EDITORIAL

Signaling pathway/molecular targets and new targeted agents under development in hepatocellular carcinoma

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Abstract

Advances in molecular cell biology over the last decade have clarified the mechanisms involved in cancer growth, invasion, and metastasis, and enabled the development of molecular-targeted agents. To date, sorafenib is the only molecular-targeted agent whose survival benefit has been demonstrated in two global phase Ⅲ randomized controlled trials, and has been approved worldwide. Phase Ⅲ clinical trials of other molecular targeted agents comparing them with sorafenib as first-line treatment agents are ongoing. Those agents target the vascular endothelial growth factor, platelet-derived growth factor receptors, as well as target the epidermal growth factor receptor, insulin-like growth factor receptor and mammalian target of rapamycin, in addition to other molecules targeting other components of the signal transduction pathways. In addition, the combination of sorafenib with standard treatment, such as resection, ablation, transarterial embolization, and hepatic arterial infusion chemotherapy are ongoing. This review outlines the main pathways involved in the development and progression of hepatocellular carcinoma and the new agents that target these pathways. Finally, the current statuses of clinical trials of new agents or combination therapy with sorafenib and standard treatment will also be discussed.

Kudo M. Signaling pathway/molecular targets and new targeted agents under development in hepatocellular carcinoma. *World J Gastroenterol* 2012; 18(42): 6005-6017 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i42/6005.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i42.6005

INTRODUCTION

Advances in molecular cell biology over the last decade have clarified the mechanisms involved in cancer growth, invasion and metastasis, and enabled the development of molecular-targeted agents, best represented by trastuzumab for breast cancer, imatinib and rituximab for hematopoietic tumors, and gefitinib and erlotinib for lung cancer. These molecular-targeted agents are broadly classified into two categories: drugs targeting cancer cell-specific molecules, and nonspecific molecular-targeted drugs for molecular biological abnormalities induced in the host stroma or blood vessels by the presence of cancer. Examples of the former approach include trastuzumab, which targets human epidermal growth factor receptor 2 (HER2), the expression of which is a poor prognostic factor for breast cancer; rituximab, which is used to treat B-cell lymphoma, targets CD20 expressed on normal and neoplastic mature B cells; while imatinib binds to the ATP-binding site of Bcr-abl, a protein that causes chronic myelogenous leukemia. However, no critical target molecules responsible for treatment response have been identified in hepatocellular carcinoma (HCC).

In recent years, clinical trials have been conducted for many agents that act on growth factor receptors, such as epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR), and intracellular signaling pathways. In addition, multi-kinase inhibitors, including sorafenib, have emerged and been evaluated. Clinical trials are ongoing to compare drugs with the same mechanism of action and to test the combined efficacy and relative merits of these drugs with existing drugs for many cancers. Since the main treatment option for metastatic, advanced stage cancers, such as breast and colorectal cancer, is systemic chemotherapy, clinical trials are ongoing to investigate how to combine molecular-targeted agents with standard therapies based on the results of long-term, large-scale clinical trials, and to identify which molecular-targeted agents should be used as initial or second-line therapy.

However, for HCC, background liver damage limits the indication for systemic chemotherapy and no anti-cancer drugs were found to be effective in large-scale randomized controlled trials except sorafenib. Now that the usefulness of sorafenib has been demonstrated in two large scale randomized clinical trials, the development of new drugs that are effective for poor-prognosis advanced HCC, who are resistant to a standard of care agent, sorafenib.

In this review, the clinical impact of sorafenib and ongoing trials of new agents or combination trials with sorafenib will be described.

SIGNALING PATHWAYS AND MOLECULAR-TARGETED AGENTS IN HCC

As in other cancers, the molecular mechanisms involved in the development and progression of HCC are complex. After hepatitis B virus and hepatitis C virus infection and alcohol or aflatoxin B1 exposure, genetic and epigenetic changes occur, including oncogene activation and tumor-suppressor gene inactivation due to inflammation-induced increase in hepatocyte turnover and oxidative stress-induced DNA damage. Through apoptosis and cell proliferation, these changes lead to the multistep development and progression of a hyperplastic to dysplastic nodule, early HCC, and advanced HCC. A number of studies have reported changes in gene expression, chromosomal amplification, mutations, deletions and copy number alterations (gain/loss), somatic mutations, CpG hypermethylation, and DNA hypomethylation, as well as molecular abnormalities, which can constitute therapeutic targets[1-5].

The binding of growth factors to their receptor proteins activates protein-phosphorylating enzymes, thus activating a cascade of proliferative signaling pathways to transmit proliferative signals into the nucleus. Growth factors, such as EGF, transforming growth factor (TGF)-α/-β, insulin-like growth factor (IGF), and VEGF, also function in liver regeneration after injury, while fibroblast growth factor (FGF) and the platelet-derived growth factor (PDGF) family are involved in liver fibrosis and HCC growth[6-8]. The receptors for these growth factors are broadly classified into G protein-coupled receptors and protein kinases. On ligand binding, these receptors activate their downstream intracellular molecules in a cascade fashion. Many of the growth factor receptors and oncogenes have tyrosine kinase activity, and the tyrosine kinases are classified into transmembrane receptor tyrosine kinases, such as the EGFR and VEGFR, and cytoplasmic non-receptor tyrosine kinases, such as Abl and Src. On the other hand, Raf, mitogen-activated protein kinase (MAPK)/extracellular signaling-regulated kinase (ERK) kinase (MEK), and mammalian target of rapamycin (mTOR) are serine/threonine kinases.

In general, the MAPK, phosphoinositide 3-kinase (PI3K)/Akt/mTOR, c-MET, IGF, Wnt-β-catenin and Hedgehog signaling pathways, and the VEGFR and PDGF receptor (PDGFR) signaling cascades show altered activity in HCC, and agents targeting these pathways are under development (Figures 1-3; Table 1)[9-12]. Many molecular-targeted agents are now under development and the target signaling pathways and growth factors are outlined below.

MAPK pathway (Ras/Raf/MEK/ERK)

The MAPK intracellular signaling pathway, which is mainly involved in cell growth and survival, and regulates cell differentiation, is upregulated in cancer cells. Therefore, this pathway has been extensively studied as a therapeutic target. The MAPK pathway is a common downstream pathway for the EGFR, PDGFR and VEGFR, and is universally used for signal transduction downstream of cytokine receptors, integrin complexes, and G-protein receptors to Ras. The MAPK pathway also plays an important role in HCC, in that its activation is reportedly involved in HCC growth and survival. The downstream ERK is activated by two upstream protein kinases, which are coupled to growth factor receptors by Ras proteins. Ras, which is activated by ligand binding, activates Raf serine/threonine kinases and MEK (MAP kinase/ERK kinase), while MEK phosphorylates and activates ERK, which phosphorylates proteins involved in cell growth, apoptosis resistance, extracellular matrix production and angiogenesis[13-15].

Raf and Ras inhibitors: Raf and Ras are proto-oncogenes. In particular, K-Ras mutations are commonly observed in many cancers, including pancreatic and colorectal cancers. One study reported that 30% of HCCs have Ras mutations[16]. To our knowledge, no agents targeting Ras are planned to enter clinical trials at the present. However, because the binding of Ras protein to the cell membrane and its functional activation require farnesylation, several farnesyltransferase inhibitors are being tested for Ras-related tumors. In addition, vaccine therapy for mutant Ras proteins is currently being tested for solid cancers, including HCC.
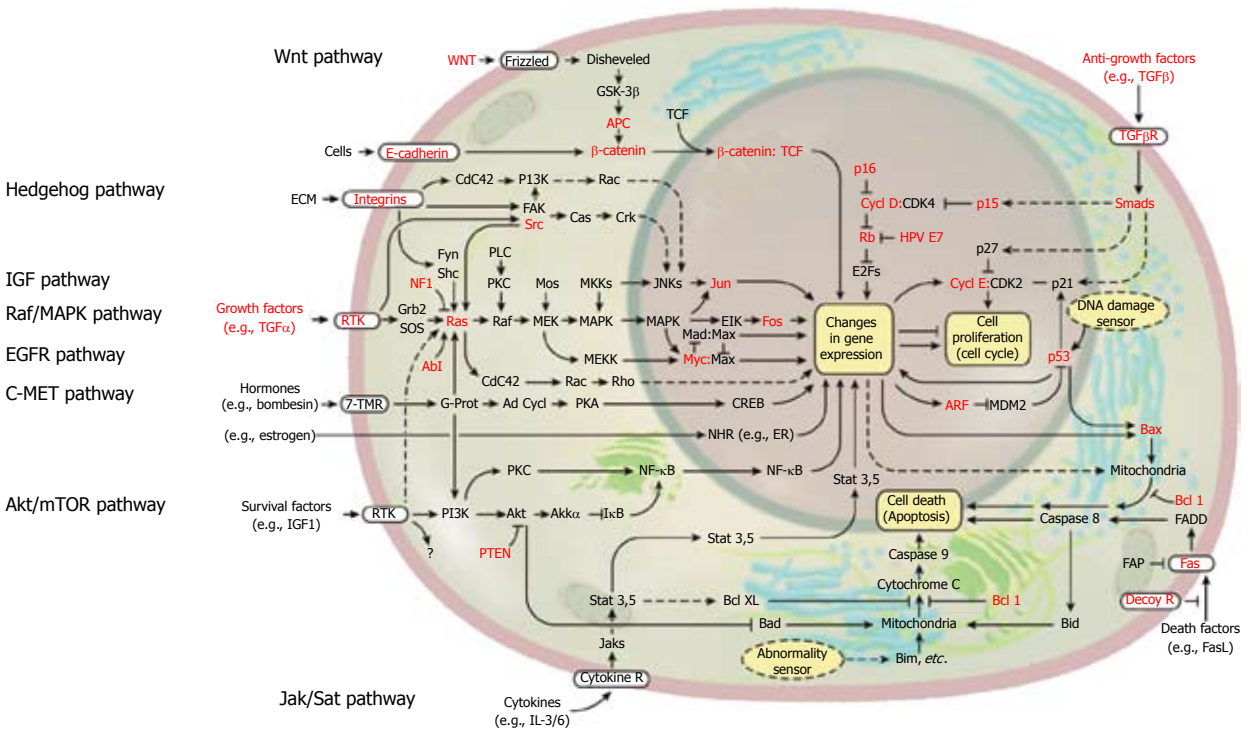
Figure 1 Signal transduction in solid cancer cells including hepatocellular carcinoma. Some of the genes known to be functionally altered are highlighted in red. These signaling pathways, including growth factor pathway, Wnt pathway, Hedgehog pathway, Akt/mammalian target of rapamycin (mTOR) pathway, and Jak/Stat pathway, can be a molecular targets for treatment of hepatocellular carcinoma. (Cited and modified from Hanahan et al[10] with permission.) EGFR: Epidermal growth factor receptor; TGF: Transforming growth factor; IGF: Insulin-like growth factor; MAPK: Mitogen-activated protein kinase; PI3K: Phosphoinositide 3-kinase; ERK: Extracellular signaling-regulated kinase; NF-κB: Nuclear factor-kappa B; IL: Interleukin.

