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TOPIC HIGHLIGHT

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Systemic therapy of hepatocellular carcinoma: Current status and future perspectives

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Abstract

The management of hepatocellular carcinoma (HCC) has substantially changed in the past few decades, the introduction of novel therapies (such as sorafenib) have improved patient survival. Nevertheless, HCC remains the third most common cause of cancer-related deaths worldwide. Decision-making largely relies on evidence-based criteria, as showed in the US and European clinical practice guidelines, which endorse five therapeutic recommendations:resection; transplantation; radiofrequency ablation; chemoembolization; and sorafenib. Many molecularly targeted agents that inhibit angiogenesis, epidermal growth factor receptor, and mammalian target of rapamycin are at different stages of clinical development in advanced HCC. Future research should continue to unravel the mechanism of hepatocarcinogenesis and to identify key relevant molecular targets for therapeutic intervention. Identification and validation of potential surrogate and predictive biomarkers hold promise to individualize patient's treatment to maximize clinical benefit and minimize the toxicity and cost of targeted agents.

Key words: Hepatocellular Carcinoma; Molecular agents; Targeted therapy; Sorafenib

Core tip: Many molecularly targeted agents that inhibit angiogenesis, epidermal growth factor receptor, and mammalian target of rapamycin are at different stages of clinical development in advanced hepatocellular carcinoma. Future research should continue to unravel the mechanism of hepatocarcinogenesis and to identify key relevant molecular targets for therapeutic intervention.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a relevant health problem, being the sixth most common cancer worldwide in terms of incidence with 626000 new cases per year, accounting for 5.7% of all new cancer cases[1]. Due to the poor prognosis of the disease, the number of deaths per year is almost the same as new cases (598000), making HCC the third most common cause of cancer-related death[1].

Prognosis and feasibility of treatments for HCC patients largely depend not only on tumor characteristics, but also on the severity of the underlying chronic liver disease that affects the majority of cases[2,3]. Outcome is significantly worse for those patients who can be treated only with palliative loco-regional treatments, such as transcatheter arterial chemo-embolization, or who are affected by advanced disease. Unfortunately, curative strategies are currently limited to a minority of patients, those who present at diagnosis with small nodules, dis-

ease confined to the liver, good performance status and well preserved liver function. The proportion of patients presenting with these characteristics is currently no more than about 30%-40%[4] . In the experience of the Cancer of the Liver Italian Program group, in a series of 650 patients diagnosed in the years 1994-1999, 59% of patients at diagnosis were not treatable by surgery or percutaneous ablation[5]. However, the proportion of small, early tumors is expected to significantly increase in the next years, together with the diffusion of surveillance procedures of high-risk patients, allowing tumor diagnosis at an earlier stage[4].

Although HCC can be considered a common cancer, evidence about best treatment options is currently based on a disappointingly limited number of randomized controlled trials, compared to many other solid tumors.

SYSTEMIC THERAPY

Unfortunately, a relevant proportion of patients successfully treated with surgical resection or local ablation therapies will experience tumor relapse. Several clinical trials have been conducted to test the efficacy of adjuvant treatments following surgical resection or complete necrosis obtained with ablation.

Several trials testing the role of interferon showed beneficial effect[5]. Most trials were characterized by a small sample size, and interferon, which is associated with significant side effects, cannot currently be considered a standard adjuvant strategy. The recent availability of target-based agents will offer a number of new approaches to test in this setting. From this point of view, the multitarget inhibitor sorafenib appears particularly promising, after the good results obtained in the treatment of advanced disease. A randomized phase Ⅲ study, the STORM trial, comparing sorafenib to placebo as adjuvant treatment for patients who have received surgical resection or local ablation is close to enrol and is currently ongoing.

Occurrence of extrahepatic disease at relapse (*e.g.*, lymph-node involvement or distant metastases) is obviously associated with a significantly worse prognosis. However, recurrence consists frequently of intrahepatic disease only, in this case it can be divided in local recurrence and distant intrahepatic recurrence. Following ablation therapy (like percutaneous ethanol injection or radiofrequency ablation), tumor can recur in the site already treated. In this case, local recurrence can be attributed to incomplete tumor necrosis obtained with the previous ablative treatment. On the contrary, intrahepatic distant recurrent disease following surgical resection or after local ablation has in principle a double etiology: it can represent intrahepatic metastasis, related to previously treated tumor, or can be expression of multicentric disease, unrelated to the primary nodule but arising in the same underlying liver disease. To date, treatment of intrahepatic distant recurrences after curative treatment of primary tumor is commonly based, similarly to primary tumor, on patient's characteristics, on liver function and on number and location of nodules. The difference between intrahepatic metastasis and multicentric occurrence, due to the objective difficulty of a correct differential diagnosis, is not accounted for in existing treatment guidelines. However, at least from a theoretical point of view, the ability to differentiate intrahepatic metastases from multicentric nodules could have relevant implications on treatment strategy. A newly diagnosed second nodule, if expression of multicentric disease, can be effectively treated with a potentially curative loco-regional approach, similarly to primary tumor. These treatments will probably be less effective if the nodule is expression of metastatic disease. In the latter case, disease should be considered at a more advanced stage, and could probably benefit, in addition or in alternative to loco-regional treatment, from a systemic treatment for advanced disease.

Until recently, systemic therapy of advanced HCC provided marginal benefit if any[6]. Systemic chemotherapy for HCC has been associated with low response rates and no survival benefit, partly because HCC is a chemotherapy-resistant tumor[7] - due to the expression of the multi-drug resistance gene *MDR-1*[7,8] - and partly due to the underlying liver cirrhosis in most patients, which prevents the administration of full dosage of many drugs. In addition, the majority of controlled clinical trials of systemic therapy in this patient population are flawed by inappropriate endpoints and controls, as well as by inadequate sample size.

