

Advances in the study of molecularly targeted agents to treat hepatocellular carcinoma

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Summary

Hepatocellular carcinoma (HCC) is a severe form of liver cancer that is found worldwide. Treatments such as liver transplantation and surgical resection and local-regional therapies such as transarterial chemoembolization have progressed considerably and play a prominent role in HCC management. After those treatments, though, systematic drug intervention is required to deal with tumor metastasis in its early stages and the high frequency of tumor recurrence and/or metastasis. The approval of sorafenib, an agent that targets receptor tyrosine kinases (RTKs), as the first effective drug for systemic treatment of HCC represents a milestone in the treatment of this disease. In addition to sorafenib, a number of agents that target various RTKs or intracellular signal transduction molecules, such as mTOR, are currently being investigated as monotherapy or combination therapy for HCC. This article reviews advances in the study of molecularly targeted agents to treat HCC.

Keywords: Hepatocellular carcinoma, molecularly targeted agents; VEGFR, EGFR, c-MET

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men, the seventh most common cancer in women, and the third most common cause of cancer deaths worldwide. HCC resulted in 696,000 deaths worldwide in 2008 (1). The condition has distinct geographical variation, with the vast majority of cases (85%) occurring in countries in East Asia and sub-Saharan Africa and lower incidence rates in Australia, Northern Europe, and the US (2). The pathogenesis of HCC is complex and not completely understood. Hepatocarcinogenesis is a multistep process involving inflammation, hyperplasia, and dysplasia that finally leads to malignant transformation. The specific sequence of genetic events that mediate these steps is only partially known (3). Chronic hepatitis B (HBV) infection and chronic hepatitis C (HCV) infection play

a key role in the onset and development of HCC; HBV is responsible for approximately half of the cases of HCC. HBV is responsible for the majority of cases in China and Africa while HCV is the major cause of HCC in Japan, the US, and parts of Europe. Other risk factors include toxins (aflatoxin B1 and alcohol), metabolic diseases (non-alcoholic fatty liver disease and diabetes), hereditary diseases (hemochromatosis), and immune-related diseases (autoimmune hepatitis and primary biliary cirrhosis) (4,5). In addition, cirrhosis is present in 67% to 80% of patients with HCC, making HCC a highly complicated disease (6,7).

Treatments for HCC include resection, liver transplantation, percutaneous ethanol injection (PEI), radiofrequency ablation (RF), transcatheter arterial chemoembolization (TACE), and sorafenib depending on the Barcelona Clinic Liver Cancer (BCLC) stage of HCC (8). However, the asymptomatic nature of early disease and the limited use of surveillance result in the disease often being diagnosed in its advanced stages in which systemic drug intervention is required. To date, sorafenib (a small-molecule kinase inhibitor) is the only standard drug therapy for patients with advanced HCC, with modest effectiveness at prolonging patients' overall

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survival (OS) for around 2-3 months (9). However, the mechanism by which sorafenib treats advanced HCC is not well known, and no biomarkers have been identified to predict the effectiveness of sorafenib in patients with HCC. In addition, the tolerance and resistance to sorafenib in some patients with HCC further limit the clinical efficacy of sorafenib.

Given the modest efficacy of sorafenib, there is still a need for a treatment of advanced HCC. The efficacy of systemic chemotherapy therapies is limited in patients with HCC because of their cirrhotic liver, potentially poor hepatic reserve, and the chemoresistance of the tumor (10). Recently, molecularly targeted drugs to treat HCC have been extensively studied. Multiple molecular pathways implicated in HCC pathogenesis, including vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), mesenchymal-epithelial transition factor (c-MET), and the mammalian target of rapamycin (mTOR) pathways, may act as potential targets for therapeutic interventions (Figure 1) (11).

This review describes the study of current kinase inhibitors besides sorafenib and their combination with other agents to treat HCC, and preclinical data and clinical data are presented (Table 1, Table S1 and S2) (<http://www.ddtjournal.com/docindex.php?year=2014&kanno=4>). A retrospective analysis

of these studies could provide a clearer understanding of the study of kinase inhibitors in HCC and facilitate further progress in the study of new kinase inhibitors.

2. Antiangiogenic agents

HCC is a highly vascularized tumor. VEGF is an angiogenic growth factor and its elevated expression is found in surgical specimens of HCC compared to non-tumoral liver tissue (12). Thus, one approach to treatment of HCC is to target angiogenic factors, such as VEGF, PDGFR, and FGFR. Sorafenib has already been approved, but many VEGFR TKIs are also being investigated, such as sunitinib, linifanib, and brivanib. Three multikinase inhibitors (sunitinib, brivanib, and linifanib) have been studied as first-line therapies in comparison to sorafenib, but all failed to achieve their primary endpoints.

2.1. Sorafenib

Sorafenib is an oral multi-kinase inhibitor that blocks multiple growth factor pathways including VEGFR-1, -2, -3, PDGFR- β , Raf, RET, and FLT-3 (13,14). To date, sorafenib is the only drug approved for the treatment of unresectable HCC, based on the results of the SHARP trial and a parallel phase III trial in the Asia-Pacific region. In the SHARP trial, sorafenib significantly prolonged the median OS of patients with advanced

