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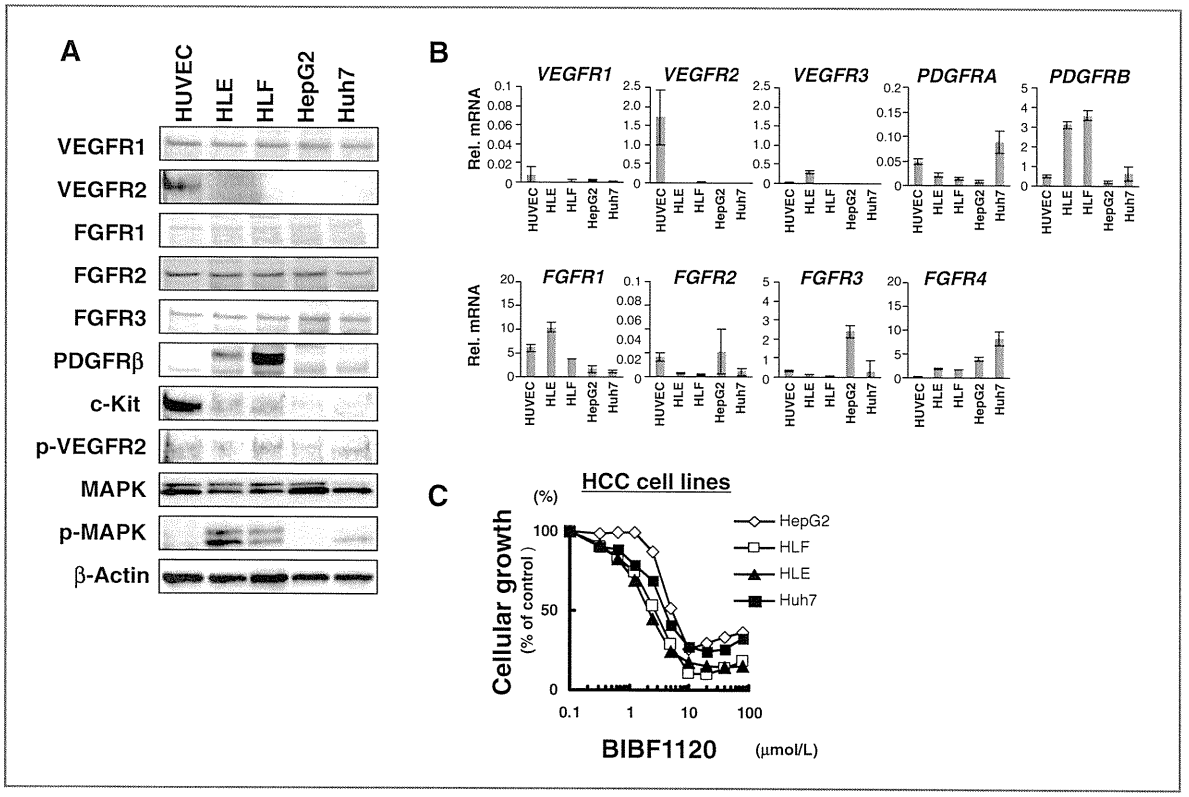

Figure 2. Expression levels of target receptors and sensitivity to BIBF 1120 in HCC cell lines. A, Western blot analysis of the expression levels of VEGFR1, VEGFR2, FGFR1, FGFR2, FGFR3, PDGFRβ, c-Kit, p-VEGFR2, MAPK, p-MAPK, and β-actin in HCC cell lines and HUVECs as a control. B, the mRNA expression levels of *VEGFR1*, *VEGFR2*, *VEGFR3*, *PDGFRA*, *PDGFRB*, *FGFR1*, *FGFR2*, *FGFR3*, and *FGFR4* were determined using real-time RT-PCR. Rel mRNA, mRNA expression levels normalized using *GAPD* (target gene/GAPD × 10^3). C, *in vitro* growth inhibitory effect of BIBF 1120 in 4 HCC cell lines by an MTT assay; bars, SD of 3 independent experiments. This assay was conducted in 3 independent experiments.

cells were orally given a low (50 mg/kg/d) or high (100 mg/kg/d) dose of BIBF 1120, or vehicle alone, for 2 weeks (Fig. 3A). The mean tumor volumes on day 14, for each group of mice, were as follows: vehicle alone, 1,367 ± 634 mm^3; 50 mg/kg/d, 488 ± 489 mm^3; and 100 mg/kg/d, 572 ± 556 mm^3. Both doses of BIBF 1120 significantly inhibited tumor growth ($T/C = 0.36$ and 0.42, respectively), indicating that BIBF 1120 has a potent antitumor activity against HCC *in vivo* (Fig. 3B). Body weight loss was not observed after the administration of BIBF 1120 at either dose (Supplementary Fig. S1). The CD31 staining of tumor tissues showed that BIBF 1120 administration also significantly inhibited tumor angiogenesis (Fig. 3C). Combined with the observation of the direct growth inhibitory activity against HCC *in vitro*, these findings suggest that the antitumor activity of BIBF 1120 *in vivo* mainly result from the drug's antiangiogenic activity, which blocks VEGF signaling.

VEGFR2$^+$pTyr$^+$ PBLs are a pharmacodynamic biomarker *in vivo*

VEGFR2$^+$CD45^{dim} PBLs are generally regarded as circulating endothelial cells (22); therefore, we hypothesized that VEGFR2$^+$CD45^{dim} PBLs might be useful as a biological biomarker of VEGFR2 TKIs. The effects of BIBF 1120 on the pTyr levels of VEGFR2$^+$CD45^{dim} PBLs and the percentage of VEGFR2$^+$pTyr$^+$ PBLs was examined *in vivo* (Fig. 4A). Murine blood samples were obtained from tumor-bearing, BIBF 1120–treated mice, as described previously. The pTyr levels of the VEGFR2$^+$CD45^{dim} PBLs were significantly inhibited by BIBF 1120 treatment, but the difference was relatively small (Fig. 4B and C). On the other hand, the percentage of VEGFR2$^+$pTyr$^+$ PBLs was markedly decreased by BIBF 1120 administration (Cont: 1.8% ± 1.1%, B50: 0.34% ± 0.21%, B100: 0.37% ± 0.29%; Fig. 5A and B). These findings raise the possibility that evaluating the VEGFR2$^+$CD45^{dim} PBLs by flow cytometry as a surrogate tissue may contribute to the proof of concept of VEGFR2-targeting drugs or the monitoring of drug effects *in vivo*. Thus, VEGFR2$^+$pTyr$^+$ PBLs might be a useful pharmacodynamic biomarker of VEGFR2 TKIs in early clinical trials.