In the following paragraphs, we briefly describe the modest results obtained in the past with hormonal therapy, with chemotherapy and with biological and biochemical treatment. Subsequently, we describe the promising results recently obtained with molecularly targeted agents.

HORMONAL THERAPY

Hormonal therapy of HCC has been investigated based on the finding that various hormone receptors are present in HCC, with a possible association between estrogen and tumor[9].

The finding that various hormone receptors are present in HCC has led many investigators to examine the role of hormone manipulation in this disease. Several lines of evidence have suggested an association between estrogen and HCC. Estrogen receptors are expressed in normal human liver, in chronic hepatitis, in benign hepatic tumour tissues, and rarely in HCC at a low concentration. In preclinical models, estrogens are involved in stimulating hepatocyte proliferation *in vitro* and may promote liver tumour growth *in vivo*. The persistent administration of estrogens, particularly in the form of oral contraceptives, has been associated with an increased incidence of hepatic adenomas and a small increased incidence of HCC. Tamoxifen, an antiestrogenic compound, has been shown to reduce the level of estrogen receptors in the liver. Tamoxifen has been extensively studied in HCC. Six large randomized studies (four of which were double-blind trials) have failed to demonstrate improved

survival with tamoxifen in advanced HCC[10,11]. The role of anti-androgen therapies has been investigated, as well, but they have also failed to improve survival in randomized studies in patients with advanced HCC[12].

In a small prospective randomized Greek study, conducted in 58 patients with advanced HCC, subcutaneous octreotide (250 μg twice daily) was associated with a median survival time of 13 mo, compared with only 4 mo in the group who received no treatment[13]. These results were really promising but later on, another placebo-controlled study randomized 70 patients with advanced HCC to receive a 2-wk course of 250 μg of short-acting octreotide twice daily, followed by a long-acting octreotide 30-mg injection once every 4 wk for six doses, or placebo[14]. Unfortunately, there was no difference in median survival time between the two groups. However, the median survival time was less than 2 mo in both groups, indicating that the study recruited patients with a very poor prognosis, who are very unlikely to derive benefit from any medical therapy. A more recent trial randomized 120 patients with advanced HCC to long-acting octreotide or placebo, with no difference in median survival (4.7 mo with octreotide and 5.3 mo with placebo)[15]. Barbare *et al*[16] reported the preliminary results of a randomized placebo-controlled trial. Two hundred 72 patients with unresectable HCC were randomized to receive either octreotide (monthly *im* injection of 30 mg of long-acting octreotide) or a placebo. Again, no survival benefit was seen in the results of the interim analysis after the occurrence of 150 deaths: the median overall survival time was 6.5 mo in the octreotide arm and 7.3 mo in the placebo arm. Finally, the results of another multicenter randomized trial assessing the combination of long-acting octreotide and tamoxifen in 109 patients with HCC were recently published, again with negative results[17].

Two studies correlated the expression of somatostatin receptors in HCC and the response to octreotide, reaching conflicting conclusions. In one study, patients with HCC expressing somatostatin receptors and randomized to receive octreotide showed a significantly improved survival compared to placebo[18], while in another study there was no relationship between expression of somatostatin receptors by HCC and response to octreotide[19].

In conclusion, octreotide does not seem to benefit patients with advanced HCC. Whether octreotide may have limited benefits in advanced HCC patients whose tumors express somatostatin receptors remains to be defined.

Although a large number of controlled and uncontrolled studies have been performed with most classes of chemotherapeutic agents, no single or combination chemotherapy regimen is particularly effective in HCC. The response rate tends to be low, and the response duration is short. The response criteria used in some of the earlier studies were poorly defined. Most of the earlier studies did not stratify patients on the basis of the severity of underlying cirrhosis or other factors, making comparison of study results difficult. More importantly, any survival benefit of systemic chemotherapy for HCC remains to be determined.

CHEMOTHERAPY

Doxorubicin is perhaps the most widely used agent in HCC. Despite the initial encouraging reports from Uganda for single-agent doxorubicin, subsequent studies have failed to confirm these data. In a large study of doxorubicin in advanced HCC, no responses were noted among 109 patients[20]. Among 475 patients who received doxorubicin in various studies, a 16% response rate was documented, with a median survival of 3-4 mo[21]. Systemic therapies that have not demonstrated improved overall survival benefits in advanced hepatocellular carcinoma.

A variety of combination chemotherapy regimens has been studied in HCC. Although a few of them have shown improved response rates, most of these have not been studied in large randomized phase Ⅲ studies. The most impressive results from phase Ⅱ studies are from the chemotherapy regimen that uses the combination of cisplatin, interferon alfa, doxorubicin, and 5-fluorouracil (PIAF)[22]. This regimen produced a partial response (PR) rate of 26%. In 9 of the 50 patients, the initially unresectable tumours became resectable after chemotherapy. In four of these patients, the resected specimens had a pathologic complete response and the alfa-fetoprotein levels fell to within the reference range. Unfortunately, this regimen was also associated with marked hematologic and gastrointestinal toxicity. Yeo *et al*[23] subsequently examined the efficacy of this regimen in a randomized phase Ⅲ study comparing PIAF with single-agent doxorubicin. A total of 188 patients with unresectable HCC were enrolled. The median survival of the doxorubicin and PIAF groups was 6.83 mo (95%CI: 4.80-9.56) and 8.67 mo (95%CI: 6.36-12.00), respectively (P = 0.83), which failed to reach statistical significance for the study primary end point.

The difficulty of developing effective chemotherapy in HCC may in part be due to the inherent resistance in the tumour conferred by the multidrug-resistant gene *MDR-1*. In addition, the underlying cirrhosis present in most patients may lead to portal hypertension with hypersplenism, platelet sequestration, varices and gastrointestinal bleeding, hepatic encephalopathy, hypoalbuminemia, differential drug binding and distribution, and altered pharmacokinetics, limiting the selection and adequate dosing of most cytotoxic agents.