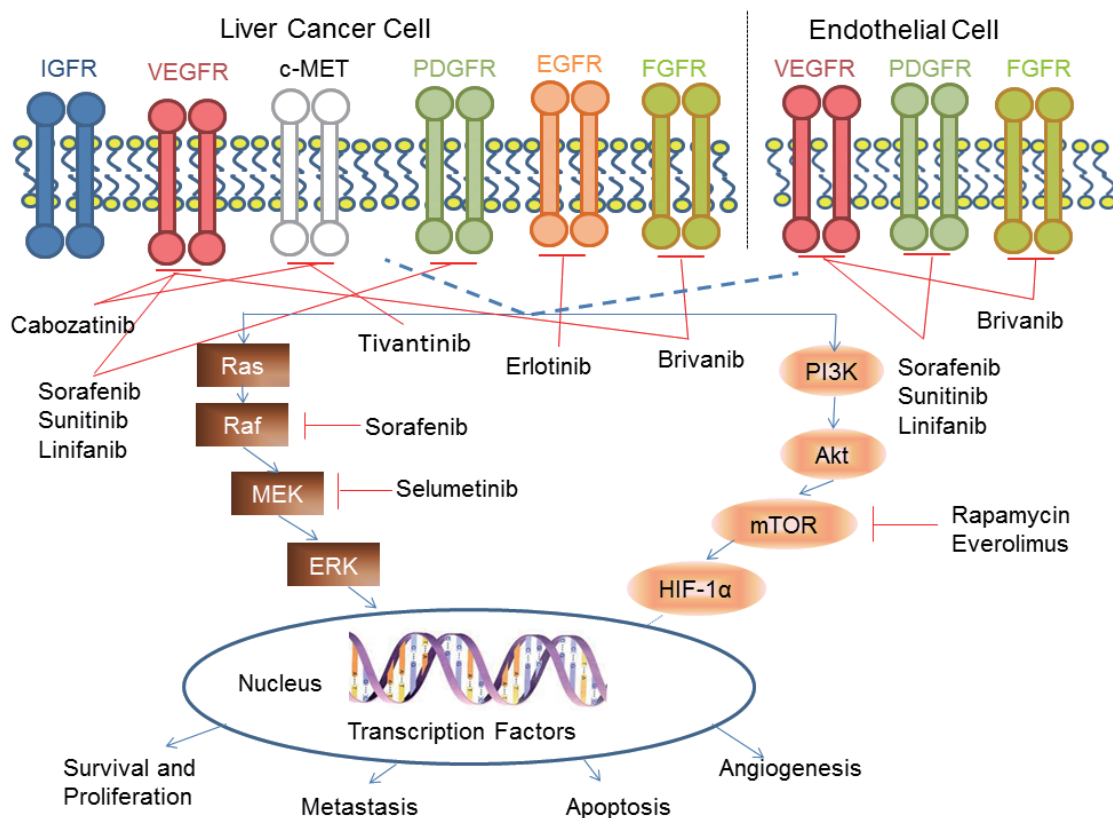


Figure 1. Signal transduction pathways implicated in HCC pathology and molecularly targeted agents that are currently being investigated.

Table 1. The current status of clinical studies of small molecule inhibitors

Agents	Targets	I Ongoing	I Completed	I Terminated	I withdrawn	II Ongoing	II Completed	II Terminated	II unknown	II suspended	III Ongoing	III Completed	III Terminated	III unknown
Sunitinib	VEGFR, PDGFR, c-KIT	1	1	1	1	1*	3	3					1	
Axitinib	VEGFR, PDGFR		1			4								
Dovitinib	VEGFR, PDGFR					1								
Linfanib	VEGFR, PDGFR		2				1				1	2	1	
Brivanib	VEGFR, FGFR						1							
Cediranib	VEGFR						1							
Pazopanib	VEGFR, PDGFR, c-Kit		2											
Lenalidomide	VEGF	1				1								
Orantinib	VEGFR-2, PDGFR, FGFR		1				1				1			
Cabozantinib	MET, VEGFR2, KIT						1				1			
Tivantinib	c-MET	1	1				1				2			
Foretinib	c-MET, VEGFR-2	1				2	4	2			1			
Erlotinib	EGFR	1	1				1							
Gefitinib	EGFR						1				1			
Everolimus	mTOR			1		5	1				1	1		
Rapamycin	mTOR	1	1			1								1
Temsirolimus	mTOR	1				1					1			
Refametinib	MEK					2								
Selumetinib	MEK						1							

*, Continuation Study

(Table 1. Continued)

Agents	Targets	II/III unknown	II/III completed	I/II ongoing	I/II completed	I/II terminated	I/II withdrawn	I/II unknown	IV ongoing
Sunitinib	VEGFR, PDGFR, c-KIT	1							
Axitinib	VEGFR, PDGFR								
Dovitinib	VEGFR, PDGFR								
Linfanib	VEGFR, PDGFR								
Brivanib	VEGFR, FGFR								
Cediranib	VEGFR								
Pazopanib	VEGFR, PDGFR, c-Kit								
Lenalidomide	VEGF								
Orantinib	VEGFR-2, PDGFR, FGFR		1						
Cabozantinib	MET, VEGFR2, KIT								
Tivantinib	c-MET								
Foretinib	c-MET, VEGFR-2					1			
Erlotinib	EGFR						1		
Gefitinib	EGFR								
Everolimus	mTOR			1	1				1
Rapamycin	mTOR		1						
Temsirolimus	mTOR			1					
Refametinib	MEK							1	
Selumetinib	MEK								

HCC from 7.9 months to 10.7 months ($p < 0.001$) and the median time to progression (TTP) from 2.8 months to 5.5 months ($p < 0.001$) (9). In the Asia-Pacific trial, the median OS and TTP were prolonged from 4.2 months to 6.5 months ($p = 0.014$) and from 1.4 months to 2.8 months ($p = 0.0005$), respectively. In both trials, the median OS of patients with advanced HCC was prolonged, but the shorter median OS in the Asia-Pacific trial may be due to differences in the state of the liver of patients in varied regions. In the Asia-Pacific trial, 73.0% of patients had baseline HBV infection compared to 12.0% in the SHARP study, whereas 8.4% of patients in the Asia-Pacific trial had baseline HCV infection compared to 30% in the SHARP trial (15).

However, the mechanism of sorafenib is still unclear and no biomarker has been identified to predict suitable patients for sorafenib treatment or the prognosis of those patients.

2.2. Sunitinib

Sunitinib is a multi-targeted receptor tyrosine kinase inhibitor of VEGFR, PDGFR, and c-KIT that was already approved for the treatment of renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumors (GIST) (16,17). In comparison to sorafenib, sunitinib has no activity against Raf but is about 10 times more active against most receptor tyrosine kinases present on the cell surface (including VEGFRs and PDGFR- β) (18). In two phase II studies of sunitinib in patients with advanced HCC, patients received 50 mg/d of sunitinib orally, 4 weeks on and 2 weeks off (19,20). Both studies found that sunitinib had a high toxicity, with treatment-related deaths occurring in 10% of patients (4, $n = 37$) in one study and grade 3/4 adverse effects (AEs) occurring in 80% of patients in the other study. The fact that sunitinib targets many different receptors may explain the high incidence of severe adverse events (SAEs) such as the classic hand-foot syndrome, stomatitis, and other dermatologic toxicities. In addition, a phase II study with continuous sunitinib treatment (37.5 mg daily) yielded promising results, with a progression-free survival at 12 weeks (PFS-12) of 33.3% (21). However, a phase III study of sunitinib malate versus sorafenib in patients with advanced HCC (patients were given 37.5 mg of sunitinib orally once a day or 400 mg of sorafenib twice a day) was terminated in 2010 because of a higher incidence of SAEs in the sunitinib arm and the fact that sunitinib did not prove to be either superior or non-inferior to sorafenib in terms of the OS of patients with advanced HCC (22). Due to the failure of the phase III trial, sunitinib is not considered as a therapeutic option for HCC anymore. Nevertheless, it may have anti-angiogenic and anti-fibrotic properties that may occasion its reuse at a lower dose to treat cirrhosis or advanced fibrosis (23).

2.3. Brivanib

Brivanib is the alanine ester of BMS-540215 and is hydrolyzed to the active moiety BMS-540215 *in vivo*. BMS-540215 has potent and selective inhibition of VEGFR and FGFR, with a high selectivity for VEGFR-2 and -3 (24). Preclinical studies have shown that brivanib has broad-spectrum *in vivo* antitumor activity at multiple dose levels (25).

A number of studies on brivanib to treat HCC have recently been conducted, and 3 relevant phase III studies yielded negative results. The phase III BRISK-PS Study involved 395 patients with advanced HCC whose condition progressed after sorafenib or who were intolerant to sorafenib (26). The patients were randomly assigned (2:1) to receive 800 mg of brivanib orally once a day plus best supportive care (BSC) or a placebo plus BSC. The median OS was 9.4 months for patients given brivanib and 8.2 months for those given the placebo ($p = 0.3307$), so there was little difference. Exploratory analyses revealed some differences between the two arms in terms of the median TTP (4.2 months for patients given brivanib vs. 2.7 months for patients given the placebo) and overall response rate (ORR, 10% for patients given brivanib vs. 2% for patients given the placebo). Notably, hyponatremia, an AE that was frequently reported in this study (occurring in 11% of patients as a grade 3 to 4 AE), has not been reported with other targeted agents, suggesting that this AE may be relatively specific to brivanib.

