Advances of Targeted Therapy for Hepatocellular Carcinoma

Mengke Niu1, Ming Yi1, Ning Li2, Kongju Wu3* and Kongming Wu1,2*

1 Department of Oncology, Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, 2 Department of Medical Oncology, The Affiliated Cancer Hospital of Zhengzhou University, Henan Cancer Hospital, Zhengzhou, China, 3 Department of Nursing, Medical School of Pingdingshan University, Pingdingshan, China

Hepatocellular carcinoma (HCC) is one of the common and fatal malignancies, which is a significant global health problem. The clinical applicability of traditional surgery and other locoregional therapies is limited, and these therapeutic strategies are far from satisfactory in improving the outcomes of advanced HCC. In the past decade, targeted therapy had made a ground-breaking progress in advanced HCC. Those targeted therapies exert antitumor effects through specific signals, including anti-angiogenesis or cell cycle progression. As a standard systemic therapy option, it tremendously improves the survival of this devastating disease. Moreover, the combination of targeted therapy with immune checkpoint inhibitor (ICI) has demonstrated more potent anticancer effects and becomes the hot topic in clinical studies. The combining medications bring about a paradigm shift in the treatment of advanced HCC. In this review, we presented all approved targeted agents for advanced HCC with an emphasis on their clinical efficacy, summarized the advances of multi-target drugs in research for HCC and potential therapeutic targets for drug development. We also discussed the exciting results of the combination between targeted therapy and ICI.

Keywords: hepatocellular carcinoma, targeted therapy, tyrosine kinase inhibitors, immune checkpoint inhibitors, clinical trials

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for approximately 75%-85% of all primary liver cancer (1). Several risk factors such as chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, autoimmune hepatitis, alcohol abuse, diabetes, obesity induce liver injury and produce an inflammatory environment, which lead to hepatocyte necrosis, repeated regeneration and chromosomal instability (2, 3). The gradual accumulation of genetic and epigenetic abnormalities in this background plays an essential role in hepatocarcinogenesis (4). As curative treatments, surgical resection, radiofrequency ablation (RFA), transarterial chemoembolization (TACE) and liver transplant (LT) prolong the survival of HCC patients at early- or intermediate-stage (5–7). However, the high incidence of recurrence indicates poor survival prospects (8–11). Besides, most of HCCs are diagnosed at an advanced stage due to its insidious onset and rapid progression (7). Palliative treatments are therefore crucial in the management of advanced HCC. The efficacy of systemic chemotherapy for advanced HCC is disappointing (12).
In recent years, molecular biology techniques are rapidly developing, such as whole exome sequencing, copy number analyses, mRNA-seq, miRNA-seq, methylyomics and proteomics (13–15). Multiplex molecular profiling of HCC deepens on the understanding of aberrant molecular events and pivotal signaling pathways associated with the development of HCC, especially tyrosine kinase-related signaling (14). In general, tyrosine kinases can be classified as receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (nRTKs) (16). RTKs transmit extracellular signals and nRTKs mediate intracellular communications (16). RTKs are receptors of a variety of subfamilies, including vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), hepatocyte growth factor receptor (HGF), Tie-2 and RET (Figure 1) (17–20). RTK consists of an extracellular domain that binds specific ligand, a transmembrane domain and an intracellular domain with tyrosine kinase activity (21). The binding of RTK to its ligand phosphorylates tyrosine residues of target protein and regulates a series of biochemical processes through corresponding downstream signaling pathways (17, 18).
Functional mutations, genomic amplification, chromosomal rearrangements and/or autocrine activation lead to oncogenic activation of RTK, ultimately leading to carcinogenesis, invasion, metastasis, and angiogenesis (17, 22, 23). The emergence of tyrosine kinase inhibitors (TKIs) has become a promising targeted therapeutic strategy (24, 25). TKIs can enter cells and interact with the intracellular domain of multiple receptors and other intracellular signaling molecules, blocking the phosphorylation of tyrosine residues and the activation of various downstream signaling pathways such as the Ras/Raf/MEK/MAPK and PI3K/AKT/mTOR (16).

Given the current investigation, multiple drugs have been approved for advanced HCC (Table 1). The emergence of targeted therapy has transformed the therapeutic landscape of advanced HCC (5, 24, 26–28). Despite advances in targeted therapy, overall response rate and 5-year survival rate remain unsatisfactory (29). The inevitable development of drug resistance and toxicity, and the absence of specific biomarkers to screen patients sensitive to these agents, have spurred the further exploration of novel therapeutic targets and strategies (29–31).

Effective combination therapy is needed due to the limited efficacy of monotherapy. Recent studies have shown that combinations of multiple therapeutic regimens demonstrated superior efficacy to monotherapy, particularly combination of targeted therapy with immune checkpoint inhibitor (ICI) (32). Notably, the approval of atezolizumab plus bevacizumab as the first-line setting for patients with unresectable or metastatic HCC alters the outlook for this disease. This review focused on the advances of targeted therapy for advanced HCC.

### Table 1: Principal clinical trials for the FDA-approval of targeted and immunotherapeutic drugs for HCC.

| Drugs | Main targets | Treatment line | Pivotal study | Study design | Results | Approval time |
|-------|--------------|----------------|---------------|--------------|---------|---------------|
| Sorafenib | VEGFRs, PDGF-β, c-Kit, FLT3, RET | First-line | NCT0105443 | Phase III, sorafenib vs. placebo | OS: 10.7 vs. 7.9 months (HR 0.69; 95% CI: 0.55-0.87, p<0.001) | 2007 |
| Lenvatinib | VEGFR1-3, FGFR1-4, PDGF-α, RET, c-Kit | First-line | NCT01761266 | Phase III, lenvatinib vs. sorafenib | OS: 13.6 vs. 12.3 months (HR 0.92; 95% CI: 0.79-1.06) | 2018 |
| Atezolizumab plus Bevacizumab | PD-L1 VEGF | First-line | NCT03434379 | Phase Ib, atezolizumab plus bevacizumab vs. sorafenib | Survival rates at 12 months: 67.2 vs. 54.6% (HR 0.59; 95% CI: 0.47-0.76, p<0.001) | 2020 |
| Regorafenib | VEGFR1-3, PDGF-β, FGFR1, Tie-2, c-Kit, RET, B-RAF | Second-line | NCT01774344 | Phase III, regorafenib vs. placebo | OS: 10.6 vs. 7.8 months (HR 0.63; 95% CI: 0.50-0.79, p<0.0001) | 2017 |
| Cabozantinib | VEGFR2, c-Met, RET, c-Kit, AXL, FLT3 | Second-line | NCT01908426 | Phase III, cabozantinib vs. placebo | OS: 10.2 vs. 8.0 months (HR 0.76; 95% CI: 0.65-0.92, p<0.0005) | 2019 |
| Ramucirumab | VEGFR2 | Second-line | NCT02435433 | Phase III, ramucirumab vs. placebo | OS: 8.5 vs. 7.3 months (HR 0.71; 95% CI: 0.531-0.949, p=0.0199) | 2019 |
| Nivolumab | PD-1 | Second-line | NCT01658878 | Phase III, nivolumab | ORR: The dose-expansion phase 20% (95% CI: 15-26) | 2017 |
| Pembrolizumab | PD-1 | Second-line | NCT02702414 | Phase II, pembrolizumab | ORR: 17% (1% complete and 16% partial responses) | 2018 |
| Nivolumab plus Ipilimumab | PD-1 CTLA-4 | Second-line | NCT01658878 | Phase III, Nivolumab plus Ipilimumab | ORR: arm A: 32% arm B: 27% arm C: 29% OS: arm A: 22.8 months arm B: 12.5 months arm C: 12.7 months | 2020 |

HCC, hepatocellular carcinoma; VEGFR, vascular endothelial growth factor receptor; PDGF, platelet-derived growth factor receptor; c-Kit, stem cell factor receptor; FLT3, FMS-like tyrosine kinase-3; RET, rearranged during transfection; OS, overall survival; ORR, objective response rate; FGFR, fibroblast growth factor receptor; PFS, progression-free survival; TTP, time to progression; PD-L1, programmed cell death ligand 1; VEGF, vascular endothelial growth factor; Tie-2, tyrosine kinase with immunoglobulin-like and epidermal growth factor homology domains; c-Met, hepatocyte growth factor receptor; PD-1, programmed cell death protein 1; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4.
APPROVED TARGETED THERAPEUTIC AGENTS FOR HCC

First-Line Setting

Sorafenib

Sorafenib is an oral multi-targeted TKI, which exerts dual antitumor effects (33). This drug not only directly suppresses tumor cells proliferation by blocking RAF/MEK/ERK and JAK/STAT signaling pathways, but also inhibits tumor angiogenesis by targeting VEGFRs, PDGFR-β, c-Kit, FLT3, RET (33, 34). In the Sorafenib HCC Assessment Randomized Protocol (SHARP) trial, in comparison to placebo arm, sorafenib arm showed prolonged overall survival (OS) (10.7 months vs 7.9 months; HR 0.69; p<0.001) and time to radiologic progression (TTP) (2.8 months vs 1.6 months; HR 0.58; p<0.001) (35). Based on the results, sorafenib was approved by FDA for the first-line treatment of advanced HCC in 2007. The similarly promising results were displayed in another phase III Oriental trial. The study also showed a significant improvement in median OS (6.5 months vs 4.2 months; HR 0.68; p=0.014) and time to progression (TTP) (2.8 months vs 1.4 months; HR 0.57; p=0.0005) in patients treated with sorafenib compared with placebo (36). Unfortunately, the treatment-related adverse events led to dose reductions in small fraction of patients and rarely needed interruptions (36).

