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Targeted and Immune-based Therapies for Hepatocellular Carcinoma

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Abstract

Treatment options for patients with hepatocellular carcinoma (HCC) are rapidly changing based on positive results from phase 3 trials of targeted and immune-based therapies. More agents designed to target specific pathways and immune checkpoints are in clinical development. Some agents have already been shown to improve outcomes of patients with HCC, as first- and second-line therapies, and are either awaiting approval by the Food and Drug Administration or have been recently approved. We summarize the targeted and immune-based agents in trials of patients with advanced HCC and discuss the future of these strategies for liver cancer.

Keywords

tumor; human; checkpoint inhibitor

HCC is the second leading cause of cancer related death worldwide with approximately 800,000 cases per year.¹ More than 80% of all HCCs occur in patients with liver diseases and cirrhosis. Most patients receive a diagnosis when their tumors are already too advanced for curative approaches such as surgical resection, orthotopic liver transplantation, or local percutaneous tumor ablation.^{2, 3} Palliative treatment options are therefore important in management of patients with HCC. First-line treatment options include trans-arterial

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approaches such as transarterial chemoembolization (TACE) or transarterial radioembolization (TARE) and systemic therapies. We review the accepted treatment options for HCC, based on recently updated AASLD and EASL guidelines.^{4, 5} We discuss standard of care options for patients with unresectable HCC, results from recent phase 3 trials, and their effects on treatment algorithms. We provide an overview of new targeted and immune-based treatment options and challenges to upcoming trials, with a focus on combination therapies.

Non-surgical and Non-transplant-based Therapies

Locoregional therapies include percutaneous local ablation, chemoembolization, radioembolization, and external radiation therapy; they are the most common first-line treatment, although 50% of patients receive systemic therapies at some point during disease progression.⁴ Their efficacy is limited by tumor size and location. Percutaneous local ablation is the only curative locoregional therapy—subtypes include percutaneous ethanol injection, radiofrequency ablation (RFA), microwave ablation, cryoablation, and irreversible electroporation (reviewed by Nault et al⁶). RFA is the most commonly used and studied percutaneous ablation technique; rates of 3-year overall survival range from 67% to 84%, rates of 3-year local recurrence range from 3.2% to 28.5%, and rates of complete response range from 90% to 98.5% (for tumors less than 3 cm).⁶ RFA is sometimes used in combination with TACE for tumors greater than 3 cm.⁷ Studies comparing RFA with liver resection for tumors of 2–3 cm are often under powered, but findings generally support the concept that rates of recurrence are similar between patients treated with RFA vs liver resection.

Arterial embolization is recommended for patients with tumors not amenable to curative resection or ablation, without extrahepatic spread and with preserved liver function.^{4, 5} Vascular invasion may be an exclusion criterion for TACE depending on tumor location. It is contraindicated for patients with decompensated cirrhosis, due to the risk of fulminant liver failure from low liver reserve. Arterial embolization techniques include conventional transarterial chemoembolization (cTACE), drug-eluting-bead transarterial chemoembolization (DEB-TACE), and trans-arterial embolization (TAE). cTACE involves an intrahepatic injection of a chemotherapy lipiodol emulsion into the tumor vasculature followed by particle embolization. This is the most commonly used TACE technique worldwide.⁸ DEB-TACE uses drug-eluting beads to deliver a high dose of doxorubicin locally, to minimize systemic toxicity. TAE deploys gelfoam to obstruct arterial perfusion, thereby causing local ischemia near the tumor without application of any cytotoxic reagents.

TARE typically involves intra-arterial infusion of yttrium-90 (Y-90), an element that emits beta radiation, has a half-life of 2.5 days, and affects only the local tumor region (reviewed by Sacco et al.⁹). Retrospective studies have found Y-90 therapy to be superior to TACE in downsizing tumors and as a bridge to transplant. However, no randomized trials have directly compared the treatments. Therefore, it is difficult to clearly define the role of TARE in the management of HCC. TARE failed to increase overall survival compared to sorafenib, the first-line systemic treatment for HCC, in patients with locally advanced HCC in 2 phase 3 trials (SARAH and SIRveNIB).^{10, 11}

Prospects for Locoregional Therapies

Locoregional therapies are important in management of patients with HCC. The SARAH and SIRveNIB trials did not find TARE to increase survival times of patients compared with systemic therapy.^{10, 11} In contrast, a recent study reported an overall survival time of 26 months for patients treated consecutively with the tyrosine kinase inhibitors sorafenib and then regorafenib.¹² These findings indicate that targeted therapies may be as effective as locoregional therapies. There have been attempts to improve outcomes of patients by adding targeted therapies to locoregional therapy. However, adding sorafenib to TACE failed to improve time to progression (SPACE trial).¹³ The combination of TACE (or RFA) and an immune checkpoint inhibitors was tested in a small pilot study,¹⁴ which demonstrated safety and early signs of efficacy. However, larger studies are needed to compare the effectiveness of combined locoregional therapies and immune-based therapies with only locoregional therapy.

First-line Treatments

Sorafenib was the only drug approved for the systemic treatment of patients with HCC until 2017. It is a tyrosine-kinase inhibitor that blocks the activities of vascular endothelial growth factor receptors (VEGFRs), platelet derived growth factor receptor (PDGFRs), and Raf family kinases. It extends the mean overall survival time of patients with advanced HCC by 2.8 months.¹⁵ Similar results have been reported in an Asian patient population.¹⁶ After positive results from a pivotal phase 3 trial (the SHARP study¹⁵) and Food and Drug Administration (FDA) approval of sorafenib for treatment of patients with HCC, several promising targeted agents were tested in phase 3 trials, from 2008 through 2017. However, these were not found to improve patient outcomes (see Table 1).