Table 1 Molecular targeted agents for hepatocellular carcinoma: Targets and development status

Agents	Targets (angiogenesis)			Targets (proliferation)				Positioning	Development status
	VEGFR	PDGFR	FGF	EGFR	Raf	mTOR	TRAIL-R2 DR5		
Sorafenib	●	●			●			1st line	Approved
Sunitinib	●	●						1st line	P III terminated
E7080	●	●	●					1st/2nd line	P II ongoing
Tigatuzumab (CS1008)							●	1st line (sorafenib combination)	P I / II ongoing
Linifanib (ABT-869)	●	●						1st line	P III ongoing
Brivanib		●			●			1st line, 2nd line, TACE adjuvant	P III ongoing
TSU-68		●	●					TACE combination	P III ongoing
Ramucirumab		●						2nd line	P III ongoing
Everolimus (RAD001)						●		2nd line	P III ongoing
Axitinib		●	●					2nd line	P III ongoing

VEGFR: Vascular endothelial growth factor receptor; PDGFR: Platelet-derived growth factor receptor; FGF: Fibroblast growth factor; EGFR: Epidermal growth factor receptor; mTOR: Mammalian target of rapamycin; TACE: Transarterial chemoembolization.

The Raf family consists of three isoforms, A-Raf, B-Raf and C-Raf/Raf-1. Genetic abnormalities, such as point mutations and gene rearrangements, have been reported in various cancers[17]; however, in HCC, ras/raf mutations are rare, and no k-ras or b-raf mutations have been detected[18]. On the other hand, wild-type Raf-1 was reported to be hyperactivated in many cancers, including HCC[19-21]. Sorafenib inhibits Raf, and has multiple characteristics in that it exhibits strong inhibitory activity against Raf-1 (C-Raf) kinase, B-Raf (wild-type B-Raf and mutant V600E B-Raf) serine/threonine kinase, the pro-angiogenic receptor tyrosine kinases VEGFR, PDGFR and FGFR1, and tyrosine kinases, such as c-kit, Flt-3 and RET, which are involved in tumor progression and overall prognosis[22].

MEK: The MEK family consists of MEK1 and MEK2 proteins, which specifically phosphorylate tyrosine and threonine residues, and phosphorylates downstream Erk1 and Erk2[23].

In an immunohistochemical study, MEK1/2 overexpression, ERK1/2 overexpression, and ERK1/2 phosphorylation were observed in 100% (46/46), 91% (42/46), and 69% (32/46) of HCCs, respectively. In ad-
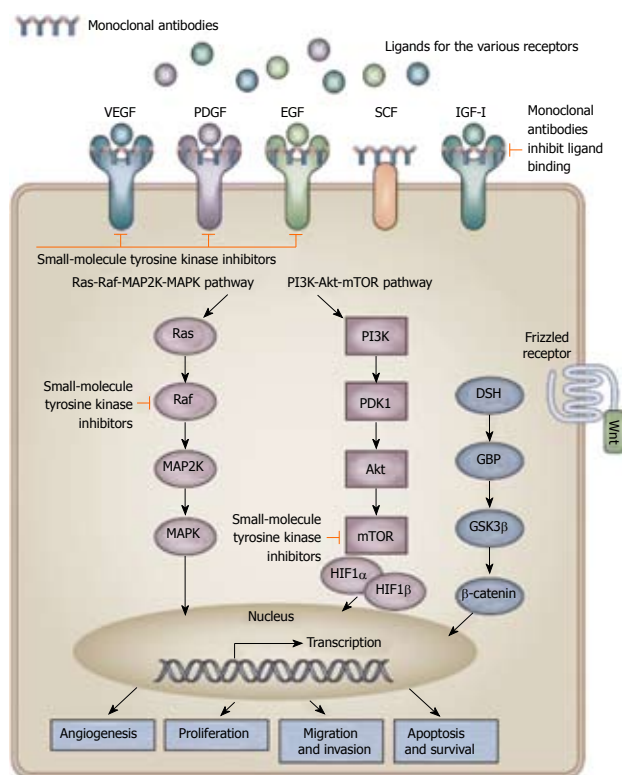
Figure 2 Signaling pathways and potential drug targets to inhibit hepatocarcinogenesis. Activation of receptor tyrosine kinases by their ligands activates downstream signaling pathways with effects on angiogenesis, proliferation, migration and invasion, and apoptosis or survival of cells. Monoclonal antibodies inhibit ligand binding to the receptor and small-molecule tyrosine kinase inhibitors inhibit propagation of the downstream signal. (Cited from Spangenberg et al[11] with permission.) IGF: Insulin-like growth factor; MAPK: Mitogen-activated protein kinase; PI3K: Phosphoinositide 3-kinase; EGF: Epidermal growth factor; VEGF: Vascular endothelial growth factor; PDGF: Platelet-derived growth factor; mTOR: Mammalian target of rapamycin; HIF: Hypoxia-inducible factor; SCF: Stem cell factor.

dition, the *in vitro* treatment of HepG2 and Hep3B cells with MEK1/2 inhibitors inhibited cell growth and up-regulated apoptosis[24].

The MEK inhibitors CI-1040, PD0325901, AZD6244, and RDEA119/BAY869766 have been tested in several cancers, including solid tumors such as HCC. Recently, the results of Phase I of AS703026 and E6201 studies against solid tumors were reported in ASCO2010. A phase Ⅱ study of AZD6244 (selumetinib, ARRY-142866) and a phase I/Ⅱ study of RDEA119/BAY869766 in combination with sorafenib are being conducted.

PI3K/Akt/mTOR pathway
The PI3K/Akt/mTORpathway also plays an important role in cell growth, survival regulation, metabolism, and anti-apoptosis. The membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP$_2$) is phosphorylated by PI3K into phosphatidylinositol 3,4,5-triphosphate (PIP$_3$), which binds to and activates the serine/threonine kinase Akt. The tumor suppressor gene product phosphatase and tensin homolog (PTEN) deleted on chromosome is antagonistic to PI3K activity. PTEN is a lipid phosphatase that

dephosphorylates inositol phosphates, such as PIP$_3$. The inactivation of PTEN through gene deletion increases PIP$_3$ levels, and activates Akt, which inhibits apoptosis, leading to the development of tumors. The serine/threonine kinase mTOR is an important mediator in the PI3K/Akt pathway, which binds intracellularly to a protein called raptor or rictor, and exists as two different complexes, complex 1 and 2 (mTORC1 and mTORC2). mTORC2 (mTOR-rictor) activates Akt, while mTORC1 (mTOR-raptor) is activated downstream of Akt; thus, both molecules regulate protein synthesis (Figures 4 and 5)[25].

Inhibiting mTOR with molecules, such as RAD001, generates additive effects that accompany upstream and downstream target inhibition. Alternatively, upstream receptor inhibition is compensated for by inhibiting the downstream pathway, even if some resistance develops against receptor inhibition regardless of initial or acquired resistance. Therefore, RAD001 is a potential targeted agent for HCC.

Besides the finding that mTOR plays a key role in cell biology, it was also demonstrated that mTOR and S6K are overexpressed in 15%-41% of HCCs. mTOR inhibitors also have antitumor effects in various HCC cell lines and animal models[26-29]. Activation of mTOR is correlated with the development of HCC and recurrence after the excision of early HCC. Regulating this specific intracellular pathway (Ras-Raf pathway) with RAD001 is potentially more effective in suppressing sorafenib-resistant tumors.

A study of 528 HCC samples showed that the expression of pAkt, PTEN, p27 and S6 ribosomal protein (pS6) was a poor prognostic factor for survival[30]. A tissue microarray analysis of HCC samples revealed that the loss of PTEN and overexpression of pAkt and p-mTOR were correlated with tumor grade, intrahepatic metastasis, vascular invasion, TNM stage, Ki-67 labeling index, and matrix metalloproteinase (MMP)-2 and -9 upregulation. Meanwhile, PTEN mRNA expression in the cancerous tissue was downregulated compared with that in the non-cancerous tissue. The levels of PTEN, MMP-2, and MMP-9 mRNA expression were correlated with tumor stage and metastasis, and the levels of PTEN and MMP-9 mRNA expression were inversely correlated[31]. In an extensive analysis of 314 HCC samples in terms of mutation analysis, DNA copy number changes, mRNA levels and immunostaining, Villanueva et al[32] found that activation of the IGF pathway, upregulation of EGF, dysregulation of PTEN, and aberrant mTOR signaling were present in half of the samples, and that inhibiting mTOR activity with everolimus was effective in improved survival and suppression of recurrence.