Lenvatinib

Lenvatinib is an oral multi-kinase inhibitor targeting VEGFR1-3, FGFR1-4, PDGFR-α, RET and c-Kit (37). Lenvatinib was approved by the FDA in 2018 as first-line treatment for advanced HCC. The approval is based on an open-label, phase III, multicenter, non-inferiority trial (38). The previous phase II clinical trial had shown positive results of lenvatinib for the treatment of HCC (39). Then, the further phase III, non-inferiority trial was performed to compare the efficacy and safety of lenvatinib versus sorafenib in HCC patients (38). As first-line treatment, lenvatinib was non-inferior to sorafenib in OS (13.6 months vs 12.3 months; HR 0.92) (38). Furthermore, lenvatinib showed a significant improvement in progression-free survival (PFS) (7.4 months vs 3.7 months; HR 0.66; p<0.0001) and objective response rate (ORR) (40.6% vs 12.4%; OR 5.01; p<0.0001) compared with sorafenib (38).

Second-Line Setting

Regorafenib

Regorafenib primarily targets VEGFR1-3, PDGFR-β, FGFR1, Tie-2, c-Kit, RET, and B-RAF (40). The FDA approved regorafenib as the second-line setting for advanced HCC in 2017 based on the results of an international, multicenter, randomized, double-blind, placebo-controlled, phase III RESORCE trial (41). The trial aimed to assess the effectiveness and safety of regorafenib in HCC patients who progressed after sorafenib treatment (41). Regorafenib increased OS to 10.6 months from 7.8 months in the placebo arm (HR 0.63; p<0.0001) (41). Regorafenib is the first systemic therapy to show survival benefit in HCC patients who progressed on sorafenib.

Cabozantinib

Cabozantinib has dual blocking effects on VEGFR2 and c-Met, which exerts anti-tumor potential by reducing angiogenesis and suppressing cell proliferation, migration and invasion (42). The drug also has targeted inhibition of RET, c-Kit, AXL, FLT3 (43). The randomized phase III clinical trial CELESTIAL enrolled 707 patients with advanced and progressed HCC who had been previously treated with sorafenib (44). Patients in cabozantinib arm showed significantly improvement of survival compared with the placebo arm (median OS: 10.2 months vs 8.0 months; HR 0.76; p=0.005. median PFS: 5.2 months vs 1.9 months; HR 0.44; p<0.001) (44). Moreover, the ORR in cabozantinib arm was 4%, higher than less than 1% in placebo arm (44). Given the survival benefits brought by cabozantinib, this drug was FDA approved as second-line setting for HCC in 2019.

Ramucirumab

Ramucirumab is a fully human IgG1 monoclonal antibody targeting VEGFR2 (45). Unlike small molecule VEGFR TKIs, ramucirumab binds to specific epitope of the extracellular domain of VEGFR2, blocking the binding of the therapeutic target to its ligand VEGF (46). A phase II study showed that ramucirumab 8 mg/kg infused intravenously every 2 weeks had anticancer activity in advanced HCC patients (47). In 2019, the FDA approved ramucirumab as monotherapy for HCC patients having alpha fetoprotein (AFP) ≥400 ng/ml and previously treated with sorafenib. The approval is based on the phase III REACH-2 clinical trial. This is the first positive phase III trial conducted in biomarker-selected HCC patients (48). Both the median OS (8.5 months vs 7.3 months; HR 0.710; p=0.0199) and PFS (2.8 months vs 1.6 months; HR 0.452; p<0.0001) were longer in ramucirumab arm than that in placebo arm (48). However, there was no statistical difference in ORR between ramucirumab arm (5%) and placebo arm (1%) (p=0.1697) (48). Ramucirumab had a manageable safety and acceptable tolerability. The incidences of serious adverse events were 35% in ramucirumab arm and 29% in placebo arm (48).

ADVANCES OF OTHER MULTI-TARGETED THERAPEUTIC AGENTS FOR HCC

Sunitinib

Sunitinib (SU011248) is an oral multi-kinase inhibitor that targets VEGFRs, PDGFRs, c-Kit, FLT3, RET and colony-stimulating factor 1 (CSF-1) (49). The multicenter phase II SAKK 77/06 trial evaluated the antitumor activity of sunitinib in advanced HCC patients (50). Patients were administrated 37.5 mg sunitinib daily until disease progression or intolerable toxicity occurred (50). The stable disease rate was 40% (50). However, another open multicenter phase II study conducted in Europe and Asia reported a low overall ORR (2.7%) in advanced unresectable HCC patients treated with sunitinib, which did not meet the primary endpoint (expected ORR was 15%) (51). In addition, 50 mg/day sunitinib showed severe toxicity (51).
Hence, phase III study of sunitinib in HCC was halted due to its toxicity.

**Brivanib**

Brivanib is a selective dual inhibitor targeting VEGFR and FGFR. Preclinical study had shown that brivanib significantly inhibited the growth of multiple HCC xenografts (52). Several clinical trials were conducted to evaluate the efficacy of brivanib in advanced HCC patients. In phase II studies, brivanib showed promising antitumor activity as first- or second-line therapy (53, 54). However, brivanib did not significantly improve OS of HCC patients as second-line therapy in phase III study, and another phase III study also did not meet the primary endpoint of OS noninferiority for brivanib versus sorafenib (55, 56).

**Vandetanib**

Vandetanib is an oral TKI targeting VEGFR, EGFR and RET. In a phase II, randomized, double-blind, placebo-controlled study, vandetanib showed a trend of improvement in PFS and OS for advanced HCC, but there was no statistically significant difference compared to the placebo arm. Also, the two arms had no difference in tumor stabilization rate (57). However, the combination of vandetanib with radiotherapy significantly enhanced radiation killing (58).

**Linifanib**

Linifanib (ABT-869) is an ATP-competitive TKI targeting all VEGFRs and PDGFR families (59). In a phase II single-arm clinical trial, linifanib showed clinical activity in advanced HCC patients who had received ≤1 systemic therapy (60). An open-label phase III clinical trial evaluated the efficacy and safety of linifanib versus sorafenib in advanced HCC patients who were not systemically treated (61). Although the linifanib arm had longer TTP, PFS and higher response rate, the study did not meet the primary endpoint, with no significant difference in OS between the linifanib and sorafenib arms (61). Moreover, patients in the linifanib arm experienced more frequent grade ≥3 adverse events (61).

**Nintedanib**

Nintedanib (BIBF 1120) is an oral triple angiokinase inhibitor targeting VEGFR1-3, FGFR, PDGFR (62). BIBF 1120 (50 or 100 mg/kg/d) showed anti-tumor and anti-angiogenic activity in HepG2 xenograft model (62). In a randomized, multicenter, open-label study of Asian patients with advanced HCC, the phase I portion, patients were divided into two groups based on baseline alanine aminotransferase/aspartate aminotransferase (ALT/AST) and Child-Pugh score (group I: ALT and AST ≤ 2 times the upper limit of normal (ULN) and Child-Pugh score 5-6; group II: ALT or AST>2 to ≤5 times the ULN or Child-Pugh score 7), and the maximum tolerated dose (MTD) of 200 mg was determined for both groups (63). The phase II portion, group I patients were randomly assigned in a 2:1 ratio to nintedanib 200 mg twice daily or sorafenib 400 mg twice daily continuously for 28 days (63). The both arms showed similar results in primary endpoint TTP (2.8 months vs 3.7 months) and the secondary endpoint OS (10.2 months vs 10.7 months) (63).

**Dovitinib**

Dovitinib is a multi-kinase inhibitor targeting VEGFR, PDGFR and FGFR. In addition to its anti-angiogenic effects, dovitinib induces dephosphorylation of retinoblastoma protein, upregulates p-histone H2A-X and p27, and downregulates p-CDK-2 and cyclin B1, thereby reducing cell proliferation and inducing tumor cell apoptosis (64). In addition, dovitinib induces apoptosis of sorafenib-resistant cell lines by inhibiting signal transducer and activator of transcription 3 (STAT3) (65). Unfortunately, a randomized, open-label, phase II study of Asian-Pacific patients reported that dovitinib did not show superior activity to sorafenib in first-line treatment of advanced HCC (66).

