Table 1. Demographic Data

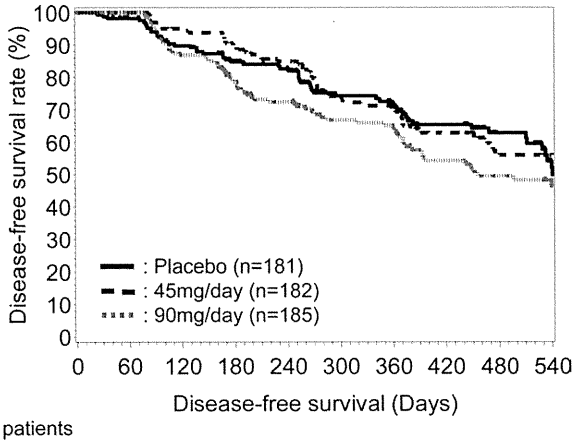
Parameter Category/mean ± SD	Placebo (n = 181)	45 mg/day (n = 182)	90 mg/day (n = 185)	Total (n = 548)	*P* Value
Gender (male/female]	108/73	117/65	117/68	342/206	0.635†
Age (y)	68.9 ± 8.1	68.2 ± 7.8	68.6 ± 7.7	68.6 ± 7.9	0.716‡
Primary or recurrence (primary/first recurrence)	144/37	144/38	144/41	432/116	0.915†
Medications given immediately before registration (local therapy/surgery)	174/7	173/9	180/5	527/21	0.534†
History of drinking (no/yes)	79/102	67/115	73/112	219/329	0.407†
Hepatitis (no/yes)	3/178	1/181	3/182	7/541	0.563†
Etiology§ (HBV/HCV/alcoholic/UK)	20/150/6/5	22/152/10/3	16/153/11/5	58/455/27/13	–
Concomitant administration of glycyrrhizic acid (no/yes)	101/80	99/83	101/84	301/247	0.958†
Liver cirrhosis (no/yes)	32/149	37/143	45/137	114/429	0.253†
Number of tumors	1.4 ± 0.7	1.4 ± 0.7	1.4 ± 0.7	1.4 ± 0.7	0.953‡
(1/2/3 ≤)	127/39/15	129/40/13	131/37/17	387/116/45	–
Diameter of tumor (mm)	20.3 ± 7.6	20.4 ± 7.9	19.3 ± 7.2	20.0 ± 7.6	0.340‡
Stage¶ (I/II/III)	81/75/25	87/74/21	93/74/18	261/223/64	0.439‖
PS (ECOG) (0/1/2)	165/14/2	171/19/1	176/7/2	512/31/5	0.295‖
Child-Pugh class** (A/B)	154/27	163/19	160/25	477/71	0.430‖
BCLC staging system (0/A/B/C)	53/115/11/2	54/117/10/1	61/109/13/2	168/341/34/5	0.862‖
Albumin (g/dL)	3.81 ± 0.50	3.83 ± 0.40	3.85 ± 0.46	3.83 ± 0.46	0.631‡
Total bilirubin (mg/dL)	0.93 ± 0.36	0.91 ± 0.35	0.86 ± 0.35	0.90 ± 0.35	0.139‡,*
Active prothrombin (%)	79.4 ± 13.9	80.0 ± 13.7	81.1 ± 15.1	80.2 ± 14.3	0.512‡
Platelet count ($\times 10^4/\mu L$)	10.66 ± 4.38	10.72 ± 5.10	11.32 ± 5.69	10.90 ± 5.08	0.389‡
AST (IU/L)	61.7 ± 28.7	71.1 ± 50.0	59.6 ± 29.8	64.1 ± 37.7	0.008‡,*
ALT (IU/L)	55.9 ± 33.4	60.8 ± 46.3	53.6 ± 38.2	56.7 ± 39.7	0.211‡
DCP (mAU/mL)††	33.7 ± 71.5	184.1 ± 1,869.5	27.4 ± 26.0	81.9 ± 1082.7	0.295‡
(<40/40 ≤/UK)	155/25/1	165/17/0	163/19/3	483/61/4	–
AFP (ng/mL)††	38.79 ± 74.42	355.50 ± 4,212.33	30.71 ± 50.25	140.86 ± 2,423.86	0.346‡
(< 100/100 ≤/UK)	164/17/0	166/15/1	178/7/0	508/39/1	–
AFP-L3 (%)††,‡‡	4.09 ± 8.96	3.46 ± 6.99	4.75 ± 10.76	4.10 ± 9.06	0.399‡
(<15.0/15.0 ≤/UK)	174/6/1	173/5/4	171/13/1	518/24/6	–