The lack of efficacy of these agents might have been due to lack of effects on tumor cells, liver toxicity, inadequate trial design, and/or improved outcomes over time in patients treated in the comparator group (sorafenib).¹⁷ It appears that the time of overall survival of patients treated with sorafenib in clinical trials increased from 10.7 months in 2008 to 15.1 months in 2013 in patients in Western regions,^{15, 18} and from 6.5 to 11 months in patients in Asia.^{16, 18} Explanations proposed by experts for this increase include better second-line treatment options, modification of inclusion criteria, and better dose adaption of sorafenib, leading to longer drug exposure.

Lenvatinib is an oral tyrosine kinase inhibitor that blocks activities of VEGFR1–3, fibroblast growth factor receptors (FGFRs) 1–4, PEGFR, RET, and KIT. Lenvatinib was tested as a first-line treatment in an open-label, phase 3, multicenter, non-inferiority trial of 954 patients with unresectable HCC, compared with sorafenib.¹⁹ Median times of overall survival were 13.6 months for lenvatinib and 12.3 months for sorafenib. Lenvatinib therefore met the criteria for non-inferiority. Of note, the rate of objective response (according to response evaluation criteria in solid tumors) was 18.8% for patients receiving lenvatinib (<1% complete response and 18% partial response) and 6.5 % for patients receiving sorafenib (<1% complete response and 6.5% partial response). The most common adverse events for

lenvatinib were hypertension, diarrhea, decreased appetite, and weight loss.¹⁹ Lenvatinib received FDA approval for treatment of chemotherapy-naïve patients in July 2018.

Immune checkpoint inhibitors are effective in treatment of several types of cancer. Nivolumab blocks a signal that prevents activated T cells from attacking the tumor. Its effects are being compared with those of sorafenib as a first-line treatment for HCC.

Second-line Therapies

Regorafenib is an oral multi-kinase inhibitor that blocks the activity of kinases that promote angiogenesis, cell proliferation, metastasis, and tumor immunity.^{20, 21} Its chemical structure is similar to that of sorafenib—it differs in only 1 fluorine carbon atom in the central phenyl ring. It was tested in 573 patients who had tolerated sorafenib but still had tumor progression, from 2013 through 2015. Patients who received regorafenib had increased median times of overall survival (10.6 months vs 7.8 months in the placebo group).²² Adverse events included hypertension, hand foot skin reactions, and diarrhea (similar to those of sorafenib). Based on these positive results regorafenib was approved by the FDA in 2017 for treatment of patients with HCC progression after tolerating sorafenib therapy. Since the approval of regorafenib, 2 more drugs showed positive results in sorafenib-treated patients and are expected to receive FDA approval.

Cabozantinib inhibits the receptors MET, VEGFR2, and RET. A phase 3 trial (CELESTIAL) that compared cabozantinib with placebo as a second-line treatment for advanced HCC (in patients with Child-Pugh score A, ECOG PS 0/1) was stopped at the second interim analysis due to the agent's efficacy. Cabozantinib increased times of overall survival to 10.2 months from 8.0 months in the placebo group.²³ Grade 3 or 4 adverse events occurred in 68% of patients in the cabozantinib group and in 36% of patients in the placebo group. The most common high-grade events were palmar–plantar erythrodysesthesia (17%), hypertension (16%), increased aspartate aminotransferase level (12%), fatigue (10%), and diarrhea (10%) in patients given cabozantinib.

Ramucirumab is a fully human monoclonal antibody (IgG1) that suppresses angiogenesis by binding to VEGFR2. Its efficacy and tolerability were initially tested as a second-line agent (post-sorafenib). However, ramucirumab failed to significantly increase times of survival vs placebo in unselected patients with HCC.²⁴ In a prespecified subgroup analysis of patients with a baseline serum concentration of alpha fetoprotein (AFP) of 400 ng/mL or greater, median times of overall survival were 7.8 months for the ramucirumab group vs 4.2 months in the placebo group.²⁴ This finding was the rationale for a phase 3 trial (REACH-2), which limited enrollment to patients with levels of AFP > 400 ng/mL. Ramucirumab increased times of overall survival from 7.3 to 8.5 months in patients with HCC.²⁵

Based on the results from this study AFP is used as a biomarker—this was the first biomarker-based study of patients with HCC. However, this study also raised several important questions: Why do only patients with increased serum levels of AFP benefit from ramucirumab and what is its mechanism? Could AFP be used as a marker of response to other

angiogenesis inhibitors for patients with HCC? Might patients with high serum levels of AFP have other features that mediate their response to ramucirumab?

Non-immune Based Agents

HCCs are recognized as hyper-vascular tumors.^{26, 27} In adults, angiogenesis occurs primarily during tumor development²⁸ and involves multiple signaling pathways.²⁹ Signaling is mediated by factors such as VEGF, PDGF, and BFGF, produced by tumor cells, immune cells, and/or stromal cells.^{30, 31} Agents designed to block the activity of VEGF (ramucirumab, bevacizumab and cabozantinib) and PDGF (MEDI-575 and preretinoin), or both (axitinib and nintedanib) are being tested in different phase trials of patients with HCC. TRC105 is a human–mouse chimeric monoclonal antibody against endoglin—a component of the transforming growth factor beta receptor (TGFBR) complex, which is highly expressed on tumor-associated endothelial cells and is required for angiogenesis.^{32, 33} By binding to endoglin, TRC105 prevents the development of new blood vessels by the tumor to slow its growth and induce tumor cells apoptosis and antibody-dependent cell-mediated cytotoxicity.