The PI3K inhibitor RG7321 and the Akt inhibitor perifosine target the PI3K/Akt/mTOR pathway and are in early stages of clinical development, while the mTOR inhibitors everolimus (RAD001), sirolimus (Rapamune), and temsirolimus (CCI-779) are at more advanced stages of development. Everolimus is used to treat sorafenib-
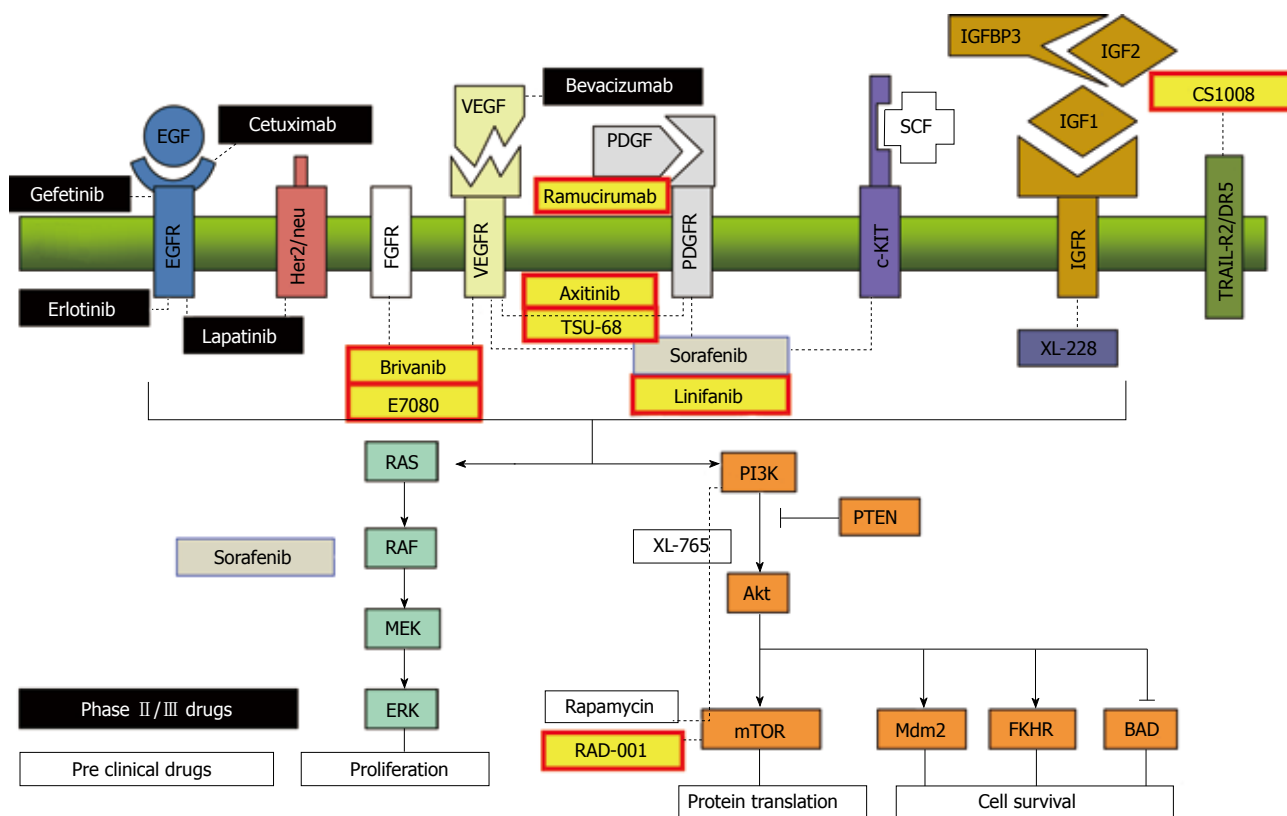
Figure 3 Target molecular and targeted agents under development. Monoclonal antibodies (VEGF: bevacizumab, EGFR: cetuxinab), tyrosine kinase inhibitors (VEGFR: sorafenib, brivanib, linifanib, axitinib, TSU-68; FGFR: E7080, brivanib), EGFR: erlotinib, lapatinib), serine/threonine kinase inhibitors (Raf: sorafenib, mTOR: rapamycin and everolimus) and agonistic ligand of TRAIL-R2/DR5 (CS1008). (Cited and modified from Villanueva *et al*[12] with permission.) IGF: Insulin-like growth factor; PI3K: Phosphoinositide 3-kinase; EGF: Epidermal growth factor; VEGF: Vascular endothelial growth factor; PDGF: Platelet-derived growth factor; mTOR: Mammalian target of rapamycin; PTEN: Phosphatase and tensin homolog; SCF: Stem cell factor; FGFR: Fibroblast growth factor receptor.
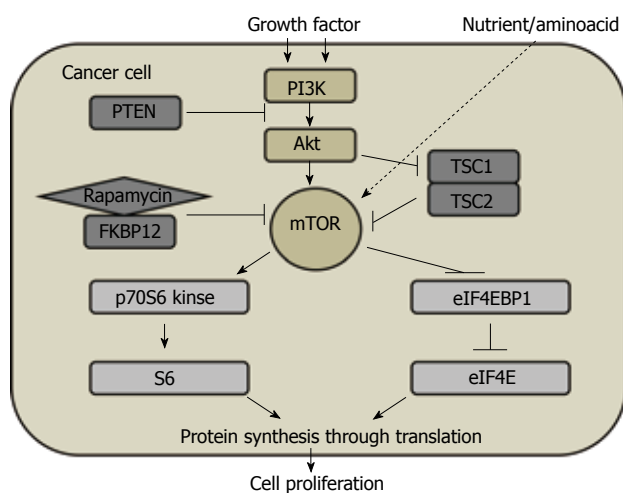

Figure 4 Phosphoinositide 3-kinase/Akt/mammalian target of rapamycin signaling pathway in cell proliferation in solid cancer. PI3K: Phosphoinositide 3-kinase; mTOR: Mammalian target of rapamycin; PTEN: Phosphatase and tensin homolog; TSC: Tuberous sclerosis; FKBP12: FK506-binding protein 12.
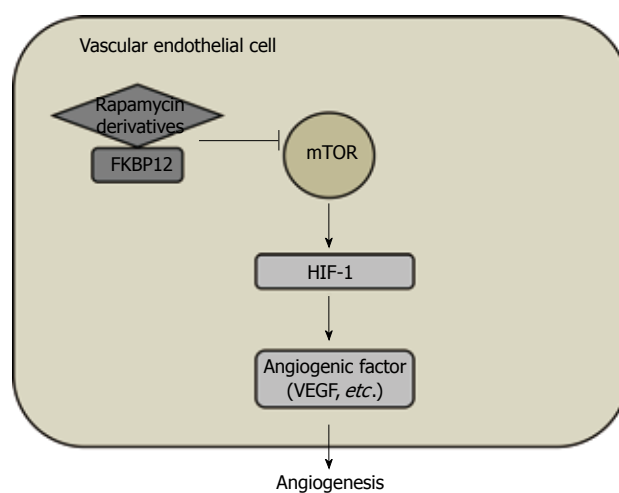

Figure 5 Mammalian target of rapamycin/hypoxia-inducible factor-1/vascular endothelial growth factor signaling pathway in angiogenesis in solid cancer. HIF: Hypoxia-inducible factor; VEGF: Vascular endothelial growth factor; mTOR: Mammalian target of rapamycin; FKBP12: FK506-binding protein 12.

intolerant patients, or for patients showing disease progression after sorafenib administration. A phase Ⅲ study to compare everolimus and a placebo (EVOLVE-1: Advanced Hepatocellular Carcinoma after Disease Progression or Intolerance to Sorafenib EverOlimus for Liver

cancer Evaluation) and a phase Ⅰ/randomized phase Ⅱ study (sorafenib + everolimus *vs* sorafenib alone) to test the efficacy and tolerance of sorafenib in combination with everolimus are underway. Since mTOR inhibitors exhibit cytostatic and antiangiogenic effects, they are

expected to be effective in combination with other angiogenesis inhibitors, such as bevacizumab, and may be appropriate for administration after transarterial chemoembolization (TACE). Furthermore, since the mTOR pathway is stimulated by factors such as EGFR, PDGFR, and TGFα, and is closely related to other signaling pathways, including the Ras/Raf/MEK/ERK pathway, they are likely to show promising efficacy when used in combination with other growth factor inhibitors[33].

VEGF/VEGFR, PDGFR, FGFR

Angiogenesis is an important event not only for HCC, but also for cancer growth and metastasis, and occurs because of complex alterations involving promoting factors such as VEGF, angiopoietin, and FGF, and inhibitory factors including thrombospondin and angiostatin, as well as the surrounding tissue. The VEGF family consists of VEGF-A, -B, -C, -D and –E, and placental growth factor (PIGF). The VEGFR family comprises VEGFR-1 (flt-1), VEGFR-2 (flk-1/KDR), and VEGFR-3 (flt-4). VEGF-A binds to VEGFR-1 and -2 and is involved in angiogenesis and the maintenance of mature blood vessels, while VEGF-C and -D mainly bind to VEGFR-3, are involved in lymphangiogenesis[34,35]. VEGF isoforms, such as VFGF$_{121}$ and VEGF$_{165}$, have been identified, and isoform subtypes also exist, such as EGF$_{166}$b. Thus, it is clear that these growth factors do not exhibit angiogenesis-promoting effects alone, and they have attracted attention as new therapeutic targets[36].

HCC typically exhibits active angiogenesis. During the progression from early to well- and moderately-differentiated HCC, angiogenesis increases and cancer cells acquire the ability to invade vessels and metastasize. Scientific and clinical studies have revealed that, during the progression from hepatitis to cirrhosis, angiogenesis and disruption of the vascular architecture are linked to the progression of HCC, and contribute to increased hepatic vascular resistance and portal hypertension, and decreased hepatocyte perfusion[37]. In addition, a meta-analysis has demonstrated that VEGF expression is a prognostic factor in HCC[38].