*$P < 0.15$.
†χ^2 test.
‡One-way analysis of variance.
§Multiple complication.
¶The General Rules for the Clinical and Pathological Study of Primary Liver Cancer, November 2000 (4th ed.).
‖Kruskal-Wallis test.
**Classified in accord with the General Rules for the Clinical and Pathological Study of Primary Liver Cancer.
††Calculated, excluding unknown cases.
‡‡Calculated, assuming that values less than the lower limit of detection were 0.
AFP, alpha-fetoprotein; AFP-L3, alpha-fetoprotein lens culinaris agglutinin fraction-3; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer Staging System; DCP, des-gamma-carboxy prothrombin; HBV, hepatitis B virus; HCV, hepatitis C virus; PS, performance status.

The first interim analysis was performed in June 2005, and no problem was found concerning safety. The second interim analysis, performed in November 2006, indicated that vitamin K2 did not prevent recurrence. The IDMC thus recommended discontinuation of the study. Data on efficacy shown in the current report were those presented at the second interim analysis, and data on safety were those obtained at termination of the study (March 2007).

Patients. Baseline characteristics of the 548 patients are summarized in Table 1. The study population was composed of 342 males (62.4%) and 206 females (37.6%), with a mean age of 68.6 years (range, 39-88). The majority (432 patients; 78.8%) were enrolled after treatment of primary HCC. Medical ablation was the dominant therapeutic modality for HCC (527 patients; 96.2%). The tumor nodule was solitary in the majority of patients (387 patients; 70.6%), and median diameter was 19 mm (range, 6-60). HCV infection (455 patients; 83.0%) and the presence of cirrhosis (429 patients; 79.0%) were both common. The majority of patients had liver function reserve in Child-Pugh class A (477 patients; 87.0%) and ECOG performance status of 0 (512 patients; 93.4%). Homogeneity was shown among the three groups for all baseline characteristics, including all stratification parameters, except total bilirubin and aspartate aminotransferase levels.

Events. During the study, HCC recurrence (i.e., intrahepatic lesions adjacent to or distant from previously treated nodules, and extrahepatic metastasis), cancer other than HCC, or death from any cause were detected in 58, 52, and 76 patients in the placebo,



No. of patients

Placebo	181	166	146	125	117	85	79	58	39	23
45mg/day	182	165	150	132	114	76	71	50	30	17
90mg/day	185	168	144	116	103	77	74	50	37	25

Fig. 2. Disease-free survival of placebo, 45-mg/day, and 90-mg/day groups.

45-mg/day, and 90-mg/day groups, respectively. Three patients developed cancer other than HCC. One patient in the placebo group developed malignant lymphoma, one patient in the 90-mg/day group developed colon cancer, and another developed lung cancer. In addition, four patients in the placebo group and one patient each in the 45-mg/day and 90-mg/day groups died without HCC recurrence. Causes of death were liver failure in four patients and acute myocardial infarction and pneumonia in one patient each. Death without HCC recurrence was treated as an event, along with HCC recurrence and development of cancer other than HCC, in DFS analysis.

Local recurrence, as defined by adjacency to a previously treated HCC nodule, is mainly the result of incomplete ablation and may have compromised the efficacy of the active drug. Whether or not recurrence was local was rigorously reviewed by the independent review committee, and HCC recurrence in 8, 6, and 11 patients in the placebo, 45-mg/day, and 90-mg/day groups, respectively, was judged to be local. Incidence of local recurrence did not differ among groups.