The phase III BRISK-FL Study tested the efficacy of brivanib versus sorafenib (27). In the study, patients with advanced HCC who had no prior systemic therapy were randomly assigned (1:1) to receive 400 mg of sorafenib orally twice a day ($n = 578$) or 800 mg of brivanib orally once a day ($n = 577$). The study did not meet its primary endpoint of OS noninferiority for brivanib versus sorafenib, with a median OS of 9.9 months for patients given sorafenib and 9.5 months for patients given brivanib. The two arms had a similar TTP and ORR. The incidence of SAEs (sorafenib:brivanib = 11.7%:11.3%) indicates that brivanib is less well-tolerated than sorafenib.

In these two phase III trials, brivanib failed to improve the OS for patients with advanced HCC but it did improve TTP and ORR, indicating that brivanib does have potential antitumor activity. Why it failed to improve the OS in both trials warrants further investigation.

In addition, the phase III Trans-Arterial Chemo-Embolization (TACE) Adjuvant HCC (BRISK TA) trial was terminated when 2 other phase III studies of brivanib in patients with advanced HCC failed to meet their OS objectives (28). In the trial, 502 patients were randomized to receive TACE + 800 mg of brivanib daily ($n = 249$, brivanib was stopped 2 days before a TACE session and restarted between day 3 and day 21 following

TACE) or TACE + a placebo ($n = 253$). However, the OS of both groups did not differ (26.4 months for patients given brivanib vs. 26.1 for patients given the placebo).

2.4. Linifanib

Linifanib is a multi-targeted receptor tyrosine kinase inhibitor that can inhibit members of VEGFR and PDGFR families with minimal activity against unrelated kinases (29,30). In a phase II trial of linifanib, 44 patients with advanced HCC who had received ≤ 1 prior systemic therapy were given linifanib orally at a dose of 0.25 mg/kg (31). The progression-free rate at 16 weeks (PFR-16) was 31.8%, with secondary endpoints of an ORR of 9.1%, TPP of 3.7 months, and OS of 9.7 months. The incidence of grade 3/4 AEs was 59.1%, with fatigue (13.6%) and hypertension (25.0%) being the most common. Results indicated that single-agent linifanib was clinically active in patients with advanced HCC with an acceptable safety profile. However, a phase III study of the efficacy and tolerance of linifanib versus sorafenib in advanced HCC was terminated in 2012 for unexplained reasons (32).

2.5. Other kinase inhibitors

Antiangiogenic agents have been described thus far, though other kinase inhibitors that target VEGFR are also being tested to treat advanced HCC. Cediranib, a VEGFR inhibitor, has completed a phase II trial involving advanced HCC, with results shown in Table S1 (<http://www.ddtjournal.com/docindex.php?year=2014&kanno=4>) (33). Pazopanib, an inhibitor that targets VEGFR and PDGFR and that has already been approved by the FDA to treat advanced RCC and advanced soft tissue sarcomas, has completed a phase I trial involving patients with HCC (Table S1) (<http://www.ddtjournal.com/docindex.php?year=2014&kanno=4>) (34). Orantinib, a receptor kinase inhibitor that targets VEGFR, PDGFR, and FGFR, has finished a phase I/II trial involving patients with advanced HCC and a phase II trial where it was combined with TACE and displayed promising antitumor ability (Table S1) (<http://www.ddtjournal.com/docindex.php?year=2014&kanno=4>) (35,36).

3. c-Met inhibitors

The hepatocyte growth factor (HGF)/c-MET signaling pathway plays a pivotal role in the development of several solid tumors, including HCC. Stimulation of the HGF/c-MET signaling pathway leads to the cascade reaction of Ras/Raf/MEK/ERK and PI3K/AKT, promoting tumor cell growth and invasion (37). In HCC, activation of the HGF/c-MET pathway is associated with an aggressive phenotype and poor prognosis (38).

3.1. Tivantinib (ARQ 197)

Tivantinib (ARQ 197) is a selective, non-ATP competitive, small-molecule c-MET inhibitor that inhibits growth and induces apoptosis in human tumor cell lines expressing c-MET (39). In a phase II study of tivantinib as a second-line treatment for advanced HCC, patients with advanced HCC and Child-Pugh class A cirrhosis who had progressed on or were unable to tolerate first-line systemic therapy were treated with tivantinib or a placebo (40). In the study, 71 patients were randomly assigned to receive tivantinib and 36 patients were randomly assigned to receive a placebo. Patients with tumors expressing high levels of c-MET ($\geq 2+$ in $\geq 50\%$ of tumor cells) had a longer median TTP when given tivantinib than when given a placebo (2.7 months for 22 patients with high levels of c-MET given tivantinib vs. 1.4 months for 15 patients with high levels of c-MET given a placebo). The encouraging results indicated that tivantinib is effective as a second-line treatment for patients with HCC expressing high levels of c-MET. Furthermore, c-MET expression may be a promising biomarker in patients with HCC. A pivotal phase III study of patients with advanced HCC expressing high levels of c-MET after sorafenib failure is currently underway (41). However, some researchers have, based on *in-vitro* studies, suggested that tivantinib is not only a c-MET inhibitor but also an antimetabolic agent that kills tumor cells independently of c-MET, contradicting the results of other studies (42,43). Further studies to clarify the mechanisms of the anti-HCC action of tivantinib are warranted.

3.2. Cabozantinib (XL184)

Cabozantinib (XL184) is an oral small-molecule tyrosine kinase inhibitor that blocks phosphorylation of c-MET and VEGFR-2 and that also has activity against AXL, RET, and KIT (44). Recently, HCC cell and mouse xenograft experiments measuring total MET and phosphorylated MET (p-MET) have indicated that high levels of p-MET are associated with resistance to adjuvant sorafenib treatment and that cabozantinib has significant antitumor activity against HCC (45). In a phase II randomized discontinuation trial, 41 patients with Child-Pugh class A advanced HCC received up to one round of prior systemic treatment before being treated with cabozantinib at a dosage of 100 mg/day for 12 weeks. The median PFS was 4.4 months, median OS was 15.1 months, and AFP response was 35% (reduction of $\geq 50\%$). In the trial, cabozantinib had encouraging clinical activity against HCC (46).

4. mTOR inhibitors

Over the past few years, several molecular pathways have been identified as contributing to the molecular

pathogenesis of HCC; the PI3K/AKT/mTOR pathway in particular plays a critical role (47,48). Upregulation of mTOR signalling has been observed in 40-45% of patients with HCC and a cell experiment indicated that elevated levels of phosphorylated mTOR were correlated with increased cell proliferation (49,50). Preclinical studies have demonstrated that mTOR inhibitors were effective at inhibiting cell proliferation, tumor growth, and metastasis in HCC tumor models (51,52).