**Donafenib**

Donafenib is a novel TKI and similar to sorafenib. In a phase Ib clinical trial, a lower dosage of donafenib showed significant anti-cancer effects (TTP was 120 days) and good safety profile in Chinese patients with advanced HCC (67). The ZGDH3 study is the first completed phase II/III clinical trial in China to evaluate the efficacy of donafenib for the first-line treatment of advanced HCC. At the 56th Annual Meeting of the American Society of Clinical Oncology (ASCO 2020), the investigators presented the latest ZGDH3 findings to the world through an oral presentation. The study results showed that the primary endpoint of OS was longer in donafenib arm than sorafenib arm (12.1 months vs 10.3 months). The donafenib arm showed a trend toward better overall safety, demonstrating the potential of donafenib in targeted therapy for HCC.

**POTENTIAL THERAPEUTIC TARGETS AND HIGHLY SELECTIVE DRUGS FOR HCC**

**EGF/EGFR**

EGFR is a PTK that binds to the ligands EGF and TGF-α to induce receptor dimerization and autophosphorylation, which trigger the downstream MAPK, PI3K, and PLCγ signaling pathways that mediate cell proliferation, survival, adhesion, migration, and differentiation (68–71). EGFR is overexpressed in human HCC cells (72). Some oncogenic mutations such as the L834R mutation lead to spontaneous EGFR dimerization (73). Erlotinib is an oral TKI that specifically blocks tyrosine kinase activity and autophosphorylation of EGFR (74). DCR of 59% was observed in a phase II study of erlotinib for advanced HCC patients who had previously allowed only one systemic or local treatment (74). Bevacizumab plus erlotinib had also shown promising biological activity in the treatment of advanced HCC. In a phase II, single-arm, single-institution, investigator-initiated study, 62.5% of patients were alive and progression free at 16 weeks after the treatment of bevacizumab plus erlotinib (75). The median PFS was 39 weeks, and the median OS was 68 weeks (75).

**FGF19/FGFR4**

FGF19 is an important driver of HCC development. It binds to FGFR4 with high affinity (76, 77). Klotho-beta is a co-receptor
for FGFR4, which is involved in the activation of FGFR1/FGFR4 (78). The FGFR1/FGFR4 pathway activates GSK3β/β-catenin, PI3K/AKT, PLCγ/DAG/PKC, RAS/RAF/MAPK signaling cascades and promotes the survival, proliferation, and metastasis of HCC (77). A phase I study evaluated the antitumor activity of fosigatinib (BLU-554), a small molecule highly selective inhibitor targeting FGFR4 (79). The ORR in patients with FGFR1-positive tumors was 17%. The median duration of response (DOR) was 5.3 months, and the median PFS was 3.3 months. However, in patients with FGFR1-negative tumors, the ORR was 0%, and the median PFS was 2.3 months (79).

**Insulin-Like Growth Factor-1 (IGF-1)/IGF-1 Receptor (IGF-1R)**

The binding of ligand IGF-1 to IGF-1R stimulates the activation and phosphorylation of tyrosine kinase, which activates downstream MAPK, AKT and STAT pathways and promotes cell proliferation, migration, stemness and survival (80). Activation of the IGF axis was observed in breast cancer, sarcoma, and non-small cell lung cancer (81). In early HCCs, IGF activity correlated with mTOR signaling and HCC cells proliferation (82). Currently, at least 4 fully human IgG1 monoclonal antibodies targeting IGF-1R have been developed, including cixutumumab (83). The drug blocks phosphorylation of tyrosine residues, mediates receptor internalization and degradation, and produces antibody-dependent complement-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) effects (84). Preclinical study had shown that IGF-1R blockade inhibited the growth of HCC, but no clinically meaningful activity was observed in the phase II study (84, 85). Besides, the combination of cixutumumab and sorafenib also did not exhibit superior clinical efficacy in unselected A3B2 twb 0.24w? patients with HCC (86). The IGF-1R is a highly selective inhibitor targeting FGFR4 (79). The ORR in patients with FGF19-negative tumors was 17%. The median duration of response (DOR) was 5.3 months, and the median PFS was 3.3 months. However, in patients with FGFR1-negative tumors, the ORR was 0%, and the median PFS was 2.3 months (79).

**c-Met**

c-Met is an RTK, and its known ligand is HGF (89). HGF induces dimerization and activation of overexpressed c-Met, which stimulates multiple downstream signaling pathways such as MAPK, PI3K, STAT and NF kappa-B (90). In preclinical models of HCC, the HGF/c-Met inhibitor MSC2156119J inhibited tumor growth and induced complete regression (91). Tivantinib (ARQ 197), an orally administered selective c-Met inhibitor, showed antitumor activity in phase I and phase II studies (92, 93). However, in phase III studies, for MET-high advanced HCC patients who previously treated with sorafenib, no significantly improved PFS and OS were observed in tivantinib arm compared to the placebo arm (94, 95). More randomized trials are necessary to determine whether tivantinib is a potential treatment for certain subgroups of patients. Tepotinib, another highly selective c-Met inhibitor, met the primary endpoint in treating sorafenib-pretreated patients with advanced HCC, with a 12-week PFS of 63.3% (96). The HGF/c-Met and VEGF/VEGFR pathways had synergistic effects in neovascularization through enhancing intracellular signaling and modulation of signaling molecules (97). A clinical study reported that advanced HCC patients treated with the anti-VEGFR2 mAb ramucirumab plus the anti-MET mAb emibetuzumab showed an 6.7% overall response rate, 60% DCR and 5.42 months PFS, which further supporting the results of preclinical study (98). In addition, other c-Met inhibitors such as foretinib and capmatinib also showed promising antitumor activity in advanced HCC (99, 100).

**Angiopoietin/Tie-2**

Ang-1 and Ang-2 are angiopoietins, which activate Tie-2 receptor and promote neovascularization (101). Trebananib is a peptide inhibitor that blocks the interaction of Ang-1 and Ang-2 with the Tie-2 receptor and reduces tumor angiogenesis (102). The efficacy of trebananib in combination with sorafenib for advanced HCC was evaluated in a phase II study (103). The primary endpoint of the study was planned to be a 4-month PFS of ≥78%. It is disappointing that the study was not met the primary endpoint (103).

**Transforming Growth Factor-β (TGF-β)/TGF-β Receptor (TGF-βR)**

TGF-β is a secreted factor that leads to decreased cell adhesion, loss of polarity and tight junctions by inducing epithelial mesenchymal transition (EMT) (104). TGF-β binds to TGF-βR and upregulates the expression of pro-angiogenic factors such as VEGF (104). TGF-β/Smad signaling promotes immune escape by impairing the function of cytotoxic T cells, DC cells and NK cells (104–106). These mechanisms contribute to HCC tumor progression. Galunisertib (LY2157299) is a small molecule inhibitor that selectively targets TGF-βR. This drug demonstrated antitumor activity for second-line treatment of HCC in a phase II study (107). TGF-β/TGFβR signaling has been reported to confer resistance to sorafenib (108). In preclinical study, galunisertib enhanced sorafenib-induced apoptosis (108).

**mTOR**

mTOR is a dual-specificity kinase that catalyzes phosphorylation on serine/threonine and tyrosine residues of its substrates (109). mTORC1 and mTORC2 are two major complexes that mediate the regulation of multiple targets by mTOR (109). mTORC1 promotes anabolism of proteins and nucleotides by upregulating the expression of metabolic genes and inhibiting catabolic processes such as autophagy (110). mTORC2 phosphorylates and activates AKT (protein kinase B), PKC (protein kinase C) and SGK (serum/glucocorticoid regulated kinase) of the AGC protein kinase family, which promotes the survival and proliferation of HCC cells (111, 112). In addition, activated AKT phosphorylates and activates mTORC1, resulting in a positive feedback pathway loop that regulates HCC cell growth (110). Preclinical studies showed that mTOR inhibitors
significantly inhibit growth and induce apoptosis of HCC cell lines (113–115). Everolimus given daily at 7.5 mg showed clinical activity in advanced HCC patients in a randomized phase I/II study (116). However, in a global multicenter randomized phase III clinical study, everolimus did not improve OS of these patients (117). Treatment of HCC patients undergoing liver transplantation with mTOR-inhibitor temsirolimus for ≥3 months improved survival outcomes, and the greatest benefit was observed in the subgroup with AFP ≥10 ng/ml (118). A phase II trial of bevacizumab plus temsirolimus for the first-line treatment of HCC reported positive results with ORR of 19% and median OS of 14 months (119). However, everolimus plus sorafenib did not demonstrate better survival benefits compared to sorafenib alone in another phase II trial (120). Combination therapy of MEK inhibitors and mTOR inhibitors exhibited enhanced antitumor effects in vivo and in vitro models of HCC (121).