Discussion

HCC is one of the most hypervascular tumors, and vascular embolization has been used as a therapeutic strategy. A recent study showed that sorafenib exhibits

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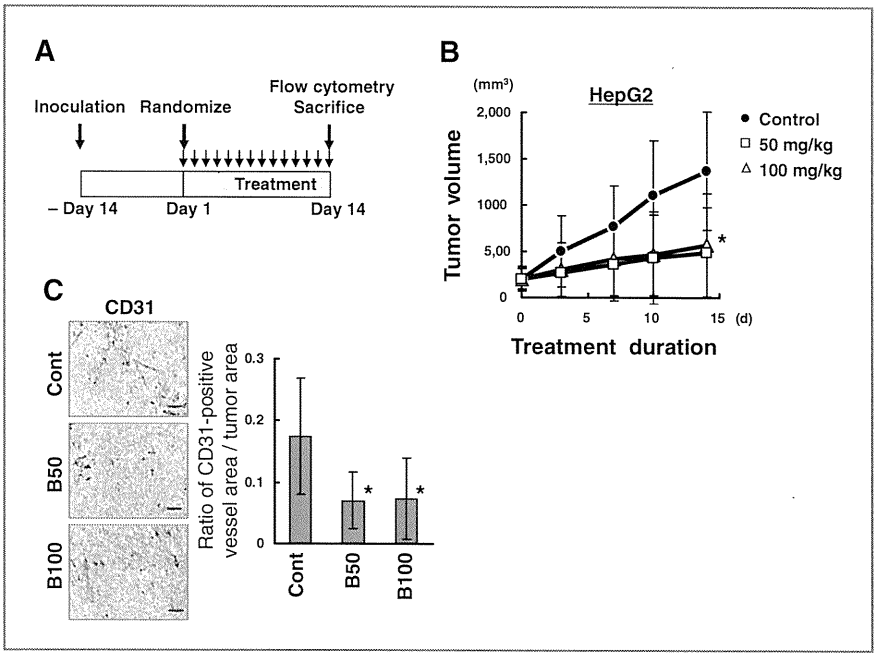

Figure 3. BIBF 1120 exhibited the antitumor and antiangiogenic effects against HCC *in vivo*. A, schema of the BIBF 1120 treatment schedules. Mice were inoculated with HepG2 cells for 14 days. The mice were then randomized into 3 groups ($n = 6$ in each group) and treated with BIBF 1120 (50 mg/kg/d, p.o.), BIBF 1120 (100 mg/kg/d, p.o.), or the vehicle control (p.o.) for 14 days. On day14, the mice were euthanized; blood was collected for the following biomarker study, and tumor specimens were collected for immunohistochemistry. B, inhibition of tumor growth by BIBF 1120 treatment. The tumor volume was assessed every 2 to 3 days ($n = 6$ in each group). Bars, SD. *, $P < 0.05$. C, inhibition of tumor angiogenesis by BIBF 1120 treatment was evaluated using the CD31 staining of tumor samples. Representative data are shown. MVD was quantified by measuring the number of CD31-positive endothelial cells in the tumors. Ten random fields per tumor sample at a magnification of ×200 were captured and saved for computer-assisted image analysis using the ImageJ software package. The *y*-axis represents the ratio of the CD31-positive vessel area/tumor area. Scale bar, 100 μm. Cont, tumor sample treated with vehicle control. B50 and B100, tumor sample treated with BIBF 1120 (50 mg/kg/d, 100 mg/kg/d, p.o.); *, $P < 0.05$.

clinical benefits in patients with advanced HCC (2, 3). This encouraging result suggests that molecular targeting drugs might be active against HCC, especially those that block VEGFR signaling. Our data showed that BIBF 1120 inhibited tumor growth and angiogenesis in HCCs *in vivo*, suggesting that BIBF 1120 may be an active and promising drug against HCC.

BIBF 1120 has a potent inhibitory effect on VEGFRs, similar to that of sorafenib and sunitinib, and it also has activities against FGFRs and Src (refs. 15, 23, 24; Supplementary Table S2). Recent evidence has shown that Src expression is elevated and active in HCC and that Src may play a key role in supporting HCC progression (25); furthermore, HBx increased the activation of the androgen receptor through c-Src kinase, which acts as a major switch in the activation of HCC (26). We conducted a Western blot analysis to detect the inhibitory effect of BIBF 1120 on Src activity, using HUVECs and HepG2, Huh7, HLE, and HLF cells (Supplementary Fig. S2). The inhibitory effect of BIBF 1120 on p-Src was observed in HUVECs and HLE and HepG2 cells, suggesting that BIBF 1120 actually has an inhibitory effect on Src. This effect may benefit HCC therapy in a manner independent of its antiangiogenic effect, although this topic needs to be further investigated. Similarly, we showed an inhibitory effect of BIBF 1120 on p-FGFR2 by using FGFR2-amplified gastric cancer cell lines (Supplementary Fig. S3). Brivanib (BMS-540215), a dual inhibitor of VEGFR and FGFR, is currently in development for the treatment of HCC and colon carcinoma, and preclinical studies have shown that FGFR signaling in HCC cells seems to be a promising therapeutic target (27, 28). These results suggest that the effect of BIBF 1120 on FGFR may contribute the antitumor effect, although further investigation is needed.

Numerous candidate biomarkers of angiogenesis have been identified, but the use of these markers for diagnosis, prognosis, and treatment monitoring remains investigational and of uncertain utility (4). Among them, biomarkers for detecting the blockade of VEGFR signaling have received particular attention because of the intimate involvement of this mechanism in drug activity of VEGFR TKIs. We have shown that VEGF-induced $VEGFR2^{+}pTyr^{+}$ PBLs in peripheral blood samples were markedly decreased by BIBF 1120 treatment *in vivo*. This analysis was done using only peripheral blood collection, VEGF stimulation, and analysis of 2-color flow cytometry; thus, this method is feasible

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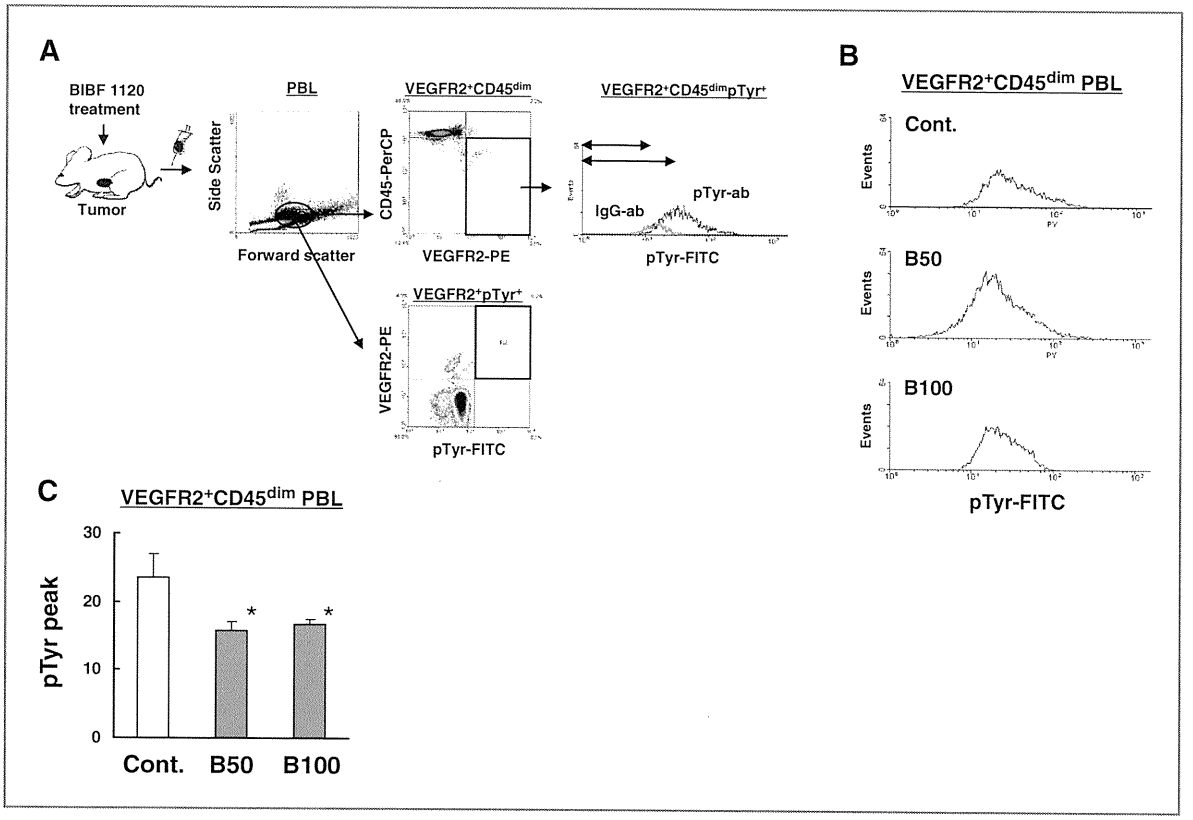