Phase Ⅱ studies have been started to test the usefulness of bevacizumab (Avastin®), which directly targets VEGF, in TACE-treated HCC, and the use of bevacizumab in combination with erlotinib (Tarceva®), an EGFR tyrosine kinase inhibitor.

Sunitinib (Sutent®) is a multi-kinase inhibitor that inhibits tyrosine kinases, such as VEGFR-1, -2, -3, PDGFR-α, -β and c-Kit. A phase Ⅱ study of sunitinib in 37 advanced HCC patients showed that the median progression-free survival (PFS) and median overall survival (OS) were 3.7 and 8 mo, respectively. In that study, adverse events included grade 3/4 thrombocytopenia in 37.8% of patients, neutropenia in 24.3%, asthenia in 13.5%, and hand-foot syndrome in 10.8%[39]. Since sunitinib has a lower IC$_{50}$ for each target than sorafenib, it is expected to exhibit greater antitumor activity. However, this factor may be responsible for the higher incidence of adverse events with sunitinib. The main evaluation item in the above phase Ⅱ trial was the response rate, which did not reach the expected value, leading to the conclusion that it was a negative study[40]. In that study, sunitinib was administered at 50 mg/d for four weeks followed by two weeks of rest per cycle[39], whereas Zhu et al[40] used a dosing schedule of 37.5 mg/d for four weeks followed by two weeks of rest per cycle, and reported that the median PFS and OS were 3.9 and 9.8 mo, respectively. An ongoing global cooperative phase Ⅲ controlled clinical trial to compare sorafenib and sunitinib head-to-head, and to seek approval for first-line indications for advanced HCC, adopted a sunitinib dosing schedule of 37.5 mg/d. However, in a "Reflection and Reaction" regarding the above trial results, Forner et al[41] cast doubt on whether the drugs at this dose could maintain tolerance and ensure efficacy. Consequently, the trial was terminated on March, 2010 because of the recommendation by data monitoring committee based on interim analysis, showing relatively high toxicity and no superior efficacy to sorafenib.

Brivanib is a kinase inhibitor that selectively inhibits VEGFR-1, -2 and -3, and FGFR-1, -2 and -3. Recent studies suggest that tumor progression following treatment with antiangiogenic agents that target the VEGF signaling pathway alone may result from either evasive or intrinsic resistance[42]. Furthermore, there is strong evidence to support the hypothesis that evasive resistance to anti-VEGF blockade is associated with reactivation of tumor angiogenesis by alternative signaling pathways. One such mechanism of resistance is the activation of the FGF signaling pathway[43,44]. Basic FGF (FGF2) is a potent angiogenic factor. Indeed, expression of FGF2 enhances growth, invasion, and angiogenesis of many tumor types[45,46]. Moreover, recent evidence has shown that FGF is overexpressed and activated in HCC, and that high FGF2 levels may predict a poor clinical outcome among patients with HCC[46].

Considering the proposed importance of FGF signaling in HCC angiogenesis, it is clear that novel antiangiogenic agents that combine inhibition of FGF receptor signaling with inhibition of VEGFR signaling might provide a potential mechanism to overcome anti-VEGF resistance in HCC (Figure 6). With this in mind, it is worthwhile considering the potential future impact of brivanib on the treatment of advanced HCC. Brivanib, a small-molecule tyrosine kinase inhibitor, is the first oral selective dual inhibitor of FGF and VEGF signaling. In multiple preclinical models of human xenograft tumors, including patient-derived HCC xenografts, brivanib has shown potent antitumor activity and no overt toxicity when dosed orally[47,48]. Furthermore, brivanib has demonstrated promising antitumor activity and acceptable tolerability in a phase Ⅱ, open-label study in patients with unresectable locally advanced or metastatic HCC[49,50]. Crucially, within this trial, brivanib showed activity both as first-line therapy (overall survival: 10 mo) or as second-
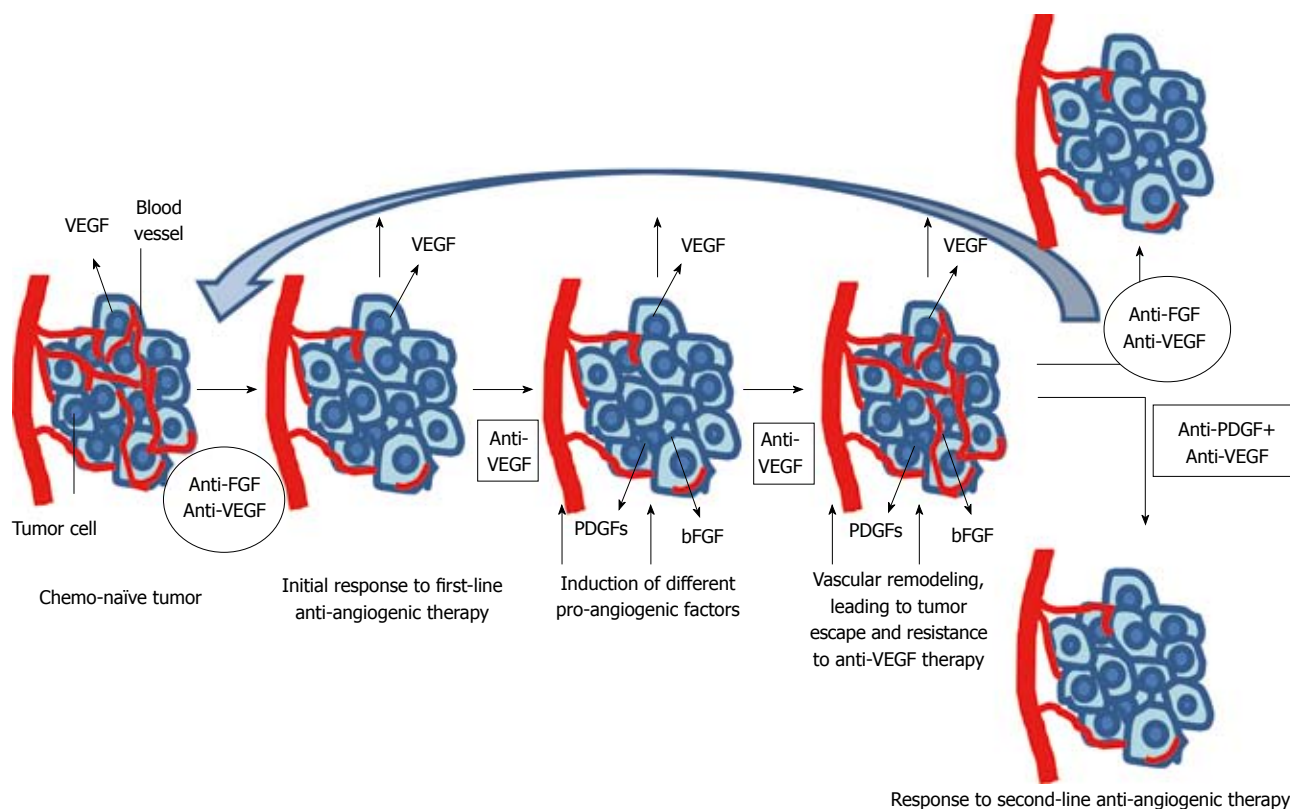
Figure 6 Brivanib may be effective for the failure or resistance of first line anti-angiogenic therapy for vascular endothelial growth factor. In addition, there is a possibility that anti-FGF agents can be first line anti-angiogenic agents. FGF: Fibroblast growth factor; VEGF: Vascular endothelial growth factor; PDGF: Platelet-derived growth factor.

line therapy in patients who had failed prior antiangio-genic treatment, primarily with sorafenib (overall survival: 9.5 mo)[50]. Of note, the incidence of all-grade hand-foot syndrome was only 8% in this study.

As for brivanib, an international global phase Ⅲ clini-cal trial to compare brivanib and sorafenib head-to-head and to seek approval for first-line therapy for advanced HCC has already been started, and the results are eagerly awaited. Since brivanib targets FGF and VEGF, and is associated with relatively mild adverse effects, a second-line study of brivanib in sorafenib-ineffective and -intol-erant patients, and a trial to evaluate the use of brivanib in combination with TACE, are underway. Depending on the results of these trials, indications for use in HCC may be obtained; therefore, positive results are eagerly anticipated.

The results have been reported for a phase Ⅱ study of brivanib in 55 patients (cohort A) who had not received systemic therapy for curatively unresectable HCC and 46 patients (cohort B) previously treated with angiogenesis inhibitors, such as sorafenib or thalidomide[49]. The me-dian TTP and OS were 2.8 mo and 10 mo, respectively, in cohort A versus 1.4 mo and 9.8 mo, respectively, in cohort B. Adverse events included fatigue (51.5%), diar-rhea (41.6%), hypertension (42.6%), anorexia (41.6%), and nausea/vomiting (40.6%/30.7%) in total. Thus, these results demonstrated the efficacy of brivanib as a second-line treatment. The results of three phase Ⅲ clinical trials,

BRISK-PS (sorafenib failure or sorafenib-intolerant pa-tients; brivanib + best supportive care (BSC) vs placebo + BSC), BRISK-FL (advanced HCC; brivanib vs sorafenib), and BRISK-TA (patients with unresectable HCC, brivanib vs placebo as post-TACE adjuvant therapy) are awaited (Table 2).

Linifanib (ABT-869), which strongly inhibits VEGFR and PDGFR, is also under global phase Ⅲ trial.

In a Japanese phase Ⅰ/Ⅱ trial of TSU-68, an oral mo-lecular inhibitor of VEGFR, PDGFR, and FGFR, to test its safety and efficacy in 35 HCC patients, the response rate was 5.6% (CR, PR, SD, PD and NE in 1, 2, 15, 16 and 1 patients, respectively), and the disease control rate was 51.4%[51]. The global phase Ⅲ trial of TACE in com-bination with TSU-68 has just started on January 2011.

