Adjuvant therapy for hepatocellular carcinoma: Current situation and prospects

Zhigang Wang, Guofeng Zhang, Jiacheng Wu, Mingku Jia*

Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, The Second Affiliated Hospital of Jilin University, Changchun, Jilin, China.

ABSTRACT: Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide, accounting for 90% of primary liver cancers, and its incidence is still increasing. While the curative treatment for HCC is surgical resection and liver transplantation, most patients are in advanced stage, and lose the chance of surgery. Other palliative treatments include radiofrequency ablation, transarterial embolization, chemotherapy, and radiotherapy. Although there are so many treatments, the prognosis of HCC is still very poor. A major obstacle for the treatment for HCC is the high frequency of tumor recurrence even after curative resection and liver transplantation. Since HCC is frequently resistant to conventional chemotherapy and radiotherapy, clinical development of novel therapeutic agents against HCC has begun in earnest. Thus far, a series of adjuvant therapies for HCC have emerged, including small molecular target agents, monocolonal antibodies, microRNA, and Chinese herbal medicine. Some agents such as sorafenib have shown an advantage in prolonging the overall survival time, and has been approved by FDA for the treatment of advanced HCC. In this article we review the current situation and prospects of adjuvant therapies for HCC.

Keywords: Hepatocellular carcinoma (HCC), adjuvant therapy, molecular targeted therapy, micro RNA (miRNA), Chinese herbal medicine

1. Introduction

Hepatocellular carcinoma (HCC) is the most common liver cancer, accounting for 90% of primary liver cancers. According to the International Agency for Research on Cancer, approximately 670,000 new cases of HCC develop per year (*1*), making it the fifth most common cancer and the third most common cause of cancer related death worldwide. The risk factors for HCC include infection with HBV or HCV, aflatoxin B1 intake, alcohol consumption, non-alcoholic fatty liver disease (NAFLD), and some hereditary diseases, including hereditary hemochromatosis (*2*). Most HCC cases occur in Eastern Asia and sub-Saharan Africa, while low rate areas are found in North America, northern Europe and Australia (*3,4*). Generally, only less than 30-40% of HCC patients are eligible for potentially curative therapies including surgical resection and transplantation because patients are often diagnosed at an advanced stage, losing the surgery opportunity (*5,6*). For other patients with unresectable HCC, transarterial chemoembolization (TACE) offers a definite survival benefit, although its use is often limited by the presence of vascular invasion or extrahepatic spread (*7,8*). Systemic therapies are consequently indicated for a substantial portion of patients with advanced HCC. There had been no proven effective systemic therapy for patients with advanced HCC until sorafenib showed a survival benefit in such patients in two randomized controlled trials (the Sorafenib HCC Assessment Randomized Protocol (SHARP), and Efficacy and Safety of Sorafenib in Patients in the Asia-Pacific Region with Advanced Hepatocellular Carcinoma: a Phase III Randomized, Double-blind, Placebo-controlled Trial (AP)) (*9,10*). And now more and more adjuvant treatments have been used in the clinic, including small molecular target agents, monocolonal antibodies, and Chinese herbal medicine. Some of these treatments have shown superiority to conventional chemotherapy, but all of these did not reach an ideal objective. In this review we will keep the focus on the current situation and prospect of those adjuvant therapies and the combination of them with other treatments such as percutaneous ablation, TACE, conventional chemotherapy, and radiotherapy.

2. Signal pathway in HCC

The key signal transduction pathways that have been

**Address correspondence to:*
Dr. Mingku Jia, Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, The Second Affiliated Hospital of Jilin University, Changchun, Jilin 130041, China.
E-mail: flybirdgang@hotail.com

implicated in the pathogenesis of HCC include those mediated by epidermal growth factor (EGF)/EGF receptor (EGFR), vascular endothelial growth factor (VEGF)/VEGF receptor (VEGFR), platelet-derived growth factor (PDGF)/PDGF receptor (PDGFR), insulin-like growth factor (IGF)/IGF receptor (IGFR), and the Ras/Raf/mitogen-extracellular activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK), Wnt/β-catenin, and phosphatidylinositol-3-kinase (PI3K)/phosphatase and the tensin homologue deleted on chromosome ten (PTEN)/Akt/mammalian target of rapamycin (mTOR) signaling pathways (Figure 1). Activation of these pathways will eventually lead to resistance to apoptosis, cell proliferation, the stimulation