**Hippo-Yes-Associated Protein (YAP)**
The Hippo-YAP pathway plays a prominent role in inhibiting tumor growth, especially in HCC (122). The core component of the Hippo signaling pathway, adaptor protein salvador homolog 1 (SAV1 or WW45), couples mammalian sterile 20-like kinase 1/2 (MST1/2)-mediated kinases large tumor suppressor homolog 1/2 (LATS1/2) phosphorylation (122). This cascade leads to downstream YAP phosphorylation and retention in the cytoplasm, followed by ubiquitination and degradation (122). When Hippo-YAP signaling is attenuated, YAP and transcriptional coactivator translocate to the nucleus and initiate transcription of pro-proliferative and apoptosis-suppressing genes (122). Hypoxia induces nuclear translocation and accumulation of YAP (123). CT-707 is a YAP signaling inhibitor that increases YAP phosphorylation and reduces nuclear accumulation. Both in vivo and in vitro HCC models have demonstrated potent anti-tumor activity of CT-707 (124).

**RAS/RAF/MEK/ERK**
Evidences suggest that the RAS/RAF/MEK/ERK pathway is hyperactive in HCC (125, 126). Activated RAS induces phosphorylation of RAF kinase, which subsequently leads to the phosphorylation of downstream signaling factors MEK and ERK. Phosphorylated ERK dimerizes and translocates to the nucleus to participate in cell proliferation and differentiation (127). Therefore, aberrant activation of the RAS/RAF/MEK/ERK pathway may be critical for the formation and maintenance of HCC. Selumetinib is a small molecule, non-ATP competitive inhibitor that selectively targets MEK1, 2 (128). Disappointingly, in a phase II study of selumetinib for the first-line treatment of advanced HCC patients, no radiographic response was observed and the TTP was short, indicating low monotherapy activity (127). The combination of sorafenib and selumetinib for advanced HCC showed encouraging antitumor activity superior to sorafenib alone in a phase Ib study, suggesting that this combination may have a synergistic effect (129). Several clinical studies had reported that HCC patients treated with the MEK1/2 inhibitor refametinib plus sorafenib had a better clinical response relative to refametinib alone, especially those with RAS mutations (130, 131).

**STAT3**
Many cancer cells harbor constitutive activation of STAT3 (132). Phosphorylated STAT3 was detected in 60% of HCC specimens (133). Several cytokines and growth factors such as IL-6, EGF, HGF are involved in the induction of STAT3 activation (134, 135). In addition, phosphorylation of tyrosine residue is critical for STAT3 dimerization, which mediates nuclear entry and DNA binding, inducing target gene transcription (132). Besides, activation of STAT3/SNAIL signaling promotes EMT, contributing to the progression of HCC (136). STAT3 inhibitor OBP-111077 showed limited preliminary efficacy in preclinical HCC models and phase I clinical trial for second-line treatment of advanced HCC (137, 138).

**Endosialin (TEM-1, CD248)**
An experiment validated the differential expression of endosialin on tumor-associated myofibroblasts and tumor vessel-associated mural cells, involving in tumor angiogenesis, adhesion to extracellular matrix (ECM) proteins and migration through matrix (139, 140). Ontuxizumab (MORAB-004-001) is a humanized anti-endosialin IgG1 monoclonal antibody. The first-in-human study of this drug was conducted in the US as an open-label phase I clinical study for patients with solid tumors who had failed standard chemotherapy. The study observed initial anticancer activity of ontuxizumab (141). A phase I study was subsequently initiated in Japan to confirm the efficacy, safety and tolerability of ontuxizumab in solid tumors. In this study, stable disease rate of 53.3% and tumor shrinkage of 33.3% were observed in HCC patients (142).

**Endoglin (CD105)**
Endoglin (CD105) is highly expressed on active endothelial cells (143). Endoglin is involved in angiogenesis, inflammation and cancer-associated fibroblast (CAF) accumulation in the tumor microenvironment (TME) (143). TRC105 is a chimeric IgG1 mAb that competitively blocks the binding of endoglin to its ligand bone morphogenetic protein (BMP) and inhibits tumor angiogenesis (144). TRC105 alone lacked significant clinical activity in the treatment of HCC (145). However, TRC105 in combination with sorafenib showed encouraging activity in first-line treatment of HCC (partial response rate was 25%) (146).

**Cyclin-Dependent Kinase 4/6 (CDK4/6)**
CDK4/6 promotes the cell cycle progression (147, 148). CDK4/6 amplification has been found in multiple malignant tumors (149–151). Palbociclib (PD-0332991) is a selective CDK4/6 inhibitor that induces reversible cell cycle arrest in human HCC lines and is efficacious in multiple preclinical models of HCC (152). In vivo model, palbociclib in combination with sorafenib was more efficacious than sorafenib alone (152). Another CDK4/6 inhibitor, ribociclib, showed similar antitumor activity in preclinical study (153).
Histone Deacetylases (HDAC)
HDAC reversibly regulates acetylation of histones and non-histones. Dysregulation and mutation of HDAC lead to abnormal cell proliferation, EMT and tumor angiogenesis (154). Resminostat is a HDAC inhibitor. In the SHELTER study, the combination of resminostat and sorafenib prolonged median TTP and OS compared with resminostat alone (155). However, in comparison of this combination with sorafenib monotherapy for East Asia advanced HCC patients, no significant efficacy advantage was observed in the combination arm (156).

COMBINATION THERAPY OF TARGETED THERAPY AND ICI
ICIs is a novel therapeutic approach that differs from conventional treatment mechanisms (157). It restores the viability of tumor-specific T cells and utilizes the host immune system to kill tumors (158, 159). Among many ICIs identified, anti-PD1/PD-L1 and anti-CTLA-4 are currently approved for clinical application, and combination treatment of anti-PD1 and anti-CTLA-4 could have synergistic effect in some kinds of cancer (160–163). PD-L1 expression and tumor mutational burden are widely used molecular marker to guide ICI therapy, but the predictive value is not consistent among different cancers (164, 165). The combination of targeted therapy with ICI shown more potent efficacy (Table 2) (32, 166).

Encouraging results from the CheckMate-040 (167) and KEYNOTE-224 (168) studies led to accelerated FDA approval of nivolumab and pembrolizumab as second-line therapy for advanced HCC. Further, combination of targeted therapy with immunotherapy becomes mainstream, especially anti-angiogenesis therapy and ICI (169). In multiple mice models, combinations of ICI with anti-angiogenesis agents significantly increase the active anti-tumor immune cell and reduce the immune inhibitory components in comparison with ICI alone. At present, it is well accepted that combination therapy of ICI and anti-angiogenesis could achieve superior efficacy to monotherapy in several types of solid cancer (170). Atezolizumab is a high-affinity human monoclonal IgG1 antibody that specifically targets PD-L1 and blocks its interaction with PD-1 and B7.1, recovering pre-existing anti-tumor immunity (164, 171). Bevacizumab is an anti-VEGF monoclonal antibody (172). In a phase II trial, 13% ORR was observed in bevacizumab-treated patients with unresectable, nonmetastatic HCC (172). Results from a multiarm phase Ib GO30140 study suggested atezolizumab plus bevacizumab had a more significant PFS benefit than atezolizumab alone (173). On May 29, 2020, the FDA approved atezolizumab plus bevacizumab as the first-line setting for patients with unresectable or metastatic HCC. Approval was granted following the results of phase III IMbrave150 trial (32). This trial assessed the efficacy of atezolizumab plus bevacizumab versus sorafenib and demonstrated that atezolizumab plus bevacizumab arm had higher 12-month OS (67.2% vs 54.6%) and longer PFS (6.8 months vs 4.3 months; HR 0.59; p<0.001) than sorafenib arm (32). The incidences of grade 3/4 adverse events were 56.5% with atezolizumab-bevacizumab and 55.1% with sorafenib (32). Approval of atezolizumab plus bevacizumab is likely to change the paradigm of the treatment of HCC. In a phase Ib study, lenvatinib plus the anti-PD-1 mAb pembrolizumab had

### TABLE 2 | Current clinical trials investigating the combination therapy of targeted agents and ICIs for HCC.

| Study design | ClinicalTrials.gov Identifier | Phase | Line | Primary end point | Study status |
|-------------|-------------------------------|-------|------|-------------------|-------------|
| SHR-1210 + Apatinib | NCT04014101 | II | First | ORR | Recruiting |
| SHR-1210 + Apatinib | NCT04014101 | II | Second | ORR | Active, not recruiting |
| AK104 + Lenvatinib | NCT04444167 | I/II | First | ORR | Recruiting |
| Nivolumab + Bevacizumab vs. Nivolumab vs. Bevacizumab | NCT04932200 | II | First | PFS/OS | Recruiting |
| Pembrolizumab + Regorafenib | NCT04968055 | II | Second | ORR | Recruiting |
| Nivolumab + Galunisertib | NCT002423943 | I/II | Second | MTD | Completed |
| Toripalimab + ATG-008 | NCT04397463 | II | Second | MTD/PD2D/ORR | Recruiting |
| HLX10 + HLX04 | NCT03973112 | II | Second | ORR | Recruiting |
| HX008 + Bevacizumab vs. HX008 + Lenvatinib | NCT04741165 | II | First | ORR | Recruiting |
| Sintilimab + Lenvatinib | NCT04042805 | II | First | ORR | Recruiting |
| Toripalimab + Lenvatinib | NCT04388078 | II | Second | ORR | Recruiting |
| Toripalimab + Bevacizumab | NCT04605796 | I/II | First | ORR/Safety | Recruiting |
| Carmelzumab + Lenvatinib | NCT04443309 | II | First | ORR | Recruiting |
| Carmelzumab + Apatinib | NCT04701060 | II | First | ORR | Recruiting |
| Tislelizumab + regorafenib vs. regorafenib | NCT04183088 | II | First | ORR/PFS | Recruiting |
| MK-1308A + Lenvatinib | NCT04743027 | II | First | ORR | Recruiting |
| Pembrolizumab + Lenvatinib vs. Lenvatinib + placebo | NCT03713993 | III | First | PFS/OS | Active, not recruiting |
| Nivolumab + Lenvatinib | NCT03841201 | II | First | ORR/Safety | Recruiting |
| PDR501 + Sorafenib | NCT02988440 | I | First | AE/DLT | Completed |
| Atezolizumab + Bevacizumab | NCT04102098 | III | First | RFS | Recruiting |
| Avelumab + Axitinib | NCT039389533 | I | First | AE | Completed |
| Atezolizumab + Cabozantinib vs. sorafenib | NCT03755791 | III | First | PFS/OS | Recruiting |
| Durvalumab + Tivozanib | NCT032170616 | I/II | First | AE | Recruiting |
| Durvalumab + Bevacizumab vs. Durvalumab | NCT0347428 | III | First | RFS | Recruiting |