Intrahepatic recurrence not adjacent to previously treated nodules may have actually been the result of a small HCC not detected at the time of initial treatment. Although such a residual tumor cannot easily be distinguished from *de novo* carcinogenesis, recurrence resulting from residual tumor is thought to occur early after treatment. Incidences of recurrence within 180 days of HCC treatment were 25, 16, and 34 in the placebo, 45-mg/day, and 90-mg/day groups, respectively ($P = 0.029$ among the groups by log-rank test).

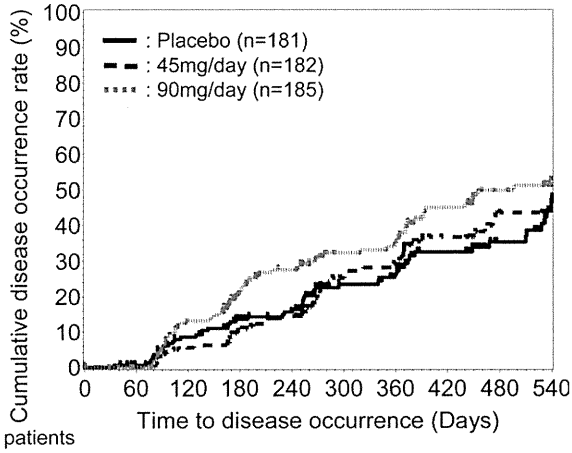
Extrahepatic metastasis also indicates the presence of surviving cancer cells. However, extrahepatic recurrence as the first manifestation of recurrence was rare in the present study and was found in only one patient each in the placebo and 90-mg/day groups.

DFS, Time to Disease Occurrence, and Overall Survival. Median DFS values were 540 and 541 days for the placebo and combined active-drug groups, respectively, as estimated by the Kaplan-Meier method. DFS rates were 69.8% (95% CI: 61.4%-76.7%) and 64.9% (58.8%-70.4%) at 1 year for placebo and combined active-drug groups, respectively. The difference in DFS was not statistically significant (HR: 1.150 [0.843-1.570]; one-sided; $P = 0.811$ by log-rank test).

The dose-response relationship was assessed between the 45-mg/day and 90-mg/day groups. Median DFS values were 560 days in the 45-mg/day group and 455 days in the 90-mg/day group (Fig. 2). DFS rates at 1 year were 68.3% (95% CI: 59.2%-75.8%) in the 45-mg/day group and 61.6% (53.0%-69.1%) in the 90-mg/day group. There was no trend toward dose-dependent increase in DFS (HR: 1.451 [1.018-2.067]; one-sided; $P = 0.982$ by log-rank test).

Analysis of DFS for per protocol population was performed among 510 patients, excluding 38 from 548 randomized patients because of major protocol violations. Similar results were obtained in the per protocol population in DFS analysis.

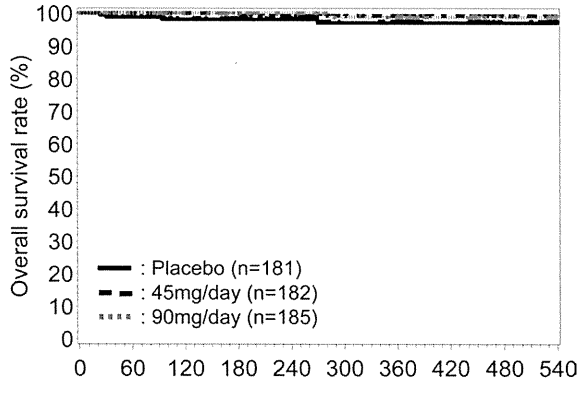
Median time to disease occurrence was 547, 560, and 496 days in the placebo, 45-mg/day, and 90-mg/day groups, respectively (Fig. 3). Cumulative disease occurrence rates at 1 year were 28.2% (95% CI:



No. of patients

Placebo	181	165	146	125	117	85	79	58	39	23
45mg/day	182	165	149	131	114	76	71	50	30	17
90mg/day	185	168	144	116	103	77	74	50	37	25

Fig. 3. Cumulative disease occurrence rate of placebo, 45-mg/day, and 90-mg/day groups.