of angiogenesis, invasiveness and metastasis. Inhibiting some of the targets of the signal pathways may exhibit an inhibitory effect on tumor progression. Some agents that have been approved have an effect to inhibit critical molecules in these signal pathways. For example, VEGFR can be inhibited by sorafenib, sunitinib, vatalanib, cederanib, linifanib, and bevacizumab. PDGFR can be inhibited by regorafenib, sorafenib, sunitinib, vatalanib, cederanib, and linifanib. FGFR can be inhibited by brivanib. c-Met can be inhibited by tivantinib and forentinib. RAF can be inhibited by sorafenib and regorafen. And mTOR can be inhibited by sorafenib andregorafen. We have generalized the relationships between signal pathways and molecule targeted agents in Figure 1 and Table 1.
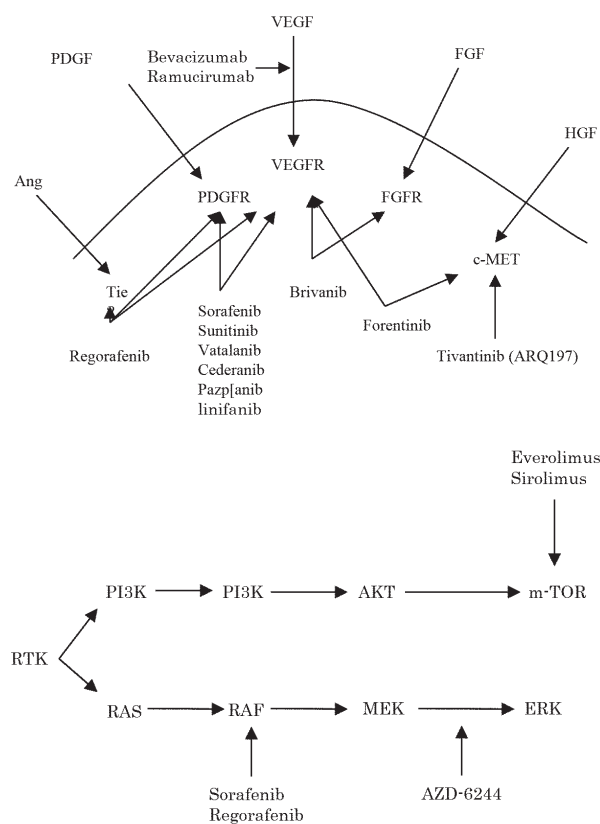
3. Small molecular target agents

3.1. *Sorafenib*

Sorafenib (Nexavar, BAY43-9006) is currently the most promising molecular targeting drug for HCC. Sorafenib is a multikinase inhibitor, which in addition to targeting Raf kinases also inhibits VEGFR-2/-3, PDGFR-β, Flt-3, and c-Kit. On the basis of two large randomized phase III studies, SHARP and AP, sorafenib has been approved by the United States (US) Food and Drug Administration (FDA) for the treatment of patients with advanced HCC (*10,11*). The two trials demonstrated that sorafenib is effective in prolonging median survival and time-to-progression in patients with advanced HCC, the overall survival times were 10.7 months (SHARP) and 6.5 months (AP), but all of them did not reach ideal results. Scientists have strived to combine sorafenib with other treatments. *i*) Sorafenib combined with radiotherapy: The median overall survival time was 15.7 months and 7.8 months in tested and control group, respectively, suggesting that the combined treatment of sorafenib and radiotherapy was feasible (*12*). *ii*) Sorafenib combined with doxorubicin: The disease control rate for 16 evaluable patients was 69% and 40% in tested and control group, respectively. Sorafenib plus doxorubicin appears to be well tolerated and more effective in the treatment of HCC than doxorubicin (*13*).

Figure 1. Signal pathways in hepatocytes and the targets of molecular target drugs.

