene mutations, which overactivate RAS.^{50–53} It has been widely proven that aberrant activation of this route in HCC tissues causes HCC, malignant transformation, and treatment resistance.^{53,54} However, RAS mutations are rare and found in less than 5% of HCC patients, despite extensive signaling activity.⁵⁵

JAK/STAT

Signal transduction via the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway begins when cytokines, hormones, or growth factors bind to cell surface receptors.^{33,56} When ligands interact with receptor-associated Janus kinases (JAKs), STAT3 and STAT5 tyrosine residues are phosphorylated. After phosphorylation, STATs form homo- or heterodimers in the nucleus. STAT dimers connect to STAT response elements (SREs) in target gene promoter regions to initiate transcription in the nucleus.^{57,58} STAT binding to DNA controls target gene transcription. Activated STATs govern cell differentiation, migration, apoptosis, and proliferation. JAK/STAT pathway dysregulation in HCC may cause uncontrollable cell proliferation and tumor growth. To prevent overactivation, STATs induce SSI-1 and SOCS1 synthesis. These negative regulators inhibit signaling by binding to phosphorylated JAKs and receptors.⁵⁹

TGF- β

By downregulating pro-proliferative transcription factors such as c-Myc, TGF- β promotes cell cycle arrest in HCC. The suppression is caused by the nuclear translocation

of Smad3, E2F4 or E2F5, and RB-related protein p107. These elements halt c-Myc synthesis and the cell cycle.^{60–62} Additionally, it modulates CDK inhibitors, which arrests G1.⁶³ To start the cell cycle, CDK2 binds to cyclin E and CDK4 or CDK6 to cyclin D.⁶⁴ TGF- β reduces the inhibitory effects of c-Myc on CDK inhibitors p21CIP1 and p15INK4B, leading to cell cycle arrest.^{65–68} Rising ROS and Nox4 levels in well-differentiated HCC cells lead to increased p21CIP1 and p15INK4B expression, which is induced by TGF- β .⁶⁹ In addition to G1 phase arrest, TGF- β promotes G2 phase arrest in HCC-cells via activating Wee1 kinase and CDK inhibitors p21CIP1 and p27KIP.⁷⁰

Tumor microenvironment (immune cells, angiogenesis, fibrosis)

Additionally, cancer cells depress the immune system in the tumor microenvironment (TME), especially when they interfere with T cell activity. One important immunomodulator, prostaglandin E2 (PGE2), influences T cells among other immune cells. Its release triggers the cyclooxygenase (COX) pathway, which has been linked to resistance to immunotherapy.^{71,72} When β -catenin signaling is triggered in cancer cells, the accumulation of conventional DC1 is inhibited.⁷³ Furthermore, kynurenine, which is generated in excess by IDO1, tryptophan 2,3-dioxygenase, and adenosine, which comes from dead tumor cells, are immunosuppressive metabolites released by cancer cells. These metabolites alter the TME, as does the loss of essential amino acids needed for T cell activity. Together, they reduce anti-tumor immunity and help

malignancies evade the immune system.⁷⁴ By encouraging the development of regulatory T cells (Tregs), preventing the growth of stem-like memory T cells, and inducing a stromal response that keeps T cells out of the tumor microenvironment, TGF- β , which is generated by cancer cells, reduces anti-tumor immunity.^{75–78} Notably, cancer cells have an independent defensive system that allows them to quickly fix membrane holes created by the deadly chemical perforin, which is produced by T cells. This defense mechanism shields the cancer cells from being killed by T cells.⁷⁹

Tissue-associated fibroblasts (CAFs), myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), and Tregs are among the immune and stromal cell subsets that influence immune evasion in the TME of HCC. Among these, CAFs are essential because they promote carcinogenesis in healthy epithelial cells and modify the extracellular matrix (ECM), especially collagen fibers. CAFs can provide tumor cells characteristics of stroma that increase their aggressiveness. They may circulate throughout the body and are derived from vascular cells, cancer cells, or activated mesenchymal stem cells in the bone marrow.

Human solid tumors have three main types of CAFs: antigen-presenting, inflammatory, and myofibroblastic CAFs.⁸⁰ Myofibroblastic CAFs release fibrosis-associated chemicals and ECM components that promote angiogenesis and tumor invasion. In the tumor microenvironment, they also inhibit CD8 T cells and other immune effector cells, which aids in immune evasion.^{81–83} C-C motif chemokine ligand 2 (CCL2), CCL12, and IL-6 are secreted by inflammatory CAFs, which may have immunosuppressive effects.^{84,85} Treg cell mobilization is triggered by antigen-presenting CAFs that express immune regulatory factors and MHC class II molecules.⁸⁶

CAFs are essential for the TME, which can be used to slow the development of HCC and liver fibrosis and might be a novel target for treatment.^{87,88} As a byproduct, CAF-produced IDO and PGE2 inhibit natural killer (NK) cells' production of TNF- α and IFN- γ , reducing their antitumor activity and promoting the growth of HCC.^{87–89} Hepatic fibroblasts may be stimulated to release pro-inflammatory cytokines by bone morphogenetic protein 4 (BMP4) in HCC, which would improve their penetration into the tumor microenvironment. As a result, BMP4 plays a crucial role in controlling CAF activity and promotes tumor growth in HCC (Table 1).⁸⁹

Current landscape of targeted therapies

Approved therapies

Tyrosine kinase inhibitors

TKIs are crucial for the treatment of advanced HCC, especially in individuals who cannot receive liver transplantation or surgery. TKIs provide a practical and

efficient method of halting the course of the illness when taken orally as systemic treatments.

Dysregulated intracellular signaling, especially from the cell surface inward, is the primary driver of important events in HCC, including tumor cell proliferation, metabolism, angiogenesis, and metastasis. Growth factors and the receptor tyrosine kinases (RTKs) that correspond to them, such as fibroblast growth factor receptor (FGFR), vascular endothelial growth factor receptor (VEGFR), hepatocyte growth factor receptor (HGFR/MET), platelet-derived growth factor receptor (PDGFR), and epidermal growth factor receptor (EGFR), mediate this signaling.¹⁰⁰ Intracellular signaling cascades, namely the PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways, are initiated when these receptors are activated by their corresponding growth factors. By encouraging cell division, survival, angiogenesis, and metastasis in HCC, these cascades drive tumor growth.¹⁰⁰

TKIs are crucial for treating HCC because they block these pathways, which stops tumor development, angiogenesis, and metastasis.¹⁰¹ This multi-targeted approach disrupts many signaling pathways that are necessary for the development and spread of tumors. TKIs have been shown in clinical studies to increase overall survival in patients with advanced HCC. VEGFR1-3, PDGFR β , KIT, RET, FLT3, and downstream kinases RAF1 and BRAF are among the targets that are inhibited by sorafenib, the first licensed systemic treatment for HCC to show a survival benefit.¹⁰² The therapeutic advantage of sorafenib in advanced HCC was shown by the phase III SHARP study, which had 602 patients with the disease and demonstrated a substantial improvement in median overall survival of 2.8 months when compared to placebo.^{18,103} Sorafenib's effectiveness in treating advanced HCC in a variety of populations was further supported by the phase III ASIA-PACIFIC research, which included 226 HCC patients from the Asia-Pacific area and validated the survival advantages shown in the SHARP trial.¹⁰⁴ There was no statistically significant difference in overall survival between lenvatinib and sorafenib in the REFLECT trial, a randomized, open-label phase III research that included 1,492 HCC patients. However, lenvatinib was approved as a first-line therapy for advanced HCC since it demonstrated a higher progression-free survival.¹⁰⁵ Lenvatinib targets VEGFR1-3, PDGFR α/β , FGFR1-4, KIT, and RET and differs greatly from sorafenib in that it does not include the fluorine atoms that sorafenib does.¹⁰² With the advent of more recent TKIs like as cabozantinib and regorafenib, the options for second-line treatment for advanced HCC have expanded, providing other approaches in the event that first-line therapy fails. Similar in structure to sorafenib, regorafenib has one extra fluorine atom, which increases its capacity to inhibit a greater variety of molecular targets implicated in the development of

Table 1. Important molecular pathways, elements, and their functions, benefits, and difficulties in the etiology and management of hepatocellular carcinoma

Pathway/Factor	Key components	Description	Function in HCC	Advantages	Challenges	References
Genetic drivers	TP53, CTNNB1, TERT, CDKN2A, SMARCA2, HGF	Tumor start and subtype development are driven by genetic changes in important oncogenes and tumor suppressor genes.	Modify survival, apoptosis, and the cell cycle; identify molecular subtypes	enables the possibility of targeted treatment and molecular categorization.	Tumor heterogeneity and several mutations remain undiagnosed.	9,90,91
Epigenetic alterations	Histone changes (acetylation, methylation), and DNA methylation	Epigenetic modifications impact tumor suppressor and oncogene activity by controlling gene expression without changing the DNA sequence.	Mute tumor suppressor genes, turn on carcinogens, and alter metabolism	reversible changes that epigenetic medications may target.	epigenetic plasticity, off-target toxicity, and global impacts.	92,93
Wnt/ β -catenin	Frizzled, Wnt ligands, GSK-3 β , APC, β -catenin, TCF/LEF	Abnormal activation of this canonical pathway, which is important in liver development, promotes immune evasion and proliferation.	Encourage resistance, angiogenesis, and proliferation via nuclear β -catenin	β -catenin inhibitors may target this subtype of HCC, which is immune-cold.	Complex regulation; direct inhibition of β -catenin is difficult.	94
PI3K/AKT/mTOR	PI3K, AKT, mTORC1/2, PIP2, PIP3, TSC2, 4E-BP1, p70S6K	Often dysregulated in HCC, this system is essential for metabolism, cell proliferation, and survival.	Promote resistance, protein synthesis, angiogenesis, and cell proliferation	mTOR inhibitors have a strong promise for treatment and are now in use.	development of resistance and impact on systemic metabolism.	95
RAS/RAF/MEK/ERK	RAS, RAF, MEK, ERK, LZTR1, CRL3 E3 ligase	LZTR1 regulates RAS activity by ubiquitination; the MAPK pathway is implicated in stress response and proliferation.	Drug resistance and proliferation are stimulated by ubiquitination via LZTR1.	Modulation of LZTR1 provides a new treatment strategy.	Uncertain regulatory mechanisms and a low mutation frequency.	96
JAK/STAT	JAKs, STAT3, STAT5, SOCS1, SSI-1	Overactivation of cytokine-mediated signaling accelerates the growth of tumors and regulates immune response and proliferation.	control immune signaling and proliferation; cancer is encouraged by dysregulation.	Drugs that block the JAK/STAT pathway are being developed.	Off-target effects are caused by broad immune functions.	97,98
TGF- β	Smad3, E2F4/5, p107, CDK2, CDK4/6, p21CIP1, p15INK4B, p27KIP1, Wee1	complex modulator of apoptosis, differentiation, and cell cycle arrest; dual function in tumor development and repression.	induce G1/G2 arrest, control transcription factors and CDKs, and encourage tumor suppression	TGF- β inhibitors have the ability to alter fibrosis and immunological responses.	Targeting is made more difficult by its dual function in tumor development and repression.	60
Tumor microenvironment	CAFs, TAMs, TANs, MDSCs, Tregs, PGE2, IDO1, adenosine, TGF- β	Tumor growth and immune escape are shaped by the intricate interactions of immune cells, fibroblasts, cytokines, and metabolic products.	alter fibrosis, angiogenesis, and immune evasion; CAFs and cytokines encourage the formation of tumors.	TME may be reshaped and immunological function restored by targeting CAFs or cytokines.	Extremely patient-specific and dynamic; difficult to successfully moderate.	99

tumors.¹⁰⁶ Cabozantinib is a dual inhibitor of VEGFR2 and the HGFR/MET, and it has a different chemical structure than sorafenib. It also targets RET, KIT, TIE2/TEK, and AXL, among other important receptors implicated in tumor development and angiogenesis.¹⁰⁷ Compared to traditional chemotherapy, new-generation TKIs often have better side-effect profiles, enhancing patient tolerance, adherence, and general quality of life. Their incorporation into treatment plans is still developing, especially when combined with immunotherapies, which have shown improved therapeutic effectiveness via mutually reinforcing processes. TKIs are being included to personalized medicine frameworks more often as our understanding of the pathophysiology of HCC grows. In order to provide more individualized and efficient

treatments, ongoing biomarker research attempts to determine which individuals are most likely to benefit from certain TKIs. Altogether, TKIs have revolutionized the way that HCC is treated by providing strong therapeutic alternatives for a condition that is often discovered at an advanced stage. Their relevance in HCC treatment is only increasing because to their multi-targeted action and their involvement in combination therapies.

Anti-angiogenic agents

HCC is distinguished by its abundant vascularization. Antiangiogenic treatments are essential for managing the disease's severe stages. In 2007, sorafenib, an oral multikinase inhibitor that targets angiogenic pathways and RAS/RAF signaling, was authorized as the first therapy for advanced HCC worldwide. After demonstrating that

lenvatinib was not inferior to sorafenib in terms of overall survival in 2018, the European Medicines Agency (EMA) and the U.S. FDA approved it. ICIs have revolutionized the treatment of HCC and other cancers. For unresectable HCC, the standard of therapy now consists of bevacizumab, a monoclonal antibody that targets VEGF, and atezolizumab, a PD-L1 inhibitor. This change was brought about by the successful results of the IMbrave150 research and the FDA's subsequent approval of this treatment strategy.^{26,108,109} Nevertheless, even while phase 3 trials of single-agent therapy in first- and second-line settings showed response rates of 15–20%,^{24,25} as first-line monotherapies for patients with HCC, single-agent PD-1 inhibitors like pembrolizumab and nivolumab have not yet shown a discernible increase in overall survival. For HCC patients who did not react to sorafenib, regorafenib was the first authorized treatment in the second-line scenario. An oral multikinase inhibitor called cabotininib, which targets the MET, AXL, RET, FLT3, and VEGF receptors, was also authorized for use in patients who had previously had sorafenib treatment. Furthermore, the FDA expedited the approval of nivolumab and pembrolizumab and nivolumab plus ipilimumab (a CTLA-4 inhibitor) for patients with advanced HCC who had previously been exposed to sorafenib.^{110,111} The FDA-approved was shown in Fig. 3.

A human IgG monoclonal antibody called Ramucirumab targets the extracellular domain of VEGFR-2. It has anticancer effects by preventing VEGFR-2 from binding to its major ligand, VEGF-A, which affects downstream signaling pathways and suppresses endothelial cell migration and proliferation.^{112–118} When compared to a placebo, ramucirumab did not substantially increase overall survival, according to the REACH trial, the first randomized, double-blind, placebo-controlled phase 3 research assessing second-line therapy after sorafenib.¹¹⁹ Ramucirumab had significant effectiveness in individuals with alpha-fetoprotein (AFP) levels higher than 400 ng/

mL, according to a subgroup analysis of the REACH study. In light of these results, the REACH-2 study was carried out to compare ramucirumab with a placebo, particularly in patients who had advanced HCC and AFP levels more than 400 ng/mL after receiving sorafenib therapy.^{22,120} Ramucirumab was adopted as a routine second-line treatment in this subgroup after the REACH-2 study showed that it gave patients with advanced HCC and increased AFP levels a survival benefit over placebo. Uncertainty surrounds the biological basis for the association between better ramucirumab outcomes and higher baseline AFP levels. AFP-high tumors may be more sensitive to VEGFR-2 inhibition because of increased VEGF signaling pathway activation and overexpression of VEGFB and placental growth factor (PGF), according to Robert et al.¹²¹ Increased activation of VEGFR1 and decreased availability of VEGFR1 for VEGFA binding may result from overexpression of VEGFB and PGF in AFP-high tumors. By rerouting VEGFA to preferentially bind VEGFR2, this competitive displacement may improve VEGFR2-mediated signaling. This has led to the hypothesis that ramucirumab may reverse this change in AFP-high HCC by inhibiting VEGFR2, which would disrupt the enhanced VEGFA signaling cascade and provide a potential reason for its effectiveness in this patient subgroup. Ramucirumab is used as a follow-up therapy after other authorized regimens and after sorafenib in clinical settings when numerous systemic treatments are available for advanced HCC. In patients with advanced HCC, there is, however, little data on the clinical effectiveness and results of ramucirumab when administered after systemic treatments other than sorafenib.

FGFR inhibitors

HCC is one of many malignancies that have been shown to have changes in the fibroblast growth factor (FGF)/FGFR signaling axis, which is categorized under class IV receptors. The growth and spread of tumors are facilitated

FDA-approved Drug for Liver cancer

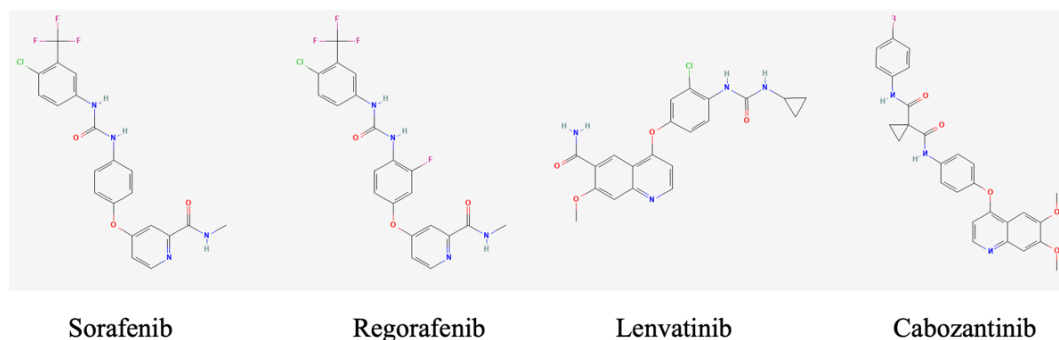


Fig. 3. FDA-approved drugs for liver cancer. This figure presents the chemical structures of four FDA-approved targeted therapies used in the treatment of liver cancer: Sorafenib, Regorafenib, Lenvatinib, and Cabozantinib. These agents are multi-kinase inhibitors that block various signaling pathways involved in tumor growth, angiogenesis, and metastasis. Their approval has significantly advanced systemic therapy options for hepatocellular carcinoma (HCC), offering improved survival outcomes for patients with advanced disease.

by the deregulation of this pathway, which is essential for controlling cell division, proliferation, and survival.^{122,123} In fact, research has shown that at least one FGF8 subfamily member and/or one FGFR are upregulated in around 82% of HCC patients, suggesting that the FGF/FGFR signaling axis is often activated in hepatocarcinogenesis.¹²⁴

While FGFR1 and FGFR2 are seldom elevated, FGFR3 and FGFR4 are the main fibroblast growth factor receptors that are overexpressed in HCC. Notably, for a number of years, FGFR3 isoforms have been suggested as attractive therapeutic targets for HCC, underscoring their potential use in focused therapy approaches.^{125,126} Recently, FGFR4 was shown to represent an oncogenic driving pathway for patients with HCC,¹²⁷ furthermore validated by proteomic analysis.¹²⁸

Along with FGF8, FGF17, and FGF18, FGF2 a soluble ligand that is normally undetectable in nonparenchymal cells or healthy liver tissue is markedly overexpressed in HCC. The genesis and progression of liver cancer are significantly influenced by FGF19 in particular. In HCC, members of the FGF1 subfamily are known to stimulate angiogenesis, invasiveness, and tumor cell proliferation mainly via autocrine signaling. Crucially, chronic liver disease also exhibits FGF1 and FGF2 expression, with greater levels corresponding to more advanced tumor stages.^{129–132} The most researched of the FGFs is FGF2, and FGFR3 seems to have the most role in the development of HCC.^{133,134} As mentioned earlier, an anti-FGF2 diabody has demonstrated promise as a treatment by preventing immunological evasion and metastasis. It showed promise in addressing important mechanisms of HCC progression by suppressing both anti-PD-L1 expression and the epithelial mesenchymal transition (EMT) in hepatoma cells.¹³⁵

The primary binding sites of FGF8, FGF17, and FGF18 within the FGF8 subfamily are FGFR2, FGFR3, and FGFR4, respectively. The tumor progresses as a result of these ligands' active promotion of angiogenesis, malignancy, and cell proliferation in HCC via paracrine signaling.¹²⁴ Interestingly, FGF8 contributes to EGFR inhibitor resistance.¹³⁶

On the other hand, it has been shown that the FGF19–FGFR4–KLB signaling axis plays a major factor in the development and spread of HCC. It is essential for controlling hepatocellular carcinoma cells' motility, invasion, EMT, survival, and proliferation.^{129,137–144}

The main member of the FGF19 subfamily associated with liver cancer is FGF19, which is an endocrine hormone. Although the liver does not normally produce FGF19 under normal physiological settings, cirrhosis, hepatocellular carcinoma, and hepatitis C have all been linked to increased production of this protein.^{129,145} Larger tumor sizes, more advanced disease stages, and early recurrence are also strongly associated with the protein level overexpression of FGF19 in HCC tissues.^{129,146,147}

Many inhibitors that target the FGF/FGFR signaling pathway have been developed because of its crucial role in the development and progression of HCC. These include more recent, more selective FGFR4 inhibitors and early broad-spectrum pan-FGFR inhibitors, some of which are now undergoing Phase I/II clinical studies (NCT04194801 and NCT02508467).

Resistance mechanisms

Primary vs. acquired resistance

Drug resistance in the treatment of HCC is often divided into two categories: acquired and primary. Primary resistance is difficult to overcome since it occurs before treatment is started and is often an inherent feature of the tumor. On the other hand, acquired resistance develops gradually over the course of therapy and is a significant management problem for advanced HCC as it reduces the long-term efficacy of systemic medicines.^{148,149} A focus of this study is the processes behind acquired resistance in HCC. Inhibition of apoptosis, dysregulated proliferation signaling pathways, altered drug transport, epigenetic modifications, changes in the TME, disruptions in drug metabolism, and enhanced DNA repair capacity are some of the contributing factors that have been identified by studies on sorafenib resistance.^{150,151} Nevertheless, little is known about the major processes and molecular targets underlying lenvatinib resistance in HCC. Since lenvatinib was just recently introduced, there are currently no trustworthy indicators to predict therapy response, and no particular drugs have been created to combat resistance. This restriction prevents lenvatinib from reaching its full clinical potential as a systemic treatment for HCC. In order to improve therapy effectiveness, it is essential to look into the underlying processes of lenvatinib resistance and find new therapeutic targets.

Tumor heterogeneity

The presence of several cell subpopulations inside a single tumor or between tumors of the same histological type is referred to as tumor heterogeneity. These cell groupings have different phenotypic characteristics and genetic compositions, which may lead to different biological activities and treatment reactions.^{152–154} Inter-tumoral heterogeneity, which describes variations across tumors from various people or between discrete tumor nodules within the same patient, is one way that tumor heterogeneity might appear at the population level. It may also manifest as intratumoral heterogeneity, in which a single tumor nodule contains a variety of cellular subpopulations.¹⁵⁵ Moreover, etiological heterogeneity refers to variations in cellular and molecular characteristics among HCCs originating from various causes for instance, tumors resulting from hepatitis virus infection may differ significantly from those associated with alcohol consumption. Finally, tumor heterogeneity can also occur over time and space, known as spatiotemporal

heterogeneity, where the tumor's characteristics change before and after treatment or across different tumor regions.^{156,157} HCC is characterized by heterogeneity, which results in a variety of cellular, molecular, functional, and lineage characteristics. It is believed to result from a confluence of environmental factors and patient genetic variation. This variability is caused by a variety of factors, including as epigenetic changes, interactions within the TME, cancer cell development and reprogramming, genomic abnormalities, and the conversion of non-cancerous cells into malignant ones. These elements work together to drive clonal development, which gives the tumor its genetic, molecular, and functional variety.^{158–160} The clinical difficulties of HCC are exacerbated by its complicated and multifactorial development, which also plays a major role in treatment resistance, tumor dormancy, and recurrence after first therapy. This variation makes therapeutic reactions more difficult and emphasizes the need for more individualized and flexible treatment plans.