ICIs, immune checkpoint inhibitors; HCC, hepatocellular carcinoma; ORR, objective response rate; PFS, progression-free survival; OS, overall survival; MTD, maximum tolerated dose; RP2D, recommended phase II dose; AE, adverse event; DLT, dose limited toxicity; RFS, recurrence-free survival.
promising anticancer activity in advanced HCC. The ORR and DOR were 46.0% and 8.6 months, respectively. The median PFS and OS were 8.6 months and 22 months, respectively (174). The combination of ramucirumab and the anti-PD-L1 mAb durvalumab also showed promising results in a phase Ia/b open-label study of advanced HCC. The ORR was 11%. The median PFS and OS were 4.4 and 10.7 months, respectively (175). SHR-1210 (anti-PD-1 antibody) 200 mg every 2 weeks plus apatinib 250 mg daily exhibited encouraging clinical activity in advanced HCC in an open, dose-escalation and extension study (176). The ORR was 30.8% and partial response was achieved in 8 of 16 evaluable HCC patients (176). Clinical trials of other targeted drugs in combination with ICIs are also underway. In ASCO 2021, the preliminary results of some ongoing clinical trials showed that combination therapies of ICIs with anlotinib had superior efficacies to monotherapies (177, 178). In addition, studies demonstrated that PARP inhibitors could also enhance the efficacy of ICIs by promoting antigen presentation and modifying immune microenvironment, leading to the enhanced tumor-killing activities of T cell (179).

CONCLUSION AND PERSPECTIVE

Advanced HCC is a major challenge in cancer treatment. Sorafenib is the first FDA-approved TKI for the first-line treatment of advanced HCC, bringing a breakthrough to the treatment challenge. Based on the promising results in clinical studies, other molecularly targeted drugs such as lenvatinib, regorafenib, cabozantinib, ramucirumab also have been approved by FDA for first- or second-line treatment of advanced HCC. However, the efficacy is far from being satisfied. Therefore, new targets are extensively explored. In addition to interfering with the interaction between PTK and ligand, blocking the downstream signaling pathway of PTK cascade also exhibits effective inhibition of HCC progression, such as mTOR inhibitors, MEK inhibitors and STAT3 inhibitors. Besides, targeted inhibitors acting on cell cycle progression also show antitumor potential in preclinical studies of HCC. Following the research advance, potential target for HCC continues to be uncovered. For example, a recent study demonstrated that p38 MAPK gamma induced mouse hepatocyte proliferation after partial hepatectomy by promoting the phosphorylation of retinoblastoma protein as CDK-like kinase. Moreover, p38 was required for the chemically induced formation of liver tumors (180). Sterol o-acyltransferase 1 (SOAT1) and carnitine palmitoyltransferase 1A (CPT1A) were found to regulate fatty acid metabolism, and simultaneously targeting SOAT1 and CPT1A demonstrated synergistic anticancer efficacy in HCC in vitro and in vivo models (181). Liu et al. applied multi-omics technology to characterize tumor microenvironment and defined HCC into three immune subtypes. Their study suggested that MMP-9 reflected immune features and might be a valuable predictor of immunotherapeutic response in HCC (182).

Despite impressive progress in targeted therapy for advanced HCC, several challenges remain. One is drug-related adverse events, which lead to dose reduction, interruption or discontinuation. Besides, drug resistance remains a major cause of the failure of targeted therapy. The underlying mechanisms may be tumor heterogeneity and clonal evolution. In addition, there is a lack of reliable biomarkers to identify the HCC patients most likely to benefit from targeted therapy. Some circulating markers, such as AFP, IL-6 and TNF-α, correlate with the treatment outcomes of HCC (183–185), but large prospective studies are required to validate the preliminary findings. How to overcome these challenges and explore low-toxic and efficient treatment strategies are the direction of effort.

Single drug activity is insufficient and a rational combination of different drugs is needed to obtain maximum benefit. The combination of targeted therapy plus ICI has attracted attention, with positive results in several clinical trials. In the future, the integration of multidisciplinary treatment approaches for advanced HCC and the development of personalized treatment plans based on the disease status of HCC will contribute to the progress of precision medicine.

AUTHOR CONTRIBUTIONS

MN drafted the manuscript and prepared the figure and tables. MY and NL helped in revising it critically for important intellectual content. KJW and KMW designed this review and figure and tables. How to incorporate the findings. How to revise the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin (2018) 68:394–424. doi: 10.3322/caac.21492
2. Yang JD, Roberts LR. Hepatocellular Carcinoma: A Global View. Nat Rev Gastroenterol Hepatol (2010) 7:448–58. doi: 10.1038/nrgastro.2010.100
3. Karagozian R, Derdák Z, Baffy G. Obesity-Associated Mechanisms of Hepatocarcinogenesis. Metabolism (2014) 63:607–17. doi: 10.1016/j.metabol.2014.01.011
4. Dal Bo M, De Mattia E, Baboci L, Mezzalira S, Cecchin E, Assaraf YG, et al. New Insights Into the Pharmacological, Immunological, and CAR-T-Cell Approaches in the Treatment of Hepatocellular Carcinoma. Drug Resist Update (2020) 51:100702. doi: 10.1016/j.drup.2020.100702
5. Forner A, Reig M, Bruix J. Hepatocellular Carcinoma. Lancet (2018) 391:1301–14. doi: 10.1016/s0140-6736(18)30010-2
6. European Organisation for Research And Treatment Of Cancer. 
EASL-EORTC Clinical Practice Guidelines: Management of Hepatocellular Carcinoma. J Hepatol (2012) 56:908–43. doi: 10.1016/j.jhep.2011.12.001

7. Yang JD, Hainaut P, Amadou A, Ploymi A, Roberts LR. A Global View of Hepatocellular Carcinoma: Trends, Risk, Prevention and Management. Nat Rev Gastroenterol Hepatol (2019) 16:589–604. doi: 10.1038/s41575-019-0186-y

8. Tabrizian P, Jibara G, Shragar B, Schwartz M, Rosayia S. Recurrence of Hepatocellular Cancer After Resection: Patterns, Treatments, and Prognosis. Ann Surg (2015) 261:947–55. doi: 10.1097/SLA.00000000000000710

9. Park W, Chung YH, Kim JA, Jin YJ, Lee D, Shim JH, et al. Recurrences of Hepatocellular Carcinoma Following Complete Remission by Transarterial Chemoembolization or Radiofrequency Therapy: Focused on the Recurrence Patterns. Hepatol Res (2013) 43:1304–12. doi: 10.1111/hepr.12083

10. Foerster F, Hoppe-Lotichius M, Vollmar J, Marquardt JU, Weinmann A, Wörns MA, et al. Long-Term Observation of Hepatocellular Carcinoma Recurrence After Liver Transplantation at a European Transplantation Centre. United Eur Gastroenterol J (2019) 7:838–49. doi: 10.1177/20506616190221803.a1

11. Portolani N, Coniglio A, Ghidoni S, Giovannelli M, Benetti A, Tiberio GA, et al. Early and Late Recurrence After Liver Resection for Hepatocellular Carcinoma: Prognostic and Therapeutic Implications. Ann Surg (2006) 243:229–35. doi: 10.1097/01.sla.0000197766.21803.a1

12. Lee JO, Lee KW, Oh DY, Kim JH, Im SA, Kim TY, et al. Combination Chemotherapy With Capcitabine and Cisplatin for Patients With Metastatic Hepatocellular Carcinoma. Ann Oncol (2009) 20:1402–7. doi: 10.1093/annonc/mdp100

13. Schulze K, Imbeaud S, Letouze E, Wörns MA, et al. New Landscapes and Horizons in Hepatocellular Carcinoma Therapy. J Hepatol (2020) 73:1137–41.e23. doi: 10.1016/j.jhep.2017.05.046