4.1. Rapamycin (sirolimus)

Rapamycin (sirolimus) is an immunosuppressant and is used to prevent rejection in organ transplants, and especially in kidney transplants. Recently, the drug has been found to be effective at inhibiting mTOR and is being studied as a treatment for HCC (52). In a phase II study of sirolimus in treatment-naïve patients with advanced HCC, 25 patients were treated with sirolimus 20 mg/week for 1 month and then 30 mg/week (53). The median TTP was 3.8 months, the OS was 6.6 months, and the ORR was 8%. These data suggest that sirolimus has antitumor action against advanced HCC, and further study is needed to investigate the efficacy of rapamycin in patients with advanced HCC.

4.2. Everolimus

Everolimus is a derivative of rapamycin and is also an mTOR inhibitor. Preclinical studies have indicated that everolimus inhibits tumour growth in xenograft models of human HCC (54). A phase I/II study of everolimus in advanced HCC has tested the toxicity and efficacy of everolimus in patients with advanced HCC and adequate hematologic, hepatic, and renal function (55). In the study, 3 patients were treated with everolimus at 5 mg/d and 25 patients were treated with everolimus at 10 mg/d. The median PFS and OS for the latter group were 3.8 months and 8.4 months, respectively. The estimated PFS at 24 weeks was 28.6%. A phase III study named the EVOLVE-1 study tested the effect of everolimus on survival in patients with advanced HCC after failure of sorafenib (56). In the study, 362 patients were randomized to receive everolimus 7.5 mg/d and 184 patients received a placebo. Both groups also received BSC. Results indicated that everolimus did not improve OS in patients with advanced HCC whose disease progressed during or after receiving sorafenib or who were intolerant of sorafenib. Despite preemptive antiviral therapy in the EVOLVE-1 study, HBV reactivation based on central laboratory findings occurred in 37.0% of patients given everolimus and 22.7% of patients given the placebo who were HBV-DNA or HBsAg-positive (or both) at the baseline. The limitation of the EVOLVE-1 study was believed to be because the study was not designed to identify a molecularly or clinically selected population that would potentially benefited

from everolimus. Furthermore, mTOR inhibitors have immunosuppressive and antitumor actions, so the potential benefits of this class of agents in the adjuvant setting are being assessed in a phase III trial of sirolimus for patients with HCC after liver transplantation (57).

5. MEK inhibitors

The Ras/Raf/MEK/ERK signaling pathway plays a pivotal role in the regulation of many cellular processes, including proliferation, survival, differentiation, apoptosis, motility, and metabolism (58). A study noted activation of this pathway in half of patients with HCC; this pathway may be involved in multistep hepatocarcinogenesis, and especially in the progression of HCC (59). MEK inhibitors can inhibit the mitogen-activated protein kinase enzymes MEK1 and/or MEK2. Hence, MEK inhibitors have potential as a treatment for HCC.

Selumetinib is a selective, non-ATP-competitive small-molecule inhibitor of MEK1/2 (60). A phase II study of selumetinib administered 100 mg of selumetinib to 19 patients twice a day for 21 days, but the study was terminated midway through since no response was radiographically evident in this group. The short TTP and no ORR indicated the minimal effectiveness of selumetinib in treating advanced HCC (61).

6. Combined therapy

Drug resistance frequently occurs with molecularly targeted cancer therapy. An important mechanism of resistance is the compensatory activation of related signaling pathways (62). To date, several molecular pathways have been identified as contributing to the molecular pathogenesis of HCC. Of these, the PI3K/AKT/mTOR and Ras/Raf/MEK/ERK pathways have been studied the most extensively. These two pathways may be activated by multiple upstream receptors (*e.g.*, VEGFR and c-MET) and inhibition of specific upstream receptors may lead to compensatory activation *via* other pathways. Various inhibitors must be combined with other therapies to more effectively treat HCC.

6.1. Combination of sorafenib and TACE

Combinational therapies with sorafenib have the potential to further improve therapeutic options for patients suffering from advanced HCC. TACE is the standard therapy for patients with HCC who are not eligible for surgery (63). The hepatic artery is embolized by selectively injecting small embolic particles coated with chemotherapeutic agents. Molecular biology studies have shown that plasma VEGF levels usually increase after TACE treatment, providing a rationale for the combination of TACE and sorafenib (64).

In a trial involving patients with HCV-related intermediate-stage HCC, 62 patients with Child-Pugh class A disease were randomized (1:1) to receive 400 mg of sorafenib twice a day or a placebo 30 days after TACE (30 mg of doxorubicin and 10 mg of mitomycin C with 10 mL of iodinated nonionic contrast media and 20 mL of iodinated oil) (65). The median TTP was 9.2 months in the sorafenib group and 4.9 months in the placebo group ($p < 0.001$). Results indicated that a conventional TACE procedure followed by sorafenib treatment resulted in a significantly longer TTP for patients with intermediate-stage HCV-related HCC.

However, another study revealed conflicting results. In a phase III study of sorafenib after TACE in Japanese and Korean patients, 458 patients with unresectable HCC, Child-Pugh class A cirrhosis, and $\geq 25\%$ tumor necrosis/shrinkage 1-3 months after 1 or 2 TACE sessions were randomized at a ratio of 1:1 to receive 400 mg sorafenib twice a day or a placebo ($> 50\%$ of patients started sorafenib > 9 weeks after TACE) (66). The median TTP was 5.4 versus 3.7 months ($p = 0.252$), and the 1-year and 2-year survival rates were 94.6% vs. 94.1% and 72.1% vs. 73.8%, respectively. This trial found that sorafenib did not significantly prolong TTP in patients who responded to TACE. Moreover, the researchers attributed their findings to the delay in starting sorafenib after TACE and/or low daily sorafenib doses.

In a propensity score matching study involving Chinese patients with advanced HCC, 198 patients were treated with TACE alone (1:1 ratio of cisplatin and iodized oil), and 82 were treated with a combination therapy of TACE and sorafenib (combined therapy group) (67). In addition, the 82 patients were matched using propensity-score matching at a 1:2 ratio with 164 patients who received TACE monotherapy. The median OS and TTP were 7.0 months vs. 4.9 months ($p = 0.003$) and 2.6 months vs. 1.9 months ($p = 0.001$), respectively. In a phase II, prospective single-arm multinational study, 192 patients with intermediate-stage, unresectable HCC received doxorubicin-based TACE (an emulsion of lipiodol 5-20 mL and doxorubicin 30-60 mg) with interrupted dosing of sorafenib (sorafenib discontinued for 3 days before and 4-7 days after TACE) and TACE/sorafenib cycles were repeated every 6-8 weeks (68). Combined TACE/sorafenib was well-tolerated, with SAEs occurring in 27.1% of patients. Median PFS and TTP were 12.8 and 13.8 months, respectively. These two studies showed that the combination of TACE and sorafenib is well-tolerated and more effective than TACE monotherapy.

Overall, the combination of TACE and sorafenib seems to be more effective than TACE monotherapy (Table S2) (<http://www.ddtjournal.com/docindex.php?year=2014&kanno=4>) (69-71). Randomized controlled trials are still needed to further confirm this effectiveness, characterize the optimal schedule of sorafenib administration and TACE, and determine which

patients are most likely to benefit from this treatment. Moreover, similar combinational strategies could be investigated with other locoregional treatments (e.g. radioembolization plus sorafenib and radiofrequency ablation plus sorafenib).