Two new chemotherapy drugs, nolatrexed - a novel thymidylate synthase inhibitor - and T138067 - a microtubule formation inhibitor - were compared to doxorubicin in two phase Ⅲ randomized studies[24,25]. Unfortunately, neither nolatrexed nor T138067 provided survival benefit compared with doxorubicin. At the present time, there is no cytotoxic drug or regimen that can be clearly defined as a standard for treating HCC, and chemotherapy should not be considered as an option for patients with HCC.

Without doubt, the development of molecularly targeted agents opened new and exciting perspectives for systemic therapy of HCC. Many molecular alterations

Table 1 Efficacy results of molecular agents for advanced hepatocellular carcinoma

Molecular agent	Study phase	Results	Ref.
Sorafenib	Ⅲ (Sharp) *vs* placebo	Median OS: 10.7 mo *vs* 7.9 mo	[31]
	Ⅲ (Asian) *vs* placebo	Median OS: 6.5 mo *vs* 4.2 mo	[32]
	Ⅱ (sorafenib + doxorubicin *vs* doxorubicin)	Median OS: 13.7 mo *vs* 6.5 mo	[33]
Bevacizumab	Ⅱ	Median OS: 12.4 mo	[43]
	Ⅱ	Median OS: 9.6 mo	[45]
	(Beva + gemox)	Median OS: 15.0 mo	[48]
	Ⅱ (Beva + erlotinib)		
Sunitinib	Ⅱ	Median OS: 9,8 mo	[49]
	Ⅱ	Median OS: 8.0 mo	[50]
	Ⅲ (Sunitinib *vs* sorafenib)	Median OS: 7.9 mo *vs* 10.2 mo	[51]
Brivanib	Ⅱ	Median OS: 9.7 mo	[52]
	Ⅲ BRISK-PS (Briv *vs* placebo)	Median OS: 9.4 mo *vs* 8.3 mo	[53]
	Ⅲ BRISK-FL(Briv *vs* sorafenib)	Median OS: 9.5 mo *vs* 9.9 mo	[54]
ABT 869 (Inifanib)	Ⅱ	Median OS: 9.7 mo	[55]
Pazopanib	Ⅰ	Median TTP 4.5 mo	[56]
AZA2171 (Cediranib)	Ⅱ	Median OS: 5.8 mo	[57]
Vatalanib (PTK787/ZK 222584)	Ⅰ-Ⅱ	Median OS: 7.3 mo	[58]
Tivantinib (ARQ 187)	Ⅱ (Tivant *vs* placebo)	Median OS 7.2 mo *vs* 3.8 wk (c-met High)	[59]
Ramucirumab	Ⅱ	PFS 4.3 mo	[60]
Everolimus	Ⅰ-Ⅱ	PFS 3.8 mo	[75]
Erlotinib	Ⅱ	Median OS: 13 mo	[67]
Gefitinib	Ⅱ	Median OS: 6.5 mo	[69]
Lapatinib	Ⅱ	Median OS: 6.2 mo	[70]
Cetuximab	Ⅱ	Median OS: 9.6 mo	[71]

OS: Overall survival; TTp: Time to progression; PFS: Progression-free survival.

have been identified in HCC and a lot of work has been done to identify the potential therapeutic targets.

In the following paragraphs, we describe the promising results recently obtained with molecularly targeted agents, in particular with sorafenib, that is the first drug with high-level evidence of efficacy in patients with advanced HCC and the future perspectives with new molecular agents.

MOLECULARLY TARGETED THERAPY

In the past few years, the mechanisms of hepato-carcinogenesis have been elucidated and the involvement of a number of pathways, including angiogenesis, aberrant signal transduction, and dysregulated cell cycle control have been demonstrated, leading to the evaluation of the activity and toxicity of some of the new molecular target agents[26] (Table 1). In chronic hepatitis and liver cirrhosis the phenotypically altered hepatocytes have high epidermal growth factor receptor (EGFR) expression and non-committal epigenetic changes (increase in transforming growth factor (TGF)-β, insulin-like growth factor-2 and Raf). These phenotypically altered hepatocytes may become dysplastic and show more committed genetic changes, *e.g.*, increased telomerase activity and varied allelic deletions, which may eventually lead to the evolution of HCC with additional genetic changes, such as an increase in c-myc and decrease in p16 expression[27].

Significant progress on the treatment of advanced HCC has been made possible by sorafenib, a novel signal transduction inhibitor that blocks tumour cell proliferation by targeting the Raf/MEK/ERK signalling pathway and exerts an antiangiogenic effect by targeting the tyrosine kinases of vascular endothelial growth factor receptor (VEGFR)-2, VEGFR-3, and platelet-derived growth factor receptor (PDGFR)-beta. In preclinical models, sorafenib exhibited antitumor activity in HCC cells and xenograft models. In a phase Ⅱ study of 137 patients with advanced HCC, sorafenib provided orally at 400 mg twice daily induced a PR in 2.2% of patients, a minor response in 5.8%, and stable disease lasting 4 mo in 34%[28]. Median time to progression (TTP) was 4.2 mo, and median overall survival (OS) was 9.2 mo. The international, phase Ⅲ, placebo-controlled sorafenib HCC Assessment Randomized Protocol trial evaluated 602 patients with advanced HCC who had not undergone prior systemic therapy to receive either sorafenib at 400 mg twice daily (299 patients) or placebo (303 patients)[29]. The primary end point of the study was OS. Patients with underlying Child-Pugh A cirrhosis accounted for 95% and 98% in