Phase 1 and 2 trials evaluated the safety and effectiveness of TRC105 as a second-line treatment for liver cancer (after sorafenib) in 2011. The combination of TRC105 and sorafenib was well tolerated at the recommended single-agent doses of both drugs. A partial response has been observed in 25% of patients; patients are still being recruited, at multiple centers, to confirm the activity of this combination³⁴. The median time of overall survival (15.5 months) exceeded the that of sorafenib (10.7 months) reported in the SHARP trial.¹⁵ Adverse events characteristic of each drug were not increased in frequency or severity when the drugs were administered concurrently. The most common TRC105-related adverse events were epistaxis, fatigue, anemia, and headache, which were previously observed. There are small molecules and tyrosine kinase inhibitors that have been tested in preclinical models and phase 1 or 2 studies with more or less success (see review³⁵). We discuss a few drugs that may be of special interest due to their potential inclusion in combination therapy with immune checkpoint inhibitors.

c-Met inhibitors

Levels of MET mRNA and protein are increased in 30%–100% of HCC tissues compared with surrounding liver tissue,^{36–39} so it could be involved in liver tumor development or progression. Tivantinib was one of the first MET inhibitors to be tested in patients with HCC. Subgroup analysis from a phase 2 study revealed a correlation between high levels of MET (based on immunohistochemistry) and improved outcome upon tivantinib treatment.⁴⁰ However, a consecutive phase 3 trial of tivantinib as a second-line treatment in patients with MET-high tumors failed to meet its primary endpoint of an increased survival.⁴¹ One possible explanation could be tivantinib's MET-independent cytotoxic activity.⁴² Other MET inhibitors are being evaluated and have produced some positive results (CELESTIAL trial),²³ increasing interest in this drug as a single agent and in combination with immunotherapy.

Immunotherapy

Immune checkpoint inhibitors

Immune checkpoints are pathways that inhibit the immune response to maintain self-tolerance and regulate the duration and amplitude of immune responses. Tumors activate immune checkpoint mechanisms to suppress the anti-tumor immune response. Binding of checkpoint proteins such as CD274 molecule (PDL1) on tumor cells to PD1 on T cells keeps the T cells from killing tumor cells. Checkpoint inhibitors are therefore being tested in clinical trials or have been approved for a number of cancers (see review⁴³). These include antibodies against cytotoxic T-lymphocyte-associated protein 4 (CTLA4) on T cells (ipilimumab, tremelimumab, and others) and antibodies that inhibit interactions between programmed cell death 1 (PDCD1, also called PD1) and PDL1, such as nivolumab, pembrolizumab, tezolizumab, and durvalumab.

Nivolumab (anti-PD1) is the first FDA-approved immune checkpoint inhibitor for HCC. In a phase 1 and 2 open-label, non-comparative trial (CheckMate040)⁴⁴ to assess the efficacy of nivolumab as an alternative second-line treatment for patients with HCC, 20% of patients had an objective response, independent of prior sorafenib treatment. Nivolumab treatment was well tolerated and patients had long-lasting responses. This was a non-randomized trial, so a phase 3 randomized trial (NCT02576509) is underway to compare nivolumab with sorafenib as a first-line treatment for advanced HCC.

Anti-CTLA4 (tremelimumab), was the first immune checkpoint inhibitor tested, in patients with hepatitis C virus (HCV) infection and HCC.⁴⁵ It was evaluated in a phase 2, non-controlled, multicenter trial of patients with HCC and chronic HCV infection who were ineligible for surgery or locoregional therapy. Three partial responses were observed among 17 evaluable patients, with an overall survival time of 8.2 months. A second trial tested whether an antigenic stimulation caused by incomplete tumor ablation via RFA or TACE could safely enhance the effects of tremelimumab.¹⁴ The rationale for this combination is based on the fact that RFA or TACE could induce immune mediated death of tumor cells, which in turn stimulated a peripheral systemic immune response, potentially amplified by immune checkpoint blockade.⁴⁶ In this pilot study,¹⁴ the combination of locoregional therapy plus tremelimumab was safe—the most common treatment related toxicity was pruritus. A partial response was observed in 5/19 of evaluable patients (26%).

Pembrolizumab, another antibody against PD1, was tested in an open-label, phase 2 trial of patients with HCC who had been treated with sorafenib and were either intolerant to this treatment or had radiographic evidence for disease progression after treatment.⁴⁷ Pembrolizumab was effective and tolerable, producing 1 complete response and 16 partial responses among 104 patients. The median time to progression and progression-free survival were both 4.9 months, and median time of overall survival was 12.9 months. Based on the efficacy of nivolumab as a second-line therapy,⁴⁴ other immune checkpoint inhibitors are being tested as first-line treatments for patients with advanced HCC. These include tislelizumab and durvalumab, which disrupt interactions between PD1 and PDL1 and are expected to produce outcomes similar to those of other agents that block this pathway.

The synergistic effects of immune checkpoint blockade and other antitumor therapies are being tested in phase 3 trials. Combined blockade of PDL1 (durvalumab) and anti-CTLA4 (tremelimumab) is hoped to activate the T-cell anti-tumor response. Reducing tumor vascularization with bevacizumab increases infiltration by immune cells and could potentiate the effects of atezolizumab. Sequential administration of an oncolytic virus followed by immune checkpoint therapy could block tumor escape mechanisms (see Table 2 for phase 3 studies evaluating combinations of antibodies against PD1, PDL1, and CTLA4).

Combining Immune and Targeted Therapies

Combination studies evaluating combined anti-CTLA4 and anti-PD1 or anti-CTLA4 and anti-PDL1 are underway.⁴⁸ Antibodies against other immune checkpoint regulators, such as LAG3, BTLA, and TI3, are in clinical trials and some of them are being evaluated in patients with HCC (see NCT03005782, NCT03489369, NCT01968109, NCT03250832, NCT03489343, NCT02817633, and NCT03099109).