No. of patients

Placebo	181	166	146	125	117	85	79	58	39	23
45mg/day	182	165	150	132	114	76	71	50	30	17
90mg/day	185	168	144	116	103	77	74	50	37	25

Fig. 4. Overall survival rate of placebo, 45-mg/day, and 90-mg/day groups.

21.4%-36.6%), 31.2% (23.7%-40.4%), and 37.7% (30.2%-46.3%), respectively.

Overall survival rates at 1 year were 97.2% (95% CI: 92.4%-99.0%), 99.2% (94.7%-99.9%), and 98.7% (91.4%-99.8%) in the placebo, 45-mg/day, and 90-mg/day groups, respectively (Fig. 4).

Subgroup Analyses. Enrollment was stratified by whether patients had been treated for primary HCC, medical ablation or surgical resection, HCV-related or -unrelated disease, and concomitant administration of glycyrrhizic acid. There was no significant difference in DFS between the placebo and combined active-drug groups in any stratification parameters (Table 2).

Safety. Safety was assessed among 539 patients. Incidences of adverse events were 88.3%, 88.3%, and 89.0% in the placebo, 45-mg/day, and 90-mg/day groups, respectively, and those of adverse drug reactions were 11.2%, 18.0%, and 15.5%, respectively (Table 3). There was no significant difference in the incidence of any adverse event or adverse drug reaction between the placebo and active-drug groups.

Discussion

In this study, we found no effect of vitamin K2 on the recurrence of HCC. Even the dose of 90 mg/day of vitamin K2, twice the recommended dose for osteoporosis, was not effective. In fact, recurrence was more frequent in the 90-mg/day than in the 45-mg/day group, though not to a statistically significant extent. There was a trend toward high AFP-L3 positivity at entry in the 90-mg/day group, including 13 patients positive for AFP-L3, compared to six and five patients in the placebo and 45-mg/day groups, respectively. AFP-L3 positivity may have indicated residual cancer cells, which may have been related to the increased incidence of recurrence. However, the results of analysis of recurrence remained similar when patients positive for AFP-L3 were excluded.

In this study, status after treatment of recurrent lesions versus naive was associated with an increased risk of recurrence (data not shown). Because this was characteristic of the original neoplasm, this was probably related not with *de novo* or secondary primary

Table 2. Subgroup Analyses of DFS by Stratification Parameter

Parameter Level	Treatment Group	N	HR	(95%CI)
Primary or recurrence HCC				
Primary	Placebo	144	1.000	
	Combined active drug	288	1.061	(0.742-1.519)
Recurrence	Placebo	37	1.000	
	Combined active drug	79	1.414	(0.751-2.664)
Medical ablation or surgical resection				
Medical ablation	Placebo	174	1.000	
	Combined active drug	353	1.152	(0.840-1.579)
Surgical resection	Placebo	7	1.000	
	Combined active drug	14	0.807	(0.113-5.745)
HCV-related disease				
Yes	Placebo	150	1.000	
	Combined active drug	305	1.214	(0.862-1.710)
No	Placebo	31	1.000	
	Combined active drug	62	0.837	(0.397-1.767)
Concomitant administration of glycyrrhizic acid				
Yes	Placebo	80	1.000	
	Combined active drug	167	1.360	(0.869-2.129)
No	Placebo	101	1.000	
	Combined active drug	200	0.958	(0.620-1.479)

DFS, disease-free survival; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio.