6.2. Combination of bevacizumab and erlotinib

EGFR is a member of the RTK family and a potent regulator of the activity of the Ras/Raf/MEK/ERK cascade (72). EGFR is highly expressed in human hepatoma cell lines, and the high expression of EGFR is associated with higher cell proliferation (73). Erlotinib is an EGFR inhibitor that inhibits the formation of phosphotyrosine residues in EGFR and the initiation of signal cascades by binding to the ATP binding site of the receptor in a reversible fashion (74). However, several phase II trials of erlotinib to treat advanced HCC have indicated that single agent erlotinib provided a modest clinical benefit (75,76), and a phase III trial of erlotinib monotherapy to treat HCC was not conducted.

As a matter of fact, the multiplicity and complexity of molecular aberrations in HCC necessitate a multi-targeted approach combined with EGFR inhibitors. Recently, several trials of erlotinib plus bevacizumab, a recombinant humanized monoclonal antibody that binds to VEGF in patients with advanced HCC, have yielded results, although some are controversial.

A phase II trial of the combination of bevacizumab and erlotinib involved 40 patients with advanced HCC who received bevacizumab 10 mg/kg every 14 days and 150 mg of oral erlotinib daily in a 28-day cycle (77). The primary endpoint of PFS at 16 weeks was 62.5%. The median PFS was 9.0 months and OS was 15.65 months. Another phase II trial of bevacizumab and erlotinib involving 59 patients with unresectable HCC administered 150 mg of oral erlotinib daily and 10 mg/kg of bevacizumab every 14 days in a 28-day cycle. The PFS at 16 weeks was 64% and SAEs occurred in 30.51% of patients (78). Both trials showed that the combination of bevacizumab and erlotinib had significant antitumor activity in patients who had advanced HCC.

However, other trials had conflicting results. A phase II study of bevacizumab plus erlotinib in patients with advanced HCC has been conducted (79). In 27 patients treated with 150 mg of erlotinib daily and 10 mg/kg of bevacizumab on days 1 and 15 every 28 days, one patient had a confirmed partial response and 11 (48%) had stable disease. Median TTP was 3.0 months and OS was 9.5 months. In addition, a phase II study of bevacizumab and erlotinib in the treatment of patients with advanced HCC not responding to sorafenib administered 10 mg/kg of bevacizumab every 2 weeks and 150 mg of erlotinib daily for a maximum of 6 cycles (80). With 10 patients recruited, the trial was halted in the first stage according to

pre-set statistical criteria. Of these 10 patients, none responded or had stable disease. The median TTP was 1.81 months and OS was 4.37 months. A phase II trial of 21 patients with metastatic or inoperable HCC who had not received local or systemic therapy administered 15 mg/kg of bevacizumab every 3 weeks and 150 mg of oral erlotinib daily. The PFS at 27 weeks was 23.8% (73). These trials showed that erlotinib combined with bevacizumab had minimal activity in patients with advanced HCC or in an unselected population with sorafenib-refractory advanced HCC.

Furthermore, a phase III trial is underway to evaluate the clinical benefit of 400 mg of sorafenib twice a day and 150 mg of erlotinib once a day versus 400 mg of sorafenib twice a day and a placebo once a day in patients with advanced HCC (87). The combination of bevacizumab and erlotinib warrants additional evaluation in randomized controlled trials.

In addition to the studies discussed thus far, some *in-vitro* studies have provided a rationale for the combination of EGFR TKIs and IGF-1R TKIs. In terms of its mechanism of action, anti-IGF-1R therapy may cause acquired resistance *via* the activation of HER3, which EGFR TKIs may inhibit (82,83).

7. Conclusion and prospects for the future

Up to now, sorafenib has been the only standard therapy for advanced HCC, with most phase III trials failing to reach their primary endpoints. The failure of other molecularly targeted drugs may be due to several reasons.

First, HCC is quite complex with a pathogenesis including hepatitis B and C, and HCC is always associated with liver cirrhosis. The heterogeneity of hepatoma cells makes therapy much more complicated and affects the performance of targeted drugs to treat HCC. Therefore, effective treatment of HCC would require the simultaneous treatment of three distinct diseases: hepatitis, cirrhosis, and cancer. In addition, many carcinogenic pathways are activated as HCC develops, but no single pathway has been identified as the most important (84). Second, most phase III trials have shown that surrogate endpoints such as TTP, PFS, and ORR inconsistently predict OS. Both the selection of eligible patients and determination of primary endpoints may affect the outcome of trials. However, there are no yet known biomarkers that can predict whether patients are sensitive to sorafenib or other molecularly targeted drugs. In the absence of well-characterized and validated predictive biomarkers, targeted agents will likely continue to have a high risk of failure if phase III trials are conducted in unselected populations. Relevant biomarkers that may predict clinical outcome in patients receiving everolimus are being assessed in the EVOLVE-1 population (56). Third, the existing TKIs used in trials to treat HCC are primarily agents optimized for the treatment of other cancers and thus

may not exhibit the best kinase inhibitory profile to counteract the signaling abnormalities that are characteristic of HCC. This limitation, coupled with the likely involvement of multi-genic lesions in HCC, may affect the performance of targeted therapies in treating HCC (85). Future studies of targeted agents to treat HCC should focus on answering these questions and particularly on identifying patient populations based on molecular classification and predictive biomarkers.

References

1. Schutte K, Bornschein J, Malferttheiner P. HCC: Epidemiological trends and risk factors. *Dig Dis*. 2009; 27:80-92.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010; 127:2893-2917.
3. Feitelson MA, Sun B, Satiroglu Tufan NL, Liu J, Pan J, Lian Z. Genetic mechanisms of hepatocarcinogenesis. *Oncogene*. 2002; 21:2593-2604.
4. Gomaa AI, Khan SA, Toledano MB, Waked I, Taylor-Robinson SD. Hepatocellular carcinoma: Epidemiology, risk factors and pathogenesis. *World J Gastroenterol*. 2008; 14:4300-4308.
5. El-Serag HB, Rudolph KL. Hepatocellular Carcinoma: Epidemiology and molecular carcinogenesis. *Gastroenterology*. 2007; 132:2557-2576.
6. Simonetti RG, Cammà C, Fiorello F, Politi F, D'Amico G, Pagliaro L. Hepatocellular carcinoma. A worldwide problem and the major risk factors. *Dig Dis Sci*. 1991; 36:962-972.
7. Unoura M, Kaneko S, Matsushita E, Shimoda A, Takeuchi M, Adachi H, Kawai H, Urabe T, Yanagi M, Matsui O. High-risk groups and screening strategies for early detection of hepatocellular carcinoma in patients with chronic liver disease. *Hepatogastroenterology*. 1993; 40:305-310.
8. Villanueva A, Hernandez-Gea V, Llovet JM. Medical therapies for hepatocellular carcinoma: A critical view of the evidence. *Nat Rev Gastroenterol Hepatol*. 2012; 10:34-42.
9. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med*. 2008; 359:378-390.
10. Marin JGG, Castaño B, Martínez-Becerra P, Rosales R, Monte MJ. Chemotherapy in the treatment of primary liver tumours. *Cancer Ther*. 2008; 6:711-728.
11. Moeini A, Cornellà H, Villanueva A. Emerging signaling pathways in hepatocellular carcinoma. *Liver Cancer*. 2012; 1:83-93.
12. An FQ, Matsuda M, Fujii H, Matsumoto Y. Expression of vascular endothelial growth factor in surgical specimens of hepatocellular carcinoma. *J Cancer Res Clin*. 2000; 126:153-160.
13. McDermott U, Sharma SV, Dowell L, *et al*. Identification of genotype-correlated sensitivity to selective kinase inhibitors by using high-throughput tumor cell line profiling. *Proc Natl Acad Sci USA*. 2007; 104:19936-19941.
14. Tai WT, Cheng AL, Shiao CW, Huang HP, Huang JW, Chen PJ, Chen KF. Signal transducer and activator of transcription 3 is a major kinase-independent target of