Other strategies to increase the anti-tumor immune response involve agents that target co-stimulatory molecules, rather than inhibitors of immune checkpoint regulators.⁴⁹ OX40, a ligand for CD134 that is expressed on subtypes of dendritic cells, is being tested, alone and in combination with 4-1BB, a transmembrane glycoprotein receptor expressed on activated T cells, in patients with HCC (NCT02315066). In mice with liver tumors induced by carbon tetrachloride and injection of transformed hepatocytes, the combination of anti-PD1 and sunitinib or liposome-loaded C6-ceramide slowed tumor growth.^{50, 51} Immune therapies are also being tested in combination with anti-angiogenic agents, tyrosine kinase inhibitors, oncolytic viruses, and local ablation techniques (see Table 3).

VEGF can have immunosuppressive effects (reviewed by Khan and Kerbel⁵²). VEGF inhibits monocytes from differentiating into dendritic cells,⁵³ inhibits differentiation of CD4⁺ and CD8⁺ cells from hematopoietic stem cells,⁵⁴ supports proliferation of T-regulatory (Treg) cells,^{55, 56} and increases accumulation of myeloid-derived suppressor cells (MDSCs)⁵⁷. Additionally, VEGF expression has been associated with expression of immune-modulating molecules on endothelial cells, including FAS ligand⁵⁸ and PDL1,⁵⁹ further enhancing the immunosuppressive tumor microenvironment. Therefore, the combination of anti-VEGF agents such as bevacizumab combined with I/O might be used to treat patients with HCC. Initial results from a phase 1 study testing the combination of atezolizumab and bevacizumab have been reported. A 62% partial response rate was observed among the first 26 enrolled patients.⁶⁰ Based on this early data, a phase 3 trial (IMbrave150) was initiated to evaluate the same combination as first-line treatment for patients with unresectable metastatic HCC (Table 2). Interesting data has recently been published from the IMmotion150 study, a randomized phase 2 study of atezolizumab alone or combined with bevacizumab versus sunitinib patients with treatment-naïve metastatic renal cell carcinoma.⁶¹ Exploratory biomarker analyses indicated that tumor mutation and neoantigen burden were not associated with progression-free survival. In contrast, angiogenesis, effector T cell and interferon-mediated responses, and gene expression signatures associated with myeloid cell-mediated inflammation were associated with progression-free survival within and among treatments, so it might be possible to identify

patients most likely to respond to anti-VEGF and immunotherapy. At least 2 other combination of anti-angiogenic agents and I/O are under investigation. In a phase 1b trial of lenvatinib plus pembrolizumab in 18 patients with unresectable HCC, 46% of patients had a partial response and 46% had stable disease, without dose-limiting toxicity.⁶² A phase 1 open-label trial is underway to test the combination of duralumab and ramucirumab in patients with locally advanced and unresected thoracic or gastrointestinal or thoracic tumors (NCT02572687).

Tyrosine kinase inhibitors might also be combined with immunotherapies. Tyrosine kinase inhibitors modify intrinsic tumor escape mechanisms whereas immunotherapies modify the tumor microenvironment. Therefore, their antitumor effects are likely to synergize. Several trials of combinations of immunotherapies and tyrosine kinase inhibitors have begun recruiting patients or are about to open (see Table 3).

Cell cycle inhibitors might also be combined with immunotherapy. Cells require CDK4 for cell cycle progression and the G1–S transition. CDK6 and CDK4 regulate the activity of the tumor suppressor protein Rb; this process is disrupted in liver cancer cells. Palbociclib, an inhibitor of CDK4 and CDK6, restores cell cycle checkpoints independently of Rb. Inhibitors of CDK4 and CDK6 also reduce intratumor Treg cells and increase antigen presentation in mouse models of breast and colon cancer, potentiating the anti-tumor effects of antibodies against PD1 and PDL1.⁶³ This combination might also be effective for treatment of HCC.

Tumor ablation can release antigens from cancer cells that increase tumor immunogenicity. Immunotherapies increase the sensitivity of T cells to antigens presented by dendritic cells. In a study of 32 patients with HCC, the combination of tremilzumab with TACE resulted in a median survival time of 12.3 months (95% CI, 9.3 to 15.4 months), disease progression within 6 months in 57.1% of patients, and disease progression within 12 months in 33.1% of patients.¹⁴ The combination of tremilzumab with TACE reduced the load of HCV and increased numbers of intratumor CD8⁺ T cells. So, these combinations appear to increase the local anti-tumor immune response, and the combination of local ablation and immunotherapy is feasible and produces an objective response. Ionized radiation modifies the tumor microenvironment and sensitizes the tumor to antibodies against PD1—at least in mouse models of head and neck squamous carcinoma.⁶⁴ Studies are underway to determine whether local Y90 therapy will have similar effects and increase the anti-tumor response in patients with HCC (Table 3).

Biomarkers for immune checkpoint inhibitors

Immune checkpoint inhibitors have an objective response rate of 20% or less.⁴³ Biomarkers are therefore needed to help identify patients most likely to respond to immune-based therapies. Expression of PDL1 on tumor cells did not correlate with responses to anti-PD1 therapy in patients with HCC,⁴⁴ even though it did correlate with outcomes of patients with other types of cancer.⁶⁵ We observed a correlation between treatment response and tumor-infiltrating T cells;¹⁴ although this observation was made in biopsies from patients already receiving treatment, so detection of these cells is not necessarily prognostic. In an analysis searching of patients treated with anti-CTLA4, a higher proportion of those with higher

levels of CD4⁺PD1⁺ T cells had a response to therapy (Agdashian, submitted). However, more data from other clinical trials are needed to confirm this observation. Researchers identified a subpopulation of HCCs with a gene expression signature indicating their susceptibility to immune checkpoint inhibitors (based on increased expression of PDL1 and PD1), and with fewer chromosome aberrations.⁶⁶

Genetically engineered T cells

Chimeric antigen receptor (CAR) contain the Fv region of immunoglobulin fused with T-cell receptor (TCR) luminal domain.⁶⁷ Autologous lymphocytes can be transfected with vectors that express CAR constructs and then infused into patients. The immunoglobulin domain can be engineered to bind any secreted or cell-surface tumor antigen, allowing T cells that express CARs to localize to tumors.⁶⁸ CD19-specific CAR-expressing T cells were approved by the FDA for treatment of patients with advanced B-cell lymphoma and acute lymphoblastic leukemia in 2017. This strategy is being tested for treatment of HCC.