14. Xu X, Zhao L, Shi Y, Zhang R, Long Q, Bai S, et al. Clonal Evolution in Liver Cancer at Single-Cell and Single-Variant Resolution. J Hepatol Oncol (2021) 14:22. doi: 10.1186/s13045-021-01036-y

15. Gotink KJ, Verheul HM. Anti-Angiogenic Tyrosine Kinase Inhibitors: What Is Their Mechanism of Action? Angiogenesis (2010) 13:1–14. doi: 10.1007/s10456-010-9160-6

16. Huseini R, Soria JC, Ishwaran H, Lees CR, Soria JC, 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 Tar (2019) 23:915–29. doi: 10.1080/14782937.2019.1685501

17. Llovet JM, Villanueva A, Lachenmayer A, Finn RS. Advances in Targeted Therapies for Hepatocellular Carcinoma in the Genomic Era. Nat Rev Clin Oncol (2015) 12:408–24. doi: 10.1038/nrclinonc.2015.103

18. Beretta M, Rinaldi L, Di Benedetto F, Llesh A, De Re V, Facchini G, et al. Angiogenesis Inhibitors for the Treatment of Hepatocellular Carcinoma. Front Pharmacol (2016) 7:428. doi: 10.3389/fphar.2016.00428

19. Chen S, Cao Q, Wen W, Wang H. Targeted Therapy for Hepatocellular Carcinoma: Challenges and Opportunities. Cancer Lett (2019) 460:1–9. doi: 10.1016/j.canlet.2019.114428

20. Schulze K, Imbeaud S, Letouze E, Wörns MA, et al. Pathways, and Therapeutic Implications for Hepatocellular Carcinoma. Mol Cancer Ther (2015) 47:505–10. doi: 10.1158/1049-5978.vna-15-0090

21. Wilhelm SM, Dumas J, Adnane L, Lynch M, Carter CA, Schütz G, et al. Discovery and Development of Sorafenib: A Multikinase Inhibitor for Treating Cancer. Nat Rev Drug Discov (2005) 4:835–44. doi: 10.1038/nrd1310

22. Chen S, Cao Q, Wen W, Wang H. Targeted Therapy for Hepatocellular Carcinoma: Challenges and Opportunities. Cancer Lett (2019) 460:1–9. doi: 10.1016/j.canlet.2019.114428

23. Schulze K, Imbeaud S, Letouze E, Wörns MA, et al. Pathways, and Therapeutic Implications for Hepatocellular Carcinoma. J Hepatol Oncol (2015) 21:1209–24. doi: 10.1038/nrclinonc.2015.103

24. Llovet JM, Villanueva A, Lachenmayer A, Finn RS. Advances in Targeted Therapies for Hepatocellular Carcinoma in the Genomic Era. Nat Rev Clin Oncol (2015) 12:408–24. doi: 10.1038/nrclinonc.2015.103

25. Beretta M, Rinaldi L, Di Benedetto F, Llesh A, De Re V, Facchini G, et al. Angiogenesis Inhibitors for the Treatment of Hepatocellular Carcinoma. Front Pharmacol (2016) 7:428. doi: 10.3389/fphar.2016.00428

26. Llovet JM, Montal R, Sia D, Finn RS. Molecular Therapies and Precision Medicine for Hepatocellular Carcinoma. Nat Rev Clin Oncol (2018) 15:599–606. doi: 10.1038/s41571-018-0034-3
44. 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 (2016) 378:54–63. doi: 10.1056/NEJMoa171002

45. 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

46. Llovet JM, Decaens T, Raoul JL, Boucher E, Kudo M, Chang C, et al. Brivanib Alaninate, a Dual Inhibitor of Vascular Endothelial Growth Factor Receptor-2 and Platelet-Derived Growth Factor-Receptor-α, as First-Line Monotherapy in Patients With Advanced Hepatocellular Carcinoma. Clin Cancer Res (2013) 19:6614–23. doi: 10.1158/1078-0432.CCR-13-1442

47. 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 Carcinoma. Clin Cancer Res (2013) 19:10383–92. doi: 10.1158/1078-0432.CCR-13-1305

48. Zhou J, Goh BC, Albert DH, Chen CS. ABT-869, a Promising Multi-Targeted Tyrosine Kinase Inhibitor: From Bench to Bedside. J Hematol Oncol (2009) 2:33. doi: 10.1186/1756-8722-2-33

49. Philip PA, Mahoney MR, Allmer C, Thomas J, Pitot HC, Kim G, et al. Phase III BRISK-PS Study. J Clin Oncol (2012) 18:2090–8. doi: 10.1200/jco.2011.34.1991

50. Park JW, Finn RS, Kim JS, Karwil M, Li RK, Ismail F, et al. Phase II, Open-Label Study of Brivanib as Second-Line Therapy in Patients With Advanced Hepatocellular Carcinoma. Clin Cancer Res (2011) 17:1973–83. doi: 10.1158/1078-0432.CCR-10-1101

51. Llovet JM, Decaens T, Raoul JL, Boucher E, Kudo M, Chang C, et al. Brivanib Alaninate in Patients With Advanced Hepatocellular Carcinoma Who Were Intolerant to Sorafenib or for Whom Sorafenib Failed: Results From the Randomized Phase III BRISK-PS Study. J Clin Oncol (2013) 31:3509–16. doi: 10.1200/jco.2012.47.3089

52. Johnson PJ, Qin S, Park JW, Poon RT, Raoul IL, Philip PA, et al. Brivanib Versus Sorafenib as First-Line Therapy in Patients With Unresectable, Advanced Hepatocellular Carcinoma: Results From the Randomized Phase III BRISK-FL Study. J Clin Oncol (2013) 31:3517–24. doi: 10.1200/jco.2012.48.4410

53. Hsu C, Yang TS, Huo TI, Hsieh RK, Yu CW, Hwang WS, et al. Vandetanib Induces Apoptosis and Overcomes Sorafenib Resistance in Hepatocellular Carcinoma. J Clin Oncol (2012) 30:2395–404. doi: 10.1200/jco.2012.43.8617

54. Xiong L, Duhoux P, Kozaitis N, Alzahabi B, et al. Clathrin Switches Transforming Growth Factor-β Receptor Signaling and Changes the Response of Cancer Cells to Chemo- and Immunotherapy. Cancer Res (2019) 79:4389–99. doi: 10.1158/0008-5472.CAN-18-3249

55. Niu et al. Targeted Therapy for HCC
Hua H, Kong Q, Yin J, Zhang J, Jiang Y. Insulin-Like Growth Factor Receptor Signaling in a Driver Event in Hepatocellular Carcinoma. Cancer Discov (2019) 9:1696–707. doi: 10.1158/2159-8290.Cd-19-0555

Hua H, Kong Q, Yin J, Jiang J, Jiang Y. Insulin-Like Growth Factor 1/2 Receptor Regulation in Hepatocellular Carcinoma With MET Overexpression. Br J Cancer (2021) 1–10. doi:10.1038/s41416-021-01334-9

Sulpice E, Ding S, Muscatelli-Groux B, Bergé M, Han ZC, Ploutz J, et al. Cross-Talk Between the VEGF-A and HGF Signalling Pathways in Endothelial Cells. Biocell (2009) 101:525–39. doi:10.1042/bioc20080221

Harding JJ, Zhu AX, Bauer TM, Choueiki TM, Drilon A, Voss MH, et al. A Phase Ib/II Study of Ramucirumab in Combination With Emibetuzumab in Patients With Advanced Cancer. Clin Cancer Res (2019) 25:5202–11. doi: 10.1158/1078-0432.Ccr-18-4010

Qin S, Chan SL, Sukeepaisarnjaroen W, Han G, Choo SP, Sriruangpong, et al. A Phase II Study of the Efficacy and Safety of the MET Inhibitor Capmatinib (INC280) in Patients With Advanced Hepatocellular Carcinoma. Ther Adv Med Oncol (2011) 15:788359198989001

doi: 10.1158/1078-0432.Ccr-18-4010

Yin Y, Hua H, Li M, Liu S, Kong Q, Shao T, et al. mTORC2 Promotes Type I Innate Resistance to Anti-MET Antibody Simultaneously Targeting TGF-β and PD-1. J Hepatol (2021) 12:80. doi: 10.1186/s13045-020-00904-3

Jams WT, Lovly CM. Molecular Pathways: Clinical Applications and Future Direction of Insulin-Like Growth Factor 1-Receptor Pathway Blockade. Clin Cancer Res (2015) 21:4270–7. doi: 10.1158/1078-0432.Ccr-15-2518

Tovar V, Alisint C, Villanueva A, Hoshida Y, Chiang DY, Sode M, et al. IGF Activation in a Molecular Subclass of Hepatocellular Carcinoma and Pre-Clinical Efficacy of IGF-IR Blockage. J Hepatol (2010) 52:550–9. doi: 10.1016/j.jhep.2010.01.015