Figure 4. Evaluation of VEGFR2$^+$CD45^{dim} PBLs as a biomarker *in vivo*. A, schema of treatment schedules of BIBF 1120 and detection methods. Peripheral blood samples obtained from BIBF 1120–treated mice were stimulated with 20 ng/mL of VEGF for 30 minutes. The cells were fixed, permeabilized, and reacted with the following antibodies: anti-mouse CD45-PerCP, anti-mouse Flk-1-PE, and anti-pTyr-FITC (fluorescein isothiocyanate). Two methods, the tyrosine phosphorylation levels of VEGFR2$^+$CD45^{dim} PBLs and the percentage of VEGFR2$^+$pTyr$^+$ PBLs, were examined. B and C, BIBF 1120 significantly inhibited the pTyr levels of VEGFR2$^+$CD45^{dim} PBLs *in vivo*. Cont, blood sample from vehicle control. B50 and B100, blood samples from BIBF 1120 (50 mg/kg/d, 100 mg/kg/d; p.o.) treatment groups; bars, SD. *, $P < 0.05$.

and specific to VEGF signaling. Our method may contribute to the proof of concept for VEGFR2 TKIs and may help to determine the biological optimal dose, especially in phase I clinical trials.

Phase II studies of BIBF 1120 against lung cancer and ovarian cancer have been completed and phase I/II study of BIBF 1120 is currently evaluated in HCC (NCT 01004003). Two large phase III clinical trials against lung cancer

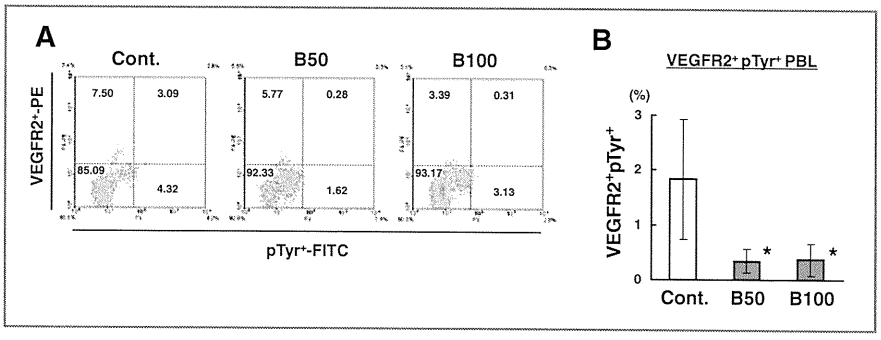

Figure 5. VEGFR2$^+$pTyr$^+$ PBLs can be used as a pharmacodynamic biomarker *in vivo*. A, the percentage of VEGFR2$^+$pTyr$^+$ PBLs obtained from BIBF 1120–treated mice. The numeral data indicate the percentage (%) in each quadrant. Representative data are shown. B, BIBF 1120 significantly inhibited the percentage of VEGFR2$^+$pTyr$^+$ PBLs. Cont, blood samples from vehicle control group ($n = 6$, not treated with drug). B50 and B100, blood samples from BIBF 1120 treatment groups ($n = 6$, 50 mg/kg/d; $n = 6$, 100 mg/kg/d; p.o.); bars, SD. *, $P < 0.05$.

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(LUME-Lung 1: docetaxel ± BIBF 1120; LUME-Lung 2: pemetrexed ± BIBF 1120) and 1 against ovarian cancer (LUME-Ovar 1: carboplatin/paclitaxel ± BIBF 1120) are now underway. We have shown that BIBF 1120 exhibited antiangiogenic and antitumor activity against HCC *in vivo*. These results may provide the scientific rationale for introducing BIBF 1120 as a treatment of HCC in the future. In addition, our approach of evaluating $VEGFR2^{+}pTyr^{+}$ PBLs in VEGFR TKI might be applicable to future phase I trials. We plan to use this method in clinical settings.

In conclusion, BIBF 1120 clearly inhibited VEGFR2 signaling in endothelial cells and exhibited relatively mild growth inhibitory effects on 4 HCC cell lines (IC_{50} values: 2–5 μmol/L) *in vitro*. BIBF 1120 exhibited potent antitumor and antiangiogenic activities against HCC *in vivo*, and the antitumor effect did not fail or show signs of weakening during the long-term administration period. In addition, $VEGFR2^{+}pTyr^{+}$ PBLs were found to be a noninvasive pharmacodynamic biomarker in a murine model.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgment

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Digestive Diseases

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Signaling Pathway and Molecular-Targeted Therapy for Hepatocellular Carcinoma

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Key Words
Hepatocellular carcinoma · Molecular-targeted agent · Sorafenib · Sunitinib · Brivanib · Complete remission

Abstract
In recent years, molecular-targeted agents have been used clinically to treat various malignant tumors. In May 2009, sorafenib (Nexavar®) was approved in Japan for 'unresectable hepatocellular carcinoma (HCC)', and was the first molecular-targeted agent for use in HCC. 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 now been approved worldwide. Phase III clinical trials of other molecular-targeted agents comparing them with sorafenib as first-line treatment agents are now 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. This review outlines the main pathways involved in the development and progression of HCC and the agents that target these pathways. Finally, current status and future perspective will also be discussed.

Introduction

The 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 non-specific 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 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 intra-

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cellular signaling pathways. In addition, multikinase inhibitors, including sorafenib, have emerged and evaluated. Clinical trials are now 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 anticancer drugs were found to be effective in large-scale randomized controlled trial 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 prognostic advanced HCC, who are resistant to a standard of care agent, sorafenib.

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. It has been shown that, after HBV/HCV 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].