Selection of the proper antigen is essential for the efficacy of CAR-expressing T cells and for avoiding diffuse liver injury. Glypican 3 (GPC3), which is specifically expressed by HCC cells,⁶⁹ is being evaluated as a CAR target. Preliminary results from a phase 1 study evaluating T cells that express GPC3-specific CARs in 13 patients in China showed that these cells slowed HCC progression.⁷⁰ The researchers are modifying their construct to increase anti-tumor activity by coexpressing a soluble PD1–CH3 fusion protein.⁷¹

In addition to GPC3, T cells designed to bind other HCC-associated antigens are under investigation (CEA, EGFR, MUC1, EPCAM, and CD133).⁷² However, these proteins do not have tumor-restricted expression. An alternate approach is to use T cells with receptors engineered to bind specific antigens.⁷³ Engineered TCRs bind cytosolic tumor-associated antigen, which T cells with CARs cannot.

It is possible to identify peptide fragments that bind to MHC class I molecules. AFP is commonly expressed by HCC cells and is a target for immunotherapy. Although AFP-specific cytotoxic T cells exist in patients, they are low in number and functionally impaired.⁷⁴ In mice, cytotoxic T cells with TCRs engineered to bind AFP with high affinity controlled growth of liver tumors.⁷⁵ An open-label phase 1 trial is underway to evaluate autologous T cells with TCRs engineered to bind antigens specifically expressed by non-small cell lung tumors or HCCs (NCT03441100). T cells with specificity for glycan-3 are in preclinical development,⁷⁶ and there is a case report of 1 patient given T cells engineered to express an HBV-specific TCR.⁷⁷

Adoptive cell therapy

HCC, like many other malignancies, has an immune-suppressive microenvironment that suppress activities of dendritic cells, tumor-infiltrating lymphocytes, and natural killer (NK) cells. Since the initial identification of lymphokine-activated killer cells in 1955,⁷⁸ researchers have speculated about the possibility of infusing patients with anti-tumor immune cells, but they could not produce a large enough quantity to have an effect. Production antibodies against CD3 (anti-CD3) and purification of IL2 in 1980 allowed for ex vivo expansion of immune cells, specifically lymphokine-activated killer cells, and

cytokine-induced killer (CIK) cells (reviewed by Gao et al.⁷⁹). Lymphokine-activated killer cells require IL2, which has significant toxicity and therefore barred clinical application. CIK cells, on the other hand, could be expanded with anti- CD3 alone and were cytotoxic via an MHC class-independent process.

CIK cells were tested as adjuvant therapy in a phase 3 trial of patients with HCC in Korea.⁸⁰ The resulting hazard ratios was 0.63 for recurrence-free survival, 0.21 for all-cause death, and 0.19 for cancer-related death. The CIK cells produced a high rate of adverse events, but no significant difference in severe adverse events. A 5-year follow-up of patients given CIK cells as adjuvant therapy produced similar outcomes. Administration of the CIK cells increased odds of recurrence-free survival by 33%, and reduced the risk of all-cause death by 67%, in a study of 226 patients with a median follow-up time of 68.5 months.⁸¹

TGFB

TGFB regulates cell activities, including proliferation, migration, adhesion, differentiation, and modulation of stroma/microenvironment.⁸² TGFB regulates survival, proliferation, and effector functions of T cells. It controls the balance of T-helper (Th) 1 vs Th2 cells to promote Th2 cell- mediated responses and inhibit M1-type macrophages while promoting differentiation of M2- type macrophages. M2-type macrophages suppress activities of CD8⁺ T cells, NK cells, and dendritic cells and increase activity of CD4⁺ regulatory T cells.^{82, 83} Interestingly, TGFB can also contribute to activation of the epithelial to mesenchymal transition, tumor invasion, and metastasis. Several TGFB pathway inhibitors have reached the clinic and are being evaluated in patients with HCC.⁸³

Galunisertib (LY2109761) is a small molecule inhibitor of TGFB that increases expression of E-cadherin by HCC cells and reduces tumor cell migration and invasion in mice.⁸⁴ In a phase 2 trial, galunisertib monotherapy increased median time of overall survival in patients with reductions in tumor biomarkers (AFP, TGFB1, CDH1) of more than 20%.⁸⁵ Patients with reductions in AFP levels had a median survival time of 93.1 weeks whereas patients without a reduction in AFP had a median survival of 29.6 weeks. Preliminary data from phase 2 trial of the combination of galunisertib and sorafenib in patients with HCC indicated that the median time to progression was 4.1 months and the median time of overall survival was 17.9 months.⁸⁵ Phase 2 trials of the combination of galunisertib with anti-PD1 (NCT02423343 and NCT02947165) are underway. M7824 is a bifunctional antibody against PDL1 and TGFB that is being tested in phase 1 trials. In mice, M7824 reduced tumor growth and and metastasis more effectively than either an antibody against PDL1 or an inhibitor of TGFB alone in the EMT-6 orthotopic breast cancer model.⁸⁶