EMT and cancer stem cell involvement

A network of transcription factors drives the EMT, which starts significant alterations in the structure and function of cells. Cells lose intercellular adhesion, epithelial polarity decreases, the cytoskeleton is rearranged, and basement membranes degrade during EMT and its opposite process, mesenchymal–epithelial transition (MET). EMT-associated transcription factors (EMT-TFs), which control gene expression to support the mesenchymal state, are essential to these changes. Depending on how they interact with elements of the signaling pathway, some EMT-TFs may change from being transcriptional repressors to activators. Mesenchymal–epithelial transition transcription factors (MET-TFs), on the other hand, support the maintenance of epithelial traits in both healthy and malignant cells. By inhibiting mesenchymal gene expression and establishing reciprocal inhibitory feedback loops with EMT-TFs, they preserve the equilibrium between mesenchymal and epithelial states.^{161–165} A number of MET-TFs, including CDH1, ZO-1, and the genes encoding claudin-4 and claudin-5 (CLDN4 and CLDN5), actively stimulate the transcription of genes that produce proteins essential to epithelial lineage determination in addition to their regulatory roles. By directly promoting the expression of the structural and functional elements of epithelial cells, these substances aid in the reinforcement of epithelial identity.^{166,167}

The complexity of the cellular transitions involved in EMT and MET is shown by these coordinated molecular events, underscoring the need of a thorough understanding of these processes in both healthy and pathological settings. EMT is mostly regulated by basic helix-loop-helix (bHLH) proteins including ZEB1 and ZEB2, as well as important transcription factors like Snail1 and Snail2

(also called Slug). Twist is another important participant that plays a major role in the activation and maintenance of the mesenchymal phenotype during EMT.^{168,169} Lymphoid enhancer binding factor 1 (LEF-1) may directly stimulate this member of the T cell factor (TCF) family.¹⁷⁰ In order to suppress the production of genes involved in cell-cell adhesion, such those producing E-cadherin, these transcription factors bind to their promoter regions. One of the main beginning events in the EMT process that propels the loss of epithelial qualities and the acquisition of mesenchymal traits is this targeted transcriptional repression. The exact control of EMT is largely dependent on the Snail family of transcriptional repressors. To inhibit the transcription of the CDH1 gene, Snail1 and Snail2 attach to its promoter region. E-cadherin, a crucial protein essential in maintaining epithelial cell–cell interaction, is encoded by CDH1, and its downregulation is a defining feature in the start of EMT.^{171,172} Breast cancer phenotypes that are prone to metastases have been associated with nuclear accumulation of Snail1 and decreased expression of E-cadherin. This change is a result of increased EMT activity, which raises the invasiveness of the tumor and increases its potential for distant dissemination.¹⁷³ A strong correlation between Snail1 overexpression and the potential for metastasis has been suggested by the discovery that circulating tumor cells from patients with metastatic HCC express Snail1 at levels up to 20 times higher than those seen in patients with nonmetastatic HCC (Table 2).¹⁷³

Emerging molecular targets

Novel RTKs and non-RTKs

c-MET, AXL, EGFR inhibitors

The most common primary liver cancer, HCC, varies significantly in both phenotype and molecular makeup. The critical roles that RTKs and non-RTKs play in promoting carcinogenic signaling pathways in HCC have been brought to light by developments in cancer genetics. Notably, because of their substantial role in tumor growth and potential for clinical intervention, c-MET, AXL, and EGFR have become important therapeutic targets.¹⁸⁴

Hepatocyte growth factor (HGF) receptor c-MET is often overexpressed or aberrantly activated in a portion of HCC patients. Its dysregulation is linked to increased angiogenesis, aggressive tumor features, and treatment resistance. The proliferation, migration, and survival of tumor cells are enhanced by the activation of the HGF/c-MET signaling axis, which sets off downstream pathways including PI3K/AKT and RAS/MAPK.¹⁸⁵ Clinical studies have shown promising outcomes for targeted inhibition of c-MET with small-molecule inhibitors such as tivantinib, cabozantinib, and tepotinib, particularly in patients with elevated c-MET expression. Of them, cabozantinib has been approved to treat advanced HCC in individuals who have already had sorafenib therapy.¹⁸⁶

Table 2. Detailed description of the causes, effectiveness, drawbacks, clinical state, and molecular precision techniques of targeted therapy for HCC

Therapy class	Description	Mechanism of action	Efficacy	Limitations & challenges	Clinical/experimental status	References
TKI – Sorafenib	multikinase inhibitor that targets the pathways involved in tumor growth and angiogenesis.	PDGFR- β , RAF, KIT, RET, FLT3, VEGFR1-3, and others are excluded.	OS improved by 2.8 months during the SHARP trial.	Resistance, little advantage, frequent negative consequences	Approved (1st-line)	174–176
TKI – Lenvatinib	TKI that is multi-targeted and has more action than sorafenib.	VEGFR1-3, FGFR1-4, PDGFR α , KIT, and RET targets	REFLECT trial: superior PFS; not inferior to sorafenib	Lack of biomarkers, hypertension, and selective advantage	Approved (1st-line)	177,178
TKI – Regorafenib	utilized as a second-line therapy; structurally similar to sorafenib.	More extensive kinase inhibition compared to sorafenib	OS improved by 2.8 months after using sorafenib in the RESORCE study.	Only after using sorafenib, toxicity	Approved (2nd-line)	179
TKI – Cabozantinib	broad-spectrum dual MET and VEGFR2 inhibitor.	Inhibits MET, VEGFR2, RET, KIT, TIE2, AXL	Trial CELESTIAL: enhanced OS and PFS	High toxicity and consequences that are not intended	Approved (2nd-line)	180
Anti-VEGFR2 – Ramucirumab	In AFP-high HCC, a monoclonal antibody that blocks VEGFR-2 works well.	inhibits VEGFA-mediated angiogenesis by binding VEGFR-2.	REACH-2 is useful for patients whose AFP is more than 400 ng/mL.	Efficacy restricted to those with elevated AFP	Approved (AFP-high, 2nd-line)	181
ICI + Anti-VEGF – Atezolizumab + Bevacizumab	In advanced HCC, immunotherapy with anti-angiogenic medication is the first-line treatment.	Combination PD-L1 inhibitor and VEGF-A inhibitor	Better OS and PFS than Sorafenib with IMbrave150	Not appropriate for those who are at risk of bleeding	Approved (1st-line)	182
FGFR4 Inhibitors – FGF401, etc.	In HCC, experimental treatments inhibit FGF19–FGFR4 signaling.	targets the FGFR4-KLB-FGF19 axis, which is implicated in metastasis and proliferation.	FGF19-overexpressing HCC shows potential in phase I/II studies.	Resistance, toxicity, and restricted patient selection	Experimental (Phase I/II)	183

As a member of the TAM (TYRO3, AXL, MER) receptor tyrosine kinase family, AXL is essential for immunological escape, metastasis, EMT, and resistance to systemic therapy. Poor clinical outcomes and a reduced response to immune checkpoint medications are associated with elevated AXL expression in HCC. Therapeutic drugs like gilteritinib and bemcentinib are now being studied in conjunction with immunotherapies and targeted treatments to boost antitumor effectiveness and overcome drug resistance in order to counteract these effects.¹⁸⁷

A fraction of HCC patients has EGFR dysregulation, which activates important signaling pathways such PI3K/AKT and RAS/RAF/MEK/ERK, hence promoting tumor development. In order to improve therapeutic outcomes, especially in patient groups defined by biomarkers, current research is investigating combination strategies, such as pairing EGFR blockade with ICIs or anti-angiogenic agents, even though EGFR inhibitors like erlotinib and gefitinib have demonstrated limited efficacy as monotherapies.¹⁸⁸

In conclusion, there is a lot of therapeutic promise for HCC when c-MET, AXL, and EGFR are targeted. However, precision medicine techniques are necessary to properly achieve therapeutic advantages. These include the creation of logical combination therapy targeted at targeting tumor heterogeneity and overcoming resistance

mechanisms, rigorous biomarker validation, and efficient patient classification.^{184,186,188}

Dual/multikinase inhibitors

As of right now, the primary molecularly targeted treatment for advanced HCC is still sorafenib (Nexavar), a multikinase inhibitor that targets RAF serine/threonine kinases, VEGFR1-3, PDGFR β , and elements of the RAS/RAF/MEK/ERK signaling cascade. An important turning point in the systemic treatment of HCC was reached in 2007 when a crucial clinical research revealed its survival advantage.^{104,189} Sorafenib has been the accepted first-line systemic treatment for advanced HCC in individuals with maintained liver function ever since it was approved. However, the development of drug resistance, frequent treatment cessation owing to side effects, and a brief duration of response restrict its therapeutic value. Many attempts over the last ten years to find strong second-line treatments after sorafenib failure or create better first-line substitutes have mostly failed.¹⁹⁰ Regulatory bodies in Europe and Asia authorized lenvatinib (Lenvima), a multikinase inhibitor that targets VEGFR1-3, FGFR1-4, PDGFR α , RET, and KIT, as an alternate first-line treatment for patients with incurable HCC in 2018. The results of a multicenter, open-label, randomized phase 3 study (NCT01761266) that contrasted lenvatinib with sorafenib served as the basis for its approval. According to the trial, lenvatinib had a better safety profile and markedly

increased time to progression (TTP), progression-free survival (PFS), and overall response rate. Nonetheless, lenvatinib's overall survival (OS) was similar to that of sorafenib.¹⁰⁵ A structurally unique multikinase inhibitor, regorafenib (Stivarga) targets a variety of kinases linked to cancer. These include oncogenic receptor tyrosine kinases like KIT, RET, and RAF; stromal kinases like PDGFR β and FGFR; and angiogenic kinases like VEGFR1-3 and TIE-2. Regorafenib's broad range of action enables it to disrupt many pathways implicated in the development, angiogenesis, and progression of tumors,^{191,192} was authorized by the US FDA in April 2017 as a backup treatment for individuals who don't react to sorafenib therapy in the first place.²⁰ Another second-line multikinase inhibitor, cabotininib, targets RET, MET, AXL, KIT, and VEGFR1-3. In January 2019, the US FDA authorized it for use in HCC patients who have already been treated with sorafenib. The findings of a worldwide, randomized, placebo-controlled phase 3 study (NCT01908426) that showed clinical improvement in patients with unresectable HCC who had received one or two previous lines of treatment, including sorafenib, provided support for its approval.²¹ As multikinase inhibitors, sorafenib, regorafenib, lenvatinib, and cabozantinib all work by inhibiting important protein kinases implicated in tumor angiogenesis (VEGFRs), oncogenic signaling (RAS, RAF, KIT, and RET), and metastasis PDGFR. Of them, regorafenib has a more favorable side effect profile and higher cytotoxic efficacy. It is not advised for people who are intolerant to sorafenib, nevertheless, because of its pharmacological resemblance to the drug¹⁹⁰; for cabozantinib, dose reduction is a frequent concern.¹⁹³ Therefore, treating patients with HCC who do not respond to current multikinase inhibitors or who relapse after these treatments is dependent on the development of tailored medicines with new mechanisms of action. These developments may help close existing treatment gaps and enhance results for this difficult patient population.

In contrast to conventional HCC treatments like sorafenib, regorafenib, and lenvatinib, researchers have created a new dual FMS-like tyrosine kinase 3 (FLT3)/Aurora kinase (AURK) multikinase inhibitor, DBPR114 (also known as BPR1K871). DBPR114 specifically targets oncogenic receptor kinases such as FLT3, AURK, KIT, and RET, while other medicines mainly target angiogenic pathways VEGFRs and the RAS/RAF/MEK/ERK cascade. DBPR114 was first created to treat FLT3 internal tandem duplication (ITD)-positive acute myeloid leukemia (AML) and FLT3 wild-type AML. Its target specificity was demonstrated by the strong growth inhibition it showed in FLT3-mutant AML cells and the low activity it displayed in FLT3-negative leukemia cell lines.¹⁹⁴ Ten times less DBPR114 was needed to provide half-maximal growth inhibition (IC₅₀) in FLT3-expressing AML cells than the well-known AURK inhibitors VX680

and barasertib, suggesting that DBPR114 is far more effective at targeting these leukemia cells.¹⁹⁴ In addition to its effectiveness in AML, DBPR114 has shown broad-spectrum anticancer action against a variety of solid tumor types, such as uterine sarcoma and malignancies of the colon, stomach, lung, and pancreas. DBPR114 shown its promise as a multipurpose anticancer drug by drastically reducing tumor volume in preclinical xenograft models, especially in colon and pancreatic malignancies.¹⁹⁴ Through mechanistic studies with HCT-116 colon cancer cells and MV4-11 leukemia cells, it was shown that DBPR114 efficiently regulates intracellular targets FLT3 and AURKA/B. Multinucleated cells accumulated after DBPR114 treatment, indicating a disruption of the mitotic checkpoint, mostly due to suppression of AURKB. This mitotic failure identifies a crucial mechanism via which DBPR114 inhibits cell division.¹⁹⁴ We looked at the possibility of using DBPR114 as a multikinase inhibitor to treat advanced HCC as a result of these results. We used six human HCC cell lines that closely resemble the genetic and histological features of actual HCC tumors in order to evaluate its therapeutic effectiveness. In order to find pharmacodynamic biomarkers that may be used as measures of target engagement and medication response, the effects of DBPR114 were examined at the cellular and molecular levels.¹⁹⁵

HER2 in cholangiocarcinoma

HCC remains the primary focus of targeted therapy research in liver cancers, intrahepatic cholangiocarcinoma (ICC) represents another important entity with distinct molecular features. Among them, HER2 amplification and overexpression have emerged as actionable alterations in a subset of ICC patients. DNA-based sequencing was performed on 283 of the 304 ICC samples that were examined. There were 271 control samples in this cohort that did not have HER2 amplification and 12 samples that did. Single-nucleotide variations, minor insertions or deletions (InDels), frameshift mutations, splice site modifications, and multi-hit mutations were among the mutations that were categorized as either somatic synonymous or non-synonymous. The five genes with the highest frequency of mutations in the HER2-amplified group were TP53 (50%), TERT (42%), ATM (33%), NPM1 (24%), and NF1 (21%). The mutational landscape of the non-HER2 amplified group, on the other hand, was different; the most frequently changed genes were TP53 (34%), KRAS (25%), ARID1A (14%), NPM1 (13%), and PBRM1 (12%).¹⁹⁶ The mutation load was considerably lower in the non-HER2-amplified group than in the HER2-amplified group ($p=0.03854$). Based on the findings of next-generation sequencing, we simultaneously analyzed samples from several HER2 IHC categories. Interestingly, the different HER2 IHC groups did not significantly vary in terms of total mutation load or mutational patterns.¹⁹⁶ Two of the 304 ICC samples that were examined had

mismatch repair (MMR) defects; they were both in the HER2 non-amplified group and both showed contemporaneous loss of MLH1 and PMS2 expression. The HER2-amplified group had a decreased prevalence of MMR changes, according to further analysis of mRNA expression for four MMR proteins and a comparison of mutation status in important MMR-related genes (MLH1, MLH3, MSH3, MSH6, PMS1, and PMS2). Remarkably, only one of the twelve HER2-amplified samples had an MSH6 mutation. Three HER2 point mutations were also found in the 283 analyzed samples by second-generation sequencing: HER2 p.S310F, p.R678Q, and p.Q711H.¹⁹⁶ ERBB2 amplification or overexpression occurs in a modest but meaningful subset of cholangiocarcinoma cases approximately 5 % in intrahepatic CCA and up to ~18–20 % in extrahepatic disease highlighting its potential as a therapeutic vulnerability.¹⁹⁷ Retrospective analyses indicate that responses to HER2-targeted therapies in CCA have generally been limited; for instance, trastuzumab-based treatments showed no objective responses in small cholangiocarcinoma cohorts, despite efficacy in gallbladder cancers (Tables S1, S2).^{198,199} Prospective trials, however, are reshaping this narrative. Dual HER2 blockade with pertuzumab and trastuzumab achieved an objective response rate (ORR) of ~23 % in advanced biliary tract cancers, while the bispecific antibody zanidatamab (Ziihera) produced ORRs of ~40–52 % in later-phase studies with FDA accelerated approval in November 2024 for previously treated HER2-positive biliary tract cancer.^{200,201} Additionally, the antibody–drug conjugate trastuzumab-deruxtecan (T-DXd) achieved ORRs around 36 % and robust disease control in phase II BTC cohorts.¹⁹⁹ In contrast, HER2 alterations are exceedingly rare in HCC, and HER2-targeted therapies have not been a focus in HCC treatment paradigms. Thus, HER2-positive CCA represents a distinct molecular subgroup amenable to precision therapy and underscores the heterogeneity between liver tumor types, with HER2 serving as a clinically impactful target in CCA but not in HCC. In comparison with HCC, where HER2 alterations are rare and not a major therapeutic focus, HER2 amplification in ICC highlights the heterogeneity of liver cancers and the need for subtype-specific targeted approaches. Thus, HER2-positive ICC represents a small but clinically relevant group of patients who may benefit from precision medicine strategies, making HER2 an emerging and distinct therapeutic target in cholangiocarcinoma.

Epigenetic targets

EZH2, HDACs, DNA methyltransferases

Methylation, phosphorylation, ubiquitination, and acetylation are the main conventional HPTMs linked to HCC.^{202,203} Acetylation and methylation are two examples of histone post-translational modifications (HPTMs) that

affect chromatin structure by controlling compaction and influencing the recruitment of transcription factors and chromatin remodeling complexes. HPTMs have direct influence on gene transcription via these methods. In addition to being crucial for the development and spread of tumors, they are also involved in key physiological functions including gene expression, DNA replication, and repair. One of the main epigenetic changes in cancer is the aberrant methylation of CpG islands, which compacts chromatin in promoter areas and causes transcriptional silence of genes.^{204–206} Methyl-binding proteins (MBPs) like MeCP2 are drawn to CpG islands inside promoter regions when methyl groups are added by DNA methyltransferases (DNMTs). By acting as molecular "bridges," these MBPs draw histone methyltransferases (HMTs) and deacetylases (HDACs) to the location. While HMTs add restrictive methylation marks, such as H3K9me3, which cause chromatin condensation, HDACs remove acetyl groups from histones. Gene expression is silenced by this densely packed chromatin structure, which prevents transcription factors from entering.²⁰⁷ For example, when the p16 promoter region is hypermethylated, its gene expression is silenced, which hinders its vital role in controlling the cell cycle. Unchecked cell growth is made easier by this absence of regulation. These epigenetic modifications may serve as early indicators of the development of cancer as they often appear in the early phases of carcinogenesis and may also be seen in precancerous lesions.²⁰⁸ Similarly, the cell's capacity to repair alkylated DNA lesions is reduced when the DNA repair gene MGMT (O6-methylguanine-DNA methyltransferase) is silenced. Because of the increased genomic instability brought on by this decline in DNA repair capabilities, mutations may flourish and cancer can be encouraged.^{209–211}

Treatment for HCC may benefit from therapeutic strategies that target epigenetic changes. By preventing HDAC activity, HDAC inhibitors like panobinostat and belinostat may restore the expression of suppressed tumor suppressor genes, which in turn prevents angiogenesis and cell proliferation in HCC. Furthermore, by inhibiting EZH2 activity, GSK126, a histone methyltransferase inhibitor, lowers the restrictive H3K27me3 histone marks. By encouraging death in tumor cells and boosting immune cells' capacity to cause cytotoxicity, this reactivation of tumor suppressor gene expression improves anticancer responses.^{212–215}

Role of non-coding RNAs (e.g., miRNAs, lncRNAs)

The enzymatic breakdown of double-stranded RNA is essential to the maturation and decay of RNA molecules.^{215,216} MicroRNAs are single-stranded, tiny RNA molecules that are spontaneously produced by living organisms. They consist of around 22 nucleotides.²¹⁷ The miRBase database now contains 1,492 human miRNA sequences. miRNAs are essential for the post-

transcriptional control of gene expression, despite the fact that they do not have coding domains. They have a role in vital biological processes and have been linked to the development and spread of a number of illnesses, including cancer.²¹⁸ Within the nucleus, RNA polymerase II normally transcribes the miRNA gene, producing a main transcript called pri-miRNA. One or more hairpin structures that will subsequently be processed into mature miRNAs are present in this initial transcript, which typically has a length of one to four kilobases.²¹⁹ Monocistronic transcripts are those that express a single mature miRNA due to the presence of a single miRNA gene downstream of a promoter. In contrast, a single parent transcript that encodes many miRNA gene products gathered together is the source of polycistronic transcripts, such the miR-17-92 cluster. This makes it possible for many functionally related miRNAs to express themselves in unison from a single promoter.²²⁰ About half of miRNA genes, sometimes referred to as intragenic miRNAs or mirtrons, are found in the introns of host genes that code for proteins and are believed to be co-regulated with the transcription of their host genes. MiRNAs present in intergenic areas, on the other hand, are probably transcribed separately, indicating that they operate as separate transcriptional units with unique regulatory factors.²²¹ Depending on where miRNAs are located in the genome, the percentage of regulatory alterations varies greatly. The main miRNA transcript (pri-miRNA) is processed into a shorter hairpin shape called precursor miRNA (pre-miRNA) in the nucleus by the microprocessor complex, which is made up of the DiGeorge syndrome critical region gene 8 (DGCR8) protein and the nuclease Drosha. Exportin-5 then carries this pre-miRNA, which is around 70 nucleotides long, to the cytoplasm. The pre-miRNA is converted into a mature miRNA-miRNA duplex of around 18–25 nucleotides by the enzyme Dicer once it is in the cytoplasm. This duplex is then ready to be incorporated into the RNA-induced silencing complex (RISC) to control gene expression.²²²

miRNAs have a dual role in carcinogenesis by interacting with a variety of molecular components in cancer and acting as both oncogenes and tumor suppressors. For instance, it has been shown that miR-429 slows the growth of endometrial cancer. By controlling the expression of DDX53, a gene linked to the advancement of cancer, it partially suppresses tumors and increases tumor cells' susceptibility to anticancer medications.²²³ Overexpression of miR-21 and miR-125b is thought to be a biomarker for ovarian cancer. Furthermore, platinum resistance in ovarian cancer is caused by downregulation of miR-125b.²²⁴ Another factor preventing cervical tumors is miR-1299. Cervical cancer has low expression of miR-1299, which is inhibited by KCNQ1OT1.²²⁵ In thyroid cancer, miR-1284 suppresses tumor growth and spread while promoting apoptosis. By downregulating

N-cadherin, a mesenchymal marker linked to enhanced cell mobility and invasiveness, and upregulating E-cadherin, a crucial epithelial marker that improves cell-cell adhesion, it suppresses the EMT.²²⁶ More crucially, miRNAs may create feedback loops with their targets. For example, in breast tumors, the loop between PAX5 and miR-142 can change the expression levels of ZEB1 and DNMT1.²²⁷ MiR-1290 and miR-29c-3p plasma levels are indicators for lung cancer.²²⁸ Additionally, miR-629 inhibits LATS2 to promote prostate tumor development.²²⁹ Because exosomes may transport miRNAs, they can also control the expression of miRNAs in human malignancies.²³⁰ MiR-1180's downregulation of FXR5 may prevent pancreatic tumor migration and metastasis.²³¹

In human malignancies, long non-coding RNAs (lncRNAs) interact with a variety of molecular targets and impact important cellular functions. For example, it has been shown that lncRNA LITATS1 inhibits the EMT and encourages the breakdown of T β RI (TGF- β receptor I), which lowers the plasticity of cancer cells and restricts their ability to spread.²³² Another element that has been shown to slow the growth of breast cancer is lncRNA MIR17HG, which increases FAM135A levels by sponging miR-454-3p.²³³ Like miRNAs, lncRNAs are primary regulators of miRNAs in cancer and may be concentrated in exosomes.²³⁴ LINC01614 modulates the SLC31A1 gene, which has been implicated as a regulator of cuproptosis in breast tumors.²³⁵ An adverse prognosis is mediated by the increased expression of HOTAIR in breast tumors, which interacts with miR-129-5p.²³⁶ Thus, in human malignancies, including ovarian tumors, lncRNAs interact with a number of biological pathways.^{237,238}

Metabolic pathways

Glutamine metabolism

A nonessential amino acid called glutamine is essential to the metabolism of HCC. It supports the fast development and multiplication of tumor cells by acting as a nitrogen donor for the production of nucleotides and amino acids and as a vital carbon source for anaplerosis, which replenishes intermediates in the tricarboxylic acid cycle.^{239–241} It also makes a substantial contribution to other biosynthetic processes, such as lipid synthesis and the creation of anti-ROS glutathione/NADPH.²⁴² Notably, the overexpression of important transporters and enzymes that promote greater glutamine uptake and use is indicative of dysregulated glutamine metabolism in HCC. Overexpression of transporters like SLC38A1 and SLC1A5 (also called ASCT2) encourages the import of glutamine into cancer cells. Furthermore, increased amounts of glutaminase isoforms GLS1 and GLS2 contribute to the TCA cycle by promoting the conversion of glutamine into glutamate, which is then converted into alpha-ketoglutarate (α -KG).^{243,244} Compared to GLS2, the isoform that is mostly expressed in normal hepatocytes,

GLS1 overexpression is especially noticeable in HCC. Glutaminases start the two-step deamination process that turns glutamine into α -KG. This metabolic change in glutaminolysis, which is often brought on by MYC activation, is linked to the development of tumors and has significant effects on patient outcomes, underscoring its potential as a target for therapy.^{245,246}

The Wnt/ β -catenin signaling system controls glutamine synthetase (GS), an essential enzyme in glutamine metabolism. Both a potential biomarker and a therapeutic target, elevated GS expression is connected to increased cell proliferation in HCC and has been related to a variety of prognostic outcomes, depending on the tumor environment.^{247,248} In fact, new research points to a multifaceted function for GS in HCC, perhaps connected to tumor behavior and cellular differentiation.^{249–251} Additionally, a number of downstream signaling pathways, most notably mTORC1, are impacted by the dysregulation of glutamine metabolism^{252,253} and mTORC2–AKT–C-MYC.²⁵⁴ The overexpression of GS and other changes in glutamine metabolism have a major impact on cellular bioenergetics and are linked to carcinogenic processes in HCC. These metabolic pathways are further modulated by the HGF signaling axis, underscoring the intricate interaction between growth factor signaling and metabolic reprogramming that propels the development of cancer.²⁵⁵