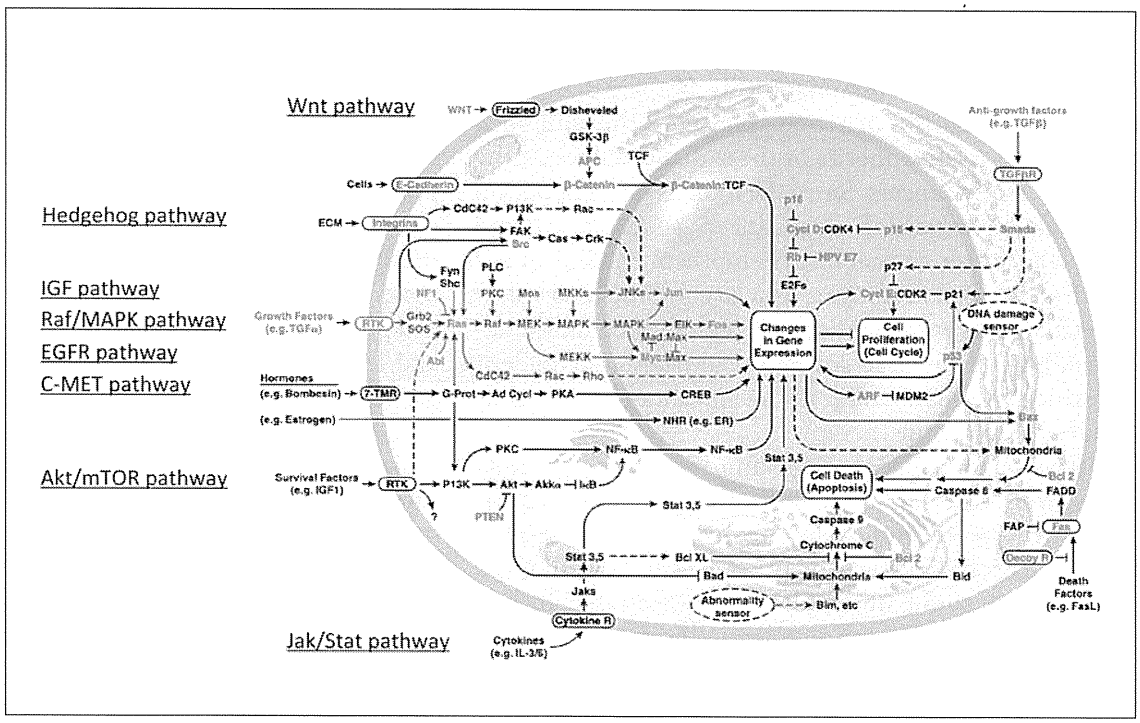
The binding of growth factors to their receptor proteins activates protein-phosphorylating enzymes, thus activating a cascade of proliferative signaling pathways to transmit proliferative signals into the nucleus. Growth factors, such as epidermal growth factor (EGF), transforming growth factor (TGF)-α/-β, insulin-like growth factor (IGF) and vascular endothelial growth factor (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, MAP kinase/ERK kinase (MEK) and mammalian target of rapamycin (mTOR) are serine/threonine kinases.

In general, the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/Akt/mTOR, c-MET, IGF, Wnt-β-catenin and Hedgehog signaling pathways, and the VEGFR and PDGFR signaling cascades show altered activity in HCC, and agents targeting these pathways are under development (fig. 1, 2; table 1) [9–11]. 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 [5]. The downstream extracellular signaling-regulated kinase (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 [12–15].

Raf and Ras Inhibitors. Raf and Ras are proto-oncogenes. In particular, K-ras mutations are commonly observed in many cancers, including pancreatic and colorec-



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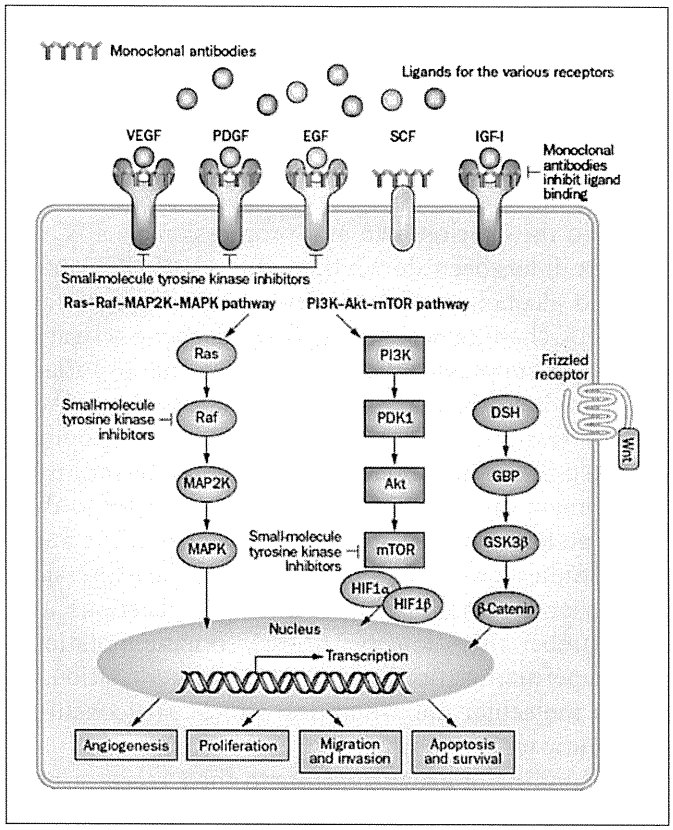

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Fig. 1. Signal transduction in solid cancer cells including HCC. Some of the genes known to be functionally altered are highlighted in red. These signaling pathways including growth factor pathway, Wnt pathway, Hedgehog pathway, Akt/mTOR pathway, and Jak/Stat pathway can be a molecular target for treatment of HCC [cited and modified from 10, with permission].

Fig. 2. Signaling pathways and molecular-targeted agents. Monoclonal antibodies (VEGFR: bevacizumab, EGFR: cetuximab), tyrosine kinase inhibitors (VEGFR: sorafenib, brivanib, linifanib, axitinib, EGFR: erlotinib, lapatinib), serine/threonine kinase inhibitors (Raf: sorafenib, mTOR: rapamycin and everolimus, PIK: KL-755) [cited and modified from 11, with permission].

Table 1. Molecular-targeted agents for HCC: targets and development status in Japan as of March 2011

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

tal 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. 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, and the in vitro treatment of HepG2 and Hep3B cells with MEK1/2 inhibitors inhibited cell growth and upregulated apoptosis [14]. The MEK inhibitors CI-1040, PD0325901, AZD6244 and RDEA119/BAY869766 have been tested in several cancers including solid tumors such as HCC. Recently, 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/mTOR pathway also plays an important role in cell growth, survival regulation, metabolism and antiapoptosis. The membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) is phosphorylated by phosphatidylinositol 3-kinase (PI3K) into phosphatidylinositol 3,4,5-triphosphate (PIP_3), which binds to and activates the serine/threonine kinase Akt. The tumor-suppressor gene product PTEN (phosphatase and tensin homolog 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 that 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 [24].