Table 1. Small molecular agents for HCC

Agent	Stage of development	Target	Overall survial time	Reference
Sorafenib	FDA approved	VEGFR/PDGFR-β/Flt-3 /c-Kit	10.7 months	*10-13*
Sunitinib	FDA approved	PDGF/VEGF/kIt/Flt/cSF-1/RET-receptor	9.3 months	*14-17*
Brivinib	Phase III	EGF/FGFR	9.2 months	*18-22*
Foretinib	Preclinical	HGF/VEGF	---	*23*
TSU-68	Phase II	VEGFR-2	13.1 months	*24*
Erlotinib	Phase II	EGFR	10.75 months	*25-28*
AZD6244	Preclinical	MEK-ERK	---	*29,30*
Linifanib	Phase II	VEGF/PDGFR	9.7 months	*31,32*
Regorafenib	Phase II	VEGFR1-3/TIE2	13.8 months	*33,34*
Tivantinib	Phase II	c-Met	7.2 months	*36*

3.2. *Sunitinib*

Sunitinib is a multikinase inhibitor that targets PDGF α and β, VEGF1.3, kit, Flt3, cSF1, and RET-receptor, and it can thereby impair tumor proliferation, pathological angiogenesis and metastasis (*14*). In a preclinical trial sunitinib moderately inhibited proliferation of the Huh7.5 cell line, upregulated p53 in the p53-wild-type cell line SK-hep-1, and increased the S-phase and the sub-G1 component of the cell cycle in the Hep3B cell line (*15*). There were two phase II trials to test the effect of sunitinib using different methods. One is 'Continuous Sunitinib Treatment in Patients with Advanced Hepatocellular Carcinoma: A Swiss Group for Clinical Cancer Research (SAKK) and Swiss Association for the Study of the Liver (SASL) Multicenter Phase II Trial (SAKK 77/06)'. In this trial, patients received continuous sunitinib treatment (37.5 mg daily). Results demonstrated one complete response and a 40% rate of stable disease as the best response achieved. The median PFS duration, disease stabilization duration, time to progression, and overall survival time were 1.5, 2.9, 1.5, and 9.3 months, respectively. The other one was 'Safety and Efficacy of Sunitinib in Patients with Advanced Hepatocellular Carcinoma: an Open-label, Multicentre, Phase II Study'. Patients were treated with repeated cycles of oral sunitinib (50 mg/day for 4 weeks, followed by 2 weeks off treatment). The result demonstrated that one (2.7%) patient experienced a confirmed partial response, giving an overall objective response rate of 2.7% (95% CI 0.1-14.2). In addition, 13 (35%) of 37 patients achieved stable disease for over 3 months. Commonly observed grade 3 and 4 adverse events included thrombocytopenia (14 of 37; 37.8%), neutropenia (9 of 37; 24.3%), asthenia (5 of 37; 13.5%), hand-foot syndrome (4 of 37; 10.8%), and anemia (4 of 37; 10.8%). There were four deaths among the 37 patients (10.8%) that were possibly related to treatment (*16,17*). On the basis of these results, the trial did not proceed to the second stage. These two trials suggested that sunitinib is a promising molecular agent with continuous oral administration, but further phase III clinical trials are needed.

3.3. *Brivinib*

Brivanib is a dual inhibitor of VEGFR and fibroblast growth factor receptor (FGFR) that are implicated in the pathogenesis of HCC (*18*). Brivanib has a single asymmetric center derived from a secondary alcohol (*19*). Three phase II open-label studies showed that the median overall survival was 10 months. Brivanib as first-line therapy demonstrates promising antitumor activity and a manageable safety profile in patients with advanced HCC (*20-22*), but the median overall survival was similar to 9.2 months observed in the phase II study of sorafenib in patients with advanced HCC and 10.7 months observed in the SHARP study.

3.4. *Foretinib*

Foretinib (XL880, GSK1363089) is a small-molecule kinase inhibitor that targets members of the hepatocyte growth factor (HGF) and VEGF receptor tyrosine kinase families, with additional inhibitory activity toward AXL, c-Kit, Flt-3, PDGFR, and Tie-2. In a preclinical trial foretinib demonstrated significant antitumor activities in patient-derived HCC xenograft models (*23*).

3.5. *TSU-68*

TSU-68 is an inhibitor of VEGFR-2, PDGFR, and FGFR. In patients with advanced HCC, in a phase I/II trial, the median overall survival was 13.1 months, showing promising preliminary efficacy and a high safety profile in patients with advanced HCC (*24*).