In addition, several phase Ⅰ/Ⅱ trials are being conduct-ed to assess kinase inhibitors such as cediranib (AZD2171), which inhibit VEGFR, PDGFR, CSF-1R (cFms), Kit, and Flt3. Furthermore, a phase Ⅲ global study of axitinib, which is currently being tested in renal cell carcinoma, has also been started as a second line agents on 2011.

EGF/EGFR
EGFR is a member of the HER family, which includes EGFR (erbB1), HER2/neu (erbB3), and HER4 (erb4). All members of this family, except HER3, have an in-tracellular tyrosine kinase domain, and the binding of a ligand to its extracellular domain triggers signal transduc-

Table 2 Ongoing clinical trials (P Ⅲ)

First line
  Comparison study between sorafenib and single agent (head to head):
    Sunitinib → endpoint not met
    Brivanib
    Linifanib
  Combination with sorafenib and another agent:
    DXR, erlotinib (SEARCH), everolimus, CS-1008, *etc.*
Second line
  Sorafenib failure:
    Brivanib, everolimus (RAD001), ramucirumab, axitinib, S-1, *etc.*
Combination with standard therapy
  Adjuvant setting after surgery or RFA: STORM
  Combination with TACE: SPACE, BRISK-TA, TACTICS, ECOG1208
  Combination with HAIC: SILIUS

TACTICS: Phase Ⅱ study: Transcatheter arterial chemoembolization therapy in combination with sorafenib (ClinicalTrials.gov ID: NCT01217034), SILIUS: Randomized controlled trial comparing efficacy of sorafenib *vs* sorafenib in combination with low dose cisplatin/fluorouracil hepatic arterial InfUSion chemotherapy in patients with advanced hepatocellular carcinoma and exploratory study of biomarker predicting its efficacy (ClinicalTrials.gov ID: NCT01214343); HAIC: Hespatic arterial infusion chemotherapy; TACE: Transarterial chemoembolization.

tion through the above-described MAPK and PI3K/Akt/mTOR pathways. Thus, these receptors are involved in cell growth, differentiation, survival, and adhesion[52]. EGFR over expression has been reported in many cancers, and in HCC. For example, Buckley *et al*[53] reported that EGFR, detected by immunohistochemical analysis, was overexpressed in 50 (66%) of 76 HCCs, and that fluorescence *in situ* hybridization showed extra EGFR gene copies in 17 (45%) of 38 HCCs.

EGFR-targeting drugs include anti-EGFR antibodies, such as cetuximab and panitumumab, and small-molecule inhibitors of EGFR tyrosine kinases, such as gefitinib *etc*, and have been used widely for the treatment of several cancers other than HCC. Unfortunately, except for phase Ⅱ trial data, there are little clinical data on the efficacy of these drugs for the treatment of HCC.

Similar to gefitinib (Iressa®), erlotinib (Tarceva®) is an oral EGFR tyrosine kinase inhibitor. Philip *et al*[54] and Yau *et al*[55] have reported the results of phase Ⅱ studies of erlotinib in HCC; the median OSs in their studies were 13 and 10.7 mo, respectively. A phase Ⅲ clinical study (SEARCH study: Sorafenib and Erlotinib, a Randomized Trial Protocol for the Treatment of Patients with Hepatocellular Carcinoma) for sorafenib in combination with erlotinib *vs* sorafenib plus placebo is ongoing. Since erlotinib is associated with a high incidence of skin rash, dry skin and gastrointestinal toxicity, such as diarrhea, the results of the SEARCH study should be evaluated to assess whether this combination therapy can be used in clinical settings. Thomas *et al*[56] conducted a phase Ⅱ clinical study of erlotinib in combination with bevacizumab in 40 advanced HCC patients, and reported promising results; the median PFS and OS were 9 mo and 15.7 mo, respectively. However, they noted frequent treatment-related grade 3/4 toxicities, including fatigue

(20%), hypertension (15%), gastrointestinal bleeding (12.5%), wound infection (5%), diarrhea (10%), elevated transaminase levels (10%), and thrombocytopenia (10%), which necessitates further evaluation for drug tolerance. Although a clinical study of erlotinib in combination with bevacizumab (OPTIMOX-3 study) was also conducted in colorectal cancer patients, no tolerance was observed, which led to a change in the protocol[57,58].

After the introduction of a number of molecular-targeted drugs, strategies for the inhibition of similar or different signaling pathways (vertical or horizontal inhibition) with several drugs have been proposed. However, the combined use of molecular-targeted agents has remained largely unsuccessful, including panitumumab in combination with bevacizumab for the treatment of colorectal cancer[59]. Similarly, the results of sorafenib in combination with bevacizumab (vertical inhibition) have been reported[60]. Although some therapeutic responses were obtained, the combination therapy resulted in greater toxicity[60], suggesting the need for detailed evaluation of the dosing regimen.

Lapatinib (Tykerb®) is a dual inhibitor of EGFR and HER-2/neu, and inhibits tumor growth by downregulating MAPK, AKT, and p70S6 kinase[61]. In Japan, lapatinib is indicated for the treatment of breast cancer. In a phase Ⅱ clinical trial of lapatinib in 26 patients with unresectable advanced HCC, the median PFS and OS were 1.9 mo and 12.6 mo, respectively, and adverse events included diarrhea (73%), nausea (54%), and skin rash (42%)[62].

Cetuximab (Erbitux®) is a human/mouse chimeric monoclonal antibody consisting of the variable region of a mouse anti-human EGFR monoclonal antibody and the human immunoglobulin G1 constant region. Cetuximab inhibits the binding of endogenous EGFR ligands, such as EGF and TGFα, to EGFR. In a phase Ⅱ clinical trial of cetuximab in 30 patients with unresectable or metastatic HCC, the median PFS and OS were 1.4 mo and 9.6 mo, respectively, and treatment-related toxicities included grade 3 hypomagnesemia (3.3%) and grade 1/2 acne-like rash (83.3%), which was observed for the duration of anti-EGFR therapy in that study[63].

The EGFR offers a very interesting therapeutic target. As described above, the use of erlotinib in combination with sorafenib is still in the research stage. However, based on the results of phase Ⅱ studies, the efficacy of cetuximab or lapatinib as a monotherapy seems to be limited, and the results of further studies evaluating their efficacy in sorafenib-refractory or -intolerant patients are awaited with interest.

Hepatocyte growth factor/c-Met pathway

Since the hepatocyte growth factor (HGF)/Met pathway is involved in tumor growth, invasion, and angiogenesis in a wide range of neoplasms, HGF and Met have recently attracted attention as therapeutic targets. HGF is a heterodimer consisting of α and β chains bound together by a disulfate bond. The α-chain contains four
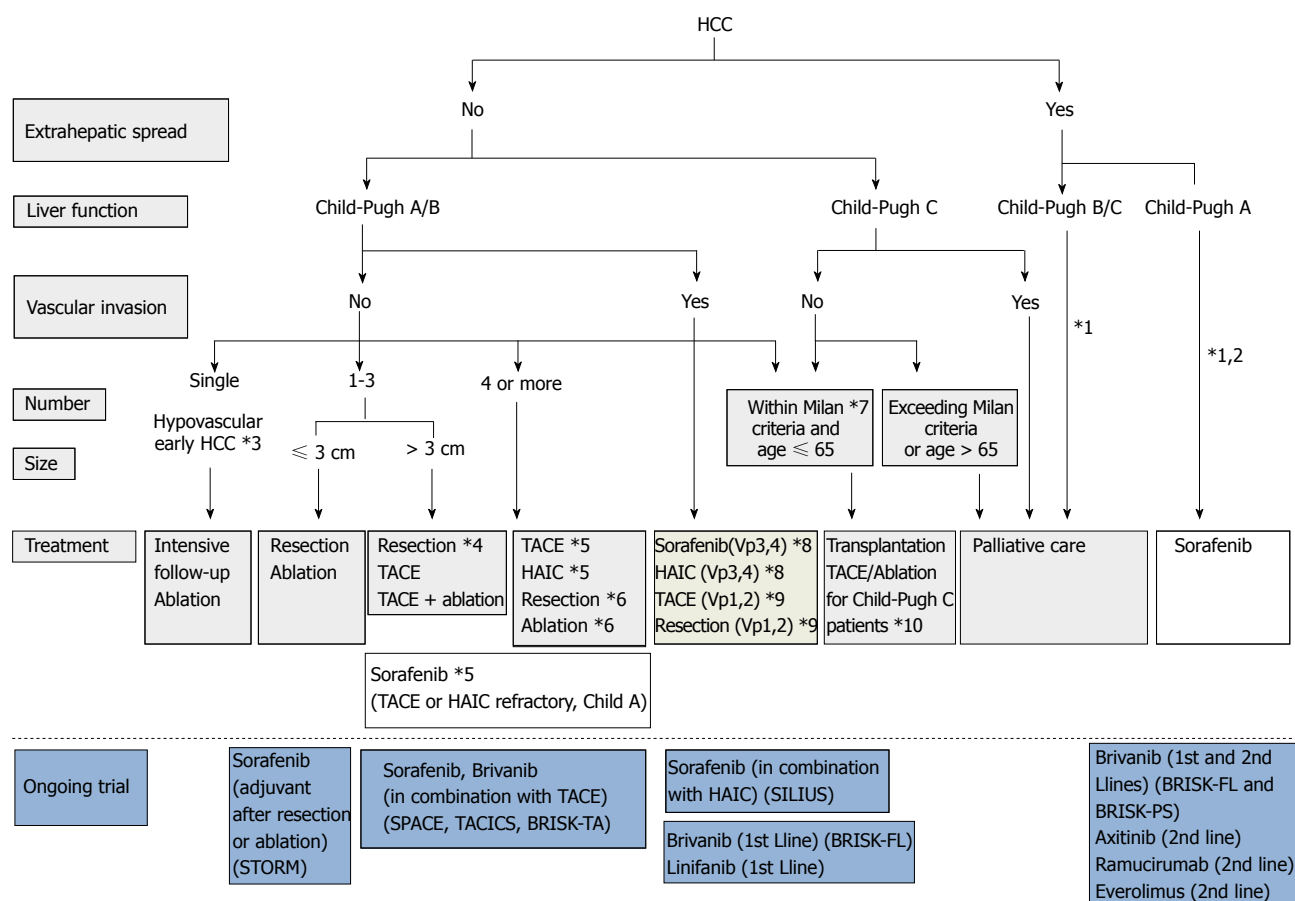
Figure 7 Consensus-based treatment algorithm for hepatocellular carcinoma proposed by Japan Society of Hepatology, revised in 2010. (Cited and modified from Kudo et al[67] with permission.) *1: Treatment should be performed as if extrahepatic spread is negative, when extrahepatic spread is not considered as a prognostic factor in Child-Pugh class A/B patients; *2: Sorafenib is the first choice of treatment in this setting as a standard of care; *3: Intensive follow-up observation is recommended for hypovascular nodules by the Japanese Evidence-Based Clinical Practice Guidelines. However, local ablation therapy is frequently performed in the following cases: (1) when the nodule is diagnosed pathologically as early hepatocellular carcinoma (HCC); (2) when the nodules show decreased uptake on Gd-EOB-MRI, or (3) when the nodules show decreased portal flow by CTAP, since these nodules frequently progress to advanced HCC; *4: Even for HCC nodules exceeding 3 cm in diameter, transcatheter arterial chemoembolization (TACE) in combination with ablation is frequently performed when resection is not indicated; *5: TACE is the first choice of treatment in this setting. Hepatic arterial infusion chemotherapy (HAIC) using an implanted port is also recommended for TACE-refractory patients. The regimen for this treatment is usually low-dose FP [5-fluorouracil (5-FU) + CDDP] or intra-arterial 5-FU infusion combined with systemic interferon therapy. Sorafenib is also recommended for TACE or HAIC-refractory patients with Child-Pugh class A liver function; *6: Resection is sometimes performed when more than 4 nodules are detected. Ablation is sometimes performed in combination with TACE; *7: Milan criteria: Tumor size $\leq$ 3 cm and tumor number $\leq$ 3, or solitary tumor $\leq$ 5 cm. Even when liver function is good (Child-Pugh A/B), transplantation is sometimes considered for frequently recurring HCC patients; *8: Sorafenib and HAIC are recommended for HCC patients with major portal invasion such as Vp3 (portal invasion in the 1st portal branch) or Vp4 (portal invasion in the main portal branch); *9: Resection and TACE are frequently performed when portal invasion is minor, such as Vp1 (portal invasion in the 3rd or more peripheral portal branch) or Vp2 (portal invasion in the 2nd portal branch); *10: Local ablation therapy or subsegmental TACE is performed even for Child-Pugh C patients when transplantation is not indicated when there is no hepatic encephalopathy, no uncontrollable ascites, and a low bilirubin level (< 3.0 mg/dL). However, it is regarded as an experimental treatment because there is no evidence of a survival benefit in Child-Pugh C patients. A prospective study is necessary to clarify this issue.