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 [25]. 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 [26]. In an extensive analysis of 314 HCC samples in terms of mutation analysis, DNA copy number changes, mRNA levels and immunostaining, Villanueva et al. [27] 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-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 TGF-α, 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 [28].

VEGF/VEGFR, PDGFR, FGFR

Angiogenesis is an important event not only for HCC but also for cancer growth and metastasis, and occurs due to complex alterations involving promoting factors such as VEGF, angiopoietin and FGF, and inhibitory factors including thrombospondin (TSP) 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, and are involved in lymphangiogenesis [29, 30]. 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 [31].

HCC typically exhibits active angiogenesis. During the progression from early to well, and to 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 [32]. In addition, a meta-analysis has demonstrated that VEGF expression is a prognostic factor in HCC [33].

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 multikinase 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 months, 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% [34]. 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 [35]. In that study, sunitinib was administered at 50 mg/day for 4 weeks followed by 2 weeks of rest per cycle [34], whereas Zhu et al. [35] used a dosing schedule of 37.5 mg/day for 4 weeks followed by 2 weeks of rest per cycle, and reported that the median PFS and OS were 3.9 and 9.8 months, 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/day. However, in a 'reflection and reaction' regarding the above trial results, Forner et al. [36] casted doubt on whether the drugs at this dose could maintain tolerance and ensure efficacy. Consequently, the trial was terminated in March 2010 because of the recommendation by data monitoring committee (DMC) 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. As for sunitinib, an international global phase III clinical 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. Japanese centers are participating in this clinical trial. Since brivanib targets FGF and VEGF, and is associated with relatively mild adverse effects, a second-line study of brivanib in sorafenib-ineffective and -intolerant 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 [37]. The median time to progression (TTP) and OS were 2.8 and 10 months, respectively, in cohort A versus 1.4 and 9.8 months, respectively, in cohort B. Adverse events included fatigue (51.5%), diarrhea (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 patients; 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. Japanese centers participated in all three trials.

In a Japanese phase I/II trial of TSU-68, an oral molecular 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% [38]. The global phase III trial of TACE in combination with TSU-68 has just started in January 2011.

In addition, several phase I/II trials are being conducted to assess kinase inhibitors such as linifanib (ABT-869) and 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 agent in 2011.

EGF/EGFR

EGFR is a member of the human epidermal growth factor receptor (HER) family that includes EGFR (erbB1), HER2/neu (erbB3) and HER4 (erb4). All members of this family, except HER3, have an intracellular tyrosine kinase domain, and the binding of a ligand to its extracellular domain triggers signal transduction through the above-described MAPK and PI3K/Akt/mTOR pathways. Thus, these receptors are involved in cell growth, differentiation, survival and adhesion [39]. EGFR overexpression has been reported in many cancers, and in HCC. For example, Buckley et al. [40] reported that EGFR, detected by immunohistochemical analysis, was overexpressed in 50 (66%) of 76 HCCs, and that fluorescence in situ hybridization (FISH) 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. [41] and Thomas et al. [42] have reported the results of phase II studies of erlotinib in HCC; the median OSs in their studies were 13 and 10.7 months, respectively. A phase III clinical study (SEARCH study: Sorafenib and Erlotinib, A Randomized Trial Protocol for the Treatment of Pa-

tients with Hepatocellular Carcinoma) for sorafenib in combination with erlotinib versus 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. [43] 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 and 15.7 months, 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 [44, 45].

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 [46]. Similarly, the results of sorafenib in combination with bevacizumab (vertical inhibition) have been reported [47]. Although some therapeutic responses were obtained, the combination therapy resulted in greater toxicity [47], 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 [48]. 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 and 12.6 months, respectively, and adverse events included diarrhea (73%), nausea (54%) and skin rash (42%) [49].

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 IgG1 constant region. Cetuximab inhibits the binding of endogenous EGFR ligands, such as EGF and TGF-α, 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 and 9.6 months, 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 [50].

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.

HGF/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 a therapeutic target. HGF is a heterodimer consisting of α- and β-chains bound together by a disulfate bond. The α-chain contains four 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 [51]. These abnormalities activate the downstream signaling cascade, leading to epithelial-mesenchymal transition and increased proliferative, migratory, invasive and metastatic potentials of cancer cells [51].

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 as a non-ATP-competitive molecule that binds near the ATP-binding site. A randomized phase II study of ARQ-197 versus 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 were reported in ASCO 2010 (abstr. No. 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. Abnormalities in IGF and IGF-1R or their overexpression have been reported in various cancers, including HCC. Their associations with disease stage, metastasis and survival [52] and

the functions of IGF and IGFR in HCC [53] have been reported.

IGF-targeting drugs are currently being developed, and mainly including 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 versus sorafenib plus BIIB022, and phase I/II studies of AVE1642 as monotherapy or in combination with sorafenib or erlotinib are ongoing.

Sorafenib: Trial Results and Clinical Experience

Clinical Results for Sorafenib in HCC

As described above, sorafenib is a multikinase inhibitor of tumor growth and angiogenesis, and exhibits a strong inhibitory effect on C- and B-Raf serine/threonine kinases (comprising the Raf/MEK/ERK pathway), VEGFR and PDGFR tyrosine kinases, and Flt-3 and c-kit [22]. To date, sorafenib is the only molecular-targeted agent approved for the treatment of HCC based on the results of two large-scale clinical trials, namely the SHARP (Sorafenib HCC Assessment Randomized Protocol) study [54] and the Asia-Pacific study [55]. The median OSs for the sorafenib group in the SHARP and Asia-Pacific studies were 10.7 months (vs. 7.9 months for the placebo group, $p < 0.001$; HR 0.69) and 6.5 months (vs. 4.2 months for the placebo group, $p = 0.014$; HR 0.68), respectively, indicating that sorafenib prolongs survival by approximately 30%. These data should compel HCC specialists to challenge their preconception that systemic anticancer drug therapy is not effective for HCC.

Current Status regarding the Use of Sorafenib in Japan

Sorafenib was approved in Japan in May 2009. A survey has confirmed that, at the time of writing (May 2011), over 10,000 patients have been prescribed sorafenib. Across several centers, 100 Japanese patients have achieved CR or near CR (superresponded PR), which was not observed in the SHARP or Asia-Pacific trials. This suggests that some Japanese patients may be very sensitive to sorafenib [56]. The reason for this or predictive biomarkers is now actively under investigation.