Lipid metabolism and fatty acid synthase

Stearoyl-CoA desaturase (SCD), fatty acid synthase (FASN), and arboxylase (ACC) are all changed in HCC.^{256,257} FASN, for instance, has been connected to the development of tumors in HCC. It has been shown that FASN loss postpones the formation of tumors, a phenomenon linked to elevated sterol regulatory element-binding protein (SREBP) activity. Genes involved in cholesterol production and de novo lipogenesis (DNL) are upregulated as a result of this increased SREBP activity, indicating a compensatory metabolic response that might affect tumor behavior.²⁵⁸ It has been shown that by inhibiting the NAD⁺/Sirtuin 1 (SIRT1) signaling pathway, mitochondrial fission activation in HCC cells increases the acetylation of sterol regulatory element-binding protein 1 (SREBP1). Key enzymes involved in DNL, including as FASN, ACC, and elongation of very long chain fatty acid protein 6 (ELOVL6), are upregulated as a result of this increased acetylation, which stabilizes and activates SREBP1. These alterations promote the development and spread of tumors by causing lipid buildup and metabolic reprogramming.²⁵⁹ The USP7/ZNF638 axis, which is linked to ubiquitination, selectively increases SREBP1c cleavage and activation as HCC advances. Important lipogenic enzymes such ACC, FASN, and SCD are upregulated as a result of this activation, which supports tumor growth and encourages lipid production.²⁶⁰ Notably, fatty acid synthesis-related long non-coding RNA (FASRL),

which is generated by upstream stimulatory factor 1 (USF1), interacts with ACC. In the end, this binding exacerbates the development of HCC by increasing fatty acid production and encouraging lipid buildup.²⁶¹ Furthermore, it has been shown that inhibiting ubiquitin-specific protease 22 (USP22) lowers HCC growth and suppresses de novo fatty acid production. ACC and ATP citrate lyase (ACLY), two important lipogenic enzymes, are downregulated as a result of the expression of peroxisome proliferator-activated receptor (PPAR), a transcription factor that controls lipid metabolism.²⁶² In HCC, ACLY, a strong modulator of Wnt/ β -catenin signaling, is also crucial for controlling the stemness and metastasis of liver tumor-initiating cells.²⁶³ According to lipidomic investigations, stearoyl-CoA desaturase 1 (SCD1) mediates the alterations in the lipid composition of HCC cells in response to matrix stiffness. Membrane fluidity is impacted by the ratio of monounsaturated fatty acids (MUFA) to saturated fatty acids (SFA), which is influenced by SCD1 expression. Increased cellular motility is made possible by this change in membrane characteristics, which also increases the invasive and metastatic potential of HCC cells.²⁶⁴

Hypoxia and HIF-1 α signaling

The activation of pro-tumor signaling pathways and oncogenes is closely linked to the overexpression of important glycolytic enzymes in HCC cells. In HCC, hypoxia-inducible factor 1 α (HIF-1 α) plays a key role in controlling glycolysis, especially in hypoxic environments. It promotes greater glucose absorption and glycolytic flux to support tumor growth and survival by increasing the transcription of many glycolytic enzymes, most notably glucose transporter 1 (GLUT1) and hexokinase 2 (HK2).^{265,266} One important transcription factor that helps cells adjust to hypoxic environments is HIF-1 α , which is also crucial for controlling glycolysis in TAMs. Research has shown that pyruvate kinase M2 (PKM2) is phosphorylated during lipopolysaccharide (LPS) stimulation, which promotes the development of a nuclear PKM2/HIF-1 α complex. In the HCC microenvironment, this complex interacts to the HIF-1 α promoter, increasing its expression and boosting glycolytic activity in TAMs.²⁶⁷ It is essential to block the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) signaling pathway since it is involved in the development of tumors and the advancement of cancer.²⁶⁸ By controlling GLUT4 and HK2, the PI3K/Akt/mTOR signaling pathway, which is important for tumor growth, has also been linked to encouraging glycolysis in HCC cells.^{269,270}

DNA damage response (DDR) targets

PARP inhibitors

DNA repair, damage tolerance, and cell-cycle checkpoint control are among the several processes that make up the DNA damage response (DDR) system. In the end,

this complex mechanism promotes cell survival by ensuring precise DNA replication and cell proliferation. The DDR pathway is essential for maintaining genomic integrity because it fixes different types of DNA damage. Chromosome fragmentation and gene loss may result from strand breaks brought on by nucleotide changes, single-strand breaks (SSBs), or double-strand breaks (DSBs). Reactive oxygen species (ROS), ionizing radiation, mechanical stress, and broken DNA replication forks are the main causes of DNA double-strand breaks.²⁷¹ The enzymes of the poly (ADP-ribose) polymerase (PARP) family, particularly PARP-1 and PARP-2, are crucial elements of the DDR. Their main responsibility is to identify SSBs and start the repair process. The recruitment and assembly of DNA repair complexes is facilitated by PARP enzymes, which swiftly relocate to the break sites and catalyze the attachment of ADP-ribose polymers to other proteins and themselves.

PARP-1 is essential for both the idea of synthetic lethality and the repair of DNA damage. DNA lesions like DSBs and SSBs trigger different repair pathways. DSBs are repaired by homologous recombination repair (HRR), while SSBs are repaired by base excision repair (BER). Cells turn to non-homologous end joining (NHEJ), an error-prone repair pathway that often causes genomic instability and cell death, when both BER and HRR are impaired, a state known as BRCAness. DNA-PKcs for the backup alternative end joining (ALT-EJ) and conventional NHEJ (C-NHEJ) pathways are important proteins in these processes. Targeting PARP-1, PARP inhibitors (PARPi) take advantage of flaws in cancers with low HR. PARP-1's activity depends on its functional domains, including the DNA binding domain (DBD) and automodification domain (AD). ROS, DNA repair proteins such as XRCC1, PALB2 (a BRCA2 partner), and RAD51 recombinase are additional significant players. Enzymes like TIP60, ATM, ATR, and checkpoint kinase 1 (CHEK1) also control cell cycle checkpoints and DNA damage signaling. While less precise than HRR, single strand annealing (SSA) also aids in DSB repair. Collectively, these mechanisms preserve the integrity of the genome, and their malfunction may be used therapeutically to create synthetic lethality, which kills cancer cells.²⁷²

ATR/CHK1 inhibitors in combination with TKIs

Significant heterogeneity and genetic instability are hallmarks of HCC, which often develops in the context of viral hepatitis infections, chronic inflammatory conditions, or liver metabolic dysfunctions. Because of the increased oxidative stress and ongoing DNA damage caused by these pathological circumstances, cancer cells are forced to rely significantly on DDR mechanisms in order to survive. The ATR (ataxia telangiectasia and Rad3-related) and CHK1 (checkpoint kinase 1) signaling axis are essential for these responses because they reduce replication stress, protect genomic integrity, and coordinate cell cycle checkpoints

and DNA repair mechanisms. This increased dependence on ATR/CHK1 signaling is a major therapeutic weakness in HCC, especially in tumors with TP53 mutations. In order to sustain survival in the face of genomic insults, these mutations impair the G1/S checkpoint, causing the cancer cells to primarily depend on the ATR and CHK1 pathways to regulate cell cycle progression through the S and G2/M phases.²⁷³ Critical cell cycle checkpoints are disrupted by inhibitors that target ATR and CHK1, which causes unrepaired DNA damage to accumulate, replication forks to collapse, and eventually apoptosis. However, the intrinsic variety of malignancies often limits their efficacy as stand-alone therapy. As a treatment strategy, combining ATR or CHK1 inhibitors with TKIs as lenvatinib or sorafenib has showed potential. TKIs increase replication stress and DNA damage while suppressing angiogenesis and signaling pathways that are critical for tumor development, such as RAF/MEK/ERK and PI3K/AKT. Increased cytotoxicity and tumor cell death are the results of this combination's dual attack on tumor cells, which overwhelms their DNA repair systems.^{274,275}

TKIs and ATR inhibitors, such as berzosertib, have been shown in preclinical research to work in concert, as shown by increased levels of DNA damage markers like γ H2AX, decreased cellular proliferation, and improved tumor growth suppression. Additionally, it has been shown that prexasertib and other inhibitors that target CHK1 enhance the therapeutic benefits of TKIs, particularly in hepatocellular carcinoma models that lack functional p53.²⁷⁶ This strategy concurrently targets several oncogenic stress pathways, offering a viable path toward the development of more potent, biomarker-guided treatments for HCC. To evaluate the safety, tolerability, and effectiveness of various combination medicines, clinical research is now being conducted (Table 3).²⁷⁷

Combination strategies

Targeted therapy + immunotherapy

In order to overcome the drawbacks of single-agent treatments and improve patient outcomes, the combination of targeted therapy and immunotherapy has emerged as a novel approach to the treatment of HCC. Notably, one of the most successful strategies is the combination of TKIs and ICIs, which has shown promising outcomes in both clinical trials and laboratory studies.^{301,302} By suppressing pathways including VEGFR, PDGFR, and other growth factors, TKIs, such as lenvatinib and sorafenib, mainly prevent tumor development by preventing angiogenesis and cell proliferation. TKIs have important immunomodulatory effects in addition to their direct anti-cancer activities. They aid in the normalization of the aberrant blood vessels in the TME, which promotes increased immune cell infiltration, especially cytotoxic CD8⁺ T cells, while

Table 3. Describe the causes, benefits, difficulties, and current clinical status of new molecular targets and treatment drugs in HCC

Target / Pathway	Therapeutic agents	Description	Mechanism	Advantages	Challenges	Clinical/ experimental status	Ref.
c-MET	Tivantinib, Cabozantinib, Tepotinib	blocks angiogenesis and tumor development by targeting HGF/c-MET signaling.	inhibits MAPK and PI3K/AKT via c-MET	Cabotanzantinib is an authorized drug that is effective in c-MET-high malignancies.	Patient stratification is necessary because to resistance.	Others are in clinical studies; cabotanzantinib has been authorized.	278,279
AXL	Bemcentinib, Gilteritinib	targets the AXL receptor to prevent EMT and immune escape.	inhibits signals mediated by TAM receptors	Possible cooperation with immunotherapies	Limited as a monotherapy; requires confirmation of biomarkers	Clinical trials ongoing	280,281
EGFR	Erlotinib, Gefitinib	inhibits the proliferation and survival signals triggered by EGFR.	suppresses PI3K/AKT, RAS/RAF/MEK/ERK	Combination tactics seem promising.	Ineffective monotherapy in individuals who were not chosen	Investigative in combination regimens	282,283
HER2 (ICC)	Trastuzumab (HER2 + ICC)	targets the subpopulation of HER2-amplified cholangiocarcinoma.	suppresses HER2 signaling	Individualized treatment for HER2 + ICC	Low rate of HER2 amplification; potential for resistance	Investigative; ICC HER2 screening	284
Multikinase Inhibitors	Sorafenib, Lenvatinib, Regorafenib, Cabozantinib	Block many kinases involved in metastasis, proliferation, and angiogenesis.	Inhibits VEGFRs, FGFRs, PDGFR, KIT, RET	Several approvals and a wider range of activities	adverse effects and agent cross-toxicity	All approved for HCC	285,286
FLT3/AURK (DBPR114)	DBPR114	A novel dual inhibitor created for AML is now being evaluated for solid tumors, such as HCC.	Inhibits FLT3, AURKA/B, KIT, RET	Strong preclinical activity and a distinct mechanism	Still in the early stages of development	Preclinical validation in HCC	287
EZH2	GSK126	restores tumor suppressor genes that have been silenced by inhibiting histone methylation.	targets H3K27me3 via inhibiting EZH2.	stimulates the immunological system and apoptosis	Effects that are not intended; intricacy of epigenetics	Preclinical and early clinical studies	288
HDACs	Panobinostat, Belinostat	HDAC inhibition prevents angiogenesis and reverses gene silencing.	reverses the deacetylation of histones	inhibits growth and increases immunological destruction	Cytotoxicity in healthy tissues; wide-ranging impact	Clinical stage	289,290
DNMTs	Decitabine, Azacitidine	inhibits DNA methylation to reactivate tumor suppressor genes that have been silenced.	Prevents DNMT-induced methylation of CpG	enhances the regulation of genomic expression	Systemic toxicity; requires the use of combinations	Clinical trials in solid tumors	291
miRNAs / lncRNAs	miR-21, miR-125b, HOTAIR (targeted via antisense approaches)	Drug resistance, apoptosis, metastasis, and gene expression are all influenced by non-coding RNAs.	mRNA post-transcriptional control	Antisense oligo targets; possibility for diagnosis	Delivery difficulties and issues with specificity	Experimental	292,293
Glutamine Metabolism	GLS1/2 inhibitors, GS modulators	Target the glutamine metabolism-dependent biosynthesis and tumor energy pathways.	inhibits the production of glutathione and α -KG	targets HCC's metabolic susceptibility	Typical tissue damage; metabolic adjustment	Preclinical models	294,295
Lipid Metabolism	FASN, ACC, SCD inhibitors	blocks the fatty acid production pathways that are essential for the development and spread of tumors.	interferes with membrane lipid remodeling and DNL	slows the growth of tumors and reverses EMT	Adaptation to tumors and off-target metabolic changes	Investigational drugs	256
HIF-1 α / Hypoxia	PX-478, Digoxin	interferes with glycolysis and signaling brought on by hypoxia in the tumor microenvironment	prevents the induction of GLUT1, HK2, and VEGF	decreases glycolysis and angiogenesis	compensating for hypoxia; limited opportunity for treatment	Early trials ongoing	296,297
PARP (DDR)	Olaparib, Niraparib	prevents PARP enzymes from repairing DNA in cancers with BRCA mutations or HR deficiencies.	creates artificial lethality	Effective in cancers that have "BRCAness" or HR-deficient	Variable DDR state in HCC	Early clinical trials	298,299
ATR / CHK1 (DDR)	Berzosertib, Prexasertib ($\pm$ TKIs)	blocks replication checkpoints, which increases DNA damage, particularly in p53-mutant HCC.	Replication stress response inhibition	TKI-synergistic; useful in high-genomic instability the HCC	Normal cell toxicity and the creation of biomarkers	Preclinical and clinical studies	300

also reducing the immunosuppressive environment produced by TAMs, Tregs, and MDSCs.^{303,304} ICIs, which include anti-PD-1 medications like pembrolizumab and nivolumab and anti-PD-L1 medications like atezolizumab, work by reactivating worn-out T cells and enhancing the body's immune response against tumors. However, the immunosuppressive TME feature of HCC often reduces their efficacy as monotherapies. TKIs and ICIs work together to improve immune cell infiltration and decrease immunosuppressive factors in the TME, which strengthens and prolongs the immune response against cancer cells.³⁰⁵ Fig. 4 presents the timeline of FDA-approved drugs and monoclonal antibodies for the treatment of HCC.

The effectiveness of this synergistic treatment approach is shown by the key IMbrave150 study. In patients with incurable HCC, the combination of bevacizumab (an anti-VEGF drug) and atezolizumab (an anti-PD-L1 antibody) produced significant survival benefits over sorafenib monotherapy in this phase III research. The combination raised the objective response rate (ORR), considerably extended the median OS and PFS, and finally established a new standard of therapy for this patient group.³⁰⁶ Clinical studies like LEAP-002 are presently evaluating other combination treatments, such as lenvatinib with pembrolizumab. According to preliminary results, this regimen may increase treatment efficacy by taking advantage of lenvatinib's ability to reduce VEGF-driven immunosuppression. It also promotes dendritic cell maturation and improves antigen presentation, which increases the immune system's capacity to target tumors. In summary, ICIs and targeted treatments work in concert to treat HCC by reviving the immune system and restoring normal blood vessel function. Treatment techniques for advanced HCC have changed as a result of

this combination approach, offering new hope for long-lasting therapeutic results and improved survival.^{307,308} To get the most benefit, current clinical studies are concentrating on enhancing patient selection and optimizing biomarker-based strategies.^{309–312}

Targeted therapy + locoregional therapy

Due in large part to the intricacy of the tumor microenvironment and the limited efficacy of single-modality methods in later stages, the combination of targeted therapies and locoregional treatments has become more popular in the management of HCC. For patients with intermediate to advanced HCC, locoregional treatments such as radiation and transarterial chemoembolization (TACE) are common; nevertheless, tumor-induced hypoxia, which stimulates angiogenesis, often reduces their efficacy. TKIs and mTOR/VEGF pathway inhibitors in particular are examples of targeted medicines that have complementing activities that may increase and prolong the therapeutic advantages of these localized treatments.^{313–315}

TACE + TKIs

By administering chemotherapy directly to the tumor and cutting off its blood supply, TACE produces ischemic tumor necrosis. Tumor recurrence may result from the hypoxic circumstances that accompany TACE, which cause an increase in pro-angiogenic factors like VEGF. In order to effectively combat this compensatory angiogenesis, TKIs, such as sorafenib, lenvatinib, and apatinib, target VEGFR, PDGFR, and other survival pathways.³¹⁶ To assess its effectiveness, this therapy combination has been investigated in a number of clinical studies, such as SPACE and TACTICS.³⁰⁵ TKI administration must be timed and sequenced precisely to optimize therapeutic efficacy, as the TACTICS study showed that starting

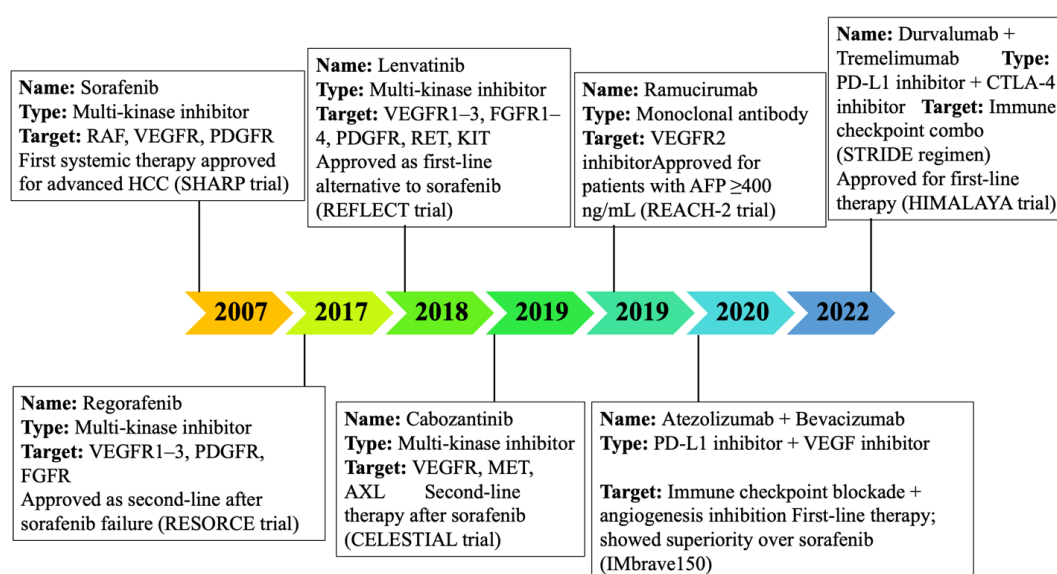


Fig. 4. Timeline overview of targeted therapies and immunotherapy combinations approved for advanced hepatocellular carcinoma (HCC).

sorafenib therapy prior to TACE significantly improved PFS.³¹⁷ Toxicology is still a problem despite encouraging outcomes, particularly hepatotoxicity and hand-foot skin responses.³¹⁸

Radiotherapy + mTOR/VEGF inhibitors

Unresectable HCC is increasingly being treated with external beam radiation treatment, particularly stereotactic body radiation therapy (SBRT). Although radiation may trigger anti-tumor immune responses and damage DNA, its efficacy is often limited by the existence of hypoxic areas within the tumor and innate tumor resistance mechanisms.³¹⁹ By disrupting the development of the cell cycle and protein synthesis, medications such as everolimus that inhibit the mTOR pathway may make tumor cells more sensitive to radiation. Similarly, VEGF inhibitors like bevacizumab aid in the normalization of tumor blood vessels, which enhances oxygen supply and, in turn, the tumor's radiation sensitivity. These combination treatments may improve overall survival outcomes and increase local tumor control, according to preclinical studies and early-phase clinical trials.^{320,321} All things considered, combining targeted medicines with locoregional therapy in HCC is a promising approach; nonetheless, successful clinical outcomes depend on patient classification, proper sequencing, and toxicity control.

Synthetic lethality and rational combinations

In cancer treatment, synthetic lethality a tactic in which the simultaneous disruption of two genes or pathways causes cell death whereas the damage of one gene or route alone is not lethal has become well-known.³²² Utilizing synthetic lethality offers a rational foundation for developing effective combination medicines, especially for patient subgroups identified by biomarkers, in HCC, which is characterized by genetic variation and treatment resistance.³²³ A well-studied example focuses on the Wnt/ β -catenin signaling system, which is abnormally triggered in around 30–40% of instances of HCC because of mutations in genes like AXIN1 or CTNNB1 (which encode β -catenin). These genetic changes provide serious treatment problems because they encourage immunological exclusion and lead to resistance to ICIs. By combining Wnt pathway inhibitors with additional targeted medicines or ICIs, synthetic lethality strategies seek to overcome this resistance. Particularly, in preclinical models of β -catenin-driven HCC, PORCN inhibitors, which prevent the production of Wnt ligands, and tankyrase inhibitors, which encourage β -catenin disintegration, have shown encouraging outcomes. Additionally, combining Wnt inhibitors with ICIs may change the immune milieu around the tumor, reviving immune surveillance in otherwise immunologically "cold" tumors.³²⁴ Several additional strategic combinations that take advantage of weaknesses resulting from DNA

damage repair (DDR) errors, epigenetic modifications, or connections between oncogenic signaling pathways are being studied in addition to targeting the Wnt pathway. PARP inhibitors, for example, may be used to specifically target hepatocellular carcinomas with homologous recombination repair deficits, such as those with BRCA mutations. The therapeutic effect is further enhanced when ATR or CHK1 inhibitors are added. Treatment regimens that combine particular inhibitors of these targets with anti-angiogenic drugs may also be beneficial for tumors displaying FGF19 amplification or MET pathway activation. The goal is to use synthetic lethality by concurrently inhibiting compensatory survival pathways.³²⁵ The use of biomarker-driven patient selection is a crucial component that makes these synthetic lethality-based strategies successful. Comprehensive molecular profiling is essential to identify individuals with actionable genetic changes or particular pathway dependencies in order to successfully transfer these tactics into therapeutic benefit. In order to classify patients and adjust treatments appropriately, methods like liquid biopsies, next-generation sequencing (NGS), and transcriptome profiling are being used more and more. For example, people with TP53 mutations or deficiencies in DDR pathways may be good candidates for therapies that target DNA repair mechanisms, whereas those with β -catenin mutations may react well to Wnt pathway inhibitors.³²⁶ Synthetic lethality has a lot of promise, but there are still a number of obstacles to overcome, including intratumoral heterogeneity, resistance that develops over time, and a lack of thorough clinical evidence on the toxicity and safety of combination treatments. However, the combination of synthetic lethality principles, accurate biomarker identification, and careful therapeutic design is gradually changing the way HCC is treated, opening the door to more accurate and successful oncology approaches in this historically challenging cancer (Table 4).³²⁷

Biomarkers and precision medicine

Precision medicine that is individualized for each patient is replacing traditional, one-size-fits-all methods to treating HCC as a result of the discovery of biomarkers. Generally speaking, biomarkers may be divided into two groups: prognostic indicators, which provide information on the overall course of the illness regardless of treatment, and predictive markers, which indicate the likelihood of responding to a specific therapy. Finding reliable biomarkers is particularly important for improving patient care because of the significant heterogeneity of HCC as well as issues including impaired liver function and common treatment resistance.³²³ Techniques for liquid biopsies, particularly the measurement of circulating tumor DNA (ctDNA), have emerged as useful non-invasive ways to evaluate tumor activity in real time. ctDNA records genetic and epigenetic alterations

in HCC, including mutations in the TERT promoter, TP53, and CTNNB1, which can guide therapy decisions. Since ctDNA allows for more frequent sampling than traditional tissue biopsies, it is especially helpful for tracking the efficacy of treatments and identifying the emergence of resistance over time.³²⁴ Because it provides comprehensive information on mutations, gene fusions, and copy number variations, tissue-based genomic profiling remains a fundamental component in cancer diagnoses. The use of comprehensive genomic panels that can identify actionable changes, like FGF19 amplification, MET overexpression, or deficiencies in DDR, is made easier by modern integrated platforms. This aids in the creation of individualized treatment regimens that are specific to the tumor biology of each patient.³³⁷ HCC is rapidly being classified into several molecular subtypes using transcriptomic profiling, each of which has its own particular immunological and metabolic characteristics. In contrast to "CTNNB1-enriched" cancers, which often show resistance to immunotherapy, tumors categorized as belonging to the "immune class," which have inflammatory gene signatures, typically react positively to ICIs. More accurate sequencing and therapy combination customization are made possible by this molecular stratification. Utilizing multi-omics and clinical information, recent developments in artificial intelligence (AI) and machine learning (ML) are being incorporated into clinical practice to reveal intricate patterns. AI-powered models can predict treatment outcomes, help find new biomarkers, and stratify patients based on risk. These technologies have the potential to improve prognosis accuracy, provide dynamic, adaptive treatment options for HCC, and improve early detection.³³⁸ Notwithstanding significant progress, a

number of obstacles still exist, including the need for standardized biomarker testing procedures, securing regulatory clearances, and guaranteeing fair access to new technologies. However, by enabling individualized, biology-driven treatment approaches, biomarker-guided precision medicine has great promise to improve outcomes in HCC with further innovation and thorough validation (Table S3).³²³

Challenges in targeted therapy

Despite substantial advancements, the clinical efficacy of targeted treatments for HCC is still limited by major hurdles. Tumor heterogeneity is a major obstacle. It may be seen inside a single tumor, where many genetically different subclones coexist, as well as across tumors, which are molded by different underlying causes such as alcohol-related liver disease, viral hepatitis, or non-alcoholic steatohepatitis (NASH). Despite early encouraging results, this clonal diversity contributes to treatment resistance and illness recurrence.^{339,340} One of the biggest challenges in treating HCC is drug resistance, which may be innate or develop with time. TKI effects are often circumvented by tumor cells via the activation of alternative signaling pathways such as PI3K/AKT, RAS/MAPK, or FGFR. Furthermore, resistance mechanisms are influenced by elements found in the tumor microenvironment, including hypoxia, immunological suppression, and increased angiogenesis. Additionally, prolonged treatment may cause EMT, which makes treatment more difficult and increases the tumor's capacity to spread.^{341,342} The absence of proven predictive biomarkers for HCC is a serious obstacle to reaching treatment precision. Patients run the danger of obtaining inefficient medicines that may potentially cause needless toxicity if reliable