- sorafenib in hepatocellular carcinoma. *J Hepatol.* 2011; 55:1041-1048.
15. Cheng AL, Kang YK, Chen Z, *et al.* Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: A phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol.* 2009; 10:25-34.
 16. Motzer RJ, Hutson TE, Tomczak P, *et al.* Sunitinib versus interferon α in metastatic renal-cell carcinoma. *N Engl J Med.* 2007; 356:115-124.
 17. Demetri GD, van Oosterom AT, Garrett CR, *et al.* Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: A randomised controlled trial. *Lancet.* 2006; 368:1329-1338.
 18. Zhu AX, Raymond E. Early development of sunitinib in hepatocellular carcinoma. *Expert Rev Anticanc.* 2009; 9:143-150.
 19. Faivre S, Raymond E, Boucher E, Douillard J, Lim HY, Kim JS, Zappa M, Lanzalone S, Lin X, Deprimo S, Harmon C, Ruiz-Garcia A, Lechuga MJ, Cheng AL. Safety and efficacy of sunitinib in patients with advanced hepatocellular carcinoma: An open-label, multicentre, phase II study. *Lancet Oncol.* 2009; 10:794-800.
 20. Barone C, Basso M, Biolato M, Pompili M, Rufini V, Miele L, Basso M, De Gaetano AM, Castaldi P, Iaculli A, Leccisotti L, Riccardi L, Grieco A. A phase II study of sunitinib in advanced hepatocellular carcinoma. *Dig Liver Dis.* 2013; 45:692-698.
 21. Koeberle D, Montemurro M, Samaras P, Majno P, Simcock M, Limacher A, Lerch S, Kovács K, Inauen R, Hess V, Saletti P, Borner M, Roth A, Bodoky G. 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). *The Oncologist.* 2010; 15: 285-292.
 22. Cheng AL, Kang YK, Lin DY, Park JW, Kudo M, Qin S, Chung HC, Song X, Xu J, Poggi G, Omata M, Pitman Lowenthal S, Lanzalone S, Yang L, Lechuga MJ, Raymond E. Sunitinib versus sorafenib in advanced hepatocellular cancer: Results of a randomized phase III trial. *J Clin Oncol.* 2013; 31:4067-4075.
 23. Majumder S, Piguet AC, Dufour JF, Chatterjee S. Study of the cellular mechanism of Sunitinib mediated inactivation of activated hepatic stellate cells and its implications in angiogenesis. *Eur J Pharmacol.* 2013; 705: 86-95.
 24. Bhide R, Cai ZW, Zhang YZ, *et al.* Discovery and preclinical studies of (R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy)propan- 2-ol (BMS-540215), an *in vivo* active potent VEGFR-2 inhibitor. *J Med Chem.* 2006; 49:2143-2146.
 25. Huynh H, Ngo VC, Fargnoli J, Ayers M, Soo KC, Koong HN, Thng CH, Ong HS, Chung A, Chow P, Pollock P, Byron S, Tran E. Brivanib alaninate, a dual inhibitor of vascular endothelial growth factor receptor and fibroblast growth factor receptor tyrosine kinases, induces growth inhibition in mouse models of human hepatocellular carcinoma. *Clin Cancer Res.* 2008; 14:6146-6153.
 26. Llovet J M, Decaens T, Raoul J L, *et al.* Brivanib in patients with advanced hepatocellular carcinoma who were intolerant to sorafenib or for whom sorafenib failed: results from the randomized phase III BRISK-PS study. *J Clin Oncol.* 2013; 31:3509-3516.
 27. Johnson P J, Qin S, Park J W, *et al.* Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: Results from the randomized phase III BRISK-FL study. *J Clin Oncol.* 2013; 31:3517-3524.
 28. Kudo M, Han G, Finn R S, *et al.* Brivanib as adjuvant therapy to transarterial chemoembolization in patients with hepatocellular carcinoma: A randomized phase 3 trial. *Hepatology.* 2014; [Epub ahead of print]
 29. Albert DH, Tapang P, Magoc TJ, *et al.* Preclinical activity of ABT-869, a multitargeted receptor tyrosine kinase inhibitor. *Mol Cancer Ther.* 2006; 5:995-1006.
 30. Guo J, Marcotte PA, McCall JO, Dai Y, Pease LJ, Michaelides MR, Davidsen SK, Glaser KB. Inhibition of phosphorylation of the colony-stimulating factor-1 receptor (c-Fms) tyrosine kinase in transfected cells by ABT-869 and other tyrosine kinase inhibitors. *Mol Cancer Ther.* 2006; 5:1007-1013.
 31. Toh HC, Chen PJ, Carr BI, Knox JJ, Gill S, Ansell P, McKeegan EM, Dowell B, Pedersen M, Qin Q, Qian J, Scappaticci FA, Ricker JL, Carlson DM, Yong WP. Phase 2 trial of linifanib (ABT-869) in patients with unresectable or metastatic hepatocellular carcinoma. *Cancer.* 2013; 119:380-387.
 32. Efficacy and Tolerability of ABT-869 Versus Sorafenib in Advanced Hepatocellular Carcinoma (HCC) <http://clinicaltrials.gov/ct2/show/NCT01009593?term=Linifanib%2C+hepatocellular+carcinoma&rank=1> (accessed July 25, 2014)
 33. Alberts SI, Fitch TR, Kim GP, Morlan BW, Dakhil SR, Gross HM, Nair S. Cediranib (AZD2171) in Patients with Advanced Hepatocellular Carcinoma: A Phase II North Central Cancer Treatment Group (NCCTG) Clinical Trial. *Am J Clin Oncol.* 2012; 35:329.
 34. Yau T, Chen PJ, Chan P, Curtis CM, Murphy PS, Suttle AB, Gauvin J, Hodge JP, Dar MM, Poon RT. Phase I dose-finding study of pazopanib in hepatocellular carcinoma: evaluation of early efficacy, pharmacokinetics, and pharmacodynamics. *Clin Cancer Res.* 2011; 17:6914-6923.
 35. Kanai F, Yoshida H, Tateishi R, *et al.* A phase I/II trial of the oral antiangiogenic agent TSU-68 in patients with advanced hepatocellular carcinoma. *Cancer Chemother Pharmacol.* 2011; 67:315-324.
 36. Inaba Y, Kanai F, Aramaki T, Yamamoto T, Tanaka T, Yamakado K, Kaneko S, Kudo M, Imanaka K, Kora S, Nishida N, Kawai N, Seki H, Matsui O, Arioka H, Arai Y. A randomised phase II study of TSU-68 in patients with hepatocellular carcinoma treated by transarterial chemoembolisation. *Eur J Cancer.* 2013; 49:2832-2840.
 37. Comoglio P M, Giordano S, Trusolino L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. *Nat Rev Dreg Discov.* 2008; 7:504-516.
 38. Kaposi-Novak P, Lee JS, Gómez-Quiroz L, Coulouarn C, Factor VM, Thorgeirsson SS. Met-regulated expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype. *J Clin Invest.* 2006; 116:1582-1595.
 39. Eathiraj S, Palma R, Volckova E, Hirschi M, France DS, Ashwell MA, Chan TC. Discovery of a novel mode of protein kinase inhibition characterized by the mechanism of inhibition of human mesenchymal-epithelial transition factor (c-Met) protein autophosphorylation by ARQ 197.