On the other hand, it has been reported that hand-foot syndrome occurs early after sorafenib administration [57] more often than was noted in the SHARP and Asia-Pacific studies, and the drug is often discontinued because of the adverse effects in many patients [57]. As demonstrated in the SHARP and Asia-Pacific studies, sorafenib is only used to achieve stable disease; therefore, it is important to improve drug efficacy by extending the period of administration for as long as possible. Therefore, it is no exaggeration to say that, in the case of sorafenib, the 'successful management of side effects' is equal to 'successful treatment'. According to the 'post-TACE phase III clinical study' [57] performed in Japan and Korea, it is strongly speculated that physicians who are unaccustomed to prescribing molecular-targeted agents and who fail to see marked efficacy, as induced by conventional chemotherapeutic agents, often do not understand the properties of this drug, and they (as well as the patients) do not fully comprehend therapeutic efficacy. Moreover, they feel too anxious about side effects

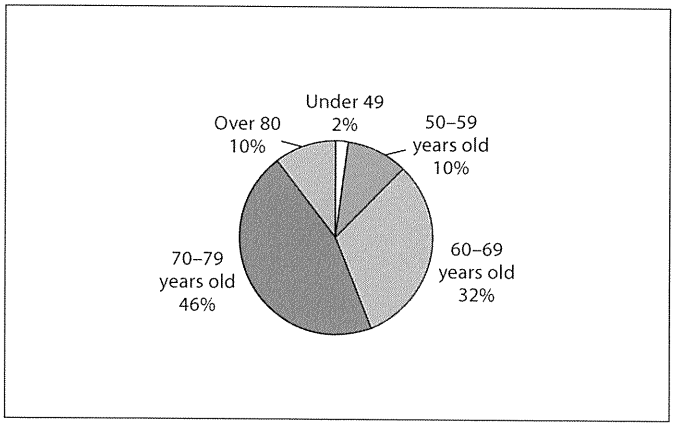

Fig. 3. Age distribution of patients treated with sorafenib.

Table 2. Patients' background treated with sorafenib (n = 113; data from Kinki University Hospital)

Age	70.1 (31–90)	
Gender	M	80 (70.8%)
	F	33 (29.2%)
Etiology	HBV	23 (20.4%)
	HCV	60 (53.1%)
	NBNC	30 (26.5%)
Child-Pugh score	5	64 (56.6%)
	6	33 (29.2%)
	7	16 (14.2%)
Child-Pugh grade	A	97 (85.8%)
	B	16 (14.2%)
Stage	III	51 (45.1%)
	IVA	28 (24.8%)
	IVB	34 (30.1%)

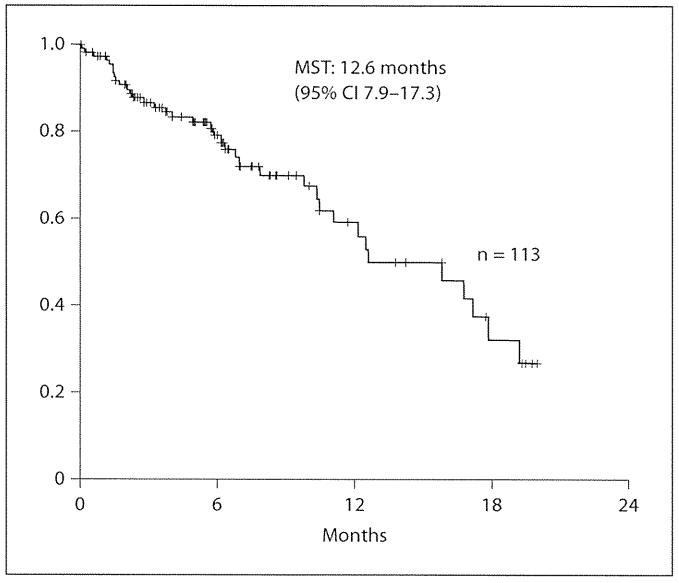

Fig. 4. Overall survival in patients treated with sorafenib.

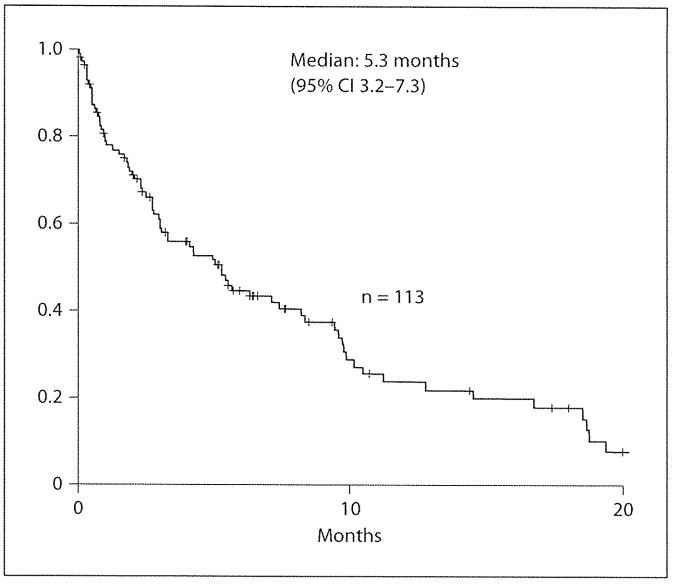

Fig. 5. Treatment duration in patients treated with sorafenib.

that have not been encountered before. These circumstances may result in treatment discontinuation in many patients. Clearly, greater awareness among physicians for therapeutic efficacy and approaches to manage adverse effects is needed to improve treatment outcomes.

Experience of Sorafenib Use at Our Institute

Since the approval of sorafenib on May 20, 2009, we have treated approximately 150 patients with sorafenib during 20 months, but few have discontinued therapy due to adverse effects or patient refusal to continue. Of these 113 patients, 2 achieved CR [56]. These 2 CR patients, in whom pulmonary, adrenal metastases and intrahepatic lesions all disappeared, survived free of recurrence for more than 3 and 2 years, respectively at the time of writing (May 2011), i.e., they are still alive at the present. In other patients who apparently achieved SD, the tumor marker levels reached a plateau after sorafenib administration, when their levels were rising rapidly before sorafenib administration. Even if hepatic lesions do not show a clear tendency to undergo necrosis or regression on CT images, three tumor markers (AFP, PIVKA-II and AFP-L3) are widely considered to serve as surrogate markers. In fact, there is very little data on serum tumor markers, except for AFP, outside Japan. Nevertheless, Japanese researchers have demonstrated the value of changes in these markers and the antitumor efficacy of sorafenib [56].

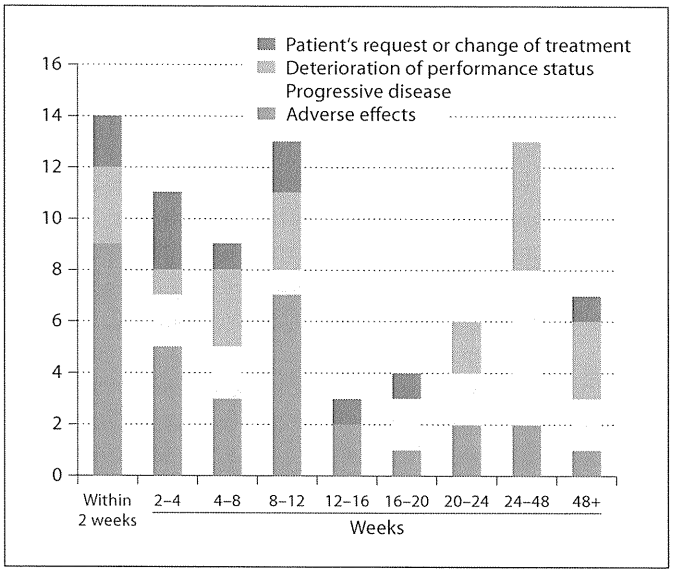

Fig. 6. Causes of discontinuation of sorafenib. Only 17 of 113 (15%) cases were terminated due to PD. Most of discontinuation of sorafenib due to adverse effects was within 12 weeks.