Table 4. Comprehensive review of combination approaches in HCC treatment, emphasizing the underlying clinical trial data, therapeutic advantages, difficulties, and processes

Combination strategy	Mechanism/Description	Advantages	Challenges	Clinical evidence	References
Targeted Therapy + Immunotherapy	mixes ICIs with TKIs to encourage vascular normalization, lower immunosuppression, and boost T cell infiltration.	Synergistic immune activation, improved response rates and survival, and a new standard of therapy for advanced HCC.	Predictive biomarker identification, immune-related adverse events, and toxicity control.	LEAP-002, IMbrave150, and continuing studies (such as lenvatinib with pembrolizumab)	282,328
Targeted Therapy + TACE	TACE causes ischemia necrosis; after TACE, TKIs inhibit VEGF-mediated rebound angiogenesis.	Prolonged PFS; effective in intermediate-stage HCC; inhibits post-TACE angiogenesis.	Risk of hepatotoxicity and HFS responses; timing and sequencing are crucial.	Current regional TACE-TKI trials, TACTICS, and SPACE	329–331
Targeted Therapy + Radiotherapy	Tumor DNA is damaged by radiation; mTOR/VEGF inhibitors promote oxygenation, which increases radiation sensitivity and the immune response.	enhanced local control, radiation sensitivity, and a combined effect on the immune system and tumor burden.	Immune suppression, cumulative toxicity, and the ideal dose/radiation schedule.	Early-stage trials; SBRT/VEGF preclinical research	332,333
Synthetic Lethality-Based Combinations	utilizes synergistic drug pairings to selectively kill tumor cells by taking advantage of tumor-specific weaknesses (such as Wnt/ β -catenin and DDR mutations).	Preclinical effectiveness in resistant and immune-excluded cancers is promising, as is precise targeting of mutation-defined subgroups.	toxicity profiles, inadequate clinical trial data, tumor heterogeneity, and biomarker validation.	Preclinical research; early-stage trials based on biomarkers (e.g., PARPi + ATRi, PORCN + ICIs)	334–336

genomic, transcriptomic, or proteomic indicators are not available to guide treatment decisions. This might eventually compromise the patients' quality of life as well as the overall value of the therapy. Considering that many patients with HCC also have underlying liver cirrhosis, toxicity is still a major issue. Systemic side effects, such as tiredness, hypertension, hemorrhage, hepatotoxicity, and gastrointestinal problems, are linked to medications such as TKIs and mTOR/VEGF inhibitors. These side effects sometimes call for dosage adjustments or even stopping treatment.^{343,344} The high expenses of targeted medicines provide significant socioeconomic obstacles to access, especially in settings with limited resources. In the absence of accurate biomarker-guided patient selection, the cost burden of some therapies may be difficult to justify, since they only provide minor gains in survival. Furthermore, preclinical model limitations still make it difficult to successfully translate treatments.^{345,346} The intricate human liver environment, including its architecture, fibrosis, immunological interactions, and tumor heterogeneity, is not well simulated by traditional two-dimensional cell cultures and mouse xenograft models. While more accurate representations are provided by sophisticated systems, such as humanized mouse models, three-dimensional co-culture models, and patient-derived organoids, these platforms still need standardization and broader usage to reach their full potential in drug discovery.^{159,347} A comprehensive research strategy that incorporates integrative investigations, real-time molecular profiling, and adaptable clinical trial designs is needed to address these intricate problems. In order to develop tailored medicines for HCC that are not only efficient but also available and fair to all patients, such tactics are crucial.³⁴⁸⁻³⁴⁹

Future directions and innovative technologies

mRNA-based therapeutics and gene editing

Therapies based on messenger RNA (mRNA) are showing promise as a novel approach to the treatment of HCC. These systems use liver-optimized lipid nanoparticles (LNPs) to transport immune-activating cytokines or tumor suppressor genes, such as p53 and PTEN, straight into cancer cells.³⁵⁰ This technique lowers the danger of insertional mutagenesis since mRNA does not integrate into the host genome. Concurrently, developments in CRISPR-Cas9 gene editing are making it possible to precisely fix oncogenic mutations, such as those seen in patient-derived HCC models in the TP53, CTNNB1, and TERT promoter genes.^{319,351} Modern gene-editing tools, such as base editors and prime editors, allow for accurate single-base changes without breaking double-strand DNA. Notwithstanding these advances, there are still significant obstacles that need to be addressed in order to effectively convert these strategies into therapeutic treatments, including obtaining targeted and effective delivery to hepatocytes, reducing side effects, and

preventing innate immune system activation.^{352,353}

Targeted protein degradation

Molecular glues and proteolysis targeting chimeras (PROTACs) are novel therapeutic strategies for focusing on proteins in HCC that have hitherto been regarded as "undruggable." Critical oncogenic drivers including β -catenin, c-MYC, and YAP/TAZ are preferentially degraded by these molecules via the ubiquitin-proteasome system.³⁵⁴ PROTACs enable the permanent removal of disease-causing proteins, in contrast to conventional inhibitors that just stop protein function. This may lessen the chance of resistance developing and need a lower dose of medication.^{355,356} In preclinical research, next-generation liver-targeted PROTACs have shown encouraging pharmacokinetic characteristics and significantly reduced systemic toxicity.³⁵⁷ These PROTACs are engineered to effectively pass through the hepatic sinusoidal endothelium and specifically break down tumor-specific proteins. To optimize clinical potential, current efforts concentrate on boosting therapeutic efficacy, improving oral bioavailability, and improving tissue selectivity.³⁵⁸

Nanotechnology in targeted drug delivery

The liver's distinct vascular anatomy and receptor-mediated endocytosis processes are being exploited by sophisticated smart nanoparticles.³⁵⁹ Highly selective tumor uptake is accomplished by functionalizing these nanocarriers with ligands that target receptors such as transferrin receptors, integrins, or the asialoglycoprotein receptor (ASGPR). In order to maximize drug concentration within the tumor and minimize systemic side effects, these nanoparticles are also designed to be stimuli-responsive, releasing their therapeutic payload in response to local triggers like acidic pH, increased enzymatic activity, or hypoxic conditions.³⁶⁰ TKIs, chemotherapeutic medications, small interfering RNAs (siRNAs), immunomodulators, or imaging probes are just a few of the substances that may be loaded into these intelligent nanocarriers to create multipurpose theranostic platforms. Real-time monitoring of medication distribution and tumor response is made possible by this dual capacity, which enables customized dosage modifications and treatment plans that are best suited to the requirements of each patient.^{361,362} Da et al developed a platelet membrane-coated nanocarrier co-loaded with anti-PD-1 antibody and Sorafenib (aPD-1-PLTM-HMSNs@Sora) for targeted delivery to HCC (Figs. 5 and 6). In vivo studies in H22 tumor-bearing mice demonstrated superior tumor suppression, with average tumor volumes reduced to 26.85% of controls. Immunofluorescence and histological analysis showed significant CD8⁺/CD4⁺ T cell infiltration and VEGF-A inhibition, while regulatory T cells were diminished.

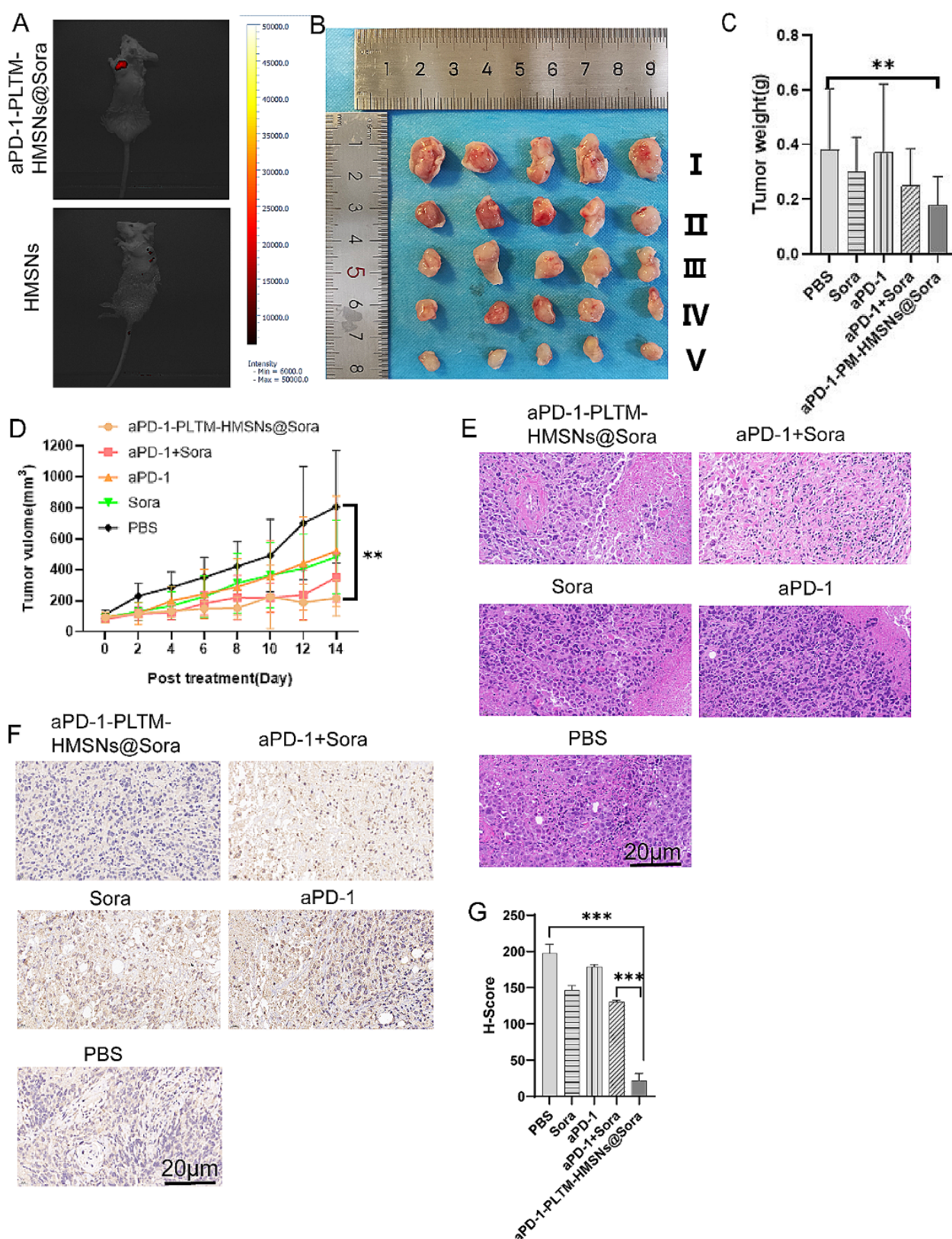


Fig. 5. Antitumor efficacy and immune activation induced by aPD-1-PLTM-HMSNs@Sora in hepatocellular carcinoma (HCC) models. Therapeutic efficacy of different treatment regimens in H22 tumor-bearing mice. (A) In vivo fluorescence imaging shows enhanced tumor targeting of Cy5.5-labeled aPD-1-PLTM-HMSNs@Sora compared to HMSNs alone. (B) Representative images of tumors harvested from five groups: I, PBS; II, Sorafenib (Sora); III, aPD-1; IV, aPD-1 + Sora; V, aPD-1-PLTM-HMSNs@Sora. (C) Tumor weight comparison among treatment groups. (D) Tumor growth curves over 14 days, showing the greatest inhibition in the aPD-1-PLTM-HMSNs@Sora group ($p < 0.01$). (E) H&E staining indicates more extensive tumor necrosis in the aPD-1-PLTM-HMSNs@Sora group. (F) Immunohistochemistry of VEGF-A expression across groups. (G) Quantitative H-score analysis of VEGF-A, with significant reduction in the aPD-1-PLTMHMSNs@Sora group ($*P < 0.001$). Under a Creative Commons Attribution 4.0 International License (CC BY 4.0).

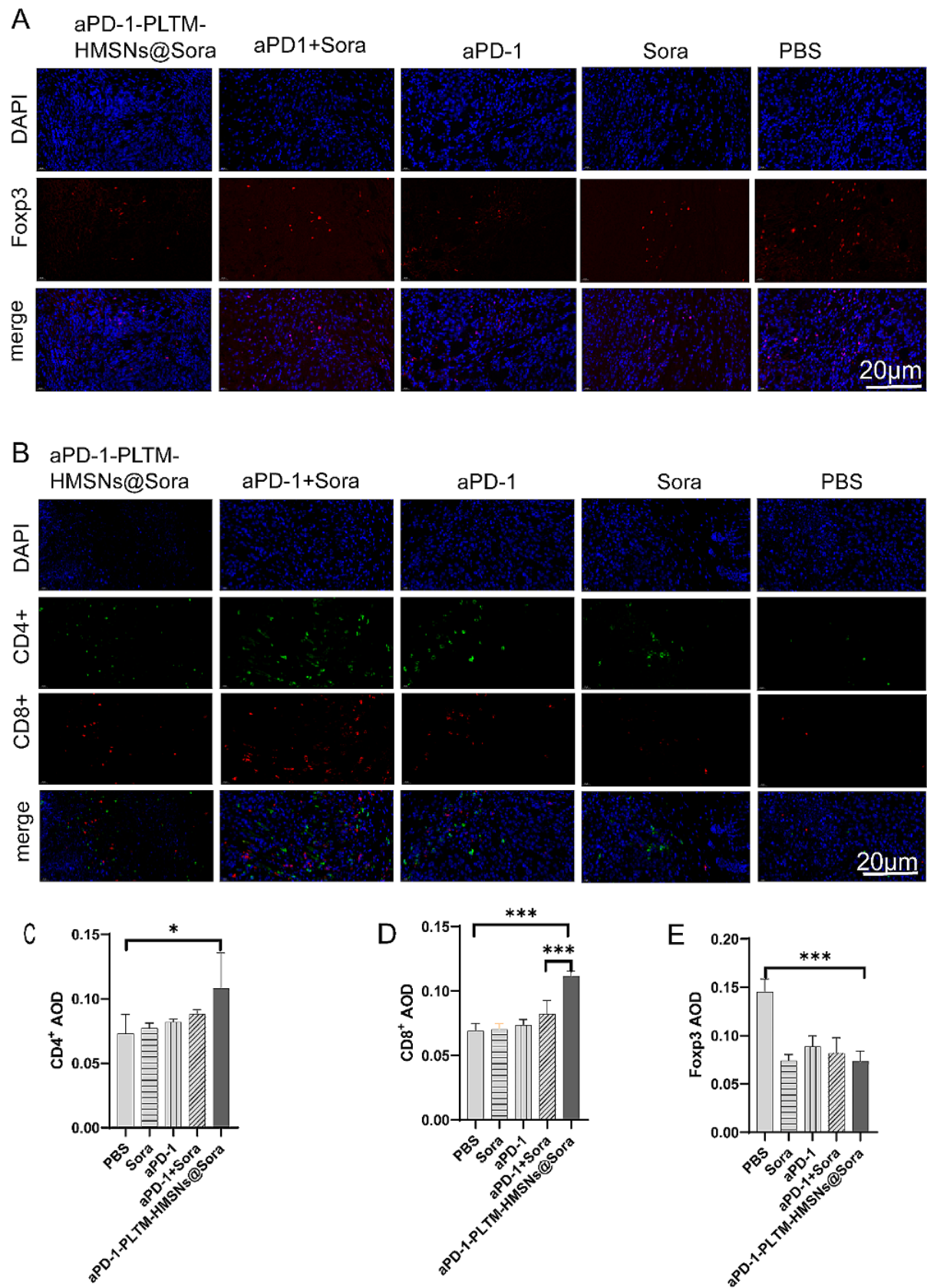


Fig. 6. aPD-1-PLTM-HMSNs@Sora enhances T cell-mediated immune response in HCC tumors. (A) Immunofluorescence staining for Foxp3⁺ regulatory T cells shows significant reduction in the aPD-1-PLTM-HMSNs@Sora group. (B) Increased infiltration of CD4⁺ and CD8⁺ T cells in tumors from aPD-1-PLTM-HMSNs@Sora-treated mice compared to other groups. (C–E) Quantification of CD4⁺ (C), CD8⁺ (D), and Foxp3⁺ (E) cells, revealing enhanced cytotoxic T cell infiltration and reduced immunosuppressive Tregs, indicating an immunostimulatory shift in the tumor microenvironment. Under a Creative Commons Attribution 4.0 International License (CC BY 4.0).

These results suggest potent synergistic antitumor activity through both direct cytotoxic and immunomodulatory mechanisms.³⁶³

Finally, we note that well-designed nanoparticles function effectively as passive delivery vehicles through the enhanced permeability and retention (EPR) effect inherent to the tumor microenvironment. Tumor vasculature is inherently leaky and characterized by poor lymphatic drainage, allowing nanoparticles typically 10–100 nm in size to selectively extravasate and accumulate in tumor tissue.^{364,365} This passive targeting enables increased local drug concentration and reduced systemic side effects. Optimizing nanoparticle physicochemical properties including size, circulation half-life, and surface chemistry can further enhance EPR-mediated accumulation and therapeutic efficacy in solid tumors.

Organoids and patient-derived xenograft (PDX) models

Evaluation of individualized treatment is changing as a result of advancements in preclinical modeling. The histological characteristics and intratumoral heterogeneity of the original tissue are preserved in patient-derived organoids (PDOs), which are generated directly from individual HCC tumors. This fidelity speeds up the creation of specialized treatment plans by enabling quick in vitro testing of both individual drugs and specific

combination therapies.³⁶⁶ In order to more precisely replicate the intricate tumor microenvironment, recent developments have established techniques that co-culture autologous immune and stromal cells with PDOs. Better treatment choices are made possible by this improved model's increased predictive ability for evaluating immunotherapy responses.³⁶⁷ Patient-derived xenograft (PDX) mice models faithfully mimic the stromal and genetic characteristics of the original tumors, allowing for thorough in vivo evaluation of medication effectiveness, the best course of therapy, and the emergence of resistance over time. These models are being used more and more in co-clinical trial settings, where knowledge from PDO and PDX research is used to inform stratification and customize therapy decisions alongside patient therapies.³⁶⁸

Conclusion and expert perspective

Recent developments in immunotherapy, biomarker discovery, and molecular oncology have significantly changed the treatment landscape for HCC^{369–372} (Fig. 7). Among the groundbreaking advancements are ICIs like atezolizumab and nivolumab, as well as multi-targeted TKIs like sorafenib, lenvatinib, and cabozantinib. A novel therapy paradigm that includes strategies that target angiogenesis, immune evasion, and tumor growth simultaneously has been introduced by the clinical

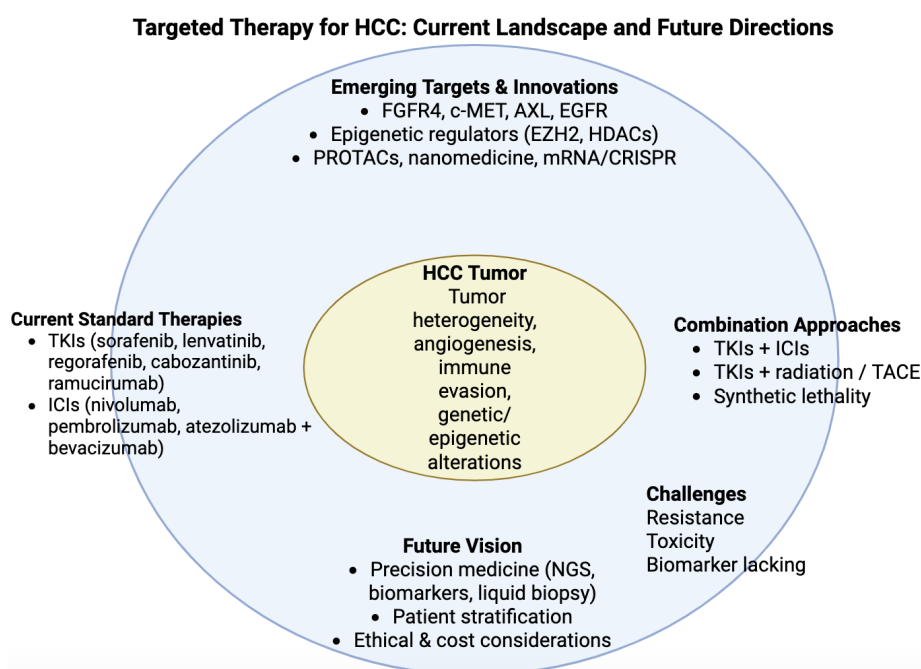


Fig. 7. Summary of Targeted Therapy for Hepatocellular Carcinoma (HCC): Current Landscape and Future Directions. This schematic illustrates the therapeutic framework for HCC. At the center lies the tumor, driven by heterogeneity, angiogenesis, and immune evasion. Surrounding layers represent: (i) **current standard therapies**, including tyrosine kinase inhibitors (sorafenib, lenvatinib, regorafenib, cabozantinib, ramucirumab) and immune checkpoint inhibitors (nivolumab, pembrolizumab, atezolizumab plus bevacizumab); (ii) **combination strategies**, such as TKIs with ICIs, TKIs with radiation or TACE, and synthetic lethality approaches, alongside key challenges of resistance, toxicity, and biomarker limitations; (iii) **emerging molecular targets and innovations**, including FGFR4, c-MET, AXL, EGFR, epigenetic regulators (EZH2, HDACs), and novel modalities such as PROTACs, nanomedicine, mRNA therapeutics, and CRISPR-based strategies; and (iv) the **future vision**, highlighting precision medicine guided by next-generation sequencing, liquid biopsy, dynamic biomarkers, and multidisciplinary collaboration. Together, these elements provide a strategic overview of current progress and future directions in the individualized treatment of liver cancer

Review Highlights

- Hepatocellular carcinoma (HCC) remains a major global health challenge with limited response to traditional chemotherapy, highlighting the need for targeted therapies focusing on key pathways like Wnt/ β -catenin, PI3K/AKT/mTOR, and RAS/RAF/MEK/ERK.
- First-line TKIs such as sorafenib and lenvatinib offer modest survival benefits, while second-line options like regorafenib, cabozantinib, and ramucirumab address resistance, particularly in patients with high AFP levels.
- Immunotherapy advancements, including ICIs (e.g., atezolizumab + bevacizumab), show promise in 20–30% of patients, emphasizing the role of the tumor microenvironment and combination strategies to overcome resistance.
- Emerging targets like FGFR4, c-MET, AXL, and epigenetic regulators (e.g., EZH2, HDACs) offer new avenues for precision medicine, with a focus on biomarker-driven approaches for improved patient stratification.
- Future directions include rational combinations (e.g., TKIs + ICIs + radiation), synthetic lethality, and dynamic molecular profiling to enhance long-term efficacy and personalize HCC treatment.

integration of these drugs, which has also improved survival results for patients with advanced HCC.^{373–}

³⁷⁵ Furthermore, a move toward more individualized treatment approaches has been fueled by a better understanding of oncogenic pathways, including as Wnt/ β -catenin, PI3K/AKT/mTOR, FGFR4, and c-MET, as well as knowledge of the tumor microenvironment and epigenetic processes. Personalized targeted treatments have the potential to become the mainstay of HCC treatment in the future. Clinicians will be able to customize therapy to the specific biology of each patient's tumor by using NGS, liquid biopsy technologies, and transcriptome profiling to find actionable mutations in real time. However, strong interdisciplinary and translational research partnerships are essential to the development of precision medicine. To bridge the gap between laboratory findings and clinical application, it is essential to integrate molecular biology, clinical oncology, computer modeling, and pharmacological innovation. Additionally, the development of dynamic predictive biomarkers will improve combination regimens, ease the monitoring of treatment resistance, and improve patient selection. Policymakers and regulatory agencies must create flexible frameworks that strike a balance between strict safety and ethical scrutiny and the quick licensing of innovative treatments in order to maintain advancement in this quickly changing sector. The privacy of genetic data, fair access to customized treatments, informed consent in biomarker-driven trials, and the

expense of new therapeutics are among the issues that need immediate attention. To preserve public confidence and promote sustainable innovation, ethical issues must be included into every step of the research and clinical implementation process. In conclusion, multimodal, tailored, and biomarker-guided techniques are the way of the future for treating liver cancer. An age of precision oncology for hepatocellular carcinoma is not only possible but also impending with sustained scientific advancement, multidisciplinary collaboration, and careful policy change.

Acknowledgements

We would like to thank the library staff at Shandong Xiehe University for their assistance in accessing key databases and resources during the literature review process. Special thanks to colleagues in the School of Medicine for insightful discussions on molecular pathways in hepatocellular carcinoma. No external editing services were used in the preparation of this manuscript.

Competing Interests

The author declares no conflicts of interest. There are no financial, personal, or professional relationships that could be perceived as influencing the content or interpretation of this review.

Ethical Approval

This article is a comprehensive review based on previously published studies and does not involve any original human or animal experimentation. Therefore, no ethical approval from an institutional review board or ethics committee was required. All referenced studies were assumed to have adhered to relevant ethical guidelines, including the Declaration of Helsinki where applicable.