kringle domains, and the β-chain contains a serine protease-like domain. Met is a receptor tyrosine kinase for the HGF ligand, and contains a semaphorin-like domain. HGF or Met overexpression, and *Met* gene mutations and duplications, have been reported in various cancers, and abnormalities due to HGF/Met pathway activation have also been noted[64]. These abnormalities activate the downstream signaling cascade, leading to epithelial-mesenchymal transition and increased proliferative, migratory, invasive, and metastatic potentials of cancer cells[64].

HGF/c-MET-targeted drugs, including kinase inhibitors, HGF inhibitors and decoy c-Met receptor molecules are being developed. Of particular interest is ARQ-197, a

c-Met receptor tyrosine kinase inhibitor, which is a non-ATP-competitive molecule that binds near the ATP-binding site. A randomized phase II study of ARQ-197 vs placebo is ongoing in patients with unresectable HCC after systemic therapy failure. In addition, the results of a phase I study of ARQ-197 in combination with sorafenib was reported in ASCO 2010 (Abstract 3024).

IGF/IGFR

The IGF/IGFR system is involved in cell growth and the chemotherapeutic response. The ligands IGF- I and - II bind to their receptors IGF-1R and IGF-2R, and are involved in DNA synthesis and cell growth. Abnormali-
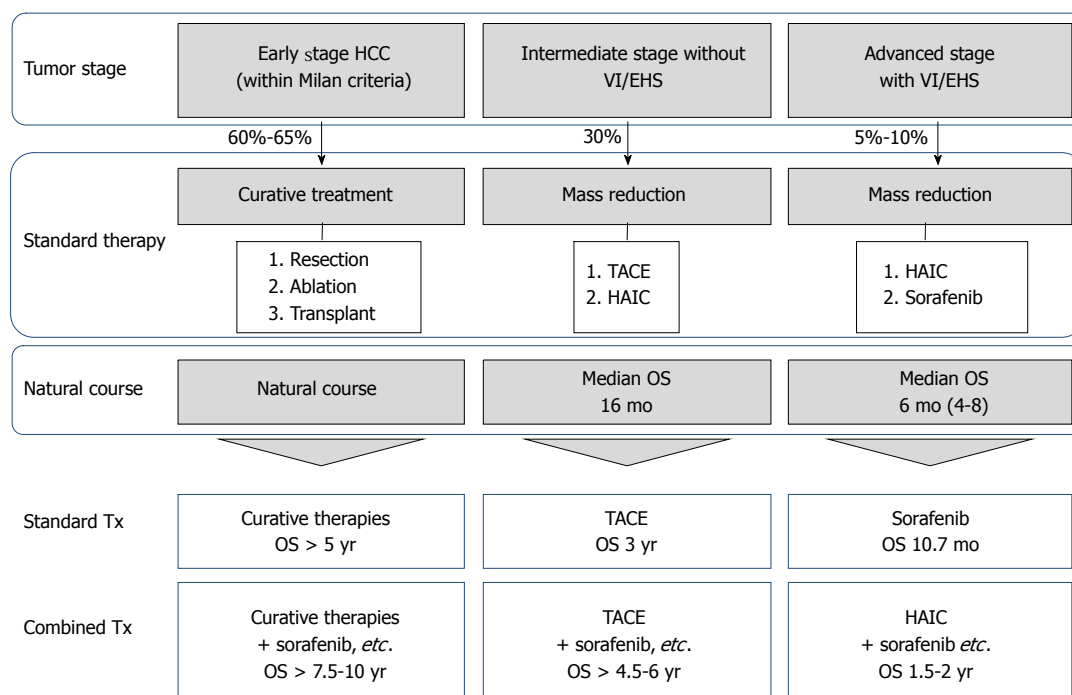
Figure 8 Outcomes of standard treatment modalities and expected future outcomes of combination therapy with molecular-targeted agents. By combining molecular targeted agents with resection or ablation, life expectancy [overall survival (OS)] is expected to be prolonged to 7.5-10 years. In addition, for intermediate stage hepatocellular carcinoma (HCC), the prognosis is expected to be improved to 4.5-6 years by combination with transarterial chemoembolization (TACE). For advanced stage HCC, the prognosis is expected to be improved to 1.5-2 years by combination with hepatic arterial infusion chemotherapy (HAIC).

Table 3 Subanalysis data of the SHARP study

		Advanced HCC with vascular invasion and/or extrahepatic spread	Advanced HCC without vascular invasion and/or extrahepatic spread
Hazard ratio		0.77 (95% CI: 0.60-0.99)	0.52 (95% CI: 0.32-0.85)
Median OS (MST) (mo)	Sorafenib	8.9 (n = 209) (95% CI: 7.6-10.3)	14.5 (n = 90) (95% CI: 14.0-N/E)
	Placebo	6.7 (n = 212) (95% CI: 5.2-8.0)	10.2 (n = 91) (95% CI: 8.6-15.5)

HCC: Hepatocellular carcinoma; OS: Overall survival; MST: Median survival time.

ties in IGF and IGF-1R, or their overexpression, have been reported in various cancers, including HCC. Their associations with disease stage, metastasis, survival[65], and the functions of IGF and IGFR in HCC[66] have been reported.

IGF-targeting drugs are currently being developed, and are mainly anti-IGF-1R antibodies, such as BⅡB022, AVE1642, and cixutumumab (IMC-A12). A phase Ⅱ study of cixutumumab, a phase Ⅰb/Ⅱ study of sorafenib *vs* sorafenib plus BⅡB022, and phase Ⅰ/Ⅱ studies of AVE1642 as monotherapy or in combination with sorafenib or erlotinib are ongoing.

COMBINATION THERAPY OF STANDARD TREATMENT WITH SORAFENIB

In addition to the pharmaceutical-sponsored clinical trials of linifanib and brivanib as first- and second-line therapy in sorafenib-refractory patients, investigator initiated trials (IIT) of sorafenib in combination with hepatic arte-rial infusion chemotherapy (SILIUS trial: trial number NCT01214343), pharmaceutical and IIT trials of sorafenib in combination with TACE [SPACE, TACICS (trial number: NCT 01217034) and BRISK-TA trials], and a trial to test the inhibitory effect of sorafenibon tumor recurrence after curative treatment (STORM trial) are ongoing. The results of these trials are eagerly awaited (Figure 7)[67,68].