Interestingly, it was previously demonstrated that the levels of PIVKA-II or DCP tend to be increased by inducing hypoxia [58]. Therefore, PIVKA-II or DCP may be a good predictive marker for evaluating the hypoxic response to antiangiogenic therapy for HCC [59].

Of 113 patients, 85.8% was Child-Pugh A stage and 53.1% of patients were HCV-related HCC (table 2). A total of 56% of patients were over 70 years of age (fig. 3). The initial status of patients treated with sorafenib is listed in table 3. Median survival time (MST) was 12.6 months (fig. 4) and median treatment duration was 5.3 months (fig. 5). The causes of discontinuation are listed in figure 6. Only 10% of the 113 patients showed PD by RECIST. However, since the speed with which the patient develops progressive disease may slow down due to tumor growth inhibition, it is very difficult to determine when to discontinue treatment because of tumor refraction. Important issues for future studies include: (1) to identify biomarkers that can predict therapeutic responses, including CR or PR, in patient groups; (2) to evaluate the role of tumor markers in the determination of therapeutic responses; (3) to establish response evaluation criteria that can determine the therapeutic responses to molecular-targeted agents, and (4) to develop effective second-line therapies after sorafenib failure (fig. 2, 7) [11, 60].

According to the consensus-based treatment algorithm by the Japan Society of Hepatology (fig. 7) [60], updated in 2010 [61], sorafenib is indicated for the treatment of patients with Child-Pugh A HCC with extrahepatic metastasis, vascular invasion or refractoriness to TACE or arterial infusion chemotherapy.

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 arterial infusion chemotherapy (SILIUS trial: trial No. NCT01214343), pharmaceutical and IIT trials of sorafenib in combination with TACE (SPACE, TACICS (trial No. NCT 01217034) and BRISK-TA trials), and a trial to test the inhibitory effect of sorafenib on tumor recurrence after curative treatment (STORM trial) are ongoing, and the results of these trials are eagerly awaited (fig. 7) [60].

The working hypotheses in these studies can be deduced by extrapolating the MST and hazard ratios in OS calculated in a subanalysis of the SHARP study (table 4). 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 4). This suggests that survival of early-stage HCC and intermediate-stage HCC may be prolonged from 5 to 7.5–10 years by using sorafenib in an adjuvant setting after curative treatment, and from 3 to 4.5–6 years by using sorafenib in combination with TACE (fig. 8) [60].

Table 3. Initial status of patients treated with sorafenib

Refractory to TACE	36
Impossible of TACE due to:	
AP shunt	2
Stenosis of artery	5
Macrovascular invasion	4
Multiple nodules at first diagnosis	3
Portal vein invasion at first diagnosis	8
Hepatic vein invasion at first diagnosis	1
Extrahepatic spread	28
Refractory to HAIC	4
Refractory to standard treatment	2
Candidate of clinical trials: SILIUS (phase I)	12
Patient's request	6
Bile duct invasion	1
Others	1

Table 4. Subanalysis data of the SHARP study (data from M. Sherman et al., ASCO 2008)

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

Summary and Future Prospects

Several clinical trials [34, 35, 37, 41, 42, 50, 62–66] of the molecular-targeted agents are ongoing. Angiogenesis-inhibiting drugs, particularly sorafenib, have been evaluated for HCC, and drugs targeting EGFR and mTOR are being developed. The results (numerical values) of phase II clinical trials show no marked differences in the

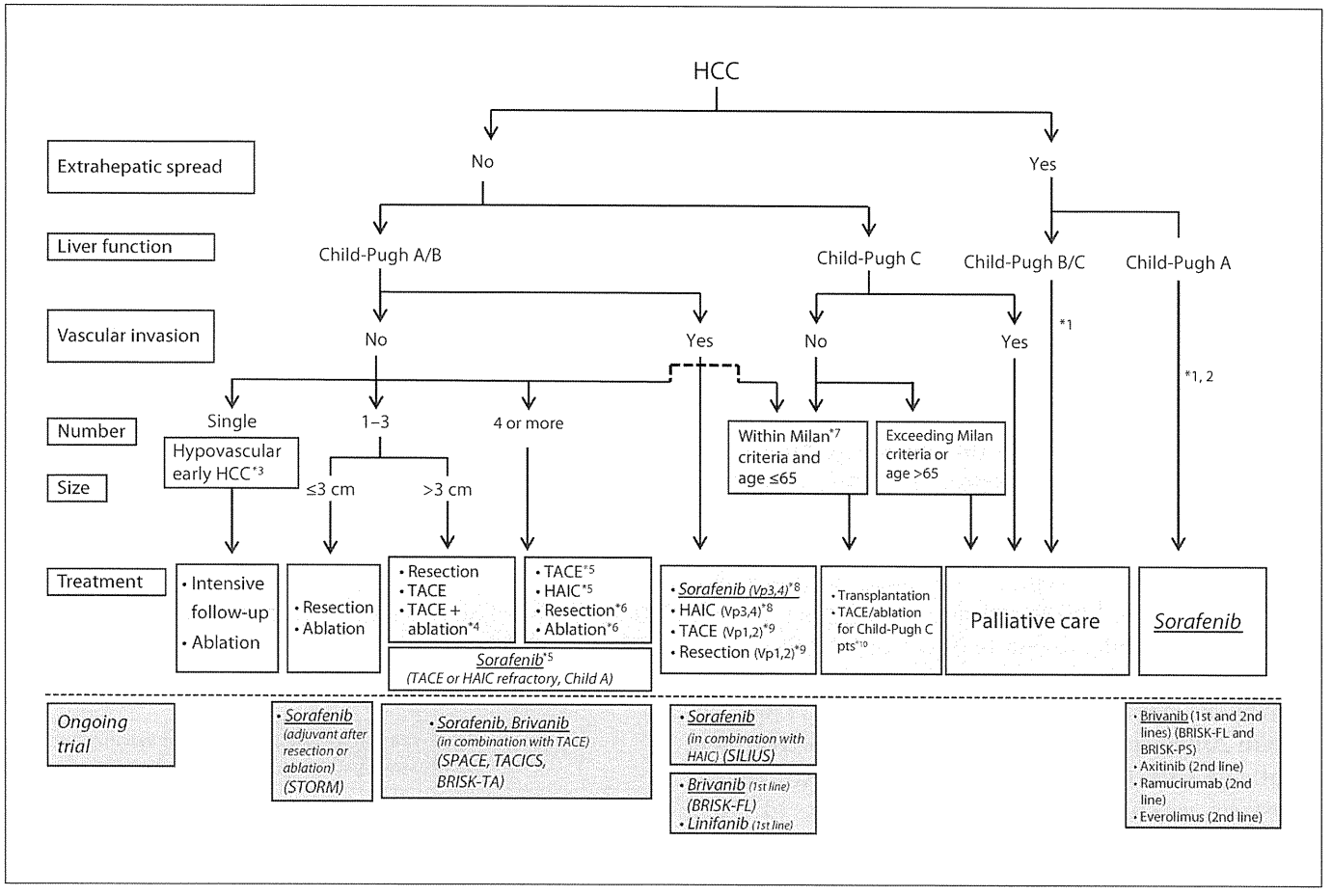

Fig. 7. Consensus-based Treatment Algorithm for Hepatocellular Carcinoma proposed by Japan Society of Hepatology (JSH) revised in 2010 [cited and modified from 60, with permission]. Footnotes: *1 = Treatment should be performed as if extrahepatic spread is negative, when extrahepatic spread is not considered 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 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, TACE in combination with ablation is frequently performed when resection is not indicated. *5 = Transcatheter arterial chemoembolization (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 (5FU + CDDP) or intra-arterial 5FU 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 ≤3 cm and tumor number ≤3, or solitary tumor ≤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 trunk). *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.