Funding

This work received no specific funding from any public, commercial, or not-for-profit sectors. The author was supported by internal resources from Shandong Xiehe University.

Supplementary files

Supplementary file 1 contains Table S1–S3.

References

1. Donne R, Lujambio A. The liver cancer immune microenvironment: therapeutic implications for hepatocellular carcinoma. *Hepatology* **2023**; 77: 1773–96. doi: 10.1002/hep.32740
2. Jemal A, Ward EM, Johnson CJ, Cronin KA, Ma J, Ryerson B, et al. Annual report to the nation on the status of cancer, 1975–2014, featuring survival. *J Natl Cancer Inst* **2017**; 109: djx030. doi: 10.1093/jnci/djx030
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* **2021**; 71: 209–49. doi: 10.3322/caac.21660
4. Wittekind C. [Pathology of liver tumors]. *Zentralbl Chir* **2000**; 125: 587–91. [German].
5. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, et al. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the Global Burden of Disease Study 2015. *JAMA Oncol* **2017**; 3: 1683–91. doi: 10.1001/jamaoncol.2017.3055
6. Sanyal A, Poklepovic A, Moyneur E, Barghout V. Population-based risk factors and resource utilization for HCC: US perspective. *Curr Med Res Opin* **2010**; 26: 2183–91. doi: 10.1185/03007995.2010.506375
7. Kanwal F, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, Richardson PA, et al. Risk of hepatocellular cancer in patients with non-alcoholic fatty liver disease. *Gastroenterology* **2018**; 155:

- 1828-37.e2. doi: 10.1053/j.gastro.2018.08.024
8. Boyault S, Rickman DS, de Reyniès A, Balabaud C, Rebouissou S, Jeannot E, et al. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* **2007**; 45: 42-52. doi: 10.1002/hep.21467
9. Zucman-Rossi J, Villanueva A, Nault JC, Llovet JM. Genetic landscape and biomarkers of hepatocellular carcinoma. *Gastroenterology* **2015**; 149: 1226-39.e4. doi: 10.1053/j.gastro.2015.05.061
10. Llovet JM, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers* **2016**; 2: 16018. doi: 10.1038/nrdp.2016.18
11. Vij M, Calderaro J. Pathologic and molecular features of hepatocellular carcinoma: an update. *World J Hepatol* **2021**; 13: 393-410. doi: 10.4254/wjh.v13.i4.393
12. Nault JC, Calderaro J, Di Tommaso L, Balabaud C, Zafrani ES, Bioulac-Sage P, et al. Telomerase reverse transcriptase promoter mutation is an early somatic genetic alteration in the transformation of premalignant nodules in hepatocellular carcinoma on cirrhosis. *Hepatology* **2014**; 60: 1983-92. doi: 10.1002/hep.27372
13. Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, et al. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nat Genet* **2012**; 44: 694-8. doi: 10.1038/ng.2256
14. Nault JC, Mallet M, Pilati C, Calderaro J, Bioulac-Sage P, Laurent C, et al. High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat Commun* **2013**; 4: 2218. doi: 10.1038/ncomms3218
15. Totoki Y, Tatsuno K, Covington KR, Ueda H, Creighton CJ, Kato M, et al. Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. *Nat Genet* **2014**; 46: 1267-73. doi: 10.1038/ng.3126
16. Llovet JM, Montal R, Sia D, Finn RS. Molecular therapies and precision medicine for hepatocellular carcinoma. *Nat Rev Clin Oncol* **2018**; 15: 599-616. doi: 10.1038/s41571-018-0073-4
17. Qin S, Bai Y, Lim HY, Thongprasert S, Chao Y, Fan J, et al. Randomized, multicenter, open-label study of oxaliplatin plus fluorouracil/leucovorin versus doxorubicin as palliative chemotherapy in patients with advanced hepatocellular carcinoma from Asia. *J Clin Oncol* **2013**; 31: 3501-8. doi: 10.1200/jco.2012.44.5643
18. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* **2008**; 359: 378-90. doi: 10.1056/NEJMoa0708857
19. Yamashita T, Kudo M, Ikeda K, Izumi N, Tateishi R, Ikeda M, et al. REFLECT-a phase 3 trial comparing efficacy and safety of lenvatinib to sorafenib for the treatment of unresectable hepatocellular carcinoma: an analysis of Japanese subset. *J Gastroenterol* **2020**; 55: 113-22. doi: 10.1007/s00535-019-01642-1
20. Bruix J, Qin S, Merle P, Granito A, Huang YH, Bodoky G, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* **2017**; 389: 56-66. doi: 10.1016/s0140-6736(16)32453-9
21. Abou-Alfa GK, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, Ryoo BY, et al. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. *N Engl J Med* **2018**; 379: 54-63. doi: 10.1056/NEJMoa1717002
22. Zhu AX, Kang YK, Yen CJ, Finn RS, Galle PR, Llovet JM, et al. Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α -fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* **2019**; 20: 282-96. doi: 10.1016/s1470-2045(18)30937-9
23. Yau T, Kang YK, Kim TY, El-Khoueiry AB, Santoro A, Sangro B, et al. Nivolumab (NIVO) + ipilimumab (IPI) combination therapy in patients (PTS) with advanced hepatocellular carcinoma (aHCC): results from CheckMate 040. *J Clin Oncol* **2019**; 37: 4012. doi: 10.1200/JCO.2019.37.15_suppl.4012
24. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol* **2018**; 19: 940-52. doi: 10.1016/s1470-2045(18)30351-6
25. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* **2017**; 389: 2492-502. doi: 10.1016/s0140-6736(17)31046-2
26. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med* **2020**; 382: 1894-905. doi: 10.1056/NEJMoa1915745
27. Zhu AX, Abbas AR, de Galarreta MR, Guan Y, Lu S, Koeppen H, et al. Molecular correlates of clinical response and resistance to atezolizumab in combination with bevacizumab in advanced hepatocellular carcinoma. *Nat Med* **2022**; 28: 1599-611. doi: 10.1038/s41591-022-01868-2
28. Shi J, Zhu X, Yang JB. Advances and challenges in molecular understanding, early detection, and targeted treatment of liver cancer. *World J Hepatol* **2025**; 17: 102273. doi: 10.4254/wjh.v17.i1.102273
29. Calderaro J, Couchy G, Imbeaud S, Amaddeo G, Letouze E, Blanc JF, et al. Histological subtypes of hepatocellular carcinoma are related to gene mutations and molecular tumour classification. *J Hepatol* **2017**; 67: 727-38. doi: 10.1016/j.jhep.2017.05.014
30. Schulze K, Imbeaud S, Letouze E, Alexandrov LB, Calderaro J, Rebouissou S, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Genet* **2015**; 47: 505-11. doi: 10.1038/ng.3252
31. Nagaraju GP, Dariya B, Kasa P, Peela S, El-Rayes BF. Epigenetics in hepatocellular carcinoma. *Semin Cancer Biol* **2022**; 86: 622-32. doi: 10.1016/j.semcancer.2021.07.017
32. Rajan PK, Udoh UA, Sanabria JD, Banerjee M, Smith G, Schade MS, et al. The role of histone acetylation-/methylation-mediated apoptotic gene regulation in hepatocellular carcinoma. *Int J Mol Sci* **2020**; 21: 8894. doi: 10.3390/ijms21238894
33. Jiang L, Meng Q, Liu L, Li W. A comprehensive review on molecular mechanisms, treatments, and brief role of natural products in hepatocellular cancer. *Nat Prod Commun* **2024**; 19: 1934578X241284873. doi: 10.1177/1934578X241284873
34. Zhao H, Ming T, Tang S, Ren S, Yang H, Liu M, et al. Wnt signaling in colorectal cancer: pathogenic role and therapeutic target. *Mol Cancer* **2022**; 21: 144. doi: 10.1186/s12943-022-01616-7
35. Gajos-Michniewicz A, Czyz M. Wnt signaling in melanoma. *Int J Mol Sci* **2020**; 21: 4852. doi: 10.3390/ijms21144852
36. Vlashi R, Zhang X, Wu M, Chen G. Wnt signaling: essential roles in osteoblast differentiation, bone metabolism and therapeutic implications for bone and skeletal disorders. *Genes Dis* **2023**; 10: 1291-317. doi: 10.1016/j.gendis.2022.07.011
37. Huang J, Gao L, Li B, Liu C, Hong S, Min J, et al. Knockdown of hypoxia-inducible factor 1 α (HIF-1 α) promotes autophagy and inhibits phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway in ovarian cancer cells. *Med Sci Monit* **2019**; 25: 4250-63. doi: 10.12659/msm.915730
38. Le TK, Dao XD, Nguyen DV, Luu DH, Bui TM, Le TH, et al. Insulin signaling and its application. *Front Endocrinol (Lausanne)* **2023**; 14: 1226655. doi: 10.3389/fendo.2023.1226655
39. Huang J, Manning BD. A complex interplay between Akt, TSC2 and the two mTOR complexes. *Biochem Soc Trans* **2009**; 37: 217-22. doi: 10.1042/bst0370217
40. Nepstad I, Hatfield KJ, Grønningsæter IS, Reikvam H. The PI3K-Akt-mTOR signaling pathway in human acute myeloid leukemia (AML) cells. *Int J Mol Sci* **2020**; 21: 2907. doi: 10.3390/ijms21082907

41. Revathidevi S, Munirajan AK. Akt in cancer: mediator and more. *Semin Cancer Biol* **2019**; 59: 80-91. doi: 10.1016/j.semcancer.2019.06.002
42. Ye G, Wang J, Xia J, Zhu C, Gu C, Li X, et al. Low protein expression of LZTR1 in hepatocellular carcinoma triggers tumorigenesis via activating the RAS/RAF/MEK/ERK signaling. *Heliyon* **2024**; 10: e32855. doi: 10.1016/j.heliyon.2024.e32855
43. Neuzillet C, Tijeras-Raballand A, de Mestier L, Cros J, Faivre S, Raymond E. MEK in cancer and cancer therapy. *Pharmacol Ther* **2014**; 141: 160-71. doi: 10.1016/j.pharmthera.2013.10.001
44. Yang X, Zheng YT, Rong W. Sevoflurane induces apoptosis and inhibits the growth and motility of colon cancer in vitro and in vivo via inactivating Ras/Raf/MEK/ERK signaling. *Life Sci* **2019**; 239: 116916. doi: 10.1016/j.lfs.2019.116916
45. Akula SM, Abrams SL, Steelman LS, Emma MR, Augello G, Cusimano A, et al. RAS/RAF/MEK/ERK, PI3K/PTEN/AKT/mTORC1 and TP53 pathways and regulatory miRs as therapeutic targets in hepatocellular carcinoma. *Expert Opin Ther Targets* **2019**; 23: 915-29. doi: 10.1080/14728222.2019.1685501
46. Steelman LS, Franklin RA, Abrams SL, Chappell W, Kempf CR, Bäscke J, et al. Roles of the Ras/Raf/MEK/ERK pathway in leukemia therapy. *Leukemia* **2011**; 25: 1080-94. doi: 10.1038/leu.2011.66
47. Wang AX, Qi XY. Targeting RAS/RAF/MEK/ERK signaling in metastatic melanoma. *IUBMB Life* **2013**; 65: 748-58. doi: 10.1002/iub.1193
48. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene* **2007**; 26: 3291-310. doi: 10.1038/sj.onc.1210422
49. Garces S, Yin CC, Patel KP, Khoury JD, Manning JT Jr, Li S, et al. Focal Rosai-Dorfman disease coexisting with lymphoma in the same anatomic site: a localized histiocytic proliferation associated with MAPK/ERK pathway activation. *Mod Pathol* **2019**; 32: 16-26. doi: 10.1038/s41379-018-0152-1
50. Guo YJ, Pan WW, Liu SB, Shen ZF, Xu Y, Hu LL. ERK/MAPK signalling pathway and tumorigenesis. *Exp Ther Med* **2020**; 19: 1997-2007. doi: 10.3892/etm.2020.8454
51. Schubert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. *Nat Rev Cancer* **2007**; 7: 295-308. doi: 10.1038/nrc2109
52. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. *Nat Rev Cancer* **2011**; 11: 761-74. doi: 10.1038/nrc3106
53. Li L, Zhao GD, Shi Z, Qi LL, Zhou LY, Fu ZX. The Ras/Raf/MEK/ERK signaling pathway and its role in the occurrence and development of HCC. *Oncol Lett* **2016**; 12: 3045-50. doi: 10.3892/ol.2016.5110
54. Zhao Z, Zhang D, Wu F, Tu J, Song J, Xu M, et al. Sophoridine suppresses lenvatinib-resistant hepatocellular carcinoma growth by inhibiting RAS/MEK/ERK axis via decreasing VEGFR2 expression. *J Cell Mol Med* **2021**; 25: 549-60. doi: 10.1111/jcmm.16108
55. Taketomi A, Shirabe K, Muto J, Yoshiya S, Motomura T, Mano Y, et al. A rare point mutation in the Ras oncogene in hepatocellular carcinoma. *Surg Today* **2013**; 43: 289-92. doi: 10.1007/s00595-012-0462-8
56. Godoi MA, Camilli AC, Gonzales KG, Costa VB, Papathanasiou E, Leite FR, et al. JAK/STAT as a potential therapeutic target for osteolytic diseases. *Int J Mol Sci* **2023**; 24: 10290. doi: 10.3390/ijms241210290
57. Kumar A, Schwab M, Laborit Labrada B, Silveira MA, Goudreault M, Fournier É, et al. SHP-1 phosphatase acts as a coactivator of PCK1 transcription to control gluconeogenesis. *J Biol Chem* **2023**; 299: 105164. doi: 10.1016/j.jbc.2023.105164
58. Su D, Zhang H, Xiong Y, Wei H, Yu Y, Li H, et al. Stratification of ovarian cancer patients from the prospect of drug target-related transcription factor protein activity: the prognostic and genomic landscape analyses. *Brief Funct Genomics* **2023**; 22: 351-65. doi: 10.1093/bfpg/elad008
59. Ilangumaran S, Gui Y, Shukla A, Ramanathan S. SOCS1 expression in cancer cells: potential roles in promoting antitumor immunity. *Front Immunol* **2024**; 15: 1362224. doi: 10.3389/fimmu.2024.1362224
60. Yan W, Rao D, Fan F, Liang H, Zhang Z, Dong H. Hepatitis B virus X protein and TGF- β : partners in the carcinogenic journey of hepatocellular carcinoma. *Front Oncol* **2024**; 14: 1407434. doi: 10.3389/fonc.2024.1407434
61. Kowalik TF. Smad about E2F. TGF β repression of c-Myc via a Smad3/E2F/p107 complex. *Mol Cell* **2002**; 10: 7-8. doi: 10.1016/s1097-2765(02)00584-1
62. Chen CR, Kang Y, Siegel PM, Massagué J. E2F4/5 and p107 as Smad cofactors linking the TGF β receptor to c-Myc repression. *Cell* **2002**; 110: 19-32. doi: 10.1016/s0092-8674(02)00801-2
63. Saltis J. TGF- β : receptors and cell cycle arrest. *Mol Cell Endocrinol* **1996**; 116: 227-32. doi: 10.1016/0303-7207(95)03721-7
64. Goel S, DeCristo MJ, McAllister SS, Zhao JJ. CDK4/6 inhibition in cancer: beyond cell cycle arrest. *Trends Cell Biol* **2018**; 28: 911-25. doi: 10.1016/j.tcb.2018.07.002
65. Claassen GF, Hann SR. A role for transcriptional repression of p21CIP1 by c-Myc in overcoming transforming growth factor beta-induced cell-cycle arrest. *Proc Natl Acad Sci U S A* **2000**; 97: 9498-503. doi: 10.1073/pnas.150006697
66. Feng XH, Liang YY, Liang M, Zhai W, Lin X. Direct Interaction of c-Myc with Smad2 and Smad3 to inhibit TGF- β -mediated induction of the CDK inhibitor p15Ink4B. *Mol Cell* **2016**; 63: 1089. doi: 10.1016/j.molcel.2016.08.027
67. Staller P, Peukert K, Kiermaier A, Seoane J, Lukas J, Karsunky H, et al. Repression of p15INK4b expression by Myc through association with Miz-1. *Nat Cell Biol* **2001**; 3: 392-9. doi: 10.1038/35070076
68. Zhang Y, Alexander PB, Wang XF. TGF- β family signaling in the control of cell proliferation and survival. *Cold Spring Harb Perspect Biol* **2017**; 9: a022145. doi: 10.1101/cshperspect.a022145
69. Senturk S, Mumcuoglu M, Gursay-Yuzugullu O, Cingoz B, Akcali KC, Ozturk M. Transforming growth factor-beta induces senescence in hepatocellular carcinoma cells and inhibits tumor growth. *Hepatology* **2010**; 52: 966-74. doi: 10.1002/hep.23769
70. Hashimoto O, Ueno T, Kimura R, Ohtsubo M, Nakamura T, Koga H, et al. Inhibition of proteasome-dependent degradation of Wee1 in G2-arrested Hep3B cells by TGF beta 1. *Mol Carcinog* **2003**; 36: 171-82. doi: 10.1002/mc.10111
71. Oura K, Morishita A, Tadokoro T, Fujita K, Tani J, Kobara H. Immune microenvironment and the effect of vascular endothelial growth factor inhibition in hepatocellular carcinoma. *Int J Mol Sci* **2024**; 25: 13590. doi: 10.3390/ijms252413590
72. Bayerl F, Meiser P, Donakonda S, Hirschberger A, Lacher SB, Pedde AM, et al. Tumor-derived prostaglandin E2 programs cDC1 dysfunction to impair intratumoral orchestration of anti-cancer T cell responses. *Immunity* **2023**; 56: 1341-58.e11. doi: 10.1016/j.immuni.2023.05.011
73. Spranger S, Gajewski TF. Impact of oncogenic pathways on evasion of antitumor immune responses. *Nat Rev Cancer* **2018**; 18: 139-47. doi: 10.1038/nrc.2017.117
74. Giles JR, Globig AM, Kaech SM, Wherry EJ. CD8+ T cells in the cancer-immunity cycle. *Immunity* **2023**; 56: 2231-53. doi: 10.1016/j.immuni.2023.09.005
75. Mucida D, Cheroutre H. TGF β and retinoic acid intersect in immune-regulation. *Cell Adh Migr* **2007**; 1: 142-4. doi: 10.4161/cam.1.3.5062
76. Castiglioni A, Yang Y, Williams K, Gogineni A, Lane RS, Wang AW, et al. Combined PD-L1/TGF β blockade allows expansion and differentiation of stem cell-like CD8 T cells in immune excluded tumors. *Nat Commun* **2023**; 14: 4703. doi: 10.1038/s41467-023-40398-4
77. Hu Y, Hudson WH, Kissick HT, Medina CB, Baptista AP, Ma C, et al. TGF- β regulates the stem-like state of PD-1+ TCF-1+ virus-specific CD8 T cells during chronic infection. *J Exp Med* **2022**; 219: e20211574. doi: 10.1084/jem.20211574
78. Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang

- Y, et al. TGF β attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature* **2018**; 554: 544-8. doi: 10.1038/nature25501
79. Ritter AT, Shtengel G, Xu CS, Weigel A, Hoffman DP, Freeman M, et al. ESCRT-mediated membrane repair protects tumor-derived cells against T cell attack. *Science* **2022**; 376: 377-82. doi: 10.1126/science.abl3855
 80. Chakravarthy A, Khan L, Bensler NP, Bose P, De Carvalho DD. TGF- β -associated extracellular matrix genes link cancer-associated fibroblasts to immune evasion and immunotherapy failure. *Nat Commun* **2018**; 9: 4692. doi: 10.1038/s41467-018-06654-8
 81. Krishnamurthy AT, Shyer JA, Thai M, Gandham V, Buechler MB, Yang YA, et al. LRRC15+ myofibroblasts dictate the stromal setpoint to suppress tumour immunity. *Nature* **2022**; 611: 148-54. doi: 10.1038/s41586-022-05272-1
 82. Dominguez CX, Müller S, Keerthivasan S, Koeppen H, Hung J, Gierke S, et al. Single-cell RNA sequencing reveals stromal evolution into LRRC15+ myofibroblasts as a determinant of patient response to cancer immunotherapy. *Cancer Discov* **2020**; 10: 232-53. doi: 10.1158/2159-8290.Cd-19-0644
 83. Feig C, Jones JO, Kraman M, Wells RJ, Deonarine A, Chan DS, et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc Natl Acad Sci U S A* **2013**; 110: 20212-7. doi: 10.1073/pnas.1320318110
 84. Koncina E, Nurmik M, Pozdeev VI, Gilson C, Tsenkova M, Begaj R, et al. IL1R1+ cancer-associated fibroblasts drive tumor development and immunosuppression in colorectal cancer. *Nat Commun* **2023**; 14: 4251. doi: 10.1038/s41467-023-39953-w
 85. Huang H, Wang Z, Zhang Y, Pradhan RN, Ganguly D, Chandra R, et al. Mesothelial cell-derived antigen-presenting cancer-associated fibroblasts induce expansion of regulatory T cells in pancreatic cancer. *Cancer Cell* **2022**; 40: 656-73.e7. doi: 10.1016/j.ccell.2022.04.011
 86. Li Y, Wang R, Xiong S, Wang X, Zhao Z, Bai S, et al. Cancer-associated fibroblasts promote the stemness of CD24+ liver cells via paracrine signaling. *J Mol Med (Berl)* **2019**; 97: 243-55. doi: 10.1007/s00109-018-1731-9
 87. Rhee H, Kim HY, Choi JH, Woo HG, Yoo JE, Nahm JH, et al. Keratin 19 expression in hepatocellular carcinoma is regulated by fibroblast-derived HGF via a MET-ERK1/2-AP1 and SP1 axis. *Cancer Res* **2018**; 78: 1619-31. doi: 10.1158/0008-5472.Can-17-0988
 88. Li T, Yang Y, Hua X, Wang G, Liu W, Jia C, et al. Hepatocellular carcinoma-associated fibroblasts trigger NK cell dysfunction via PGE2 and IDO. *Cancer Lett* **2012**; 318: 154-61. doi: 10.1016/j.canlet.2011.12.020
 89. Mano Y, Yoshio S, Shoji H, Tomonari S, Aoki Y, Aoyanagi N, et al. Bone morphogenetic protein 4 provides cancer-supportive phenotypes to liver fibroblasts in patients with hepatocellular carcinoma. *J Gastroenterol* **2019**; 54: 1007-18. doi: 10.1007/s00535-019-01579-5
 90. Rashid S, Sun Y, Ali Khan Saddozai U, Hayyat S, Munir MU, Akbar MU, et al. Circulating tumor DNA and its role in detection, prognosis and therapeutics of hepatocellular carcinoma. *Chin J Cancer Res* **2024**; 36: 195-214. doi: 10.21147/j.issn.1000-9604.2024.02.07
 91. Campani C, Zucman-Rossi J, Nault JC. Genetics of hepatocellular carcinoma: from tumor to circulating DNA. *Cancers (Basel)* **2023**; 15: 817. doi: 10.3390/cancers15030817
 92. Martinez-Useros J, Martin-Galan M, Florez-Cespedes M, Garcia-Foncillas J. Epigenetics of most aggressive solid tumors: pathways, targets and treatments. *Cancers (Basel)* **2021**; 13: 3209. doi: 10.3390/cancers13133209
 93. Mesgari H, Esmalian S, Nasiri K, Ghasemzadeh S, Doroudgar P, Payandeh Z. Epigenetic regulation in oral squamous cell carcinoma microenvironment: a comprehensive review. *Cancers (Basel)* **2023**; 15: 5600. doi: 10.3390/cancers15235600
 94. Leung RW, Lee TK. Wnt/ β -catenin signaling as a driver of stemness and metabolic reprogramming in hepatocellular carcinoma. *Cancers (Basel)* **2022**; 14: 5468. doi: 10.3390/cancers14215468
 95. Mei L, Sun H, Yan Y, Ji H, Su Q, Chang L, et al. mTOR signaling: roles in hepatitis B virus infection and hepatocellular carcinoma. *Int J Biol Sci* **2024**; 20: 4178-89. doi: 10.7150/ijbs.95894
 96. Wang Q, Feng J, Tang L. Non-coding RNA related to MAPK signaling pathway in liver cancer. *Int J Mol Sci* **2022**; 23: 11908. doi: 10.3390/ijms231911908
 97. Masuzaki R, Kanda T, Sasaki R, Matsumoto N, Nirei K, Ogawa M, et al. Suppressors of cytokine signaling and hepatocellular carcinoma. *Cancers (Basel)* **2022**; 14: 2549. doi: 10.3390/cancers14102549
 98. Körholz J, Chen LS, Strauss T, Schuetz C, Dalpke AH. One gene to rule them all - clinical perspectives of a potent suppressor of cytokine signaling - SOCS1. *Front Immunol* **2024**; 15: 1385190. doi: 10.3389/fimmu.2024.1385190
 99. Li Z, Zhang Z, Fang L, Zhao J, Niu Z, Chen H, et al. Tumor microenvironment composition and related therapy in hepatocellular carcinoma. *J Hepatocell Carcinoma* **2023**; 10: 2083-99. doi: 10.2147/jhc.S436962
 100. Garcia-Lezana T, Lopez-Canovas JL, Villanueva A. Signaling pathways in hepatocellular carcinoma. *Adv Cancer Res* **2021**; 149: 63-101. doi: 10.1016/bs.acr.2020.10.002
 101. Qin S, Li A, Yi M, Yu S, Zhang M, Wu K. Recent advances on anti-angiogenesis receptor tyrosine kinase inhibitors in cancer therapy. *J Hematol Oncol* **2019**; 12: 27. doi: 10.1186/s13045-019-0718-5
 102. Dipasquale A, Marinello A, Santoro A. A comparison of lenvatinib versus sorafenib in the first-line treatment of unresectable hepatocellular carcinoma: selection criteria to guide physician's choice in a new therapeutic scenario. *J Hepatocell Carcinoma* **2021**; 8: 241-51. doi: 10.2147/jhc.S270532
 103. Gawi Ermi A, Sarkar D. Resistance to tyrosine kinase inhibitors in hepatocellular carcinoma (HCC): clinical implications and potential strategies to overcome the resistance. *Cancers (Basel)* **2024**; 16: 3944. doi: 10.3390/cancers16233944
 104. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, 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: 25-34. doi: 10.1016/s1470-2045(08)70285-7
 105. Kudo M, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* **2018**; 391: 1163-73. doi: 10.1016/s0140-6736(18)30207-1
 106. Ettrich TJ, Seufferlein T. Regorafenib. *Recent Results Cancer Res* **2018**; 211: 45-56. doi: 10.1007/978-3-319-91442-8_3
 107. Yakes FM, Chen J, Tan J, Yamaguchi K, Shi Y, Yu P, et al. Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. *Mol Cancer Ther* **2011**; 10: 2298-308. doi: 10.1158/1535-7163.Mct-11-0264
 108. Chen MH, Lu SN, Chen CH, Lin PC, Jiang JK, D'Yachkova Y, et al. How may ramucirumab help improve treatment outcome for patients with gastrointestinal cancers? *Cancers (Basel)* **2021**; 13: 3536. doi: 10.3390/cancers13143536
 109. Casak SJ, Donoghue M, Fashoyin-Aje L, Jiang X, Rodriguez L, Shen YL, et al. FDA approval summary: atezolizumab plus bevacizumab for the treatment of patients with advanced unresectable or metastatic hepatocellular carcinoma. *Clin Cancer Res* **2021**; 27: 1836-41. doi: 10.1158/1078-0432.Ccr-20-3407
 110. Saung MT, Pelosof L, Casak S, Donoghue M, Lemery S, Yuan M, et al. FDA approval summary: nivolumab plus ipilimumab for the treatment of patients with hepatocellular carcinoma previously treated with sorafenib. *Oncologist* **2021**; 26: 797-806. doi: 10.1002/onco.13819
 111. Zhu AX, Finn RS, Edeline J, Cattani S, Ogasawara S, Palmer D, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a