3.6. *Erlotinib*

Erlotinib (Tarceva, OSI-774; OSI Pharmaceuticals, Melville, NY, USA) is an orally active, potent selective inhibitor of the EGFR/HER-1-related tyrosine kinase enzyme. Erlotinib inhibits EGF-dependent proliferation of cancer cells at submicromolar concentrations and blocks cell-cycle progression in the G1 phase. In a phase I/II trial, the median overall survival was 10.75 months. Results of this study indicated that single-agent erlotinib is well tolerated and has a modest disease-control benefit in HCC (*25*). In other three phase II studies of erlotinibin plus bevacizumab in patients with advanced HCC, the median overall survival was 10.7 months, 13.7 months, and 9.5 months, showing that the combination of bevacizumab with erlotinib achieved encouraging results in patients with advanced HCC. Current results may help to guide future HCC studies (*26-28*).

3.7. *AZD6244*

AZD6244 is an oral tyrosine kinase inhibitor of the MEK-ERK pathway in patients with advanced HCC (*29*). A preclinical trial combining AZD6244 with sorafenib tested the effect of AZD6244. In this trial, patient-derived HCC xenografts were treated with (*i*) sorafenib, (*ii*) AZD6244, and (*iii*) sorafenib plus AZD6244. Results showed that pharmacological inhibition of the MEK/ERK pathway by AZD6244 enhanced the anti-tumor effect of sorafenib in both orthotopic and ectopic models of HCC (*30*).

3.8. *Linifanib*

Linifanib (ABT-869) is a selective inhibitor of VEGFR and PDGFR. A phase II study showed that when advanced HCC patients received oral linifanib at a daily dose of 25 mg/kg, the estimated progression-free rate at

16 weeks was 31.8%, the median overall survival was 9.7 months, suggesting that single-agent linifanib was clinically active in patients with advanced HCC (*31*). When combined with rapamycin in a preclinical study, the result showed that either linifanib or rapamycin could significantly reduce tumor burden, while combination treatment reduced tumors to the lowest volume, and was significantly better than single agent treatment. These results suggested that HCC could potentially be treated with the combination treatment of ABT-869 and rapamycin (*32*).

3.9. Regorafenib

Regorafenib, a sorafenib analog, has a distinct biochemical kinase inhibition profile and pharmacologic characteristics, including potent inhibition of several angiogenic, stromal, and oncogenic kinases, and broad spectrum activity against several experimental tumors (*33*). Regorafenib targets kinases involved in angiogenesis (*e.g.* VEGFR1-3 and TIE2), oncogenesis (*e.g.* c-kit, RET, and wild-type and V600-mutated BRAF) and the tumor microenvironment (*e.g.* PDGFR and FGFR). In a phase II study, median overall survival was 13.8 months. The present study suggests that, in patients with advanced HCC that has progressed following first-line treatment with sorafenib, regorafenib can be beneficial. The mechanism by which regorafenib may overcome resistance to sorafenib remains to be investigated in future studies (*34*).

3.10. Tivantinib

Tivantinib (ARQ 197) is a selective oral inhibitor of c-Met (*35*). A controlled phase II study showed that when tivantinib (ARQ 197) was used in patients who had received previous sorafenib treatment, it still had a promising result. The median overall survival was 7.2 months. Therefore, tivantinib could provide an option for a second-line treatment of patients with advanced HCC and well-compensated liver cirrhosis, particularly for patients with c-Met-high expression tumors (*36*).

4. Monocolonal antibodies

4.1. Bebacizumab

Bevacizumab is a humanized recombinant monoclonal antibody that binds all isoforms of circulating VEGF-A, the main ligand of VEGFR. It is approved for treatment of several advanced solid tumors. Preclinical studies demonstrated it extended the time to progression of HCC xenografts in mouse models and significantly decreased microvessel density (*37*). In two phase II studies, bevacizumab showed good clinical and biologic activity, indicating that it is a hopeful molecular agent for advanced HCC (*38*). In addition some studies have examined the efficacies of treatments combining bevacizumab with other drugs. Some regimens achieved promising results, including: *i*) Combining bevacizumab with erlotinib, *ii*) Combining bevacizumab with rapamycin, *iii*) Combining bevacizumab with TACE, *iv*) Combining bevacizumab with capecitabine, and *v*) Combining bevacizumab with oxalioplation. It showed that bevacizumab combined with erlotinib has the longest OS, perhaps because it inhibits both VEGF and EGF. The result of all the treatments are shown in Table 2 (*39-43,45,46*).