the sorafenib and placebo groups, respectively. Median OS was 10.7 mo in the sorafenib group and 7.9 mo in the placebo group (HR = 0.69; P = 0.001). The median TTP was 5.5 mo in the sorafenib group and 2.8 mo in the placebo group (P = 0.001). In another Asian-Pacific randomized phase Ⅲ study, sorafenib also demonstrated improved OS in patients with advanced HCC, mostly in patients with hepatitis B virus infection[30]. OS was 6.5 mo in the sorafenib group *vs* 4.2 mo in the placebo group (HR = 0.68; P = 0.014). The safety profiles of sorafenib seem favorable; however, grade Ⅲ diarrheal, hand-and-foot skin reaction, and fatigue were observed. The successful development of sorafenib has validated the use of molecularly targeted agents in HCC. This is the first agent ever to have shown improved survival benefits in this disease. It highlights the importance of selecting the right patient population (good performance status and preserved hepatic function) for clinical trial design. The major benefits of sorafenib are mainly manifested as disease stabilization rather than radiologic response. However, many questions remained unanswered: what is the mechanism of action mediating the clinical benefits of sorafenib? Who are at risk for developing toxicities? What is the escape and resistance mechanism of sorafenib failure? Will sorafenib benefit patients with worsening underlying cirrhosis? Will sorafenib prove to be beneficial in patients in earlier stages of disease that is, after surgical resection, high-risk transplantation, or radiofrequency ablation, as well as transarterial chemoembolization? Some of these questions are addressed in ongoing and planned clinical trials as BOOST trial.

Sorafenib-based regimens under development

Abou-Alfa *et al*[31] reported their experience from a randomized, double-blinded, phase Ⅱ study comparing Doxorubicin in combination with sorafenib *vs* doxorubicin with placebo in patients with advanced HCC. Patients had Eastern Cooperative Oncology Group performance status of 0-2, Child-Pugh A cirrhosis, and no prior systemic therapy. They received Doxorubicin at 60 mg/m^2 intravenously every 21 d (cycle) plus either sorafenib at 400 mg orally twice daily or placebo, for a maximum of six cycles of doxorubicin. Patients could continue with single-agent sorafenib or placebo afterward. The primary end point was TTP by independent review. Ninety-six patients were randomized in this study. Following complete accrual, an unplanned early analysis for efficacy was performed by the independent data monitoring committee, so the trial was halted. The 2 patients remaining in the placebo group at that time were offered sorafenib. Based on 51 progressions, 63 deaths, and 70 events for progression-free survival, median time to progression was 6.4 mo in the sorafenib-doxorubicin group (95%CI: 4.8-9.2), and 2.8 mo (95%CI: 1.6-5) in the doxorubicin-placebo monotherapy group (P = 0.02). Median overall survival was 13.7 mo (95%CI: 8.9-not reached) and 6.5 mo (95%CI: 4.5-9.9; P = 0.006), and progression-free survival was 6.0 mo (95%CI: 4.6-8.6) and 2.7 mo (95%CI: 1.4-2.8) in these groups, respectively (P = 0.006). Toxicity profiles were similar to those for the single agents.

Among patients with advanced HCC, treatment with sorafenib plus doxorubicin compared with doxorubicin monotherapy resulted in greater median time to progression, overall survival, and progression-free survival. The degree to which this improvement may represent synergism between sorafenib and doxorubicin remains to be defined. The combination of sorafenib and doxorubicin is not yet indicated for routine clinical use.

Because of the lack of consensus on the best chemotherapeutic agents/regimens in HCC and the safety concerns including cardiac toxicity for doxorubicin, other investigators are investigating the efficacy and tolerability of combining sorafenib with capecitabine and oxaliplatin or gemcitabine and cisplatin in advanced HCC. Given the complexity of hepatocarcinogenesis and heterogeneity of HCC, targeting HCC by means of a combination of sorafenib and another agent inhibiting a distinct pathway represents an appealing strategy. On the basis of this rationale, preclinical data, phase I experience, and single-agent activity and tolerability in HCC, a randomized international phase Ⅲ study comparing sorafenib plus erlotinib *vs* sorafenib plus placebo as first-line treatment in advanced HCC is ongoing. The primary end point of the study is OS. Other sorafenib-based combinations, including mTOR inhibitors and insulin growth factor receptor (IGF-R) inhibitors, are at an early stage of development.

Antiangiogenic agents and TKI-inhibitors

HCCs are vascular tumours, and increased levels of vascular endothelial growth factor (VEGF) and microvessel density have been observed[34,35]. High VEGF expression has been associated with worse survival[36-38]. Therefore, inhibition of angiogenesis represents a potential therapeutic target in HCC, and several antiangiogenic agents have entered clinical studies in HCC.

Bevacizumab: Bevacizumab is a recombinant humanized monoclonal antibody that targets VEGF. In addition to its direct antiangiogenic effects, Bevacizumab may enhance chemotherapy administration by "normalizing" tumour vasculature and lowering the increased interstitial pressure in tumours[39,40]. Several studies have explored the use of Bevacizumab either as a single agent or in combination with cytotoxic or molecularly targeted agents in patients with advanced HCC. Siegel *et al*[41] reported their experience using single-agent bevacizumab in HCC in a phase Ⅱ study. Two dosages of bevacizumab, 5 and 10 mg/kg administered intravenously once every 2 wk, were tested in patients with HCC with no overt extrahepatic metastases or invasion of major blood vessels. Of the 46 patients with data available for efficacy, 6 had objective responses (13%; 95%CI: 3-23), and 65% were progression free at 6 mo. Median progression-free survival (PFS) time was 6.9 mo (95%CI: 6.5-9.1), and median survival was 12.4 mo (95%CI: 9.4-19.9). Malk *et al*[42] also reported their early experience using Bevacizumab as a single agent in HCC