- J Biol Chem. 2011; 286:20666-20676.
40. Santoro A, Rimassa L, Borbath I, *et al.* Tivantinib for second-line treatment of advanced hepatocellular carcinoma: A randomised, placebo-controlled phase 2 study. *Lancet Oncol.* 2013; 14:55-63.
 41. A Randomized Double-blind, Placebo-controlled Japanese Phase III Trial of ARQ 197 in Hepatocellular Carcinoma (HCC) (JET-HCC). <http://clinicaltrials.gov/ct2/show/NCT02029157?term=Tivantinib%2C+hepatocellular+carcinoma&rank=5> (accessed July 25,2014).
 42. Katayama R, Aoyama A, Yamori T, Qi J, Oh-hara T, Song Y, Engelman JA, Fujita N. Cytotoxic activity of tivantinib (ARQ 197) is not due solely to c-MET inhibition. *Cancer Res.* 2013; 73:3087-3096.
 43. Basilico C1, Pennacchietti S, Vigna E, Chiriaco C, Arena S, Bardelli A, Valdembri D, Serini G, Michieli P. Tivantinib (ARQ197) displays cytotoxic activity that is independent of its ability to bind MET. *Clin Cancer Res.* 2013; 19:2381-2392.
 44. Yakes FM, Chen J, Tan J, *et al.* Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. *Mol Cancer Ther.* 2011; 10:2298-2308.
 45. Xiang Q, Chen W, Ren M, Wang J, Zhang H, Deng DY, Zhang L, Shang C, Chen Y. Cabozantinib suppresses tumor growth and metastasis in hepatocellular carcinoma by a dual blockade of VEGFR2 and MET. *Clin Cancer Res.* 2014; 20:2959-2970.
 46. Verslype C, Cohn A L, Kelley R K, *et al.* Activity of cabozantinib (XL184) in hepatocellular carcinoma: Results from a phase II randomized discontinuation trial (RDT). *J Clin Oncol.* 2012; 30:4007.
 47. Bjornsti M-A, Houghton PJ. The TOR pathway: a target for cancer therapy. *Nat Rev Cancer.* 2004; 4:335-348.
 48. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev.* 2004; 18:1926-1945.
 49. Sahin F, Kannangai R, Adegbola O, Wang J, Su G, Torbenson M. mTOR and P70 S6 kinase expression in primary liver neoplasms. *Clin Cancer Res.* 2004; 10:8421-8425.
 50. Sieghart W, Fuereder T, Schmid K, Cejka D, Werzowa J, Wrba F, Wang X, Gruber D, Rasoul-Rockenschaub S, Peck-Radosavljevic M, Wacheck V. Mammalian target of rapamycin pathway activity in hepatocellular carcinomas of patients undergoing liver transplantation. *Transplantation.* 2007; 83:425-432.
 51. Varma S, Khandelwal RL. Effects of rapamycin on cell proliferation and phosphorylation of mTOR and p70(S6K) in HepG2 and HepG2 cells overexpressing constitutively active Akt/PKB. *Biochim Biophys Acta.* 2007; 1770:71-78.
 52. Huynh H, Chow KH, Soo KC, Toh HC, Choo SP, Foo KF, Poon D, Ngo VC, Tran E. RAD001 (everolimus) inhibits tumour growth in xenograft models of human hepatocellular carcinoma. *J Cell Mol Med.* 2009; 13:1371-1380.
 53. Decaens T, Luciani A, Itti E, Hulin A, Roudot-Thoraval F, Laurent A, Zafrani ES, Mallat A, Duvoux C. Phase II study of sirolimus in treatment-naïve patients with advanced hepatocellular carcinoma. *Dig Liver Dis.* 2012; 44:610-616.
 54. Huynh H, Chow KH, Soo KC, Toh HC, Choo SP, Foo KF, Poon D, Ngo VC, Tran E. RAD001 (everolimus) inhibits tumour growth in xenograft models of human hepatocellular carcinoma. *J Cell Mol Med.* 2009; 13:1371-1380.
 55. Zhu A X, Abrams T A, Miksad R, *et al.* Phase 1/2 study of everolimus in advanced hepatocellular carcinoma. *Cancer.* 2011; 117:5094-5102.
 56. Zhu A X, Kudo M, Assenat E, *et al.* Effect of everolimus on survival in advanced hepatocellular carcinoma after failure of sorafenib: The EVOLVE-1 Randomized Clinical Trial. *JAMA.* 2014; 312:57-67.
 57. Immunosuppression in Patients Undergoing Liver Transplantation for Hepatocellular Carcinoma. <http://clinicaltrials.gov/ct2/show/NCT00355862?term=NCT00355862&rank=1> (accessed July 25,2014)
 58. Blum R, Cox AD, Kloog Y. Inhibitors of chronically active RAS: Potential for treatment of human malignancies. *Recent Pat Anticancer Drug Discov.* 2008; 3:31-47.
 59. Ito Y, Sasaki Y, Horimoto M, Wada S, Tanaka Y, Kasahara A, Ueki T, Hirano T, Yamamoto H, Fujimoto J, Okamoto E, Hayashi N, Hori M. Activation of mitogen-activated protein kinases/extracellular signal-regulated kinases in human hepatocellular carcinoma. *Hepatology.* 1998; 27:951-958.
 60. Davies BR, Logie A, McKay JS, Martin P, Steele S, Jenkins R, Cockerill M, Cartlidge S, Smith PD. AZD6244 (ARRY-142886), a potent inhibitor of mitogen-activated protein kinase/extracellular signal regulated kinase kinase 1/2 kinases: Mechanism of action *in vivo*, pharmacokinetic/pharmacodynamics relationship, and potential for combination in preclinical models. *Mol Cancer Ther.* 2007; 6:2209-2219.
 61. O'Neil BH, Goff LW, Kauh JS, Strosberg JR, Bekaii-Saab TS, Lee RM, Kazi A, Moore DT, Learoyd M, Lush RM, Sebti SM, Sullivan DM. Phase II study of the mitogen-activated protein kinase 1/2 inhibitor selumetinib in patients with advanced hepatocellular carcinoma. *J Clin Oncol.* 2011; 29:2350-2356.
 62. Ou DL, Lee BS, Chang YC, Lin LI, Liou JY, Hsu C, Cheng AL. Potentiating the efficacy of molecular targeted therapy for hepatocellular carcinoma by inhibiting the insulin-like growth factor pathway. *PLoS one.* 2013; 8: e66589.
 63. Bruix J, Sala M, Llovet J M. Chemoembolization for hepatocellular carcinoma. *Gastroenterology.* 2004; 127:S179-S188.
 64. Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. *Mol Cancer Ther.* 2008; 7:3129-3140.
 65. Sansonno D, Lauletta G, Russi S, Conteduca V, Sansonno L, Dammacco F. Transarterial chemoembolization plus sorafenib: a sequential therapeutic scheme for HCV-related intermediate-stage hepatocellular carcinoma: a randomized clinical trial. *The oncologist.* 2012; 17:359-366.
 66. Kudo M, Imanaka K, Chida N, *et al.* Phase III study of sorafenib after transarterial chemoembolisation in Japanese and Korean patients with unresectable hepatocellular carcinoma. *Eur J Cancer.* 2011; 47:2117-2127.
 67. Hu H, Duan Z, Long X, Hertzanzu Y, Shi H, Liu S, Yang Z. Sorafenib Combined with Transarterial Chemoembolization versus Transarterial Chemoembolization Alone for Advanced-Stage Hepatocellular Carcinoma: A Propensity Score Matching Study. *PLoS one.* 2014; 9:e96620.
 68. Chao Y, Chung YH, Han G, Yoon JH, Yang J, Wang J, Shao GL, Kim BI, Lee TY. The combination of TACE