- non-randomised, open-label phase 2 trial. *Lancet Oncol* **2018**; 19: 940-52. doi: 10.1016/s1470-2045(18)30351-6
112. Spratlin JL, Cohen RB, Eadens M, Gore L, Camidge DR, Diab S, et al. Phase I pharmacologic and biologic study of ramucirumab (IMC-1121B), a fully human immunoglobulin G1 monoclonal antibody targeting the vascular endothelial growth factor receptor-2. *J Clin Oncol* **2010**; 28: 780-7. doi: 10.1200/jco.2009.23.7537
 113. Zhu AX, Duda DG, Sahani DV, Jain RK. HCC and angiogenesis: possible targets and future directions. *Nat Rev Clin Oncol* **2011**; 8: 292-301. doi: 10.1038/nrclinonc.2011.30
 114. Huang J, Zhang X, Tang Q, Zhang F, Li Y, Feng Z, et al. Prognostic significance and potential therapeutic target of VEGFR2 in hepatocellular carcinoma. *J Clin Pathol* **2011**; 64: 343-8. doi: 10.1136/jcp.2010.085142
 115. Zhu AX, Finn RS, Mulcahy M, Gurtler J, Sun W, Schwartz JD, et al. A phase II and biomarker study of ramucirumab, a human monoclonal antibody targeting the VEGF receptor-2, as first-line monotherapy in patients with advanced hepatocellular cancer. *Clin Cancer Res* **2013**; 19: 6614-23. doi: 10.1158/1078-0432.Ccr-13-1442
 116. Meng W, Li X, Bai Z, Li Y, Yuan J, Liu T, et al. Silencing alpha-fetoprotein inhibits VEGF and MMP-2/9 production in human hepatocellular carcinoma cell. *PLoS One* **2014**; 9: e90660. doi: 10.1371/journal.pone.0090660
 117. Chen LT, Oh DY, Ryu MH, Yeh KH, Yeo W, Carlesi R, et al. Anti-angiogenic therapy in patients with advanced gastric and gastroesophageal junction cancer: a systematic review. *Cancer Res Treat* **2017**; 49: 851-68. doi: 10.4143/crt.2016.176
 118. Roviello G, Sohbani N, Petrioli R, Rodriquenz MG. Ramucirumab as a second line therapy for advanced HCC: a significant achievement or a wasted opportunity for personalised therapy? *Invest New Drugs* **2019**; 37: 1274-88. doi: 10.1007/s10637-019-00760-0
 119. Zhu AX, Park JO, Ryoo BY, Yen CJ, Poon R, Pastorelli D, et al. Ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib (REACH): a randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol* **2015**; 16: 859-70. doi: 10.1016/s1470-2045(15)00050-9
 120. Kanogawa N, Ogasawara S, Maruta S, Iino Y, Obu M, Ishino T, et al. Use of ramucirumab for various treatment lines in real-world practice of patients with advanced hepatocellular carcinoma. *BMC Gastroenterol* **2023**; 23: 70. doi: 10.1186/s12876-023-02674-x
 121. Montal R, Andreu-Oller C, Bassaganyas L, Esteban-Fabré R, Moran S, Montironi C, et al. Molecular portrait of high alpha-fetoprotein in hepatocellular carcinoma: implications for biomarker-driven clinical trials. *Br J Cancer* **2019**; 121: 340-3. doi: 10.1038/s41416-019-0513-7
 122. Pessino G, Scotti C, Maggi M, Immuno-Hub C. Hepatocellular carcinoma: old and emerging therapeutic targets. *Cancers (Basel)* **2024**; 16: 901. doi: 10.3390/cancers16050901
 123. Wang Y, Liu D, Zhang T, Xia L. FGF/FGFR signaling in hepatocellular carcinoma: from carcinogenesis to recent therapeutic intervention. *Cancers (Basel)* **2021**; 13: 1360. doi: 10.3390/cancers13061360
 124. Gauglhofer C, Sagmeister S, Schrottmaier W, Fischer C, Rodgarkia-Dara C, Mohr T, et al. Up-regulation of the fibroblast growth factor 8 subfamily in human hepatocellular carcinoma for cell survival and neoangiogenesis. *Hepatology* **2011**; 53: 854-64. doi: 10.1002/hep.24099
 125. Qiu WH, Zhou BS, Chu PG, Chen WG, Chung C, Shih J, et al. Over-expression of fibroblast growth factor receptor 3 in human hepatocellular carcinoma. *World J Gastroenterol* **2005**; 11: 5266-72. doi: 10.3748/wjg.v11.i34.5266
 126. Paur J, Nika L, Maier C, Moscu-Gregor A, Kostka J, Huber D, et al. Fibroblast growth factor receptor 3 isoforms: novel therapeutic targets for hepatocellular carcinoma? *Hepatology* **2015**; 62: 1767-78. doi: 10.1002/hep.28023
 127. Raja A, Park I, Haq F, Ahn SM. FGF19-FGFR4 signaling in hepatocellular carcinoma. *Cells* **2019**; 8: 536. doi: 10.3390/cells8060536
 128. Elmas A, Lujambio A, Huang KL. Proteomic analyses identify therapeutic targets in hepatocellular carcinoma. *Front Oncol* **2022**; 12: 814120. doi: 10.3389/fonc.2022.814120
 129. Wang H, Yang J, Zhang K, Liu J, Li Y, Su W, et al. Advances of fibroblast growth factor/receptor signaling pathway in hepatocellular carcinoma and its pharmacotherapeutic targets. *Front Pharmacol* **2021**; 12: 650388. doi: 10.3389/fphar.2021.650388
 130. Asada N, Tanaka Y, Hayashido Y, Toratani S, Kan M, Kitamoto M, et al. Expression of fibroblast growth factor receptor genes in human hepatoma-derived cell lines. *In Vitro Cell Dev Biol Anim* **2003**; 39: 321-8. doi: 10.1290/1543-706x(2003)039<0321:Eofgr>2.0.Co;2
 131. Sandhu DS, Baichoo E, Roberts LR. Fibroblast growth factor signaling in liver carcinogenesis. *Hepatology* **2014**; 59: 1166-73. doi: 10.1002/hep.26679
 132. Cheng AL, Shen YC, Zhu AX. Targeting fibroblast growth factor receptor signaling in hepatocellular carcinoma. *Oncology* **2011**; 81: 372-80. doi: 10.1159/000335472
 133. Poon RT, Ng IO, Lau C, Yu WC, Fan ST, Wong J. Correlation of serum basic fibroblast growth factor levels with clinicopathologic features and postoperative recurrence in hepatocellular carcinoma. *Am J Surg* **2001**; 182: 298-304. doi: 10.1016/s0002-9610(01)00708-5
 134. Midorikawa Y, Ishikawa S, Iwanari H, Imamura T, Sakamoto H, Miyazono K, et al. Glypican-3, overexpressed in hepatocellular carcinoma, modulates FGF2 and BMP-7 signaling. *Int J Cancer* **2003**; 103: 455-65. doi: 10.1002/ijc.10856
 135. Sun H, Song X, Li C, Li Q, Liu S, Deng N. Humanized disulfide-stabilized diabody against fibroblast growth factor-2 inhibits PD-L1 expression and epithelial-mesenchymal transition in hepatoma cells through STAT3. *IUBMB Life* **2023**; 75: 957-68. doi: 10.1002/iub.2766
 136. Pei Y, Sun X, Guo X, Yin H, Wang L, Tian F, et al. FGF8 promotes cell proliferation and resistance to EGFR inhibitors via upregulation of EGFR in human hepatocellular carcinoma cells. *Oncol Rep* **2017**; 38: 2205-10. doi: 10.3892/or.2017.5887
 137. Lin ZZ, Hsu C, Jeng YM, Hu FC, Pan HW, Wu YM, et al. Klotho-beta and fibroblast growth factor 19 expression correlates with early recurrence of resectable hepatocellular carcinoma. *Liver Int* **2019**; 39: 1682-91. doi: 10.1111/liv.14055
 138. Sheu MJ, Hsieh MJ, Chiang WL, Yang SF, Lee HL, Lee LM, et al. Fibroblast growth factor receptor 4 polymorphism is associated with liver cirrhosis in hepatocarcinoma. *PLoS One* **2015**; 10: e0122961. doi: 10.1371/journal.pone.0122961
 139. Gu Q, Zhang B, Sun H, Xu Q, Tan Y, Wang G, et al. Genomic characterization of a large panel of patient-derived hepatocellular carcinoma xenograft tumor models for preclinical development. *Oncotarget* **2015**; 6: 20160-76. doi: 10.18632/oncotarget.3969
 140. Liu WY, Xie DM, Zhu GQ, Huang GQ, Lin YQ, Wang LR, et al. Targeting fibroblast growth factor 19 in liver disease: a potential biomarker and therapeutic target. *Expert Opin Ther Targets* **2015**; 19: 675-85. doi: 10.1517/14728222.2014.997711
 141. Lin BC, Desnoyers LR. FGF19 and cancer. *Adv Exp Med Biol* **2012**; 728: 183-94. doi: 10.1007/978-1-4614-0887-1_12
 142. French DM, Lin BC, Wang M, Adams C, Shek T, Hötzel K, et al. Targeting FGFR4 inhibits hepatocellular carcinoma in preclinical mouse models. *PLoS One* **2012**; 7: e36713. doi: 10.1371/journal.pone.0036713
 143. Ho HK, Pok S, Streit S, Ruhe JE, Hart S, Lim KS, et al. Fibroblast growth factor receptor 4 regulates proliferation, anti-apoptosis and alpha-fetoprotein secretion during hepatocellular carcinoma progression and represents a potential target for therapeutic intervention. *J Hepatol* **2009**; 50: 118-27. doi: 10.1016/j.jhep.2008.08.015
 144. Yang H, Fang F, Chang R, Yang L. MicroRNA-140-5p suppresses tumor growth and metastasis by targeting transforming growth factor β receptor 1 and fibroblast growth factor 9 in hepatocellular carcinoma. *Hepatology* **2013**; 58: 205-17. doi: 10.1002/hep.26315

145. Naugler WE, Tarlow BD, Fedorov LM, Taylor M, Pelz C, Li B, et al. Fibroblast growth factor signaling controls liver size in mice with humanized livers. *Gastroenterology* **2015**; 149: 728-40.e15. doi: 10.1053/j.gastro.2015.05.043
146. Sawey ET, Chanrion M, Cai C, Wu G, Zhang J, Zender L, et al. Identification of a therapeutic strategy targeting amplified FGF19 in liver cancer by oncogenomic screening. *Cancer Cell* **2011**; 19: 347-58. doi: 10.1016/j.ccr.2011.01.040
147. Miura S, Mitsuhashi N, Shimizu H, Kimura F, Yoshidome H, Otsuka M, et al. Fibroblast growth factor 19 expression correlates with tumor progression and poorer prognosis of hepatocellular carcinoma. *BMC Cancer* **2012**; 12: 56. doi: 10.1186/1471-2407-12-56
148. Qin Y, Han S, Yu Y, Qi D, Ran M, Yang M, et al. Lenvatinib in hepatocellular carcinoma: resistance mechanisms and strategies for improved efficacy. *Liver Int* **2024**; 44: 1808-31. doi: 10.1111/liv.15953
149. Kudo M. Lenvatinib may drastically change the treatment landscape of hepatocellular carcinoma. *Liver Cancer* **2018**; 7: 1-19. doi: 10.1159/000487148
150. Hu B, Zou T, Qin W, Shen X, Su Y, Li J, et al. Inhibition of EGFR overcomes acquired lenvatinib resistance driven by STAT3-ABCB1 signaling in hepatocellular carcinoma. *Cancer Res* **2022**; 82: 3845-57. doi: 10.1158/0008-5472.Can-21-4140
151. Ao J, Chiba T, Shibata S, Kurosugi A, Qiang N, Ma Y, et al. Acquisition of mesenchymal-like phenotypes and overproduction of angiogenic factors in lenvatinib-resistant hepatocellular carcinoma cells. *Biochem Biophys Res Commun* **2021**; 549: 171-8. doi: 10.1016/j.bbrc.2021.02.097
152. Safri F, Nguyen R, Zerehpooeshneschi S, George J, Qiao L. Heterogeneity of hepatocellular carcinoma: from mechanisms to clinical implications. *Cancer Gene Ther* **2024**; 31: 1105-12. doi: 10.1038/s41417-024-00764-w
153. Chan LK, Tsui YM, Ho DW, Ng IO. Cellular heterogeneity and plasticity in liver cancer. *Semin Cancer Biol* **2022**; 82: 134-49. doi: 10.1016/j.semcancer.2021.02.015
154. Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol* **2018**; 15: 81-94. doi: 10.1038/nrclinonc.2017.166
155. Dong LQ, Peng LH, Ma LJ, Liu DB, Zhang S, Luo SZ, et al. Heterogeneous immunogenomic features and distinct escape mechanisms in multifocal hepatocellular carcinoma. *J Hepatol* **2020**; 72: 896-908. doi: 10.1016/j.jhep.2019.12.014
156. Wang YF, Yuan SX, Jiang H, Li ZX, Yin HZ, Tan J, et al. Spatial maps of hepatocellular carcinoma transcriptomes reveal spatial expression patterns in tumor immune microenvironment. *Theranostics* **2022**; 12: 4163-80. doi: 10.7150/thno.71873
157. Friemel J, Frick L, Unger K, Egger M, Parrotta R, Böge YT, et al. Characterization of HCC mouse models: towards an etiology-oriented subtyping approach. *Mol Cancer Res* **2019**; 17: 1493-502. doi: 10.1158/1541-7786.Mcr-18-1045
158. Liu J, Dang H, Wang XW. The significance of intertumor and intratumor heterogeneity in liver cancer. *Exp Mol Med* **2018**; 50: e416. doi: 10.1038/emmm.2017.165
159. Kalasekar SM, VanSant-Webb CH, Evason KJ. Intratumor heterogeneity in hepatocellular carcinoma: challenges and opportunities. *Cancers (Basel)* **2021**; 13: 5524. doi: 10.3390/cancers13215524
160. Torbenson MS. Hepatocellular carcinoma: making sense of morphological heterogeneity, growth patterns, and subtypes. *Hum Pathol* **2021**; 112: 86-101. doi: 10.1016/j.humpath.2020.12.009
161. Bhat GR, Sethi I, Sadida HQ, Rah B, Mir R, Algehainy N, et al. Cancer cell plasticity: from cellular, molecular, and genetic mechanisms to tumor heterogeneity and drug resistance. *Cancer Metastasis Rev* **2024**; 43: 197-228. doi: 10.1007/s10555-024-10172-z
162. Watanabe K, Villarreal-Ponce A, Sun P, Salmans ML, Fallahi M, Andersen B, et al. Mammary morphogenesis and regeneration require the inhibition of EMT at terminal end buds by Ovol2 transcriptional repressor. *Dev Cell* **2014**; 29: 59-74. doi: 10.1016/j.devcel.2014.03.006
163. Hong T, Watanabe K, Ta CH, Villarreal-Ponce A, Nie Q, Dai X. An Ovol2-Zeb1 mutual inhibitory circuit governs bidirectional and multi-step transition between epithelial and mesenchymal states. *PLoS Comput Biol* **2015**; 11: e1004569. doi: 10.1371/journal.pcbi.1004569
164. Mooney SM, Talebian V, Jolly MK, Jia D, Gromala M, Levine H, et al. The GRHL2/ZEB feedback loop-a key axis in the regulation of EMT in breast cancer. *J Cell Biochem* **2017**; 118: 2559-70. doi: 10.1002/jcb.25974
165. Haensel D, Sun P, MacLean AL, Ma X, Zhou Y, Stemmler MP, et al. An Ovol2-Zeb1 transcriptional circuit regulates epithelial directional migration and proliferation. *EMBO Rep* **2019**; 20: e46273. doi: 10.15252/embr.201846273
166. Yu Q, Shi H, Ding Z, Wang Z, Yao H, Lin R. The E3 ubiquitin ligase TRIM31 attenuates NLRP3 inflammasome activation in *Helicobacter pylori*-associated gastritis by regulating ROS and autophagy. *Cell Commun Signal* **2023**; 21: 1. doi: 10.1186/s12964-022-00954-9
167. Ma J, Wang P, Liu Y, Zhao L, Li Z, Xue Y. Krüppel-like factor 4 regulates blood-tumor barrier permeability via ZO-1, occludin and claudin-5. *J Cell Physiol* **2014**; 229: 916-26. doi: 10.1002/jcp.24523
168. Peinado H, Portillo F, Cano A. Transcriptional regulation of cadherins during development and carcinogenesis. *Int J Dev Biol* **2004**; 48: 365-75. doi: 10.1387/ijdb.041794hp
169. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* **2004**; 117: 927-39. doi: 10.1016/j.cell.2004.06.006
170. Nawshad A, Hay ED. TGFβ3 signaling activates transcription of the LEF1 gene to induce epithelial mesenchymal transformation during mouse palate development. *J Cell Biol* **2003**; 163: 1291-301. doi: 10.1083/jcb.200306024
171. Battle E, Sancho E, Francí C, Domínguez D, Monfar M, Baulida J, et al. The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nat Cell Biol* **2000**; 2: 84-9. doi: 10.1038/35000034
172. Cano A, Pérez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, et al. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol* **2000**; 2: 76-83. doi: 10.1038/35000025
173. Yook JI, Li XY, Ota I, Hu C, Kim HS, Kim NH, et al. A Wnt-Axin2-GSK3β cascade regulates Snail1 activity in breast cancer cells. *Nat Cell Biol* **2006**; 8: 1398-406. doi: 10.1038/ncb1508
174. Jiang L, Li L, Liu Y, Lu L, Zhan M, Yuan S, et al. Drug resistance mechanism of kinase inhibitors in the treatment of hepatocellular carcinoma. *Front Pharmacol* **2023**; 14: 1097277. doi: 10.3389/fphar.2023.1097277
175. Huynh H. Molecularly targeted therapy in hepatocellular carcinoma. *Biochem Pharmacol* **2010**; 80: 550-60. doi: 10.1016/j.bcp.2010.03.034
176. Zheng J, Wang S, Xia L, Sun Z, Chan KM, Bernards R, et al. Hepatocellular carcinoma: signaling pathways and therapeutic advances. *Signal Transduct Target Ther* **2025**; 10: 35. doi: 10.1038/s41392-024-02075-w
177. Guo J, Zhao J, Xu Q, Huang D. Resistance of lenvatinib in hepatocellular carcinoma. *Curr Cancer Drug Targets* **2022**; 22: 865-78. doi: 10.2174/1568009622666220428111327
178. Matsuki M, Hoshi T, Yamamoto Y, Ikemori-Kawada M, Minoshima Y, Funahashi Y, et al. Lenvatinib inhibits angiogenesis and tumor fibroblast growth factor signaling pathways in human hepatocellular carcinoma models. *Cancer Med* **2018**; 7: 2641-53. doi: 10.1002/cam4.1517
179. Long H, Zhou J, Zhou C, Xie S, Wang J, Tan M, et al. Proteomic characterization of liver cancer cells treated with clinical targeted drugs for hepatocellular carcinoma. *Biomedicines* **2025**; 13: 152. doi: 10.3390/biomedicines13010152
180. Santoni M, Iacovelli R, Colonna V, Klinz S, Mauri G, Nuti M.