4.2. Glypican-3 (GPC3)

GPC3 is a member of the glypican family. Glypicans are proteoglycans that are attached to the cell surface by a glycosyl-phosphatidylinositol anchor. They regulate the signaling activity of several growth factors, including Wnts. This regulation is based on the ability of glypicans to stimulate or inhibit the interaction of these growth factors with their respective signaling receptors. It has been clearly established that whereas GPC3 is expressed by most HCCs, this glypican is not detected in normal and cirrhotic liver, or in benign hepatic lesions (*47*). Phase I study results showed that the GPC3-derived peptide vaccine was well tolerated (*48*). Another phase I study also showed that GPC3-derived peptide vaccination was well-tolerated (*49*), GPC3 is a good target for the treatment of HCC, but it still needs further investigation.

5. Micro RNA (miRNA)

In recent years, several studies revealed that the expression of miRNA is deregulated in human HCC in comparison with matched non-neoplastic tissue. These miRNAs were most likely involved in liver tumorigenesis. Most of these miRNAs are downregulated in HCC, such as miRNA-503, miRNA-29C, miRNA-22,

Table 2. The effect and target of bevacizumab combined with other drug

Combination	Phase	Target	OS	reference
Bevacizumab plus erlotinib	Phase II	VEGF and EGF	15.6 month	*39,42,46*
Bevacizumab plus rapamycin	Phase I	VEGF	---	*40*
Bevacizumab plus TACE	Phase II	VEGF	10.8 month	*41,43*
Bevacizumab capecitabine and oxalipation	Phase II	VEGF	9.8 months	*44*
Bevacizumab plus capecitabine	Phase II	VEGF	5.9 months	*45*

Table 3. The effect and target of five Chinese herbal medicine for HCC

Agent	Effect	Target	reference
Silibinin	Inhibits HCC proliferation, survival and metastasis	ERK1/2, PTEN	*56,57*
Berberine	Inhibits HCC proliferation, survival, angiogenesis, and metastasis	VGEF	*58,59*
Quercetin	Inhibits HCC proliferation and survival	caspase-3, -9	*60,61*
Tanshinone II-A	Inhibits HCC proliferation, survival, and metastasis	↓Bcl-2; ↓c-myc; ↑Fas; ↑Bax; ↑p53; ↓MMP-2, -9	*62,63*
Celastrol	Inhibits HCC proliferation, and survival	↑caspase-8; ↑cleaved-bid; ↑caspase-9; ↑caspase-3; ↑PARP; ↓STAT3	*64,65*

miRNA-122, and miRNA-375 (*50-54*). If we can restore expression of the downregulated miRNAs, they can be used for treatment of HCC.

6. Chinese herbal medicine

Chinese herbal medicine has been used for HCC for a long time, and there is a wealth of experience in preventing and treating HCC (*55*). Some Chinese herbal medicine has been proved effective for HCC, for example silibinin, berberine, quercetin, tanshinone II-A, and celastrol. We have summarized the effects and the possible targets in Table 3 (*56-65*). Chinese herbal medicine has a promising prospective, but due to its lack of powerful proof, it needs a lot of preclinical, and clinical studies to support it.

7. Conclusion

In conclusion, adjuvant treatments including small molecular agents, monoclonal antibodies, miRNA, and Chinese herbal medicines have effects on advanced HCC. Some treatments have shown promising results, for example, bevacizumab plus erlotinib can prolong the overall survial time to 15.6 months, but are still be a long distance from people's anticipation. In the future, there may be three ways to improve the situation. *i*) Some new target agent will be used in the clinic. *ii*) A Combination of existing treatment, including small molecular agents, monoclonal antibody, miRNA, Chinese herbal medicine, TACE, conventional chemotherapy, radiotherapy, and radiofrequency ablation. *iii*) Use above mentioned treatment especially molecular target agents early after a patient with early HCC has radical surgery, or before the surgery, but all need to be tested in clinical studies.

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