Qu X, Wu Z, Dong W, Zhang T, Wang L, Pang Z, et al. Update of IGF-1 Receptor Inhibitor (Ganitumab, Dalotuzumab, Cixutumumab, Terapotumumab and Figitumumab) Effects on Cancer Therapy. Oncotarget (2017) 8:29501–18. doi: 10.18632/oncotarget.15704

Abou-Alfa GK, Caparros M, O’reilly EM, Ma J, Chou JF, Gansukh B, et al. A Phase II Study of Cixutumumab (IMC-A12, NSC424460) in Advanced Hepatocellular Carcinoma. J Hepatol (2014) 60:3139–24. doi: 10.1016/j.jhep.2013.09.008

Zhang YC, Zhang Y, Song AL, Kou ZM, Li XS. Effect of Blocking IGF-1 Receptor on Growth of Human Hepatocellular Carcinoma Cells. World J Gastroenterol (2006) 12:3977–82. doi: 10.3748/wjg.v12.i25.3977

El-Khoueiry AB, O’Donnell R, Semrad TJ, Mack P, Blanchard S,Bahary N, et al. A Phase I Trial of Escalating Doses of Cixutumumab (IMC-A12) and Sorafenib in the Treatment of Advanced Hepatocellular Carcinoma. Cancer Chemother Pharmacol (2018) 81:957–63. doi: 10.1007/s00280-018-3553-4

George B, George SK, Shi W, Haque A, Shi P, Eskandari G, et al. Dual Inhibition of IGF-IR and ALK as an Effective Strategy to Eradicate NPD-ALK(+) T-Cell Lymphoma. J Hepatol Oncol (2019) 12.80. doi: 10.1186/s13045-019-0768-8

Lee JS, Kang JH, Boo HJ, Hwang SJ, Hong S, Lee SC, et al. STAT3-Mediated IGF-2 Secretion in the Tumour Microenvironment Elicits Innate Resistance to Anti-IGF-1R Antibody. Nat Commun (2015) 6:6999. doi: 10.1038/ncomms9499

Bouattou M, Raymond E, Qin S, Cheng AL, Stammberger U, Locatelli G, et al. Recent Developments of C-Met as a Therapeutic Target in Hepatocellular Carcinoma. Hepatology (2018) 67:1132–49. doi: 10.1002/hep.29496

Garavoji A, Giovannetti E, Biscagio P, Peters GJ. C-Met as a Target for Personalized Therapy. Transl Oncogen (2015) 7:13–31. doi: 10.4137/tog.50354

Baldt F, Friese-Hamim M, Ihling C, Wilm C, Blaukat A. The C-Met Inhibitor MSC2156119J Effectively Inhibits Tumor Growth in Liver Cancer Models. Cancers (Basel) (2014) 6:1736–52. doi: 10.3390/cancers6031736

Santoro A, Simonelli M, Rodriguez-Lopez C, Zucali P, Camacho LH, Granito A, et al. A Phase-1b Study of Tivantinib (ARQ 197) in Adult Patients With Hepatocellular Carcinoma and Cirrhosis. Br J Cancer (2013) 108:21–44. doi: 10.1038/bjc.2012.556

Santoro A, Rimassa L, Borbath I, Daniele B, Salvagini S, Van Laethem JL, et al. Tivantinib for Second-Line Treatment of Advanced Hepatocarcinoma: A Randomised, Placebo-Controlled Phase 2 Study. Lancet Oncol (2013) 14:35–63. doi: 10.1016/S1470-2045(12)70490-4

Rimassa L, Assenat E, Peck-Radosavljevic M, Pracht M, Zagolov V, Mathurin P, et al. Tivantinib for Second-Line Treatment of MET-High, Advanced Hepatocellular Carcinoma (METIV-HCC): A Final Analysis of a Phase A, Randomised, Placebo-Controlled Study. Lancet Oncol (2018) 19:682–93. doi: 10.1016/S1470-2045(18)30146-3

Kudo M, Morimochi M, Morishita Y, Izumi N, Takayama T, Yoshiji H, et al. A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Study of Tivantinib in Japanese Patients With MET-High Hepatocellular Carcinoma. Cancer Sci (2020) 111:3759–69. doi: 10.1111/cas.14582

Decaens T, Barone C, Assenat E, Wermke M, Fasolo A, Merle P, et al. Phase 1b/2 Trial of Tivantinib in Sorafenibpretreated Advanced Hepatocellular Carcinoma With MET Overexpression. Br J Cancer (2021) 1–10. doi:10.1038/s41416-021-01334-9

Hui IC, Giovannetti E, Biasco G, Peters GJ. C-Met as a Target for Personalized Therapy. Targeted Therapy for HCC
Xie Z, Wang J, Liu M, Chen D, Qiu C, Sun K. CC-223 Blocks Mtorc1/C2 Activation and Inhibits Human Hepatocellular Carcinoma Cells In Vitro and In Vivo. PloS One (2017) 12:e0173522. doi: 10.1371/journal.pone.0173522

Shah HS, Chen CY, Dai CY, Hsiao CF, Lin YJ, Su WC, et al. Randomised Clinical Trial: Comparison of Two Everolimus Dosing Schedules in Patients With Advanced Hepatocellular Carcinoma. Aliment Pharmacol Ther (2013) 37:62–73. doi: 10.1111/apt.12132

Zhu H, Wang DD, Yuan T, Yan FJ, Zeng CM, Dai XY, et al. Multikinase Activation and Inhibits Human Hepatocellular Carcinoma Cells In Vivo. Cancer Cell (2008) 14:223–34. doi: 10.1016/j.ccr.2008.03.012

Knox JJ, Qin R, Strosberg JR, Tan B, Kaubisch A, El-Khoueiry AB, et al. A Randomized Phase II Trial of Sorafenib: The EVOLVE-1 Randomized Clinical Trial. JAMA (2006) 295:1117. doi: 10.1001/jama.2006.7189

Koeberle D, Dufour JF, Demeter G, Li Q, Ribi K, Samaras P, et al. Sorafenib Inhibition of Ras and Jak/Stat Pathways in Human HCC. Oncogene (2011) 29:2350–8. doi: 10.1038/onc.2009.98

Lim HY, Heo J, Choi HJ, Lin CY, Yoon JH, Hsu C, et al. A Phase II Study of Selumetinib in Patients With Unresectable Hepatocellular Carcinoma. Clin Cancer Res (2014) 20:5976–85. doi: 10.1158/1078-0432.Ccr-13-3445

Lim HY, Merle P, Weiss KH, Yau T, Ross P, Marzaferro V, et al. Phase II Studies With Refametinib or Refametinib Plus Sorafenib in Patients With Ras-Mutated Hepatocellular Carcinoma. Clin Cancer Res (2018) 24:4650–61. doi: 10.1158/1078-0432.Ccr-17-3588

Al Zaid Siddiquee K, Turkson J. STAT3 as a Target for Inducing Apoptosis in Solid and Hematological Tumors. Cell Res (2008) 18:254–67. doi: 10.1038/cr.2008.18

He G, Yu GY, Temkin V, Ogata H, Kunzten C, Sakurai T, et al. Hepatocyte IKKbeta/NF-kappaB Inhibits Tumor Promotion and Progression by Preventing Oxidative Stress-Driven STAT3 Activation. Cancer Cell (2010) 17:286–97. doi: 10.1016/j.ccr.2009.12.048

Takeda A, Akira S. STAT Family of Transcription Factors in Cytokine-Mediated Biological Responses. Cytokine Growth Factor Rev (2000) 11:199–207. doi: 10.1016/s1359-6101(00)00005-8

Wan S, Zhao E, Kryczek I, Vatan L, Sadovskaya A, Ludema G, et al. Tumor-Associated Macrophages Produce interleukin-6 and Signal via STAT3 To Promote Expansion of Human Hepatocellular Cancers Stem Cells. Gastroenterology (2014) 147:1393–404. doi: 10.1053/j.gastro.2014.08.039

Yin X, Zhang BH, Zheng SS, Gao DM, Qiu SJ, Wu WZ, et al. Coexpression of Genes Oct4 and Nanog Initiates Stem Cell Characteristics in Hepatocellular Carcinoma and Promotes Epithelial-Mesenchymal Transition Through Activation of Stat3/Snail Signaling. J Hematol Oncol (2015) 8:23. doi: 10.1186/s13045-015-0119-3

Lin L, Amin R, Gallicano GI, Glasgow E, Jorguinosi W, Jessup JM, et al. The STAT3 Inhibitor NSC 74859 Is Effective in Hepatocellular Cancers With Disrupted TGF-Beta Signaling. Oncogene (2009) 28:961–72. doi: 10.1053/j.onc.2008.448

Yoo C, Kang J, Lim HY, Kim JH, Lee MA, Lee KH, et al. Phase I Dose-Finding Study of OPB-111077, a Novel STAT3 Inhibitor, in Patients With Advanced Hepatocellular Carcinoma. Cancer Treat Rev (2013) 39:510–8. doi: 10.1016/j.ctrv.2013.08.026