- Antitumor effects of the multi-target tyrosine kinase inhibitor cabozantinib: a comprehensive review of the preclinical evidence. *Expert Rev Anticancer Ther* **2021**; 21: 1029-54. doi: 10.1080/14737140.2021.1919090
181. Taha AM, Aboulwafa MM, Zedan H, Helmy OM. Ramucirumab combination with sorafenib enhances the inhibitory effect of sorafenib on HepG2 cancer cells. *Sci Rep* **2022**; 12: 17889. doi: 10.1038/s41598-022-21582-w
 182. Kudo M. Combination immunotherapy with anti-VEGF/TKI for hepatocellular carcinoma: present and future perspective. *Hepatobiliary Surg Nutr* **2021**; 10: 241-5. doi: 10.21037/hbsn-20-707
 183. Xie H, Alem Glison DM, Kim RD. FGFR4 inhibitors for the treatment of hepatocellular carcinoma: a synopsis of therapeutic potential. *Expert Opin Investig Drugs* **2022**; 31: 393-400. doi: 10.1080/13543784.2022.2017879
 184. Hsu CH, Huang YH, Lin SM, Hsu C. AXL and MET in hepatocellular carcinoma: a systematic literature review. *Liver Cancer* **2022**; 11: 94-112. doi: 10.1159/000520501
 185. Yao S, Liu X, Feng Y, Li Y, Xiao X, Han Y, et al. Unveiling the role of HGF/c-Met signaling in non-small cell lung cancer tumor microenvironment. *Int J Mol Sci* **2024**; 25: 9101. doi: 10.3390/ijms25169101
 186. Fu J, Su X, Li Z, Deng L, Liu X, Feng X, et al. HGF/c-Met pathway in cancer: from molecular characterization to clinical evidence. *Oncogene* **2021**; 40: 4625-51. doi: 10.1038/s41388-021-01863-w
 187. Centuori SM, Bauman JE. c-Met signaling as a therapeutic target in head and neck cancer. *Cancer J* **2022**; 28: 346-53. doi: 10.1097/ppo.0000000000000619
 188. Yadav M, Sharma A, Patne K, Tabasum S, Suryavanshi J, Rawat L, et al. AXL signaling in cancer: from molecular insights to targeted therapies. *Signal Transduct Target Ther* **2025**; 10: 37. doi: 10.1038/s41392-024-02121-7
 189. Lai YL, Wang KH, Hsieh HP, Yen WC. Novel FLT3/AURK multikinase inhibitor is efficacious against sorafenib-refractory and sorafenib-resistant hepatocellular carcinoma. *J Biomed Sci* **2022**; 29: 5. doi: 10.1186/s12929-022-00788-0
 190. Deng GL, Zeng S, Shen H. Chemotherapy and target therapy for hepatocellular carcinoma: new advances and challenges. *World J Hepatol* **2015**; 7: 787-98. doi: 10.4254/wjh.v7.i5.787
 191. Wilhelm SM, Dumas J, Adnane L, Lynch M, Carter CA, Schütz G, et al. Regorafenib (BAY 73-4506): a new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. *Int J Cancer* **2011**; 129: 245-55. doi: 10.1002/ijc.25864
 192. Tai WT, Chu PY, Shiao CW, Chen YL, Li YS, Hung MH, et al. STAT3 mediates regorafenib-induced apoptosis in hepatocellular carcinoma. *Clin Cancer Res* **2014**; 20: 5768-76. doi: 10.1158/1078-0432.Ccr-14-0725
 193. Debaillon Vesque A, Decraecker M, Blanc JF. Profile of cabozantinib for the treatment of hepatocellular carcinoma: patient selection and special considerations. *J Hepatocell Carcinoma* **2020**; 7: 91-9. doi: 10.2147/jhc.S195570
 194. Hsu YC, Coumar MS, Wang WC, Shiao HY, Ke YY, Lin WH, et al. Discovery of BPR1K871, a quinazoline based, multi-kinase inhibitor for the treatment of AML and solid tumors: rational design, synthesis, in vitro and in vivo evaluation. *Oncotarget* **2016**; 7: 86239-56. doi: 10.18632/oncotarget.13369
 195. Chen B, Sirota M, Fan-Minogue H, Hadley D, Butte AJ. Relating hepatocellular carcinoma tumor samples and cell lines using gene expression data in translational research. *BMC Med Genomics* **2015**; 8: S5. doi: 10.1186/1755-8794-8-s2-s5
 196. DuPrie ML, Palacio T, Calil FA, Kolodner RD, Putnam CD. Mlh1 interacts with both Msh2 and Msh6 for recruitment during mismatch repair. *DNA Repair (Amst)* **2022**; 119: 103405. doi: 10.1016/j.dnarep.2022.103405
 197. Munugala N, Maithel SK, Shroff RT. Novel biomarkers and the future of targeted therapies in cholangiocarcinoma: a narrative review. *Hepatobiliary Surg Nutr* **2022**; 11: 253-66. doi: 10.21037/hbsn-20-475
 198. Javle M, Churi C, Kang HC, Shroff R, Janku F, Surapaneni R, et al. HER2/neu-directed therapy for biliary tract cancer. *J Hematol Oncol* **2015**; 8: 58. doi: 10.1186/s13045-015-0155-z
 199. Hadfield MJ, DeCarli K, Bash K, Sun G, Almhanna K. Current and emerging therapeutic targets for the treatment of cholangiocarcinoma: an updated review. *Int J Mol Sci* **2023**; 25: 543. doi: 10.3390/ijms25010543
 200. Opreescu Macovei AM, Venter DP, Makkai GG, Valcea S, Venter MD, Tulin A, et al. Options in targeted therapy for advanced cholangiocarcinoma: a 2024 update. *Cureus* **2024**; 16: e59793. doi: 10.7759/cureus.59793
 201. Ayasun R, Ozer M, Sahin I. The role of HER2 status in the biliary tract cancers. *Cancers (Basel)* **2023**; 15: 2628. doi: 10.3390/cancers15092628
 202. Wang Z, Liu Z, Lv M, Luan Z, Li T, Hu J. Novel histone modifications and liver cancer: emerging frontiers in epigenetic regulation. *Clin Epigenetics* **2025**; 17: 30. doi: 10.1186/s13148-025-01838-8
 203. Braghini MR, Lo Re O, Romito I, Fernandez-Barrena MG, Barbaro B, Pomella S, et al. Epigenetic remodelling in human hepatocellular carcinoma. *J Exp Clin Cancer Res* **2022**; 41: 107. doi: 10.1186/s13046-022-02297-2
 204. Herman JG, Latif F, Weng Y, Lerman MI, Zbar B, Liu S, et al. Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. *Proc Natl Acad Sci U S A* **1994**; 91: 9700-4. doi: 10.1073/pnas.91.21.9700
 205. Esteller M, Silva JM, Dominguez G, Bonilla F, Matias-Guiu X, Lema E, et al. Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. *J Natl Cancer Inst* **2000**; 92: 564-9. doi: 10.1093/jnci/92.7.564
 206. Esteller M, Avizienyte E, Corn PG, Lothe RA, Baylin SB, Aaltonen LA, et al. Epigenetic inactivation of LKB1 in primary tumors associated with the Peutz-Jeghers syndrome. *Oncogene* **2000**; 19: 164-8. doi: 10.1038/sj.onc.1203227
 207. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet* **2002**; 3: 415-28. doi: 10.1038/nrg816
 208. Nuovo GJ, Plaia TW, Belinsky SA, Baylin SB, Herman JG. In situ detection of the hypermethylation-induced inactivation of the p16 gene as an early event in oncogenesis. *Proc Natl Acad Sci U S A* **1999**; 96: 12754-9. doi: 10.1073/pnas.96.22.12754
 209. Esteller M, Risques RA, Toyota M, Capella G, Moreno V, Peinado MA, et al. Promoter hypermethylation of the DNA repair gene O6-methylguanine-DNA methyltransferase is associated with the presence of G:C to A:T transition mutations in p53 in human colorectal tumorigenesis. *Cancer Res* **2001**; 61: 4689-92.
 210. Esteller M, Hamilton SR, Burger PC, Baylin SB, Herman JG. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Res* **1999**; 59: 793-7.
 211. van Attikum H, Gasser SM. Crosstalk between histone modifications during the DNA damage response. *Trends Cell Biol* **2009**; 19: 207-17. doi: 10.1016/j.tcb.2009.03.001
 212. Bitzer M, Horger M, Giannini EG, Ganten TM, Wörns MA, Siveke JT, et al. Resminostat plus sorafenib as second-line therapy of advanced hepatocellular carcinoma - the SHELTER study. *J Hepatol* **2016**; 65: 280-8. doi: 10.1016/j.jhep.2016.02.043
 213. Bugide S, Green MR, Wajapeyee N. Inhibition of enhancer of zeste homolog 2 (EZH2) induces natural killer cell-mediated eradication of hepatocellular carcinoma cells. *Proc Natl Acad Sci U S A* **2018**; 115: E3509-18. doi: 10.1073/pnas.1802691115
 214. Hong YK, Li Y, Pandit H, Li S, Pulliam Z, Zheng Q, et al. Epigenetic modulation enhances immunotherapy for hepatocellular carcinoma. *Cell Immunol* **2019**; 336: 66-74. doi: 10.1016/j.cellimm.2018.12.010
 215. Toh TB, Lim JJ, Chow EK. Epigenetics of hepatocellular carcinoma. *Clin Transl Med* **2019**; 8: 13. doi: 10.1186/s40169-019-0230-0
 216. Shi Z, Nicholson RH, Jaggi R, Nicholson AW. Characterization of *Aquifex aeolicus* ribonuclease III and the reactivity epitopes of its

- pre-ribosomal RNA substrates. *Nucleic Acids Res* **2011**; 39: 2756-68. doi: 10.1093/nar/gkq1030
217. Borel F, Konstantinova P, Jansen PL. Diagnostic and therapeutic potential of miRNA signatures in patients with hepatocellular carcinoma. *J Hepatol* **2012**; 56: 1371-83. doi: 10.1016/j.jhep.2011.11.026
 218. Bushati N, Cohen SM. MicroRNA functions. *Annu Rev Cell Dev Biol* **2007**; 23: 175-205. doi: 10.1146/annurev.cellbio.23.090506.123406
 219. Saini HK, Griffiths-Jones S, Enright AJ. Genomic analysis of human microRNA transcripts. *Proc Natl Acad Sci U S A* **2007**; 104: 17719-24. doi: 10.1073/pnas.0703890104
 220. Lee Y, Jeon K, Lee JT, Kim S, Kim VN. MicroRNA maturation: stepwise processing and subcellular localization. *EMBO J* **2002**; 21: 4663-70. doi: 10.1093/emboj/cdf476
 221. Griffiths-Jones S. Annotating noncoding RNA genes. *Annu Rev Genomics Hum Genet* **2007**; 8: 279-98. doi: 10.1146/annurev.genom.8.080706.092419
 222. Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* **2000**; 404: 293-6. doi: 10.1038/35005107
 223. Lee KJ, Singh N, Bizuneh M, Kim NH, Kim HS, Kim Y, et al. miR-429 suppresses endometrial cancer progression and drug resistance via DDX53. *J Pers Med* **2023**; 13: 1302. doi: 10.3390/jpm13091302
 224. Habel A, Nassar F, Itani M, Bouaziz H, Hadj-Ahmed M, Msheik Z, et al. miR-21 and miR-125b as theranostic biomarkers for epithelial ovarian cancer in Tunisian women. *Afr Health Sci* **2023**; 23: 256-64. doi: 10.4314/ahs.v23i2.29
 225. Yang Q, Fu J, Wang M, Fang Y, Fu J. MiR-1299 is regulated by KCNQ1OT1 and inhibits cervical cancer progression. *Cell Mol Biol (Noisy-le-grand)* **2023**; 69: 166-73. doi: 10.14715/cmb/2023.69.10.24
 226. Zhang L, Ge R, Cheng A, Hu T. MiR-1284 suppresses the proliferation and migration of thyroid cancer. *Cell Mol Biol (Noisy-le-grand)* **2023**; 69: 246-9. doi: 10.14715/cmb/2023.69.8.38
 227. Chen ZH, Chen YB, Yue HR, Zhou XJ, Ma HY, Wang X, et al. PAX5-miR-142 feedback loop promotes breast cancer proliferation by regulating DNMT1 and ZEB1. *Mol Med* **2023**; 29: 89. doi: 10.1186/s10020-023-00681-y
 228. Zhang Q, Zheng K, Gao Y, Zhao S, Zhao Y, Li W, et al. Plasma exosomal miR-1290 and miR-29c-3p as diagnostic biomarkers for lung cancer. *Heliyon* **2023**; 9: e21059. doi: 10.1016/j.heliyon.2023.e21059
 229. Li Y, Zeng S, Cao L. Mir-629 repressed LATS2 expression and promoted the proliferation of prostate cancer cells. *Horm Metab Res* **2023**; 55: 573-9. doi: 10.1055/a-2065-0954
 230. Liu MX, Chu KM. MiR-410-3p suppresses primary gastric cancer and exosomes regulate endogenous expression of miR-410-3p. *Am J Cancer Res* **2023**; 13: 2670-80.
 231. Xie H, Li J, Lu M, Zhang R, Mao H. MiR-1180 targets FXYD5 to regulate pancreatic cancer cells migration and invasion. *Mol Biotechnol* **2024**; 66: 3182-94. doi: 10.1007/s12033-023-00923-8
 232. Fan C, Wang Q, Kuipers TB, Cats D, Iyengar PV, Hagenaars SC, et al. LncRNA LITAT1 suppresses TGF- β -induced EMT and cancer cell plasticity by potentiating T β RI degradation. *EMBO J* **2023**; 42: e112806. doi: 10.15252/embj.2022112806
 233. Xu J, Hu M, Gao Y, Wang Y, Yuan X, Yang Y, et al. LncRNA MIR17HG suppresses breast cancer proliferation and migration as ceRNA to target FAM135A by sponging miR-454-3p. *Mol Biotechnol* **2023**; 65: 2071-85. doi: 10.1007/s12033-023-00706-1
 234. Shi L, Li B, Zhang Y, Chen Y, Tan J, Chen Y, et al. Exosomal lncRNA Mir100hg derived from cancer stem cells enhance glycolysis and promote metastasis of lung adenocarcinoma through microRNA-15a-5p/31-5p. *Cell Commun Signal* **2023**; 21: 248. doi: 10.1186/s12964-023-01281-3
 235. Wu JH, Cheng TC, Zhu B, Gao HY, Zheng L, Chen WX. Identification of cuproptosis-related gene SLC31A1 and upstream lncRNA-miRNA regulatory axis in breast cancer. *Sci Rep* **2023**; 13: 18390. doi: 10.1038/s41598-023-45761-5
 236. Qian L, Li L, Li Y, Li S, Zhang B, Zhu Y, et al. LncRNA HOTAIR as a ceRNA is related to breast cancer risk and prognosis. *Breast Cancer Res Treat* **2023**; 200: 375-90. doi: 10.1007/s10549-023-06982-4
 237. Huang K, Chen X, Geng Z, Xiong X, Cong Y, Pan X, et al. LncRNA SLC25A21-AS1 increases the chemosensitivity and inhibits the progression of ovarian cancer by upregulating the expression of KCNK4. *Funct Integr Genomics* **2023**; 23: 110. doi: 10.1007/s10142-023-01035-x
 238. Jin Y, Qiu J, Lu X, Ma Y, Li G. LncRNA CACNA1G-AS1 up-regulates FTH1 to inhibit ferroptosis and promote malignant phenotypes in ovarian cancer cells. *Oncol Res* **2023**; 31: 169-79. doi: 10.32604/or.2023.027815
 239. Park S, Hall MN. Metabolic reprogramming in hepatocellular carcinoma: mechanisms and therapeutic implications. *Exp Mol Med* **2025**; 57: 515-23. doi: 10.1038/s12276-025-01415-2
 240. Wise DR, Thompson CB. Glutamine addiction: a new therapeutic target in cancer. *Trends Biochem Sci* **2010**; 35: 427-33. doi: 10.1016/j.tibs.2010.05.003
 241. Yang L, Venneti S, Nagrath D. Glutaminolysis: a hallmark of cancer metabolism. *Annu Rev Biomed Eng* **2017**; 19: 163-94. doi: 10.1146/annurev-bioeng-071516-044546
 242. Yang WH, Qiu Y, Stamatatos O, Janowitz T, Lukey MJ. Enhancing the efficacy of glutamine metabolism inhibitors in cancer therapy. *Trends Cancer* **2021**; 7: 790-804. doi: 10.1016/j.trecan.2021.04.003
 243. Björnson E, Mukhopadhyay B, Asplund A, Pristovsek N, Cinar R, Romeo S, et al. Stratification of hepatocellular carcinoma patients based on acetate utilization. *Cell Rep* **2015**; 13: 2014-26. doi: 10.1016/j.celrep.2015.10.045
 244. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab* **2008**; 7: 11-20. doi: 10.1016/j.cmet.2007.10.002
 245. Zhu WW, Lu M, Wang XY, Zhou X, Gao C, Qin LX. The fuel and engine: the roles of reprogrammed metabolism in metastasis of primary liver cancer. *Genes Dis* **2020**; 7: 299-307. doi: 10.1016/j.gendis.2020.01.016
 246. Yu D, Shi X, Meng G, Chen J, Yan C, Jiang Y, et al. Kidney-type glutaminase (GLS1) is a biomarker for pathologic diagnosis and prognosis of hepatocellular carcinoma. *Oncotarget* **2015**; 6: 7619-31. doi: 10.18632/oncotarget.3196
 247. Long J, Wang H, Lang Z, Wang T, Long M, Wang B. Expression level of glutamine synthetase is increased in hepatocellular carcinoma and liver tissue with cirrhosis and chronic hepatitis B. *Hepatol Int* **2011**; 5: 698-706. doi: 10.1007/s12072-010-9230-2
 248. Liu P, Lu D, Al-Ameri A, Wei X, Ling S, Li J, et al. Glutamine synthetase promotes tumor invasion in hepatocellular carcinoma through mediating epithelial-mesenchymal transition. *Hepatol Res* **2020**; 50: 246-57. doi: 10.1111/hepr.13433
 249. Sohn BH, Park IY, Shin JH, Yim SY, Lee JS. Glutamine synthetase mediates sorafenib sensitivity in β -catenin-active hepatocellular carcinoma cells. *Exp Mol Med* **2018**; 50: e421. doi: 10.1038/emmm.2017.174
 250. Tsujikawa H, Masugi Y, Yamazaki K, Itano O, Kitagawa Y, Sakamoto M. Immunohistochemical molecular analysis indicates hepatocellular carcinoma subgroups that reflect tumor aggressiveness. *Hum Pathol* **2016**; 50: 24-33. doi: 10.1016/j.humpath.2015.10.014
 251. Zhang B, Liu K, Zhang J, Dong L, Jin Z, Zhang X, et al. Glutamine synthetase predicts adjuvant TACE response in hepatocellular carcinoma. *Int J Clin Exp Med* **2015**; 8: 20722-31.
 252. Wang YS, Du L, Liang X, Meng P, Bi L, Wang YL, et al. Sirtuin 4 depletion promotes hepatocellular carcinoma tumorigenesis through regulating adenosine-monophosphate-activated protein kinase α /mammalian target of rapamycin axis in mice. *Hepatology* **2019**; 69: 1614-31. doi: 10.1002/hep.30421
 253. Adebayo Michael AO, Ko S, Tao J, Moghe A, Yang H, Xu M, et al. Inhibiting glutamine-dependent mTORC1 activation ameliorates liver cancers driven by β -catenin mutations. *Cell Metab* **2019**; 29: 1135-50.e6. doi: 10.1016/j.cmet.2019.01.002

254. Wei Y, Tang X, Ren Y, Yang Y, Song F, Fu J, et al. An RNA-RNA crosstalk network involving HMGB1 and RICTOR facilitates hepatocellular carcinoma tumorigenesis by promoting glutamine metabolism and impedes immunotherapy by PD-L1+exosomes activity. *Signal Transduct Target Ther* **2021**; 6: 421. doi: 10.1038/s41392-021-00801-2
255. Huang X, Gan G, Wang X, Xu T, Xie W. The HGF-MET axis coordinates liver cancer metabolism and autophagy for chemotherapeutic resistance. *Autophagy* **2019**; 15: 1258-79. doi: 10.1080/15548627.2019.1580105
256. Cheng Y, He J, Zuo B, He Y. Role of lipid metabolism in hepatocellular carcinoma. *Discov Oncol* **2024**; 15: 206. doi: 10.1007/s12672-024-01069-y
257. Batchuluun B, Pinkosky SL, Steinberg GR. Lipogenesis inhibitors: therapeutic opportunities and challenges. *Nat Rev Drug Discov* **2022**; 21: 283-305. doi: 10.1038/s41573-021-00367-2
258. Che L, Chi W, Qiao Y, Zhang J, Song X, Liu Y, et al. Cholesterol biosynthesis supports the growth of hepatocarcinoma lesions depleted of fatty acid synthase in mice and humans. *Gut* **2020**; 69: 177-86. doi: 10.1136/gutjnl-2018-317581
259. Wu D, Yang Y, Hou Y, Zhao Z, Liang N, Yuan P, et al. Increased mitochondrial fission drives the reprogramming of fatty acid metabolism in hepatocellular carcinoma cells through suppression of sirtuin 1. *Cancer Commun (Lond)* **2022**; 42: 37-55. doi: 10.1002/cac2.12247
260. Ni W, Lin S, Bian S, Zheng W, Qu L, Fan Y, et al. USP7 mediates pathological hepatic de novo lipogenesis through promoting stabilization and transcription of ZNF638. *Cell Death Dis* **2020**; 11: 843. doi: 10.1038/s41419-020-03075-8
261. Ye Z, Xiong Y, Peng W, Wei W, Huang L, Yue J, et al. Manipulation of PD-L1 endosomal trafficking promotes anticancer immunity. *Adv Sci (Weinh)* **2023**; 10: e2206411. doi: 10.1002/adv.202206411
262. Ning Z, Guo X, Liu X, Lu C, Wang A, Wang X, et al. USP22 regulates lipidome accumulation by stabilizing PPAR γ in hepatocellular carcinoma. *Nat Commun* **2022**; 13: 2187. doi: 10.1038/s41467-022-29846-9
263. Han Q, Chen CA, Yang W, Liang D, Lv HW, Lv GS, et al. ATP-citrate lyase regulates stemness and metastasis in hepatocellular carcinoma via the Wnt/ β -catenin signaling pathway. *Hepatobiliary Pancreat Dis Int* **2021**; 20: 251-61. doi: 10.1016/j.hbpd.2020.05.010
264. Liu HH, Xu Y, Li CJ, Hsu SJ, Lin XH, Zhang R, et al. An SCD1-dependent mechanoresponsive pathway promotes HCC invasion and metastasis through lipid metabolic reprogramming. *Mol Ther* **2022**; 30: 2554-67. doi: 10.1016/j.ymthe.2022.03.015
265. Gao B, Lu Y, Lai X, Xu X, Gou S, Yang Z, et al. Metabolic reprogramming in hepatocellular carcinoma: mechanisms of immune evasion and therapeutic implications. *Front Immunol* **2025**; 16: 1592837. doi: 10.3389/fimmu.2025.1592837
266. Dang CV. MYC on the path to cancer. *Cell* **2012**; 149: 22-35. doi: 10.1016/j.cell.2012.03.003
267. Zhang Z, Deng X, Liu Y, Liu Y, Sun L, Chen F. PKM2, function and expression and regulation. *Cell Biosci* **2019**; 9: 52. doi: 10.1186/s13578-019-0317-8
268. Wong KK, Engelman JA, Cantley LC. Targeting the PI3K signaling pathway in cancer. *Curr Opin Genet Dev* **2010**; 20: 87-90. doi: 10.1016/j.gde.2009.11.002
269. Kohn AD, Summers SA, Birnbaum MJ, Roth RA. Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. *J Biol Chem* **1996**; 271: 31372-8. doi: 10.1074/jbc.271.49.31372
270. Robey RB, Hay N. Mitochondrial hexokinases, novel mediators of the antiapoptotic effects of growth factors and Akt. *Oncogene* **2006**; 25: 4683-96. doi: 10.1038/sj.onc.1209595
271. Arai H, Elliott A, Xiu J, Wang J, Battaglin F, Kawanishi N, et al. The landscape of alterations in DNA damage response pathways in colorectal cancer. *Clin Cancer Res* **2021**; 27: 3234-42. doi: 10.1158/1078-0432.Ccr-20-3635
272. Catalano F, Borea R, Puglisi S, Boutros A, Gandini A, Cremante M, et al. Targeting the DNA damage response pathway as a novel therapeutic strategy in colorectal cancer. *Cancers (Basel)* **2022**; 14: 1388. doi: 10.3390/cancers14061388
273. Karnitz LM, Zou L. Molecular pathways: targeting ATR in cancer therapy. *Clin Cancer Res* **2015**; 21: 4780-5. doi: 10.1158/1078-0432.Ccr-15-0479
274. Lowery CD, VanWye AB, Dowless M, Blosser W, Falcon BL, Stewart J, et al. The checkpoint kinase 1 inhibitor prexasertib induces regression of preclinical models of human neuroblastoma. *Clin Cancer Res* **2017**; 23: 4354-63. doi: 10.1158/1078-0432.Ccr-16-2876
275. Parmar K, Kochupurakkal BS, Lazaro JB, Wang ZC, Palakurthi S, Kirschmeier PT, et al. The CHK1 inhibitor prexasertib exhibits monotherapy activity in high-grade serous ovarian cancer models and sensitizes to PARP inhibition. *Clin Cancer Res* **2019**; 25: 6127-40. doi: 10.1158/1078-0432.Ccr-19-0448
276. Do KT, Kochupurakkal B, Kelland S, de Jonge A, Hedglin J, Powers A, et al. Phase 1 combination study of the CHK1 inhibitor prexasertib and the PARP inhibitor olaparib in high-grade serous ovarian cancer and other solid tumors. *Clin Cancer Res* **2021**; 27: 4710-6. doi: 10.1158/1078-0432.Ccr-21-1279
277. Lowery CD, Dowless M, Renschler M, Blosser W, VanWye AB, Stephens JR, et al. Broad-spectrum activity of the checkpoint kinase 1 inhibitor prexasertib as a single agent or chemopotentiator across a range of preclinical pediatric tumor models. *Clin Cancer Res* **2019**; 25: 2278-89. doi: 10.1158/1078-0432.Ccr-18-2728
278. Yao Y, Dou C, Lu Z, Zheng X, Liu Q. MACC1 suppresses cell apoptosis in hepatocellular carcinoma by targeting the HGF/c-MET/AKT pathway. *Cell Physiol Biochem* **2015**; 35: 983-96. doi: 10.1159/000369754
279. Fu R, Jiang S, Li J, Chen H, Zhang X. Activation of the HGF/c-MET axis promotes lenvatinib resistance in hepatocellular carcinoma cells with high c-MET expression. *Med Oncol* **2020**; 37: 24. doi: 10.1007/s12032-020-01350-4
280. Liu Y, Xu L, Dou Y, He Y. AXL: shapers of tumor progression and immunosuppressive microenvironments. *Mol Cancer* **2025**; 24: 11. doi: 10.1186/s12943-024-02210-9
281. Engelsen AS, Lotsberg ML, Abou Khouzam R, Thierry JP, Lorens JB, Chouaib S, et al. Dissecting the role of AXL in cancer immune escape and resistance to immune checkpoint inhibition. *Front Immunol* **2022**; 13: 869676. doi: 10.3389/fimmu.2022.869676
282. Luo X, He X, Zhang X, Zhao X, Zhang Y, Shi Y, et al. Hepatocellular carcinoma: signaling pathways, targeted therapy, and immunotherapy. *MedComm (2020)* **2024**; 5: e474. doi: 10.1002/mco2.474
283. Al-Awadhi SS, Patil P, Shetty P, Shetty PK, Shetty RA, Shetty VV. Potential role of epidermal growth factor receptors (EGFR) signaling in the pathogenesis and management of hepatocellular carcinoma. *Bioimpacts* **2025**; 15: 30905. doi: 10.34172/bi.30905
284. Cheng TC, Huang BM, Liao YC, Chang HS, Tu SH, Ho YS, et al. Fibroblast growth factor receptor four inhibitor FGF401 improves the efficacy of trastuzumab in FGFR4-overexpressing breast cancer cells. *Int J Cancer* **2025**; 156: 1606-20. doi: 10.1002/ijc.35271
285. Das D, Xie L, Qiao D, Jia J, Hong J. Discovery of novel, orally bioavailable phenylacetamide derivatives as multikinase inhibitors and in vivo efficacy study in hepatocellular carcinoma animal models. *Bioorg Med Chem Lett* **2024**; 113: 129971. doi: 10.1016/j.bmcl.2024.129971
286. Ladd AD, Duarte S, Sahin I, Zarrinpar A. Mechanisms of drug resistance in HCC. *Hepatology* **2024**; 79: 926-40. doi: 10.1097/hep.0000000000000237
287. Becht R, Kielbowski K, Wasilewicz MP. New opportunities in the systemic treatment of hepatocellular carcinoma-today and tomorrow. *Int J Mol Sci* **2024**; 25: 1456. doi: 10.3390/ijms25031456
288. Pinton G, Perucca M, Gigliotti V, Mantovani E, Clemente N, Malecka J, et al. EZH2-mediated H3K27 trimethylation in the liver of mice is an early epigenetic event induced by high-fat diet exposure. *Nutrients* **2024**; 16: 3260. doi: 10.3390/nu16193260
289. Garmpis N, Damaskos C, Garmpi A, Georgakopoulou VE, Sarantis P, Antoniou EA, et al. Histone deacetylase inhibitors