in a phase Ⅱ study. The combination of Bevacizumab with cytotoxic agents was also evaluated in three phase Ⅱ studies. Zhu *et al*[43] completed a phase Ⅱ study that used bevacizumab in combination with gemcitabine and oxaliplatin in advanced HCC. This regimen had moderate antitumor activity in HCC with an overall response rate of 20% in evaluable patients. An additional 27% of patients had stable disease with a median duration of 9 mo (range, 4.5-13.7 mo). The median OS was 9.6 mo and the median PFS was 5.3 mo. The combination of Bevacizumab with capecitabine and oxaliplatin or with capecitabine alone in patients with advanced HCC was also reported[44,45]. Thomas *et al*[46] reported their single-center phase Ⅱ experience using the combination of Bevacizumab and Erlotinib in patients with advanced HCC. Bevacizumab was provided at 10 mg/kg intravenously once every 14 d and Erlotinib at 150 mg orally daily. Of the 40 patients with efficacy data available, a 25% response rate was observed. The median PFS was 9 mo and OS was 15 mo. The above studies demonstrated early evidence of antitumor activity of Bevacizumab in HCC. Despite the overall good tolerability profiles, the risk of bleeding, hypertension, and thromboembolic events remain to be further characterized. Moreover, as a result of the nonrandomized nature, small sample size, and patient selection bias inherent in single-arm studies, the relative contributions, if any, from any chemotherapy regimens or erlotinib remain unknown and warrant further investigations.

Sunitinib: Sunitinib is an oral multikinase inhibitor that targets receptor tyrosine kinases including VEGFR-1, VEGFR-2, PDGFR-a/b, c-KIT, FLT3, and RET kinases. Zhu *et al*[47] performed a study in patients with advanced HCC that used sunitinib at 37.5 mg orally once daily on a standard 4-wk-on, 2-wk-off regimen (6 wk per cycle). The primary end point of the study was PFS. Of the 34 patients enrolled, one patient had a PR of 20 mo duration, and an additional 10 patients (38.5%) had stable disease of at least 12 wk duration. The median PFS was 3.9 mo and OS was 9.8 mo. In another European/Asian phase Ⅱ study, sunitinib was administered at 50 mg daily for 4 wk every 6 wk to patients with unresectable HCC[48]. The primary end point of the study was overall response rate according to Response Evaluation Criteria in Solid Tumours criteria. Of the 37 patients enrolled, one patient (2.7%) experienced PR, and 13 patients (35%) had stable disease as their best response. The median OS was 8.0 mo and PFS was 3.7 mo. Preliminary results from two other phase Ⅱ studies were also presented, one that used 37.5 mg for a 4-wk-on, 2-wk-off schedule, and the other with 37.5 mg continuous daily dosing.

In terms of toxicity, the studies that used the lower dose (37.5 mg) reported acceptable safety profiles. The most common adverse events included hematologic toxicities, fatigue, and an increase in transaminase. Grade 3 or 4 adverse events occurred in no more than 20% of the patients in any category. At the higher dose of 50 mg daily, sunitinib treatment led to more pronounced grade 3-4 toxicities and a higher death rate of 10% in this patient population.

Although the lower dose at 37.5 mg seems to be more tolerable, it remains uncertain whether the continuous or intermittent schedule is better. A randomized phase Ⅲ study comparing sunitinib at 37.5 mg continuous daily dosing *vs* sorafenib at 400 mg twice daily in advanced HCC was presented at ASCO meeting in 2011 and sunitinib failed its primary OS endpoint, indeed the median OS was 7.9 mo for sunitinib *vs* 10.2 mo for sorafenib[49].

Brivanib: Brivanib alaninate is a dual inhibitor of VEGFR and fibroblast growth factor receptor (FGFR)-signaling pathways that can induce tumour growth inhibition in mouse HCC xenograft model. A phase Ⅱ study was conducted to assess the efficacy and safety of brivanib in patients with unresectable locally advanced or metastatic HCC who had received either no prior systemic therapy (cohort A) or one prior regimen of angiogenesis inhibitor (cohort B)[50]. This phase Ⅱ open-label study assessed brivanib as second-line therapy in patients with advanced Brivanib was administered orally at a dose of 800 mg once daily. The primary objectives were tumor response rate, time to response, duration of response, progression-free survival, OS, disease control rate, TTP, and safety and tolerability. Forty-six patients were treated. Best responses to treatment with brivanib (N¼ 46 patients) using modified World Health Organization criteria were partial responses for two patients (4.3%), stable disease for 19 patients (41.3%), and progressive disease for 19 patients (41.3%). The tumor response rate was 4.3%; the disease control rate was 45.7%. Median OS was 9.79 mo. Median TTP as assessed by study investigators following second-line treatment with brivanib was 2.7 mo. The most common adverse events were fatigue, decreased appetite, nausea, diarrhea, and hypertension. In conclusion Brivanib had a manageable safety profile and is one of the first agents to show promising antitumor activity in advanced HCC patients treated with prior sorafenib. Large randomized phase Ⅲ Brivanib Study in Patients at Risk (BRISK) HCC program trials have been conducted to evaluate the role of brivanib in advanced HCC (BRISK-FL, BRISK-PS and BRISK-APS). The BRISK-PS trial evaluated brivanib *vs* placebo in patients who had failed or were intolerant to sorafenib therapy. This study did not meet its primary end point of improving OS, but treatment with brivanib showed improvements in the response rate[51]. The BRISK-FL trial directly compared the clinical outcomes of brivanib *vs* sorafenib in patients with advanced HCC who received no prior systemic therapy. The median OS was 9.5 mo in the brivanib ar m compared with 9.9 mo in the sorafenib arm, which was not a statistically significant difference. No significant survival differences were observed between subgroups based on geographic regions, cause of HCC or disease severity. The study did not meet its primary OS objective based upon a non-inferiority statistical design[52].