Oncolytic viruses

Oncolytic viruses specifically infect and kill cancer cells. Several are in clinical trials of patients with solid tumors, including HCC.⁸⁷ JX-594 (also known as Pexa-Vec) engineered strain of vaccinia poxvirus from which the thymidine kinase gene was deleted, which limits viral replication to cells with high levels of thymidine kinase, such as cancer cells with mutations in RAS or p53. The virus also expresses colony stimulating factor 2, which recruits anti-tumor immune cells. The agent had a good safety profile in a non-comparative,

open-label phase 1 trial of 22 patients with primary or metastatic liver cancer.⁸⁸ In a phase 2 trial of 20 patients with sorafenib-refractory HCC, 81% had a response to a 3-week course of Pexa-Vec followed by sorafenib.⁸⁹ A randomized, open-label, phase 3 trial (PHOCUS; NCT02562755) is underway to compare sorafenib alone vs Pexa-Vec with sorafenib in patients with advanced HCC.⁹⁰

Targeting the tumor microenvironment

Immune-based approaches for treatment of HCC are aimed primarily at immune effector cell functions rather than the tumor immune microenvironment, which hosts different immune cells with immunosuppressive functions. The tumor immune microenvironment affects response to immune-based therapies. Tumors are broadly assigned into categories of infiltrated-excluded, infiltrated-inflamed, or infiltrated with tertiary lymphocyte structures.⁹¹ Infiltrated-inflamed tumors are most susceptible to respond to immune checkpoint therapy. Antibodies against CTLA4 lead to a T-cell enriched tumor phenotype.¹⁴

An extensive analysis of 10,000 tumors comprising 33 diverse cancer types (including HCC) revealed 6 immune subsets of cancers.⁹² Most HCCs were characterized as lymphocyte depleted, but tumors of high-macrophage or inflammatory subtypes (a high ratio of Th1:Th2 cells) had a high number of Th17 cells and a balanced ratio of macrophages to lymphocytes. Technologies such as RNA-seq combined with CIBERSORT⁹³ and XCell,⁹⁴ in combination with mass-spectrometry analysis of single cells,⁹⁵ allow for multiplex high-dimensional analysis of immune cell populations in tumors.

MDSC could be a therapeutic target,⁹⁶ and different approaches are being tested.⁹⁷ Phosphodiesterase-5 (PDE5) inhibitors downregulate tumor-associated MDSC.⁹⁸ Although these inhibitors downregulate expression of arginase and nitrous oxide synthetase, their precise mechanism of MDSC suppression requires further investigation. In mice with subcutaneous orthotopic tumors, PDE5 inhibitors increase the CIK cell-mediated anti-tumor response (Yu et al, manuscript in revision).

Treg cells also suppress the anti-HCC immune response, either be part of the effects of MDSCs or independently.⁹⁹ High numbers of Treg cells in HCCs correlate with poor outcomes of patients, so inhibiting these cells could be an important goal of immunotherapy.¹⁰⁰ Treg cells can indirectly suppress T-effector cells via indoleamine 2,3-dioxygenase (IDO), released by dendritic cells.¹⁰¹ Additionally, tumor associated macrophages (TAM) are another drugable target.⁵¹

Metabolic factors such as fatty acids, which are increased in livers of patients with non-alcoholic fatty liver disease, reduce the activity of CD4⁺ T cells and ultimately also change the tumor microenvironment.¹⁰² Tryptophan, in addition to its role in neuronal signaling, also regulates the immune response. Kynurenine is a tryptophan metabolite (produced during 90% of tryptophan metabolism¹⁰³)—the combination of tryptophan depletion and kynurenine accumulation impairs activities of CD4⁺ and CD8⁺ T cells and NK cells. Alternatively, the suppressive activities of Treg cells and MDSC are increased following tryptophan depletion. IDO is involved in conversion of tryptophan to kynurenine. IDO1 is

expressed by multiple cell types, but not T cells, in response to interferon. HCC cells are believed to overexpress IDO1 to escape the anti-tumor immune response.¹⁰⁴

Several IDO1 inhibitors have been developed,¹⁰⁵ including indoximod, epacadostat, and BMS-986205. IDO1 inhibitors alone are ineffective as anti-tumor agents, but they are being tested in combination with immune-based therapies. The rationale is to induce inflammation in the tumor microenvironment and thereby boosts the efficacy of checkpoint inhibitors. Although there was some evidence for efficacy from a phase 2 trial of patients with HCC (NCT0207312), in phase 3 trials (ECHO-301, KEYNOTE-252; NCT02752074), the combination of pembrolizumab and epacadostat did not increase time of progression-free survival in patients with metastatic melanoma. So, it is not clear if IDO inhibitors will be effective as anticancer therapies. Agents are also being developed to inhibit A3 adenosine receptors—a G-protein- coupled receptors that are involved in inhibition of neutrophil degranulation in neutrophil-mediated tissue injury. The A3 adenosine receptor is highly expressed by inflammatory and cancer cells. CF102 targets this receptor and is being tested in trials of patients with HCC and non-alcoholic steatohepatitis.¹⁰⁶

The intestinal microbiome can also affect hepatocarcinogenesis. Yu et al¹⁰⁷ demonstrated that endotoxin promotes hepatocarcinogenesis in mice. Depletion of gut endotoxin with oral antibiotics reduced diethylnitrosamine-induced hepatic inflammation, but administration of lipopolysaccharide (LPS, endotoxin) restored it. Endotoxin-induced hepatocarcinogenesis appears to be mediated through TLR4. TLR4-knockout mice were resistant to diethylnitrosamine-induced hepatic inflammation, but reconstitution of TLR4-expressing myeloid cells restored the hepatic inflammation phenotype. Dapito et al¹⁰⁸ reported similar findings.