Christian S, Winkler R, Helfrich I, Boos AM, Eschedorf D, et al. Endostatin (Tem1) Is a Marker of Tumor-Associated Myofibroblasts and Tumor-Vessel-Associated Mural Cells. Ann J Pathol (2008) 172:486–94. doi: 10.2353/ajpath.2008.070623

Tomkowicz B, Bybinski K, Foley B, Elbel W, Kline R, Routahir E, et al. Interaction of Endostatin/TEM1 With Extracellular Matrix Proteins Mediates Cell Adhesion and Migration. Proc Natl Acad Sci USA (2007) 104:17965–70. doi: 10.1073/pnas.0705647104

Diaz LA Jr, Coughlin CM, Weil SC, Fishel J, Gouinier MM, Lawrence S, et al. A First-in-Human Phase I Study of MORAb-004, A Monoclonal Antibody to Endostatin in Patients With Advanced Solid Tumors. Clin Cancer Res (2015) 21:1281–8. doi: 10.1158/1078-0432.Ccr-14-1829

Dai T, Aramaki T, Yasui H, Muro K, Ikeda M, Okusaka T, et al. A Phase I Dose Escalation Study of Selumetinib (AZD6244, ARRY-142886) In Combination With Sorafenib in Hepatocellular Carcinoma. Invest New Drugs (2019) 37:1061–74. doi: 10.1007/s10637-018-0713-7

Ollauri-Ibáñez C, Ayauso-Ilógó B, Perciacco M. Hot and Cold Tumors: Is Endoglin (CD105) a Potential Target for Vessel Normalization? Cancers (Basel) (2021) 13:1552. doi: 10.3390/cancers13071552

Nolan-Stevaux O, Zhong W, Culp S, Shaffer K, Hoover J, Wickramasinghe D, et al. Endoglin Requirement for BMP9 Signaling in Endothelial Cells Reveals New Mechanism of Action for Selective Anti-Endoglin Antibodies. PloS One (2012) 7:e50920. doi: 10.1371/journal.pone.0050920

Duffy AG, Ulahannan SV, Cao L, Rahma OE, Makarova-Rusher O, Cao L, et al. Phase II Study of TRC105 in Patients With Hepatocellular Carcinoma Who Have Progressed on Sorafenib. United Eur Gastroenterol J (2019) 3:453–61. doi: 10.1177/2050641618835877

Ollauri-Ibáñez C, Ayauso-Ilógó B, Perciacco M. Hot and Cold Tumors: Is Endoglin (CD105) a Potential Target for Vessel Normalization? Cancers (Basel) (2021) 13:1552. doi: 10.3390/cancers13071552

Nolan-Stevaux O, Zhong W, Culp S, Shaffer K, Hoover J, Wickramasinghe D, et al. Endoglin Requirement for BMP9 Signaling in Endothelial Cells Reveals New Mechanism of Action for Selective Anti-Endoglin Antibodies. PloS One (2012) 7:e50920. doi: 10.1371/journal.pone.0050920

Duffy AG, Ulahannan SV, Cao L, Rahma OE, Makarova-Rusher O, Cao L, et al. Phase I Preliminary Phase II Study of TRC105 in Combination With Sorafenib in Hepatocellular Carcinoma. Clin Cancer Res (2017) 23:4633–41. doi: 10.1158/1078-0432.Ccr-16-1371

Asghar U, Witkiewicz AK, Turner NC, Knudsen ES. The History and Future of Targeting Cyclin-Dependent Kinases in Cancer Therapy. Nat Rev Drug Discov (2015) 14:330–46. doi: 10.1038/nrd4504

Li T, Zhang C, Hassan S, Liu X, Song F, Chen K, et al. Histone Deacetylase 6 in Cancer. J Hematol Oncol (2018) 11:11. doi: 10.1186/s13045-018-0654-9

Khitab ZA, Matsumi H, Valentine M, Shapiro DN, Sherr CJ, Look AT. Coamplification of the CDK4 Gene With MDM2 and Gli1 in Human
150. Park S, Lee J, Do IG, Jang J, Rho K, Ahn S, et al. Aberrant CDK4 Amplification in Refractory Rhodomyelocarcinoma as Identified by Genomic Profiling. *Sci Rep* (2014) 4:4362. doi: 10.1038/srep04362

151. Xu H, Yu S, Liu Q, Yuan X, Mani S, Pestell RG, et al. Recent Advances of Highly Selective CDK4/6 Inhibitors in Breast Cancer. *J Hematol Oncol* (2017) 10:97. doi: 10.1186/s13045-017-0467-2

152. Bollard J, Miguela V, Ruiz de Galarreta M, Venkatesh A, Bian CB, Roberto Xu H, Yu S, Liu Q, Yuan X, Mani S, Pestell RG, et al. Recent Advances of Highly Selective CDK4/6 Inhibitors in Breast Cancer. *J Hematol Oncol* (2017) 10:97. doi: 10.1186/s13045-017-0467-2

153. Reiter FP, Denk G, Ziesch A, Ofner A, Wimmer R, Hohenester S, et al.

154. Li Y, Seto E. HDACs and HDAC Inhibitors in Cancer Development and Progression. *Cold Spring Harbor Perspect Med* (2017) 9:021652. doi: 10.1186/s13045-019-09458-8

155. Bitzer M, Horger M, Giannini EG, Ganten TM, Wörns MA, Siveke JT, et al.

156. Yau T, Kang YK, Kim TY, El-Khoueiry AB, Santoro A, Sangro B, et al.

157. Zhang L, Ding J, Li HY, Wang ZH, Wu K. ImmunoTherapy for Advanced Hepatocellular Carcinoma. *Cancer Res* (2017) 77:10984. doi: 10.1158/0008-5472.can-17-10984

158. Luo XY, Wu KM, He XX. Advances in Drug Development for Hepatocellular Carcinoma: Clinical Trials and Potential Therapeutic Targets. *J Exp Clin Cancer Res* (2021) 40:172. doi: 10.1186/s13046-021-01968-w

159. Mi Y, Jiao D, Qin S, Chu Q, Wu K, Li A. Synergistic Effect of Immune Checkpoint Blockade and Anti-Angiogenesis in Cancer Treatment. *Mol Cancer* (2019) 18:60. doi: 10.1186/s12943-019-0974-6

160. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive Correlates of Response to the Anti-PD-L1 Antibody MPD3280A in Cancer Patients. *Nature* (2014) 515:563–7. doi: 10.1038/nature14011

161. Siegel AB, Cohen EL, Ocean A, Leher D, Goldenberg A, Knox J, et al. Phase II Trial Evaluating the Clinical and Biologic Effects of Bevacizumab in Unresectable Hepatocellular Carcinoma. *J Clin Oncol* (2008) 26:2992–8. doi: 10.1200/jco.2007.15.9947

162. Lee MS, Ryu BY, Hsu CH, Numata K, Stein S, Verret W, et al. Atezolizumab With or Without Bevacizumab in Unresectable Hepatocellular Carcinoma (GO30104): An Open-Label, Multicentre, Phase Ib Study. *Lancet Oncol* (2021) 22:808–20. doi: 10.1016/j.locho.2020.10.035-x

163. Finn RS, Ikeda M, Zhu AX, Sung MW, Baron AD, Kudo M, et al. Phase Ib Study of Lenvatinib Plus Pembrolizumab in Patients With Unresectable Hepatocellular Carcinoma. *J Clin Oncol* (2020) 38:2960–70. doi: 10.1200/jco.2020.00808

164. Yi M, Jiao D, Xu H, Liu Q, Zhao W, Han X, et al. Biomarkers for Predicting Efficacy of PD-1/PD-L1 Inhibitors. *Exp Hematol Oncol* (2019) 8:41. doi: 10.1186/s13046-019-00458-8

165. Bang YJ, Golan T, Dahan L, Fu S, Moreno V, Park K, et al. Ramucirumab and Durvalumab for Previously Treated, Advanced Non-Small-Cell Lung Cancer, Gastric/Gastro-Oesophageal Junction Adenocarcinoma, or Hepatocellular Carcinoma: An Open-Label, Phase I/II Study (JUDI). *Eur J Cancer* (2020) 137:272–84. doi: 10.1016/j.ejca.2020.06.007

166. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, et al. Atezolizumab Plus Bevacizumab as Second-Line Therapy of Advanced Hepatocellular Carcinoma-The SHELTER Study. *Gut* (2019) 68:705. doi: 10.1053/j.gut.2019.10.021

167. Luo XY, Wu KM, He XX. Advances in Drug Development for Hepatocellular Carcinoma: Clinical Trials and Potential Therapeutic Targets. *J Exp Clin Cancer Res* (2021) 40:172. doi: 10.1186/s13046-021-01968-w
185. Tan W, Luo X, Li W, Zhong J, Cao J, Zhu S, et al. TNF-α Is a Potential Therapeutic Target to Overcome Sorafenib Resistance in Hepatocellular Carcinoma. *EBioMedicine* (2019) 40:446–56. doi: 10.1016/j.ebiom.2018.12.047

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