ABT-869: ABT-869 (inifanib) is an orally active, potent, and selective inhibitor of VEGFR and PDGFR. Preliminary results from an open-label, multicenter phase Ⅱ study of ABT-869 in advanced HCC were reported[53]. ABT-869 was provided at 0.25 mg/kg daily in Child-Pugh A or once every other day in Child-Pugh B patients until disease progressed or toxicity became intolerable. The primary end point was the progression-free rate at 16 wk. Of the 44 patients enrolled, 34 had data available for analysis (28 with Child Pugh A and 6 with Child Pugh B cirrhosis). The estimated response rate was 8.7% (95%CI: 1.1-28) for the 23 patients with Child A cirrhosis. For all 34 patients, median TTP was 112 d (95%CI: 110-not estimable), median PFS was 112 d (95%CI: 61-168), and median OS was 295 d (95%CI: 182-333). The most common adverse events for all patients were hypertension (41%), fatigue (47%), diarrheal (38%), rash (35%), proteinuria (24%), vomiting (24%), cough (24%), and oedema peripheral (24%). The most common grade 3-4 adverse events were hypertension (20.6%) and fatigue (11.8%). The early evidence of efficacy and tolerable safety profiles has encouraged further development of ABT-869 in HCC.

Pazopanib: Pazopanib is an oral angiogenesis inhibitor targeting VEGFR, PDGFR, and c-Kit. Reports from a phase I study to determine the maximum tolerated dose (MTD), safety, pharmacokinetics, pharmacodynamics, and efficacy of pazopanib in patients with locally unresectable and/or advanced HCC were presented[54]. Eligibility criteria included unresectable and/or metastatic HCC with at least one target lesion, recovery from prior systemic regimens, Eastern Cooperative Oncology Group performance status of 0 or 1, Child Pugh A, and adequate organ function. Doses of pazopanib were escalated from 200 mg once daily to 800 mg daily in a 3 + 3 design. In the 27 Asian patients enrolled, MTD was determined to be 600 mg once daily. PR was observed in two patients (7%; one at 800 mg, one at 600 mg) and stable disease of 4 mo in 11 patients (41%). Median TTP at the MTD was 137.5 d (range, 4-280 d). Changes in tumour dynamic contrast-enhanced magnetic resonance imaging parameters were seen after repeated dose pazopanib administration.

Cediranib (AZD2171): Cediranib is a potent oral pan-VEGF receptor tyrosine kinase inhibitor with activity against plateletderived growth factor receptors and c-Kit. AZD2171 is a potent inhibitor of both KDR (IC_{50} = 0.002 lM) and Flt-1 (IC_{50} = 0.005 lM), and shows activity against c-kit, platelet-derived growth factor receptor beta (PDGFRb) and Flt-4 at nanomolar concentrations. Alberts *et al*[55] reported their experiences of toxicity and efficacy of AZD2171 from a phase Ⅱ study in patients with advanced HCC, the median OS was 5.8 mo. No patients experienced confirmed response. The median time to progression was 2.8 mo.

Vatalanib (PTK787/ZK 222584): Vatalanib is an oral angiogenesis inhibitor targeting all known VEGFR tyrosine kinases, including VEGFR-1/flt-1, VEGFR-2/KDR, and VEGFR-3/Flt-4, PDGFR, and the c-kit with a higher selectivity for VEGFR-2. Koch *et al*[56] reported the early experience of an open-label, multicenter phase I study to characterize the safety, tolerability, and pharmacokinetic profile of PTK787 administered once daily at a dose of 750-1250 mg in patients with unresectable HCC. Patients were stratified into three groups with mild, moderate, and severe hepatic dysfunction, respectively, on the basis of total bilirubin and aspartate aminotransferase/alanine aminotransferase levels. The maximal tolerated dose of PTK787 was defined as 750 mg daily. Of patients in all groups, 18 had efficacy data available. No complete response or PR was observed. Nine patients had a best response of stable disease, and nine had progressive disease. There are no studies planned to develop this agent in the treatment of HCC at this time.

Tivantinib (ARQ 187): Tivantinib is a selective, oral inhibitor of c-Met, the tyrosine kinase receptor for hepatocyte growth factor involved in tumor cell migration, invasion, proliferation and angiogenesis. Tivantinib has shown promising results in HCC in phase I studies as monotherapy and in combination with sorafenib. A phase Ⅱ study was published this year, this multi-center randomized clinical trial enrolled patients with unresectable HCC, 1 failed systemic therapy, ECOG PS < 2. Child-Pugh B-C were excluded. Patients were randomized 2:1 to oral tivantinib [360 mg *bid* (A), 240 mg *bid* (B)] or placebo (P), stratifying by PS and vascular invasion. Treatment continued until disease progression (PD) or unacceptable toxicity[57]. RECIST 1.1 response was evaluated by CT/MRI every 6 wk. Crossover to open-label T was allowed after PD. Primary endpoint was TTP in the intent-to-treat (ITT) population by central radiology review. Other endpoints included disease control rate (DCR), PFS, OS, efficacy in Met+ (Met ⩾ 2+ in > 50% of tumor at immunohistochemistry) pts, safety. Major TTP, DCR and PFS benefits were obtained in Met+ patients, with preliminary OS trend favoring Tivantinib (HR = 0.47) and no detrimental effect in Met- patients. Disease control rate (95%CI) in tivantinib/placebo was 44 (31-56)/31(16-48)% for ITT and 50(28-72)/20(4-48)% in Met+ patients. Most common AEs in Tivantinib were asthenia (26.8%), NEUT (25.4%), low appetite (25.4%); most common drug-related AEs were NEUT (25.4%), anemia (15.5%). Most frequent drug-related serious AE was neutropenic sepsis (4.2%). Efficacy was similar in A/B with less frequent NEUT in B (21.1%/6.1%). Compared to Placebo, tivantinib significantly benefited second-line HCC patients, especially if Met+, with manageable safety profile at 240 mg BID. A phase Ⅲ, randomised, double blind study of tivantinib in subjects with met-diagnostic high inoperable HCC treated with one prior systemic therapy is ongoing.