- in the treatment of hepatocellular carcinoma: current evidence and future opportunities. *J Pers Med* **2021**; 11: 223. doi: 10.3390/jpm11030223
290. Kielbowski K, Szwedkowicz A, Plewa P, Bakinowska E, Becht R, Pawlik A. Anticancer properties of histone deacetylase inhibitors - what is their potential? *Expert Rev Anticancer Ther* **2025**; 25: 105-20. doi: 10.1080/14737140.2025.2452338
 291. Cai H, Li X, Liu Y, Ke J, Liu K, Xie Y, et al. Decitabine-based nanoparticles for enhanced immunotherapy of hepatocellular carcinoma via DNA hypermethylation reversal. *Chem Eng J* **2024**; 492: 152175. doi: 10.1016/j.cej.2024.152175
 292. Song G, Yu X, Shi H, Sun B, Amateau S. miRNAs in HCC, pathogenesis, and targets. *Hepatology* **2024**. doi: 10.1097/hep.0000000000001177
 293. Ismail M, Fadul MM, Taha R, Siddig O, Elhafiz M, Yousef BA, et al. Dynamic role of exosomal long non-coding RNA in liver diseases: pathogenesis and diagnostic aspects. *Hepatol Int* **2024**; 18: 1715-30. doi: 10.1007/s12072-024-10722-1
 294. Hyroššová P, Milošević M, Škoda J, Vachtenheim J Jr, Rohlena J, Rohlenová K. Effects of metabolic cancer therapy on tumor microenvironment. *Front Oncol* **2022**; 12: 1046630. doi: 10.3389/fonc.2022.1046630
 295. Yoo HC, Han JM. Amino acid metabolism in cancer drug resistance. *Cells* **2022**; 11: 140. doi: 10.3390/cells11010140
 296. Kong X, Liu W, Zhang X, Zhou C, Sun X, Cheng L, et al. HIF-1 α inhibition in macrophages preserves acute liver failure by reducing IL-1 β production. *FASEB J* **2023**; 37: e23140. doi: 10.1096/fj.202300428RR
 297. Luo M, Li T, Sang H. The role of hypoxia-inducible factor 1 α in hepatic lipid metabolism. *J Mol Med (Berl)* **2023**; 101: 487-500. doi: 10.1007/s00109-023-02308-5
 298. Lin Z, Wang L, Xing Z, Wang F, Cheng X. Update on combination strategies of PARP inhibitors. *Cancer Control* **2024**; 31: 10732748241298329. doi: 10.1177/10732748241298329
 299. Yang XD, Kong FE, Qi L, Lin JX, Yan Q, Loong JH, et al. PARP inhibitor olaparib overcomes sorafenib resistance through reshaping the pluripotent transcriptome in hepatocellular carcinoma. *Mol Cancer* **2021**; 20: 20. doi: 10.1186/s12943-021-01315-9
 300. Gorecki L, Andrs M, Korabecny J. Clinical candidates targeting the ATR-CHK1-WEE1 axis in cancer. *Cancers (Basel)* **2021**; 13: 795. doi: 10.3390/cancers13040795
 301. Marzi L, Mega A, Gitto S, Pelizzaro F, Seeber A, Spizzo G. Impact and novel perspective of immune checkpoint inhibitors in patients with early and intermediate stage HCC. *Cancers (Basel)* **2022**; 14: 3332. doi: 10.3390/cancers14143332
 302. Shen W, Chen Y, Lei P, Sheldon M, Sun Y, Yao F, et al. Immunotherapeutic approaches for treating hepatocellular carcinoma. *Cancers (Basel)* **2022**; 14: 5013. doi: 10.3390/cancers14205013
 303. Brackenier C, Kinget L, Cappuyns S, Verslype C, Beuselinck B, Dekervel J. Unraveling the synergy between atezolizumab and bevacizumab for the treatment of hepatocellular carcinoma. *Cancers (Basel)* **2023**; 15: 348. doi: 10.3390/cancers15020348
 304. Kudo M. Scientific rationale for combined immunotherapy with PD-1/PD-L1 antibodies and VEGF inhibitors in advanced hepatocellular carcinoma. *Cancers (Basel)* **2020**; 12: 1089. doi: 10.3390/cancers12051089
 305. Krupa K, Fudalej M, Cencelewicz-Lesikow A, Badowska-Kozakiewicz A, Czerw A, Deptała A. Current treatment methods in hepatocellular carcinoma. *Cancers (Basel)* **2024**; 16: 4059. doi: 10.3390/cancers16234059
 306. Salani F, Genovesi V, Vivaldi C, Massa V, Cesario S, Bernardini L, et al. Primary resistance to immunotherapy-based regimens in first line hepatocellular carcinoma: perspectives on jumping the hurdle. *Cancers (Basel)* **2022**; 14: 4896. doi: 10.3390/cancers14194896
 307. Aram C, Alijanizadeh P, Saleki K, Karami L. Development of an ancestral DC and TLR4-inducing multi-epitope peptide vaccine against the spike protein of SARS-CoV and SARS-CoV-2 using the advanced immunoinformatics approaches. *Biochem Biophys Rep* **2024**; 39: 101745. doi: 10.1016/j.bbrep.2024.101745
 308. Kamali MJ, Saeedi F, Khoshghiafeh A, Aghajani Mir M, Aram C, Ahmadifard M. Therapeutic targeting of triple-negative breast cancer: a multi-model evaluation of LNA-anti-miR-19b-3p and small molecule inhibitors. *Comput Biol Med* **2025**; 196: 110771. doi: 10.1016/j.combiomed.2025.110771
 309. Federico P, Petrillo A, Giordano P, Bosso D, Fabbrocini A, Ottaviano M, et al. Immune checkpoint inhibitors in hepatocellular carcinoma: current status and novel perspectives. *Cancers (Basel)* **2020**; 12: 3025. doi: 10.3390/cancers12103025
 310. Chen Y, Hu H, Yuan X, Fan X, Zhang C. Advances in immune checkpoint inhibitors for advanced hepatocellular carcinoma. *Front Immunol* **2022**; 13: 896752. doi: 10.3389/fimmu.2022.896752
 311. Xing R, Gao J, Cui Q, Wang Q. Strategies to improve the antitumor effect of immunotherapy for hepatocellular carcinoma. *Front Immunol* **2021**; 12: 783236. doi: 10.3389/fimmu.2021.783236
 312. Wu D, Fan Y, Zhang M, Wang X, He X, Guo X, et al. Tumor-suppressing multi-enterobacteria and PD-1/PD-L1 immune checkpoint inhibitor combination improves the outcome of hepatocellular carcinoma therapy. *Front Immunol* **2025**; 16: 1598436. doi: 10.3389/fimmu.2025.1598436
 313. Cervello M, Emma MR, Augello G, Cusimano A, Giannitrapani L, Soresi M, et al. New landscapes and horizons in hepatocellular carcinoma therapy. *Aging (Albany NY)* **2020**; 12: 3053-94. doi: 10.18632/aging.102777
 314. Tan X, Li M, Zhao Z. Treatment options for non-colorectal cancer liver metastases. *J Cancer Treatment Diagn* **2020**; 4: 1-5.
 315. Satapathy T, Diwakar MK. Molecular targets and nano-technological approaches in the treatment of hepatic carcinoma. *Curr Cancer Drug Targets* **2024**. doi: 10.2174/0115680096336494240915164019
 316. Saleki K, Aram C, Alijanizadeh P, Khanmirzaei MH, Vaziri Z, Ramzankhah M, et al. Matrix metalloproteinase/Fas ligand (MMP/FasL) interaction dynamics in COVID-19: an in-silico study and neuroimmune perspective. *Heliyon* **2024**; 10: e30898. doi: 10.1016/j.heliyon.2024.e30898
 317. Juthani R, Malalur P, Manne A, Mittra A. The combined use of lenvatinib and locoregional therapies for the management of hepatocellular carcinoma. *Cancers (Basel)* **2025**; 17: 1572. doi: 10.3390/cancers17091572
 318. Lu MC, Huang WY, Fan HL, Chen TW, Chang WC, Lin HH, et al. Beneficial effect of combining radiotherapy and transarterial chemoembolization on patient survival in hepatocellular carcinomas and macrovascular invasion treated with sorafenib. *Cancers (Basel)* **2023**; 15: 2687. doi: 10.3390/cancers15102687
 319. Chakraborty E, Sarkar D. Emerging therapies for hepatocellular carcinoma (HCC). *Cancers (Basel)* **2022**; 14: 2798. doi: 10.3390/cancers14112798
 320. Wu FD, Zhou HF, Yang W, Zhu D, Wu BF, Shi HB, et al. Transarterial chemoembolization combined with lenvatinib and sintilimab vs lenvatinib alone in intermediate-advanced hepatocellular carcinoma. *World J Gastrointest Oncol* **2025**; 17: 96267. doi: 10.4251/wjgo.v17.i1.96267
 321. Guo Y, Li RC, Xia WL, Yang X, Zhu WB, Li FT, et al. Immune effect and prognosis of transcatheter arterial chemoembolization and tyrosine kinase inhibitors therapy in patients with hepatocellular carcinoma. *World J Gastrointest Oncol* **2024**; 16: 3256-69. doi: 10.4251/wjgo.v16.i7.3256
 322. Alishvandi A, Barancheshmeh M, Firuzpour F, Aram C, Kamali MJ, Keikha M. Decoding virulence and resistance in *Klebsiella pneumoniae*: pharmacological insights, immunological dynamics, and in silico therapeutic strategies. *Microb Pathog* **2025**; 205: 107691. doi: 10.1016/j.micpath.2025.107691
 323. Morita M, Nishida N, Aoki T, Chishina H, Takita M, Ida H, et al. Role of β -catenin activation in the tumor immune microenvironment and immunotherapy of hepatocellular carcinoma. *Cancers (Basel)* **2023**; 15: 2311. doi: 10.3390/cancers15082311
 324. Aoki T, Nishida N, Kudo M. Clinical significance of the duality

- of Wnt/ β -catenin signaling in human hepatocellular carcinoma. *Cancers (Basel)* **2022**; 14: 444. doi: 10.3390/cancers14020444
325. Ma L, Wang X, Jia T, Wei W, Chua MS, So S. Tankyrase inhibitors attenuate Wnt/ β -catenin signaling and inhibit growth of hepatocellular carcinoma cells. *Oncotarget* **2015**; 6: 25390-401. doi: 10.18632/oncotarget.4455
 326. Zhang R, Li S, Schippers K, Eimers B, Niu J, Hornung BV, et al. Unraveling the impact of AXIN1 mutations on HCC development: insights from CRISPR/Cas9 repaired AXIN1-mutant liver cancer cell lines. *PLoS One* **2024**; 19: e0304607. doi: 10.1371/journal.pone.0304607
 327. Thorvaldsen TE, Pedersen NM, Wenzel EM, Stenmark H. Differential roles of AXIN1 and AXIN2 in tankyrase inhibitor-induced formation of degradasomes and β -catenin degradation. *PLoS One* **2017**; 12: e0170508. doi: 10.1371/journal.pone.0170508
 328. Dong H, Zhang Z, Ni M, Xu X, Luo Y, Wang Y, et al. The trend of the treatment of advanced hepatocellular carcinoma: combination of immunotherapy and targeted therapy. *Curr Treat Options Oncol* **2024**; 25: 1239-56. doi: 10.1007/s11864-024-01246-9
 329. Guo Y, Pan Z, Kan X, Li T, Gong B, Li Y, et al. Immunotherapy improved the efficacy of TACE or TACE plus MTTs in HCC patients: a meta-analysis. *Int Immunopharmacol* **2025**; 147: 114006. doi: 10.1016/j.intimp.2024.114006
 330. Feng J, Zhao Y, Zhai L, Zhou J. Efficacy and safety of transarterial chemoembolization combined with targeted therapy and immunotherapy versus with targeted monotherapy in unresectable hepatocellular carcinoma: a systematic review and meta-analysis. *Medicine (Baltimore)* **2024**; 103: e38037. doi: 10.1097/md.00000000000038037
 331. Fang H, Ke Q, Wu S, Tu Q, Wang L. Immune-targeted therapy with transarterial chemo(embolization) for unresectable HCC: a systematic review and meta-analysis. *Front Immunol* **2024**; 15: 1421520. doi: 10.3389/fimmu.2024.1421520
 332. Li Z, Zhai Y, Wu F, Cao D, Ye F, Song Y, et al. Radiotherapy with targeted therapy or immune checkpoint inhibitors for hepatocellular carcinoma with hepatic vein and/or inferior vena cava tumor thrombi. *J Hepatocell Carcinoma* **2024**; 11: 1481-93. doi: 10.2147/jhc.S464140
 333. Liu QJ, Zhang JC, Wang YF, Zou MH, Zhou WX, Lu Y, et al. Correlation of radiotherapy, targeted therapy, and immunotherapy with hepatocellular carcinoma recurrence. *World J Gastrointest Oncol* **2025**; 17: 107815. doi: 10.4251/wjgo.v17.i7.107815
 334. Tang L, Chen R, Xu X. Synthetic lethality: a promising therapeutic strategy for hepatocellular carcinoma. *Cancer Lett* **2020**; 476: 120-8. doi: 10.1016/j.canlet.2020.02.016
 335. Fang M, Lin Y, Xue C, Sheng K, Guo Z, Han Y, et al. The AKT inhibitor AZD5363 elicits synthetic lethality in ARID1A-deficient gastric cancer cells via induction of pyroptosis. *Br J Cancer* **2024**; 131: 1080-91. doi: 10.1038/s41416-024-02778-5
 336. Sun C, Jing W, Xiong G, Ma D, Lin Y, Lv X, et al. Inhibiting Src-mediated PARP1 tyrosine phosphorylation confers synthetic lethality to PARP1 inhibition in HCC. *Cancer Lett* **2022**; 526: 180-92. doi: 10.1016/j.canlet.2021.11.005
 337. Kaur A, Lim JY, Sepmaniam S, Patnaik S, Harmston N, Lee MA, et al. Wnt inhibition creates a BRCA-like state in Wnt-addicted cancer. *EMBO Mol Med* **2021**; 13: e13349. doi: 10.15252/emmm.202013349
 338. Wang W, Liu P, Lavrijsen M, Li S, Zhang R, Li S, et al. Evaluation of AXIN1 and AXIN2 as targets of tankyrase inhibition in hepatocellular carcinoma cell lines. *Sci Rep* **2021**; 11: 7470. doi: 10.1038/s41598-021-87091-4
 339. Phoolchund AG, Khakoo SI. MASLD and the development of HCC: pathogenesis and therapeutic challenges. *Cancers (Basel)* **2024**; 16: 259. doi: 10.3390/cancers16020259
 340. Sas Z, Cendrowicz E, Weinhäuser I, Rygiel TP. Tumor microenvironment of hepatocellular carcinoma: challenges and opportunities for new treatment options. *Int J Mol Sci* **2022**; 23: 3778. doi: 10.3390/ijms23073778
 341. Edeline J, Meyer T, Blanc JF, Raoul JL. New challenges facing systemic therapies of advanced HCC in the era of different first-line immunotherapy-based combinations. *Cancers (Basel)* **2022**; 14: 5868. doi: 10.3390/cancers14235868
 342. Rizzo A, Ricci AD. Challenges and future trends of hepatocellular carcinoma immunotherapy. *Int J Mol Sci* **2022**; 23: 11363. doi: 10.3390/ijms231911363
 343. Geh D, Manas DM, Reeves HL. Hepatocellular carcinoma in non-alcoholic fatty liver disease-a review of an emerging challenge facing clinicians. *Hepatobiliary Surg Nutr* **2021**; 10: 59-75. doi: 10.21037/hbsn.2019.08.08
 344. Yeh H, Chiang CC, Yen TH. Hepatocellular carcinoma in patients with renal dysfunction: pathophysiology, prognosis, and treatment challenges. *World J Gastroenterol* **2021**; 27: 4104-42. doi: 10.3748/wjg.v27.i26.4104
 345. Zheng P, Xu D, Cai Y, Zhu L, Xiao Q, Peng W, et al. A multi-omic analysis reveals that Gamabufotalin exerts anti-hepatocellular carcinoma effects by regulating amino acid metabolism through targeting STAMBPL1. *Phytomedicine* **2024**; 135: 156094. doi: 10.1016/j.phymed.2024.156094
 346. Zhang X, Zhao Z, Wang F, Chen Z. Bakuchiol Induces Apoptosis in Human Hepatocellular Carcinoma Cells HepG2 via Enhancing Bcl-2/Bax/Cyc-t/Caspase-3 Signaling Pathway. *Int J Pharmacol* **2024**; 20: 862-73. doi: 10.3923/ijp.2024.862.873
 347. Xia T, Zhao B, Li B, Lei Y, Song Y, Wang Y, et al. MRI-based radiomics and deep learning in biological characteristics and prognosis of hepatocellular carcinoma: opportunities and challenges. *J Magn Reson Imaging* **2024**; 59: 767-83. doi: 10.1002/jmri.28982
 348. Chen F, Zhang K, Wang M, He Z, Yu B, Wang X, et al. VEGF-FGF Signaling Activates Quiescent CD63+ Liver Stem Cells to Proliferate and Differentiate. *Adv Sci (Weinh)* **2024**; 11: e2308711. doi: 10.1002/advs.202308711
 349. Chen F, Wang Z, Yao H, Liu Q, Gan Y, Xu S, et al. Large-scale manufacturing of human gallbladder epithelial cell products and derived hepatocytes via a chemically defined approach. *Trends Biotechnol* **2025**; 43: 2646-64. doi: 10.1016/j.tibtech.2025.04.009
 350. Leighton LJ, Gee YJ, Madugalle SU, Victorova M, Carrods NL, Bridle KR, et al. Efficient delivery of mRNA-LNPs in primary and secondary liver cancer. *bioRxiv* [Preprint]. March 19, 2025. Available from: <https://www.biorxiv.org/content/10.1101/2025.03.18.643845v1>.
 351. Xing L, Wang ZK, Li DM, Li J, Liu M. RNA-based therapies in hepatocellular carcinoma: state of the art and clinical perspectives. *Hepatoma Res* **2024**; 10: 24. doi: 10.20517/2394-5079.2024.25
 352. Younis MA, Harashima H. Understanding gene involvement in hepatocellular carcinoma: implications for gene therapy and personalized medicine. *Pharmgenomics Pers Med* **2024**; 17: 193-213. doi: 10.2147/pgpm.S431346
 353. Hu F, Yang H, Qiu L, Wang X, Ren Z, Wei S, et al. Innovation networks in the advanced medical equipment industry: supporting regional digital health systems from a local-national perspective. *Front Public Health* **2025**; 13: 1635475. doi: 10.3389/fpubh.2025.1635475.
 354. Sincere NI, Anand K, Ashique S, Yang J, You C. PROTACs: emerging targeted protein degradation approaches for advanced druggable strategies. *Molecules* **2023**; 28: 4014. doi: 10.3390/molecules28104014
 355. Kumar V, Rahman M, Gahtori P, Al-Abbasi F, Anwar F, Kim HS. Current status and future directions of hepatocellular carcinoma-targeted nanoparticles and nanomedicine. *Expert Opin Drug Deliv* **2021**; 18: 673-94. doi: 10.1080/17425247.2021.1860939
 356. Liu Y, Wu Y, Li Z, Wan D, Pan J. Targeted drug delivery strategies for the treatment of hepatocellular carcinoma. *Molecules* **2024**; 29: 4405. doi: 10.3390/molecules29184405
 357. Wang Z, Li Y, Wang X, Zhang W, Chen Y, Lu X, et al. Precision Strike Strategy for Liver Diseases Trilogy with Xiao-Chai-Hu Decoction: A Meta-Analysis with Machine Learning. *Phytomedicine* **2025**; 142: 156796. doi: 10.1016/j.phymed.2025.156796
 358. Ma L, Zhang K, Huang Z, Guo Y, Liu N, Chen J, et al. Development

- of novel silicon-based hydrophobic tags (SiHyT) for targeted proteins degradation. *J Med Chem* **2024**; 67: 21344-63. doi: 10.1021/acs.jmedchem.4c02273
359. Firuzpour F, Saleki K, Aram C, Rezaei N. Nanocarriers in glioblastoma treatment: a neuroimmunological perspective. *Rev Neurosci* **2025**; 36: 431-53. doi: 10.1515/revneuro-2024-0097
 360. Mukherjee B, Das L, Bhattacharya S, Chakraborty A, Chakraborty S, Al Hoque A, et al. Future direction of nanotherapy in the management of hepatocellular carcinoma. In: *Nanotherapeutics for the Treatment of Hepatocellular Carcinoma*. Bentham Science Publishers; **2022**. p. 490-525.
 361. Alhalimi A, Beg S, Kohli K, Waris M, Singh T. Nanotechnology based approach for hepatocellular carcinoma targeting. *Curr Drug Targets* **2021**; 22: 779-92. doi: 10.2174/1389450121999201209194524
 362. Escutia-Gutiérrez R, Sandoval-Rodríguez A, Zamudio-Ojeda A, Guevara-Martínez SJ, Armendáriz-Borunda J. Advances of nanotechnology in the diagnosis and treatment of hepatocellular carcinoma. *J Clin Med* **2023**; 12: 6867. doi: 10.3390/jcm12216867
 363. Da X, Cao B, Mo J, Xiang Y, Hu H, Qiu C, et al. Inhibition of growth of hepatocellular carcinoma by co-delivery of anti-PD-1 antibody and sorafenib using biomimetic nano-platelets. *BMC Cancer* **2024**; 24: 273. doi: 10.1186/s12885-024-12006-1
 364. Roma-Rodrigues C, Pombo I, Raposo L, Pedrosa P, Fernandes AR, Baptista PV. Nanotheranostics targeting the tumor microenvironment. *Front Bioeng Biotechnol* **2019**; 7: 197. doi: 10.3389/fbioe.2019.00197
 365. Ogawara K, Yoshizawa Y, Un K, Araki T, Kimura T, Higaki K. Nanoparticle-based passive drug targeting to tumors: considerations and implications for optimization. *Biol Pharm Bull* **2013**; 36: 698-702. doi: 10.1248/bpb.b13-00015
 366. Hou X, Du C, Lu L, Yuan S, Zhan M, You P, et al. Opportunities and challenges of patient-derived models in cancer research: patient-derived xenografts, patient-derived organoid and patient-derived cells. *World J Surg Oncol* **2022**; 20: 37. doi: 10.1186/s12957-022-02510-8
 367. Gao J, Lan J, Liao H, Yang F, Qiu P, Jin F, et al. Promising preclinical patient-derived organoid (PDO) and xenograft (PDX) models in upper gastrointestinal cancers: progress and challenges. *BMC Cancer* **2023**; 23: 1205. doi: 10.1186/s12885-023-11434-9
 368. Chen K, Li Y, Wang B, Yan X, Tao Y, Song W, et al. Patient-derived models facilitate precision medicine in liver cancer by remodeling cell-matrix interaction. *Front Immunol* **2023**; 14: 1101324. doi: 10.3389/fimmu.2023.1101324
 369. Hu F, Yang H, Qiu L, Wang X, Ren Z, Wei S, et al. Innovation networks in the advanced medical equipment industry: supporting regional digital health systems from a local-national perspective. *Front Public Health* **2025**; 13: 1635475. doi: 10.3389/fpubh.2025.1635475
 370. Liu G, Long J, Liu C, Chen J. Development and verification of a nomogram for predicting portal vein tumor thrombosis in hepatocellular carcinoma. *Am J Transl Res* **2024**; 16: 7511-20. doi:10.62347/PLQF5135
 371. He L, Yang H, Tang J, Liu Z, Chen Y, Lu B, et al. Intestinal probiotics *E. coli* Nissle 1917 as a targeted vehicle for delivery of p53 and Tum-5 to solid tumors for cancer therapy. *J Biol Eng* **2019**; 13: 58. doi: 10.1186/s13036-019-0189-9
 372. Ning Q, Yang T, Guo X, Huang Y, Gao Y, Liu M, et al. CHB patients with rtA181T-mutated HBV infection are associated with higher risk hepatocellular carcinoma due to increases in mutation rates of tumour suppressor genes. *J Viral Hepat* **2023**; 30: 951-8. doi: 10.1111/jvh.13886
 373. Wang Y, Wang Q, Yang TW, Yin JM, Wei F, Liu H, et al. Analysis of Immune and Inflammatory Microenvironment Characteristics of Noncancer End-Stage Liver Disease. *J Interferon Cytokine Res* **2023**; 43: 86-97. doi: 10.1089/jir.2022.0172
 374. Chen Y, Li L, Chen X, Yan Q, Hu X. The Efficacy of Decision Aids on Enhancing Early Cancer Screening: A Meta-Analysis of Randomized Controlled Trials. *Worldviews Evid Based Nurs* **2025**; 22: e70048. doi: 10.1111/wvn.70048
 375. Zhang S, Li J, Zhang Y, Hu X. Effects of advance care planning for patients with advanced cancer: A meta-analysis of randomized controlled studies. *Int J Nurs Stud* **2025**; 168: 105096. doi: 10.1016/j.ijnurstu.2025.105096