Germ-free mice develop fewer and smaller DEN induced liver tumors, supporting the hypothesis that intestinal dysbiosis can contribute to hepatocarcinogenesis.¹⁰⁹ Surprisingly, gut sterilization at later stages of tumor development had a stronger antitumor effect than at early stages, and the resident liver cells that express TLR4 appear to promote tumorigenesis. These data indicate that antibiotics might prevent HCC in high-risk populations, but human studies are needed. Probiotics might help restore a healthy microbiota in patients at risk for HCC. Probiotics were reported to reduce the burden of LPS-induced tumors in mice.¹⁰⁹ Studies are needed to determine the optimal time for probiotic initiation and appropriate doses.

Yoshimoto et al¹¹⁰ have challenged the involvement of LPS in liver carcinogenesis. *Tlr4*^{-/-} mice had a slight increase in tumor burden instead of a decrease, indicating that LPS did not promote hepatocarcinogenesis. Feces from mice fed a high-fat diet, which leads to fatty liver and liver tumors, have increased levels of gram-positive bacteria compared to mice fed a regular chow diet. Vancomycin blocks HCC development, so gram-positive bacteria-mediated conversion of primary bile acids to secondary bile acids might prevent tumorigenesis. Studies have investigated mechanisms by which vancomycin prevents tumorigenesis.¹¹¹ The antibiotic-mediated loss of gram-positive bacteria results in a higher ratio of primary to secondary bile acid in the enteric circulation. This induces expression of CXCL16 on liver endothelial sinusoidal cells, which recruits NKT cells to the liver and

elimination of malignant cells. Elimination of gram-positive bacteria from the gut prevents not only hepatocarcinogenesis, but also development of melanoma and B-cell lymphomas in mice. The gut microbiome affects the efficacy of immune checkpoint inhibitors, possibly by modulating bile acid composition. These pathways might be manipulated for treatment of HCC.

Future Directions

With the recent approval of new drugs for the treatment of patients with HCC, several positive results from phase 3 studies, and advances in immunotherapy, there are more treatment options for patients with liver cancer. Trials are underway that combine targeted therapies with immune-based therapies (see Figure 1). However, more studies are needed to identify the best combinations and to determine their optimal timing, such as adjuvant therapies or first-line or second-line agents. Advances in DNA and RNA sequencing techniques have provided insights into mechanisms of hepatocarcinogenesis and HCC progression that could identify additional targets. Intelligent trial design and clever correlative studies that include paired tumor biopsies will help us identify the best therapeutic approaches and improve outcomes of patients with HCC.

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Abbreviations:

CAR	Chimeric antigen receptor
CIK	Cytokine-induced killer
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
FGFR	Fibroblast growth factor receptor
GPC3	Glypican 3
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
IDO	indoleamine 2,3-dioxygenase
MDSC	Myeloid-derived suppressor cell
NK	Natural killer
PDGF	Platelet-derived growth factor
PDCD1 or PD1	programmed cell death 1
TGFB	Transforming growth factor beta 1

Treg T-regulatory
VEGFR VEGF receptor

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Figure 1. Combination Therapy Options.

Different FDA-approved and experimental single agent anti-HCC therapies are categorized by their mechanism. The aim is to combine two (or three) therapies that are either in the same class (e.g. anti-PD1 and anti-CTLA4) or across different classes of mechanism (e.g. immune checkpoint blockade and molecular targeting therapy) to achieve synergy.

Table 1.

Phase 3 Studies

Trial	Drugs	n	Median time of overall survival (months)	Hazard ratio (95 % CI)	P value	Positive result	Reference
First-line							
SHARP						YES	15
	Sorafenib	299	10.7	0.69	<.001		
	Placebo	303	7.9	(0.55–0.87)			
Asian-Pacific						YES	16
	Sorafenib	150	6.5	0.68	.01		
	Placebo	76	4.2	(0.5–0.93)			
SUN1170						NO	112
	Sunitinib	530	7.9	1.3	.001		
	Sorafenib	544	10.2	(1.13–1.5)			
LiGHT						NO	113
	Linifanib	514	9.1	1.046	NS		
	Sorafenib	521	9.8	(0.896–1.221)			
BRISK-FL						NO	114
	Brivanib	577	9.5	1.07	.31		
	Sorafenib	578	9.9	(0.94–1.23)			
SEARCH						NO	115
	Sorafenib + Everolimus	362	9.5	0.92	.2		
	Sorafenib	358	8.5	(0.781–1.106)			
REFLECT/Study304						YES	19
	Lenvatinib	478	13.6	0.92	<.05		
	Sorafenib	476	12.3	(0.79–			
				1.06)			
CALGB 80802						NO	116
	Sorafenib + doxo	173	9.3	1.06	NS		
	Sorafenib	173	10.5	(0.8–1.4)			
SILIUS						NO	117
	Sorafenib + HIAC	88	16.9	1.2	NS		
	Sorafenib	102	16.1	(0.8–1.6)			

Trial	Drugs	n	Median time of overall survival (months)	Hazard ratio	P value	Positive result	Reference
				(95 % CI)			
SARAH						NO	10
	SIRT (Y-90)	237	8	1.15	NS		
	Sorafenib	222	9.9	(0.94–1.41)			
NCT 01287585						NO	118
	ADI-PEG20	424	7.8	1.17			
	Placebo	211	7.4				
SIRveNIB						NO	11
	SIRT (Y-90)	182	8.8	1.12			
	Sorafenib	178	10	(0.88–1.42)	NS		
Second-line							
BRISK-PS						NO	119
	Brivanib	263	9.4	0.89	.33		
	Placebo	132	8.2	(0.69–1.15)			
EVOLVE-1						NO	120
	Everolimus	362	7.6	1.05	.68		
	Placebo	184	7.3	(0.86–1.27)			
REACH						NO	24
	Ramucirumab	283	9.2	0.86	.13		
	Placebo	282	7.6	(0.72–1.05)			
RESORCE						YES	22
	Regorafenib	379	10.6	0.63	<.001		
	Placebo	194	7.8	(0.50–0.79)			
METIV-HCC						NO	121
	Tivantinib	226	8.4	0.97	NS		
	Placebo	114	9.1	(0.75–1.25)			
S-CUBE							122
	S-1	223	11.1	0.86	.220		
	Placebo	111	11.2	0.67–1.10			
CELESTIAL						YES	23
	Cabozantinib	467	10.2	0.76	.0049		
	Placebo	237	8.0	(0.63–0.92)			
REACH II						YES	25