The working hypotheses in these studies can be deduced by extrapolating the median survival time (MST) and hazard ratios in overall survival (OS) calculated in a subanalysis of the SHARP study (Table 3). The results obtained suggest that starting treatment with molecular-targeted drugs at an earlier tumor stage in combination with standard treatment options such as resection, ablation, TACE, or hepatic arterial infusion chemotherapy can improve the prognosis of HCC. Thus, sorafenib has the potential to induce a paradigm shift in the treatment of HCC. For example, in a subanalysis of the SHARP trial, the hazard ratios for OS and MST ratio in intermediate stage HCC without vascular invasion or extrahepatic spread were 0.52 and 1.50, respectively (Table 3). This

suggests that survival of early stage HCC and intermediate stage HCC may be prolonged from 5 years to 7.5-10 years by using sorafenib in an adjuvant setting after curative treatment, and from 3 years to 4.5-6 years by using sorafenib in combination with TACE (Figure 8)[68].

CONCLUSION

Several clinical trials[39,40,49,54,63,69-74] of the molecular-targeted agents are ongoing. Angiogenesis-inhibiting drugs, particularly sorafenib, have been established for HCC, and drugs targeting several molecules are being developed.

Although sorafenib was recently approved, many issues remain to be addressed, including: (1) how to determine and define refractoriness; and (2) whether to continue TACE or hepatic arterial infusion chemotherapy (a de facto standard in Japan) in patients with TACE-refractory HCCs or portal tumor thrombi before starting sorafenib therapy. We strongly recommend that, based on the molecular-targeted agents currently under development, clinical studies (including IITs) should be conducted aggressively, and therapeutic strategies should be devised to resolve the limitations of currently used therapeutic approaches and to improve the therapeutic outcomes.

The introduction of sorafenib to treat HCC in 2007 in Western countries and in 2009 in Japan was undoubtedly the real beginning of a paradigm shift of HCC treatment, representing a significant breakthrough for HCC treatment not previously experienced for this unique tumor. Further development of survival benefit in HCC patients with new targeted agents are greatly expected.

REFERENCES

1   **Farazi PA**, DePinho RA. Hepatocellular carcinoma pathogenesis: from genes to environment. *Nat Rev Cancer* 2006; **6**: 674-687

2   **Mínguez B**, Tovar V, Chiang D, Villanueva A, Llovet JM. Pathogenesis of hepatocellular carcinoma and molecular therapies. *Curr Opin Gastroenterol* 2009; **25**: 186-194

3   **Villanueva A**, Newell P, Chiang DY, Friedman SL, Llovet JM. Genomics and signaling pathways in hepatocellular carcinoma. *Semin Liver Dis* 2007; **27**: 55-76

4   **Laurent-Puig P**, Zucman-Rossi J. Genetics of hepatocellular tumors. *Oncogene* 2006; **25**: 3778-3786

5   **Llovet JM**, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. *Hepatology* 2008; **48**: 1312-1327

6   **Höpfner M**, Schuppan D, Scherübl H. Growth factor receptors and related signalling pathways as targets for novel treatment strategies of hepatocellular cancer. *World J Gastroenterol* 2008; **14**: 1-14

7   **Campbell JS**, Hughes SD, Gilbertson DG, Palmer TE, Holdren MS, Haran AC, Odell MM, Bauer RL, Ren HP, Haugen HS, Yeh MM, Fausto N. Platelet-derived growth factor C induces liver fibrosis, steatosis, and hepatocellular carcinoma. *Proc Natl Acad Sci USA* 2005; **102**: 3389-3394

8   **Ogasawara S**, Yano H, Iemura A, Hisaka T, Kojiro M. Expressions of basic fibroblast growth factor and its receptors and their relationship to proliferation of human hepatocellular carcinoma cell lines. *Hepatology* 1996; **24**: 198-205

9   **Kudo M**. Hepatocellular carcinoma 2009 and beyond: from the surveillance to molecular targeted therapy. *Oncology* 2008; **75** Suppl 1: 1-12

10 **Hanahan D**, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57-70

11 **Spangenberg HC**, Thimme R, Blum HE. Targeted therapy for hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 423-432

12 **Villanueva A**, Toffanin S, Llovet JM. Linking molecular classification of hepatocellular carcinoma and personalized medicine: preliminary steps. *Curr Opin Oncol* 2008; **20**: 444-453

13 **Roberts PJ**, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene* 2007; **26**: 3291-3310

14 **Schmidt CM**, McKillop IH, Cahill PA, Sitzmann JV. Increased MAPK expression and activity in primary human hepatocellular carcinoma. *Biochem Biophys Res Commun* 1997; **236**: 54-58

15 **Calvisi DF**, Ladu S, Gorden A, Farina M, Conner EA, Lee JS, Factor VM, Thorgeirsson SS. Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. *Gastroenterology* 2006; **130**: 1117-1128

16 **Bos JL**. ras oncogenes in human cancer: a review. *Cancer Res* 1989; **49**: 4682-4689

17 **Beeram M**, Patnaik A, Rowinsky EK. Raf: a strategic target for therapeutic development against cancer. *J Clin Oncol* 2005; **23**: 6771-6790

18 **Tannapfel A**, Sommerer F, Benicke M, Katalinic A, Uhlmann D, Witzigmann H, Hauss J, Wittekind C. Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. *Gut* 2003; **52**: 706-712

19 **Jenke HS**, Deml E, Oesterle D. C-raf expression in early rat liver tumorigenesis after promotion with polychlorinated biphenyls or phenobarbital. *Xenobiotica* 1994; **24**: 569-580

20 **Beer DG**, Neveu MJ, Paul DL, Rapp UR, Pitot HC. Expression of the c-raf protooncogene, gamma-glutamyltranspeptidase, and gap junction protein in rat liver neoplasms. *Cancer Res* 1988; **48**: 1610-1617

21 **Hwang YH**, Choi JY, Kim S, Chung ES, Kim T, Koh SS, Lee B, Bae SH, Kim J, Park YM. Over-expression of c-raf-1 proto-oncogene in liver cirrhosis and hepatocellular carcinoma. *Hepatol Res* 2004; **29**: 113-121

22 **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

23 **Ohren JF**, Chen H, Pavlovsky A, Whitehead C, Zhang E, Kuffa P, Yan C, McConnell P, Spessard C, Banotai C, Mueller WT, Delaney A, Omer C, Sebolt-Leopold J, Dudley DT, Leung IK, Flamme C, Warmus J, Kaufman M, Barrett S, Tecle H, Hasemann CA. Structures of human MAP kinase kinase 1 (MEK1) and MEK2 describe novel noncompetitive kinase inhibition. *Nat Struct Mol Biol* 2004; **11**: 1192-1197

24 **Huynh H**, Nguyen TT, Chow KH, Tan PH, Soo KC, Tran E. Over-expression of the mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK in hepatocellular carcinoma: its role in tumor progression and apoptosis. *BMC Gastroenterol* 2003; **3**: 19

25 **Engelman JA**. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer* 2009; **9**: 550-562

26 **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

27 **Semela D**, Piguet AC, Kolev M, Schmitter K, Hlushchuk R, Djonov V, Stoupis C, Dufour JF. Vascular remodeling and antitumoral effects of mTOR inhibition in a rat model of hepatocellular carcinoma. *J Hepatol* 2007; **46**: 840-848

28 **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

29 **Zhou P**, Kudo M, Minami Y, Chung H, Inoue T, Fukunaga T, Maekawa K. What is the best time to evaluate treatment response after radiofrequency ablation of hepatocellular carcinoma using contrast-enhanced sonography? *Oncology* 2007; **72** Suppl 1: 92-97

30 **Zhou L**, Huang Y, Li J, Wang Z. The mTOR pathway is associated with the poor prognosis of human hepatocellular carcinoma. *Med Oncol* 2010; **27**: 255-261

31 **Chen JS**, Wang Q, Fu XH, Huang XH, Chen XL, Cao LQ, Chen LZ, Tan HX, Li W, Bi J, Zhang LJ. Involvement of PI3K/PTEN/AKT/mTOR pathway in invasion and metastasis in hepatocellular carcinoma: Association with MMP-9. *Hepatol Res* 2009; **39**: 177-186

32 **Villanueva A**, Chiang DY, Newell P, Peix J, Thung S, Alsinet C, Tovar V, Roayaie S, Minguez B, Sole M, Battiston C, Van Laarhoven S, Fiel MI, Di Feo A, Hoshida Y, Yea S, Toffanin S, Ramos A, Martignetti JA, Mazzaferro V, Bruix J, Waxman S, Schwartz M, Meyerson M, Friedman SL, Llovet JM. Pivotal role of mTOR signaling in hepatocellular carcinoma. *Gastroenterology* 2008; **135**: 1972-1983

33 **Treiber G**. mTOR inhibitors for hepatocellular cancer: a forward-moving target. *Expert Rev Anticancer Ther* 2009; **9**: 247-261

34 **Ferrara N**, Davis-Smyth T. The biology of vascular endothelial growth factor. *Endocr Rev* 1997; **18**: 4-25

35 **Griffioen AW**, Molema G. Angiogenesis: potentials for pharmacologic intervention in the treatment of cancer, cardiovascular diseases, and chronic inflammation. *Pharmacol Rev* 2000; **52**: 237-268

36 **Harper SJ**, Bates DO. VEGF-A splicing: the key to anti-angiogenic therapeutics? *Nat Rev Cancer* 2008; **8**: 880-887

37 **Fernández M**, Semela D, Bruix J, Colle I, Pinzani M, Bosch J. Angiogenesis in liver disease. *J Hepatol* 2009; **50**: 604-620

38 **Schoenleber SJ**, Kurtz DM, Talwalkar JA, Roberts LR, Gores GJ. Prognostic role of vascular endothelial growth factor in hepatocellular carcinoma: systematic review and meta-analysis. *Br J Cancer* 2009; **100**: 1385-1392

39 **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

40 **Zhu AX**, Sahani DV, Duda DG, di Tomaso E, Ancukiewicz M, Catalano OA, Sindhwani V, Blaszkowsky LS, Yoon SS, Lahdenranta J, Bhargava P, Meyerhardt J, Clark JW, Kwak EL, Hezel AF, Miksad R, Abrams TA, Enzinger PC, Fuchs CS, Ryan DP, Jain RK. Efficacy, safety, and potential biomarkers of sunitinib monotherapy in advanced hepatocellular carcinoma: a phase II study. *J Clin Oncol* 2009; **27**: 3027-3035