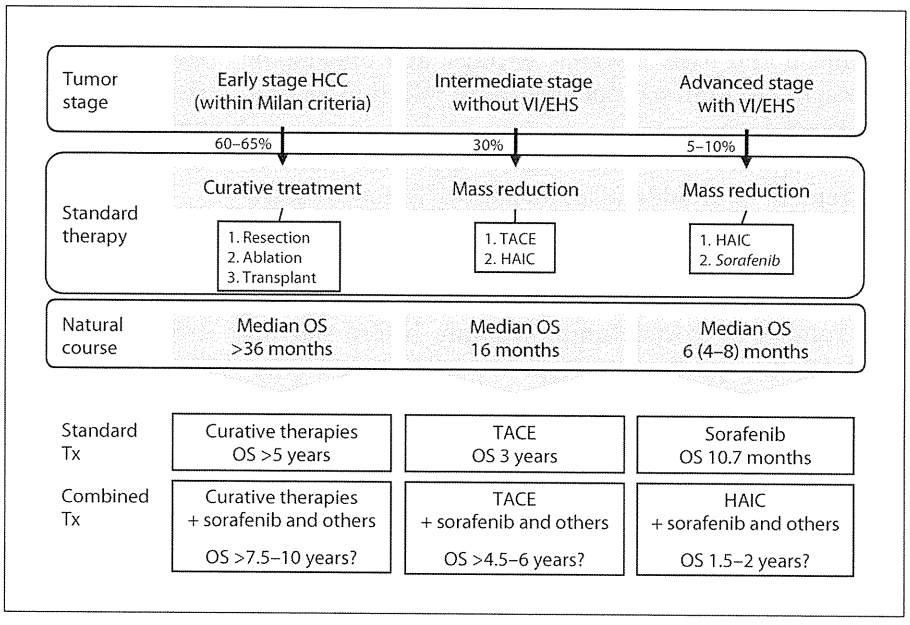

Fig. 8. Outcomes of standard treatment modalities and expected future outcomes of combination therapy with molecular-targeted agents. By combining molecular-targeted agents with resection or ablation, life expectancy is expected to be prolonged to 7.5–10 years. In addition, for intermediate-stage HCC, the prognosis is expected to be improved to 4.5–6 years by combination with TACE. OS = Overall survival.

Table 5. Ongoing clinical trials (PIII)

First line
Comparison study between sorafenib and single agent (head to head):
Sunitinib → endpoint did not meet!
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 versus 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 = hepatic arterial infusion chemotherapy.

therapeutic efficacy evaluated by TTP or PFS. However, phase II studies may be subject to patient selection bias and cannot be compared with the results of other trials. Thus, when determining the therapeutic efficacy of drugs, we should review the efficacy of the respective drugs, and consider where the theoretical target molecules are present and what combinations of drugs have a theoretical rationale, and thus evaluate options for monotherapy and combination therapy based on the efficacy and safety data obtained from phase III clinical trials.

Molecular-targeted agents that have been introduced into clinical use in recent years are approved for the treatment of specific cancer and are then frequently used to treat other cancers. Although not discussed here, studies to identify predictors of efficacy (i.e., biomarkers) for angiogenesis inhibitors and EGFR tyrosine kinase inhibitors, and factors involved in drug resistance, are making steady progress, and the associated therapeutic strategies are undergoing major changes. Therefore, even in the treatment of HCC, it is necessary for HCC specialists to expand their knowledge of and techniques for applying existing treatment modalities (resection, ablation, TACE, arterial infusion chemotherapy) to physically remove, destroy or necrotize the tumor, and to better understand clinical oncology, particularly the role and mechanisms of action of molecular-targeted agents. We are entering an era in which physicians treating HCC should pay close attention to the development of therapeutic agents not only for HCC but also for other cancers, and be aware of

the use of molecular-targeted agents for treating cancers in clinical and basic research settings, and understand approaches to limit or control adverse effects associated with these drugs.

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 tumors or portal tumor thrombi before starting sorafenib therapy. For oncology, in particular, the Pharmaceuticals and Medical Devices Agency (PMDA) in Japan has approved several drugs based on results from global clinical trials and from Japanese phase I study data alone. 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 (table 5).

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.

Disclosure Statement

The author has no conflict of interest to declare.

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Des-γ-Carboxyprothrombin May Be a Promising Biomarker to Determine the Therapeutic Efficacy of Sorafenib for Hepatocellular Carcinoma

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Key Words

Des-γ-carboxyprothrombin · Protein-induced vitamin K absence II · Hepatocellular carcinoma · Antiangiogenic therapy · Hypoxia · Sorafenib

Abstract

Objective: The purpose of this study was to evaluate the role of des-γ-carboxyprothrombin (DCP) as a marker for the efficacy of sorafenib therapy for hepatocellular carcinoma (HCC). ***Methods:*** Patients with advanced HCC treated with sorafenib were retrospectively evaluated, focusing on DCP levels and clinical characteristics. ***Results:*** 50 patients with advanced HCC were treated with sorafenib alone. In 25 of these patients, the serum levels of DCP were evaluated twice (pretreatment and within 2 weeks after starting therapy). The time to progression was significantly longer in patients in whom the DCP level at 2 weeks after starting sorafenib was ≥2-fold higher than the pretreatment levels, as compared with patients without an increase in DCP ($p = 0.0296$). ***Conclusions:*** The serum level of DCP is a surrogate marker for tissue hypoxia and can be a predictive marker to assess the tumor response to sorafenib therapy.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in Japan and is the fifth most common cancer worldwide [1, 2]. It is well known that HCC is less sensitive to chemotherapeutic agents than other tumors. Furthermore, because of pancytopenia and poor hepatic preservation caused by underlying hepatic cirrhosis, systemic chemotherapy is unsuitable for patients with HCC. Thus, locoregional therapies such as hepatic resection, radiofrequency ablation and transcatheter chemoembolization (TACE) have been developed and are widely used. However, more effective systemic chemotherapy is necessary for patients who are refractory to locoregional therapy or who progress to advanced stage cancer with extrahepatic spread and/or vascular invasion.

Sorafenib (Nexavar®; Bayer HealthCare Pharmaceuticals-Onyx Pharmaceuticals) is a small molecule that inhibits tumor proliferation and angiogenesis. It inhibits serine-threonine kinase Raf-1, a member of the RAF/MEK/ERK signaling pathway, and several receptor tyrosine kinases involved in neovascularization and tumor progression, including vascular endothelial growth

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