Trial	Drugs	n	Median time of overall survival (months)	Hazard ratio	P value	Positive result	Reference
				(95 % CI)			
	Ramucirumab	197	8.5	0.71	.0199		
	Placebo	95	7.3	(0.531–0.949)			
			Recurrence free survival (months)				
Adjuvant							
<i>Surgery or RFA</i>							
STORM						NO	123
	Sorafenib	556	RFS 8.5	0.891	.12		
	Placebo	558	RFS: 8.4 (RF)	(0.735–1.081)			
Adjuvant CIK						NO	80
	CIK	115	RFS 44.0		.010		
	No treatment	114	RFS: 30.0				
<i>TACE</i>							
SPACE						NO	13
	Sorafenib	154	time to progression, 169 days	0.797	.072		
	Placebo	153	Time to	(0.588–			
			progression, 166 days	1.08)			
							124
BRISK-TA *	Brivanib	249	26.4	0.9	NS	NO	
	Placebo	253	26.1	(0.66–0.23)			

NS, not significant

* early termination

Table 2.

Phase 3 Trials of Immune Checkpoint Inhibitors

Treatment groups	Line of therapy	Patients	Estimated study completion date
Nivolumab vs sorafenib (CheckMate-459)	1 st line	726	2018
Tremelimumab plus durvalumab vs durvalumab vs sorafenib (HIMALAYA)	1 st line	1200	2021
Pexa vec (oncolytic virus) plus sorafenib vs sorafenib (PHOCUS)	1 st line	600	2019
Atezolizumab plus bevacizumab vs sorafenib (IMbrave 150)	1 st line	480	2022
Tislelizumab (BGB-A317) vs sorafenib	1 st line	600	2022
Pembrolizumab vs placebo (KEYNOTE-240)	2 nd line	408	2019

Table 3.

Combination Studies of Immune-based and Targeted Therapies

Anti PDI/PDL1	Combination partner	Mechanism	Patients	ClinicalTrials.gov number
Nivolumab	galunisertib	TGFB inhibitor	75	02423343
Nivolumab	ipilimumab	anti-CTLA4	45	03222076
Nivolumab	bevacizumab	anti-VEGF	12	03382886
Nivolumab	lenvatinib	anti-VEGFR1,2, and 3	26	03418922
Nivolumab	cabozantinib	TKI	25, unspecified	03299946, 01658878
Nivolumab	trans-arterial tirapazamine embolization	hypoxia-activated toxin	80	03259867
Nivolumab	TACE	tumor ischemia	49	03572582
Nivolumab	deb TACE	tumor ischemia	14	03143270
Nivolumab	vorolanib (x-82)	anti-VEGR and PDGFR	56	03511222
Nivolumab	mogamulizumab	anti-CCR4	114	02705105
Nivolumab	CC-122	pleiotropic pathway modifier	50	02859324
Nivolumab	Y-90	radiation	35, 40	02837029, 03033446
Nivolumab	BMS-986183	undisclosed	25	02828124
Nivolumab	PEXA-Vec	oncolytic virus	30	03071094
Nivolumab	ipilimumab + cabozantinib	anti-CTLA4, TKI	620	01658878
Atezolizumab	codrituzumab	anti-glypican 3		Japan
Atezolizumab	bevacizumab	anti-VEGF	480	03434379
Averumab (PDL1)	axitinib	TKI	20	03289533
Pembrolizumab	XL888	Hsp90 inhibitor	50	03095781
Pembrolizumab	Lenvatinib	anti-VEGFR1,2,3	30	03006926
Pembrolizumab	Bavituximab	anti-phosphatidylserine	34	03519997
Pembrolizumab	Regorafenib	TKI	40	03347292
Pembrolizumab	SBRT	radiation	30	03316872
Pembrolizumab	Y90	local radiation	30	03099564
Pembrolizumab	talimogene laherparepvec (intratumoral)	oncolytic virus	244	02509507
Pembrolizumab	vorolanib (x-82)	TKI	56	03511222
Pembrolizumab	trans-arterial tirapazamine embolization	hypoxia-activated toxin	80	03259867
Pembrolizumab	p53MVA	P53-expressing oncolytic virus	19	02432963

Anti PDI/PDL1	Combination partner	Mechanism	Patients	ClinicalTrials.gov number
Pembrolizumab	autologous tumor-infiltrating lymphocyte		332	01174121
Durvalumab	ramucirumab	anti-VEGFR2	114	02572687
Durvalumab	tremelimumab	anti-VEGF	440, 1200	02519348, 03298451
Durvalumab	tremelimumab plus radiation	anti-VEGF	70	03482102
Durvalumab	cabozantinib	TKI	30	03539822
Durvalumab	guadecitabine	DNMT inhibitor	90	03257761
Durvalumab	ramucirumab	Anti-VEGFR2	114	02572687
Durvalumab	TACE	Tumor ischemia	90	02821754
PDR001	INC280	C-met inhibitor	87	02795429
PDR001	FGF401	FGFR4 inhibitor	238	02325739
PDR001	sorafenib	TKI	50	02988440

TKI, tyrosine kinase inhibitor

DNMT, DNA-methyltransferase

FGFR4, fibroblast growth factor receptor 4

CCR4, CC chemokine receptor 4

Hsp90, heat shock protein 90