41 **Forner A**, Llovet JM, Bruix J. Sunitinib and the benefits of a negative study. *Lancet Oncol* 2009; **10**: 743-744

42 **Bergers G**, Hanahan D. Modes of resistance to anti-angiogenic therapy. *Nat Rev Cancer* 2008; **8**: 592-603

43 **Batchelor TT**, Sorensen AG, di Tomaso E, Zhang WT, Duda DG, Cohen KS, Kozak KR, Cahill DP, Chen PJ, Zhu M, Ancukiewicz M, Mrugala MM, Plotkin S, Drappatz J, Louis DN, Ivy P, Scadden DT, Benner T, Loeffler JS, Wen PY, Jain RK. AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. *Cancer Cell* 2007; **11**: 83-95

44 **Kopetz S**, Hoff PM, Eng MJ. Angiogenic cytokines are increased prior to disease progression in metastatic colorectal cncer patients treated with bebacizumab. Proceedings of ASCO Gastrointestinal Cancers Symposium; 2009 Jan 15-17; San Francisco, USA. Abstract 292

45 **El-Assal ON**, Yamanoi A, Ono T, Kohno H, Nagasue N. The clinicopathological significance of heparanase and basic fibroblast growth factor expressions in hepatocellular carcinoma. *Clin Cancer Res* 2001; **7**: 1299-1305

46 **Poon RT**, Ng IO, Lau C, Yu WC, Fan ST, Wong J. Correlation of serum basic fibroblast growth factor levels with clinicopathologic features and postoperative recurrence in hepatocellular carcinoma. *Am J Surg* 2001; **182**: 298-304

47 **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

48 **Bhide RS**, Lombardo LJ, Hunt JT, Cai ZW, Barrish JC, Galbraith S, Jeyaseelan R, Mortillo S, Wautlet BS, Krishnan B, Kukral D, Malone H, Lewin AC, Henley BJ, Fargnoli J. The antiangiogenic activity in xenograft models of brivanib, a dual inhibitor of vascular endothelial growth factor receptor-2 and fibroblast growth factor receptor-1 kinases. *Mol Cancer Ther* 2010; **9**: 369-378

49 **Raoul JL**, Flinn RS, Kang YK, Park JW, Harris R, Coric V, Donic M, Walters I. An open-label phase II study of first- and second-line treatment with Brivanib in patients with hepatocellular carcinoma (HCC). *J Clin Oncol* 2009; **27**: Abstr 4577

50 **Raoul JL**, Finn RS, Kang WK, Park JW, Harris R, Coric V. Phase 2 study of first- and second-line treatment with brivanib in patients with hepatocellular carcinoma. *ILCA* 2009: Abstract P-0111

51 **Kanai F**, Yoshida H, Tateishi R, Shiina S, Ikeda M, Okusaka T, Kondo Y, Tagawa K, Omata M, TSU-68 HCC study group. Final results of a phse I/II trial of the oral anti-angiogenesis inhibitor TSU-68 in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2008; **26** (235 suppl): abstract 4589

52 **Ciardiello F**, Tortora G. EGFR antagonists in cancer treatment. *N Engl J Med* 2008; **358**: 1160-1174

53 **Buckley AF**, Burgart LJ, Sahai V, Kakar S. Epidermal growth factor receptor expression and gene copy number in conventional hepatocellular carcinoma. *Am J Clin Pathol* 2008; **129**: 245-251

54 **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

55 **Yau T**, Chan P, Ng KK, Chok SH, Cheung TT, Fan ST, Poon RT. Phase 2 open-label study of single-agent sorafenib in treating advanced hepatocellular carcinoma in a hepatitis B-endemic Asian population: presence of lung metastasis predicts poor response. *Cancer* 2009; **115**: 428-436

56 **Thomas MB**, Morris JS, Chadha R, Iwasaki M, Kaur H, Lin E, et al. Phase II trial of the combination of bevacizumab and erlotinib in patients who have advanced hepatocellular carcinoma. *J Clin Oncol* 2009; **27**: 843-850

57 **Tournigand C**, Lledo G, Delord J, Andre T, Maindrault-Goebel F, Louvet C, W. Scheithauer, A. de Gramont. Modified folfox7/bevacizumab or modified Xelox/bevacizumab with or without erlotinib in first-line metastatic colorectal cancer (MCRC): Results of the feasibility phase of the DREAM-OPTIMOX3 study (GERCOR). *J Clinical Oncol* 2007; **25**: 4097

58 **Tournigand C**, Samson B, Scheithauer W, Louvet C, Andre T, Lledo G, Latreille J, Viret F, Chibaudel B, de Gramont A. mFOLFOX-bevacizumab or XELOX-bevacizumab then be-

vacizumab alone or with erlotinib in first-line treatment of patients with metastatic colorectal cancer (mCRC): Interim safety analysis of DREAM study. *J Clin Oncol* 2009; **27**: Abstract 4077

59 **Hecht JR**, Mitchell E, Chidiac T, Scroggin C, Hagenstad C, Spigel D, Marshall J, Cohn A, McCollum D, Stella P, Deeter R, Shahin S, Amado RG. A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. *J Clin Oncol* 2009; **27**: 672-680

60 **Azad NS**, Posadas EM, Kwitkowski VE, Steinberg SM, Jain L, Annunziata CM, Minasian L, Sarosy G, Kotz HL, Premkumar A, Cao L, McNally D, Chow C, Chen HX, Wright JJ, Figg WD, Kohn EC. Combination targeted therapy with sorafenib and bevacizumab results in enhanced toxicity and antitumor activity. *J Clin Oncol* 2008; **26**: 3709-3714

61 **Burris HA**, Hurwitz HI, Dees EC, Dowlati A, Blackwell KL, O'Neil B, Marcom PK, Ellis MJ, Overmoyer B, Jones SF, Harris JL, Smith DA, Koch KM, Stead A, Mangum S, Spector NL. Phase I safety, pharmacokinetics, and clinical activity study of lapatinib (GW572016), a reversible dual inhibitor of epidermal growth factor receptor tyrosine kinases, in heavily pretreated patients with metastatic carcinomas. *J Clin Oncol* 2005; **23**: 5305-5313

62 **Bekaii-Saab T**, Markowitz J, Prescott N, Sadee W, Heerema N, Wei L, Dai Z, Papp A, Campbell A, Culler K, Balint C, O' Neil B, Lee RM, Zalupski M, Dancey J, Chen H, Grever M, Eng C, Villalona-Calero M. A multi-institutional phase II study of the efficacy and tolerability of lapatinib in patients with advanced hepatocellular carcinomas. *Clin Cancer Res* 2009; **15**: 5895-5901

63 **Zhu AX**, Stuart K, Blaszkowsky LS, Muzikansky A, Reitberg DP, Clark JW, Enzinger PC, Bhargava P, Meyerhardt JA, Horgan K, Fuchs CS, Ryan DP. Phase 2 study of cetuximab in patients with advanced hepatocellular carcinoma. *Cancer* 2007; **110**: 581-589

64 **Comoglio PM**, Giordano S, Trusolino L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. *Nat Rev Drug Discov* 2008; **7**: 504-516

65 **Scharf JG**, Braulke T. The role of the IGF axis in hepatocarcinogenesis. *Horm Metab Res* 2003; **35**: 685-693

66 **Chen YW**, Boyartchuk V, Lewis BC. Differential roles of insulin-like growth factor receptor- and insulin receptor-mediated signaling in the phenotypes of hepatocellular carcinoma cells. *Neoplasia* 2009; **11**: 835-845

67 **Kudo M**, Izumi N, Kokudo N, Matsui O, Sakamoto M, Nakashima O, Kojiro M, Makuuchi M. Management of hepatocellular carcinoma in Japan: Consensus-Based Clinical Practice Guidelines proposed by the Japan Society of Hepatology (JSH) 2010 updated version. *Dig Dis* 2011; **29**: 339-364

68 **Kudo M**. The 2008 Okuda lecture: Management of hepatocellular carcinoma: from surveillance to molecular targeted therapy. *J Gastroenterol Hepatol* 2010; **25**: 439-452

69 **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

70 **Abou-Alfa GK**, Schwartz L, Ricci S, Amadori D, Santoro A, Figer A, De Greve J, Douillard JY, Lathia C, Schwartz B, Taylor I, Moscovici M, Saltz LB. Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2006; **24**: 4293-4300

71 **Toh H**, Chen PJ, Carr BI, Knox JJ, Gill S, Steinberg J, Carlson DM, Qian J, Qin Q, Yong W. A phase II study of ABT-869 in hepatocellular carcinoma (HCC): Interim analysis. *J Clin Oncol* 2009; **27**: Abstr 4581

72 **Siegel AB**, Cohen EI, Ocean A, Lehrer D, Goldenberg A, Knox JJ, Chen H, Clark-Garvey S, Weinberg A, Mandeli J, Christos P, Mazumdar M, Popa E, Brown RS, Rafii S, Schwartz JD. Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. *J Clin Oncol* 2008; **26**: 2992-2998

73 **O'Dwyer PJ**, Giantonio BJ, Levy DE, Kauh JS, Fitzgerald DB, Benson AB. Gefitinib in advanced unresectable hepatocellular carcinoma: Results from the Eastern Cooperative Oncology Group's Study E1203. *J Clin Oncol* 2006; **24**: Abstr 4143

74 **Ramanathan RK**, Belani CP, Singh DA, Tanaka M, Lenz HJ, Yen Y, Kindler HL, Iqbal S, Longmate J, Mack PC, Lurje G, Gandour-Edwards R, Dancey J, Gandara DR. A phase II study of lapatinib in patients with advanced biliary tree and hepatocellular cancer. *Cancer Chemother Pharmacol* 2009; **64**: 777-783