Ramucirumab: The monoclonal antibody ramucirumab

is a specific inhibitor of VEGFR-2. A phase Ⅱ study of 42 patients with advanced HCC and primarily well-preserved liver function (75% C-P A status) showed that first-line ramucirumab monotherapy produced a disease control rate of 50% and a median PFS of 4.3 mo[58]. This positive study prompted the phase Ⅲ REACH trial in HCC, comparing ramucirumab/supportive care with placebo/supportive care for second-line treatment after sorafenib, the results of which will be available in the coming months.

EGFR inhibitors

The expression of several EGF family members, specifically EGF, TGF-α, and heparinbinding epidermal growth factor, as well as EGFR, has been described in several HCC cell lines and tissues[59-64]. Multiple strategies to target EGFR signaling pathways have been developed, and two classes of anti-EGFR agents have established clinical activity in cancer: monoclonal antibodies that competitively inhibit extracellular endogenous ligand binding, and small molecules that inhibit the intracellular tyrosine kinase domain. Other than the modest activity with erlotinib, the rest of the EGFR inhibitors failed to show any activity as single agents in advanced HCC.

EGFR tyrosine kinase inhibitors

Two phase Ⅱ clinical studies have evaluated the safety and efficacy of Erlotinib provided at 150 mg daily in patients with advanced HCC. In the study by Philip *et al*[65], 3 (9%) of 38 patients experienced PR, and 12 patients (32%) were free of progression of disease at 6 mo. Median OS time for this cohort was 13 mo. In another report by Thomas *et al*[66], 17 (43%) of 40 patients achieved PFS at 16 wk, and the PFS rate at 24 wk was 28%. No PR or complete response was observed in this study. The median time to failure, defined as either disease progression or death, was 13.3 wk. The median time of OS was 25.0 wk (95%CI: 17.9-42.3) from the date of Erlotinib therapy initiation. In the Eastern Cooperative Oncology Group's E1203 study, Gefitinib provided at 250 mg daily was examined in a single-arm phase Ⅱ study[67]. A two-stage design was used, and 31 patients were accrued to the first stage. One patient had PR and seven patients had stable disease. The median PFS was 2.8 mo (95%CI: 1.5-3.9) and median OS was 6.5 mo (95%CI: 4.4-8.9). The criterion for second stage accrual was not met, and the authors concluded that gefitinib as a single agent was not active in advanced HCC. Lapatinib, a selective dual inhibitor of both EGFR and HER-2/NEU tyrosine kinases, also demonstrated modest activity in HCC. Among the 40 patients with advanced HCC, the response rate was 5%, PFS 2.3 (95%CI: 1.7-5.6) mo, and OS of 6.2 (95%CI: 5.1-infinity) mo[68].

Monoclonal antibodies against EGFR

Cetuximab, a chimeric monoclonal antibody against EGFR, was tested in two phase Ⅱ studies in patients with advanced HCC. In a phase Ⅱ study, 30 patients with advanced HCC were enrolled[69]. The initial dose of cetuximab was 400 mg/m^2 provided intravenously, followed by weekly intravenous infusions at 250 mg/m^2. No responses were seen. Five patients had stable disease (median time, 4.2 mo; range, 2.8-4.2 mo). The median OS was 9.6 mo (95%CI: 4.3-12.1) and the median PFS was 1.4 mo (95%CI: 1.2-2.6). Cetuximab trough concentrations were not notably altered in patients with Child-Pugh A and B cirrhosis. The combination of cetuximab with gemcitabine and oxaliplatin (GEMOX) was evaluated in a phase Ⅱ study. All patients received cetuximab at an initial dose of 400 mg/m^2 followed by 250 mg/m^2 weekly, gemcitabine 1000 mg/m^2 on day 1, and oxaliplatin at 100 mg/m^2 on day 2, repeated every 14 d until disease progression or limiting toxicity. Of the 45 patients enrolled, the confirmed response rate was 20% and disease stabilization rate was 40%. The median PFS and OS were 4.7 mo and 9.5 mo, respectively. The 1-year survival rate was 40%. Given the reported antitumor activity of GEMOX in prior phase Ⅱ studies and the lack of activity of cetuximab as single agents, the relative contribution of cetuximab to this regimen remains to be defined[70].

The combination of cetuximab with capecitabine and oxaliplatin was evaluated in a single-arm phase Ⅱ study[71]. Patients received capecitabine at 850 mg/m^2 twice daily for 14 d, oxaliplatin on day 1 at 130 mg/m^2 intravenously, and cetuximab at 400 mg/m^2 on day 1 followed by 250 mg/m^2 weekly in a 21-d cycle. Of the 25 patients enrolled, data for efficacy were available for 20 patients. Response rate was 10% (95%CI: 1-33), and TTP was 4.3 mo (95%CI: 2.3-5.0). Although most patients tolerated the treatment well, diarrheal and electrolyte abnormalities including hypomagnesemia and hypocalcemia were more pronounced in this population.

mTOR inhibitors

mTOR functions to regulate protein translation, angiogenesis, and cell-cycle progression in many cancers, including HCC. Preclinical data have demonstrated that mTOR inhibitors were effective in inhibiting cell growth and tumour vascularity in HCC cell lines and HCC tumour models. The importance of the mTOR pathway in HCC was examined in a comprehensive study with 314 HCC and 37 nontumoral tissues that used a series of molecular techniques to assess mutation, DNA copy number changes, messenger RNA and gene expression, and protein activation[72]. Aberrant mTOR signalling (p-RPS6) was present in half of the cases and chromosomal gains in rapamycininsensitive companion of mTOR (RICTOR) (25% of patients), and positive p-RPS6 staining correlated with HCC recurrence after resection.