- (transcatheter arterial chemobolization) and sorafenib is well-tolerated and effective in Asian Patients with hepatocellular carcinoma: Final results of the START trial. *Int J Cancer*. 2014; [Epub ahead of print]
69. Pawlik TM, Reyes DK, Cosgrove D, Kamel IR, Bhagat N, Geschwind JF. Phase II trial of sorafenib combined with concurrent transarterial chemoembolization with drug-eluting beads for hepatocellular carcinoma. *J Clin Oncol*. 2011; 29:3960-3967.
 70. Park JW, Koh YH, Kim HB, Kim HY, An S, Choi JI, Woo SM, Nam BH. Phase II study of concurrent transarterial chemoembolization and sorafenib in patients with unresectable hepatocellular carcinoma. *J Hepatol*. 2012; 56:1336-1342.
 71. Zhao Y, Wang WJ, Guan S, Li HL, Xu RC, Wu JB, Liu JS, Li HP, Bai W, Yin ZX, Fan DM, Zhang ZL, Han GH. Sorafenib combined with transarterial chemoembolization for the treatment of advanced hepatocellular carcinoma: A large-scale multicenter study of 222 patients. *Ann Oncol*. 2013; 24:1786-1792.
 72. Bublil EM, Yarden Y. The EGF receptor family: spearheading a merger of signaling and therapeutics. *Curr Opin Cell Biol*. 2007; 19:124-134.
 73. Govindarajan R, Siegel E, Makhoul I, Williamson S. Bevacizumab and erlotinib in previously untreated inoperable and metastatic hepatocellular carcinoma. *Am J Clin Oncol*. 2013; 36:254-257.
 74. Raymond E, Faivre S, Armand J P. Epidermal growth factor receptor tyrosine kinase as a target for anticancer therapy. *Drugs*. 2000; 60:15-23.
 75. Philip PA, Mahoney MR, Allmer C, Thomas J, Pitot HC, Kim G, Donehower RC, Fitch T, Picus J, Erlichman C. Phase II study of Erlotinib (OSI-774) in patients with advanced hepatocellular cancer. *J Clin Oncol*. 2005; 23:6657-6663.
 76. Thomas MB, Chadha R, Glover K, Wang X, Morris J, Brown T, Rashid A, Dancey J, Abbruzzese JL. Phase 2 study of erlotinib in patients with unresectable hepatocellular carcinoma. *Cancer*. 2007; 110:1059-1067.
 77. Thomas MB, Morris JS, Chadha R, Iwasaki M, Kaur H, Lin E, Kaseb A, Glover K, Davila M, Abbruzzese J. Phase II trial of the combination of bevacizumab and erlotinib in patients who have advanced hepatocellular carcinoma. *J Clin Oncol*. 2009; 27:843-850.
 78. Efficacy and Safety Study of Bevacizumab and Erlotinib to Treat Primary Liver Cancer That Cannot be Removed By Surgery. <http://clinicaltrials.gov/ct2/show/NCT00242502?term=NCT00242502&rank=1> (accessed July 25,2014).
 79. Philip PA, Mahoney MR, Holen KD, Northfelt DW, Pitot HC, Picus J, Flynn PJ, Erlichman C. Phase 2 study of bevacizumab plus erlotinib in patients with advanced hepatocellular cancer. *Cancer*. 2012; 118:2424-2430.
 80. Yau T, Wong H, Chan P, Yao TJ, Pang R, Cheung TT, Fan ST, Poon RT. Phase II study of bevacizumab and erlotinib in the treatment of advanced hepatocellular carcinoma patients with sorafenib-refractory disease. *Invest New Drug*. 2012; 30:2384-2390.
 81. Nexavar-Tarceva Combination Therapy for First Line Treatment of Patients Diagnosed With Hepatocellular Carcinoma (SEARCH). <http://clinicaltrials.gov/ct2/show/NCT00901901?term=erlotinib%2C+hepatocellular+carcinoma&rank=6> (accessed July 25,2014).
 82. Desbois-Mouthon C, Baron A, Blivet-Van Eggelpoël MJ, Fartoux L, Venot C, Bladt F, Housset C, Rosmorduc O. Insulin-like growth factor-1 receptor inhibition induces a resistance mechanism *via* the epidermal growth factor receptor/HER3/AKT signaling pathway: rational basis for cotargeting insulin-like growth factor-1 receptor and epidermal growth factor receptor in hepatocellular carcinoma. *Clin Cancer Res*. 2009; 15:5445-5456.
 83. Ou DL, Lee BS, Chang YC, Lin LI, Liou JY, Hsu C, Cheng AL. Potentiating the efficacy of molecular targeted therapy for hepatocellular carcinoma by inhibiting the insulin-like growth factor pathway. *PloS one*. 2013; 8:e66589.
 84. Bagi C M, Gebhard D F, Andresen C J. Antitumor effect of vascular endothelial growth factor inhibitor sunitinib in preclinical models of hepatocellular carcinoma. *Eur J Gastroen Hepat*. 2012; 24:563-574.
 85. Ho HK, Chua BT, Wong W, Lim KS, Teo V, Ong HT, Chen X, Zhang W, Hui KM, Go ML, Ullrich A. Benzylidene-indolinones are effective as multi-targeted kinase inhibitor therapeutics against hepatocellular carcinoma. *Mol Oncol*. 2014; [Epub ahead of print]

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