A number of mTOR inhibitors (sirolimus, temsirolimus, and everolimus) are available clinically. Retrospective studies in patients who underwent liver transplantation for HCC have shown that patients who received sirolimus for immunosuppression had a much

lower rate of tumour recurrence than those who received calcineurin inhibitors. Clinical studies with mTOR inhibitors alone and in combination with either targeted agents or chemotherapeutic agents in advanced HCC are at an early stage of clinical development. Chen *et al*[73] recently reported their early experience of a randomized phase I pharmacokinetic study of everolimus in advanced HCC. Two different schedules were tested: continuous daily dosing and once-weekly dosing. A total of 36 patients were enrolled. Dose-limiting toxicities observed included hyperbilirubinemia, high levels of alanine aminotransferase, thrombocytopenia, infection, diarrheal, and cardiac ischemia. The MTD for weekly and daily dosing schedules was determined to be 70 and 7.5 mg, respectively. Interestingly, reactivation of hepatitis B and C virus was observed in four and one patients, respectively. The disease control rate of 31 evaluable patients was 61% (10 of 16) and 46.7% (7 of 15, including one case of PR) of patients receiving daily and weekly treatment, respectively.

In patients with advanced HCC, everolimus produced a median PFS of 3.8 mo and a disease control rate of 44% in phase Ⅰ/Ⅱ testing. Consequently, a phase Ⅲ EVOLVE-1 trial to compare everolimus with BSC in patients with HCC who progressed on or after sorafenib or who were intolerant to sorafenib, has been completed and everolimus did not show survival benefit for patients as announced in a press release on August 7 2013[74].

MEK inhibitor

HCC is characterized by frequent MEK/ERK activation in the absence of RAS or RAF mutation. A multicenter, singlearm phase Ⅱ study with a two-stage design was conducted with AZD6244, a specific inhibitor of MEK, in advanced HCC[75]. The primary end point was response rate. AZD6244 was administered orally at a dose of 100 mg twice a day. Of the 19 patients enrolled, 16 had response data available. Despite the good tolerability of AZD6244, it showed minimal activity in advanced HCC. No response was observed, and stable disease was observed in 37.5% of the patients. The median TTP was only 8 wk (95%CI: 6.6-11.1).

Monoclonal antibodies against GPC-3

Glypican-3 (GPC-3) is a member of the glypican family, a group of heparan sulfate proteoglycans linked to the cell surface through a glycosylphosphatidylinositol (GPI) anchor Glypicans play an important role in cell growth, differentiation, and migration[76,77].

GPC-3 protein is expressed in a wide variety of tissues during development, but the expression in most adult tissues is suppressed by the methylation of DNA within its promoter region[78]. Recently, it was shown that GPC-3 is highly expressed, both at the mRNA and protein level, in HCC[79]. Immunohistochemical studies have shown that GPC-3 is expressed in approximately 70%-100% of surgically removed or biopsied HCC tissues, whereas it is not detectable in adjacent non-tumoral lesions[80]. GPC-3 may promote hepatocellular carcinoma growth by stimulating the canonical Wnt pathway and it also interacts with the IGFII-IGF1R pathway. Others have suggested that it may play a role in FGF signaling as well. GPC-3 therefore may represent a specific tumor marker and a potential target for therapy in HCC[81].

GC33 (RO5137382) is a recombinant, humanized monoclonal antibody that binds to human GPC-3 with high affinity. The nonclinical pharmacological assessments have shown that GC33 elicits antibody dependent cellular cytotoxicity (ADCC) through human peripheral blood mononuclear cells as well as mouse effector cells against GPC-3-expressing human HCC and hepatoblastoma cell lines *in vitro*. It also showed anti-tumor activities in several mouse xenograft models inoculated with human HCC cell lines expressing GPC3.

Activity is proportional to cell surface expression of the target across 3 xenograft models, and is associated with macrophage infiltration into the xenografts. Direct activity of GC33 *in vitro* was not observed, suggesting that the relevant mechanism of action (MoA) is *via* ADCC. Two phase I studies are being conducted in the United States: GC-001US (GC33 monotherapy), and GC-002US (GC33/Sorefanib combination) and one study in Japan: GC-003JP (GC33monotherapy). The dose escalation phase of GC-001US has completed accrual at planned doses up to and including 20 mg/kg per week. Is now ongoing a phase Ⅱ trial, in second line setting, designed to establish the efficacy of GC33 compared to placebo in patients whose hepatocellular cancer tumor expresses the GPC-3 protein.

CONCLUSION

Despite decades of efforts by many investigators, no studies with systemic chemotherapy or hormone therapy have demonstrated improved survival in patients with advanced HCC. sorafenib has emerged as the new standard treatment for advanced HCC also patients with advanced HCC who failed first-line therapy could have substantially improved prognosis if they had Child-Pugh A liver reserves or were potentially eligible for clinical trials[82].

Many molecularly targeted agents are at different stages of clinical development in HCC, and several agents, including Sunitinib, Brivanib,Tivantinib and Everolimus are being tested in phase Ⅲ studies. Combining targeted agents that inhibit different pathways in hepatocarcinogenesis is an area of active investigation.

Future research should continue to unravel the mechanism of hepatocarcinogenesis and to identify key relevant molecular targets for therapeutic intervention. While we are developing other antiangiogenic and targeted agents in HCC, it is imperative that we continue our efforts to identify and validate surrogate and predictive biomarkers that would be helpful to predict clinical efficacy, toxicity, and resistance to these agents.

After decades of disappointing results for systemic treatment of HCC, exciting developments are expected in this once neglected field of clinical oncology research.

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