Table 3. Summary of Adverse Events (Safety Analysis Set)

			Incidence			
	Treatment Group	N	Case	%	(95% CI)	*P* Value*
Adverse event	Placebo	179	158	88.3	(82.6-92.6)	–
	45 mg/day	179	158	88.3	(82.6-92.6)	1.000
	90 mg/day	181	161	89.0	(83.5-93.1)	0.869
Adverse drug reaction†	Placebo	179	20	11.2	(7.0-16.7)	–
	45 mg/day	179	32	18.0	(12.6-24.3)	0.098
	90 mg/day	181	28	15.5	(10.5-21.6)	0.278
Serious adverse event	Placebo	179	52	29.1	(22.5-36.3)	–
	45 mg/day	179	40	22.4	(16.5-29.2)	0.183
	90 mg/day	181	48	26.5	(20.2-33.6)	0.638
Serious adverse drug reaction†	Placebo	179	1	0.6	(0.0-3.1)	–
	45 mg/day	179	3	1.7	(0.3-4.8)	0.622
	90 mg/day	181	2	1.1	(0.1-3.9)	1.000

*Comparison with placebo group by Fisher's exact test.
†Among adverse events, causal relationship of something other than "not related" to the study drug.

HCC, but with recurrence resulting from microscopic residual cancer or intrahepatic metastasis. On the other hand, other factors, such as alcohol consumption, low albumin concentration, and high total bilirubin concentration, were also associated with risk of recurrence (data not shown). These are also risk factors of primary HCC development among chronic hepatitis patients, and we consider them to indicate the risk of *de novo* carcinogenesis. In other words, we observed two types of HCC "recurrence," intrahepatic metastasis and *de novo* HCC, although it may be difficult to distinguish them in each case. Previous reports suggested the possibility that vitamin K may be effective against both types of HCC recurrence.[23] However, it is also possible that the effect of vitamin K on HCC recurrence is limited to either inhibition of tumor cell growth or reduction of *de novo* carcinogenesis. We performed subgroup analyses by stratifying patients, based on several tumor-related factors, and evaluated the effect of vitamin K on HCC recurrence in each stratum, but recurrence was decreased in none (data not shown).

Prevention of *de novo* hepatocarcinogenesis by vitamin K was first reported by Habu et al.[9] among cirrhotic women who took vitamin K2 to prevent osteoporosis. In the present study, HCC recurrence resulting from metachronous *de novo* carcinogenesis should have been reduced by vitamin K2. However, such an effect may have been obscured in the overall analysis because of the presence of recurrence resulting from intrahepatic metastases. In the subgroup analysis among patients with decreased platelet count, HCC recurrence was marginally reduced in the 45-mg/day group, compared to the placebo group (data not shown). However, no effect was observed with the dose of 90 mg/day. High-dose vitamin K is unlikely to induce hepatocarcinogenesis, because no carcinogenicity has been reported for this vitamin. However, the growth of HCC cells may be dependent on vitamin K. Vitamin K deficiency has been reported in HCC tissues,[31] but it is not known whether replacement of vitamin K facilitates or suppresses tumor growth *in vivo*. Caution is needed in the administration of high-dose vitamin K to HCC patients at high risk of intrahepatic metastasis. The estimated 30% risk reduction of recurrence was not confirmed, and the effect of vitamin K on recurrence, if any, might be observed only in carefully selected patients in a very large-scale trial. If effects of vitamin K2 on HCC prevention are to be further investigated, a preferable endpoint would be the suppression of primary HCC in patients with cirrhosis or advanced fibrosis using the dose of 45 mg/day.

Poon et al.[5] reported that intrahepatic recurrence were classified into early (<1 year) and late (>1 year) recurrences, which seemed to correspond to intrahepatic metastasis and be multicentric in origin, respectively. The present study was terminated approximately 1.5 years after the start of enrollment, according to the recommendation of IDMC. If we are to assume that vitamin K2 at 45 mg/day reduced *de novo* carcinogenesis, it may have been necessary to observe for recurrence for more than 2 years.

Conclusion

In conclusion, the efficacy of vitamin K2 in suppressing HCC recurrence was not confirmed in this double-blind, randomized, controlled study.

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Phase III study of sorafenib after transarterial chemoembolisation in Japanese and Korean patients with unresectable hepatocellular carcinoma ☆

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ABSTRACT

Background: In Japan and South Korea, transarterial chemoembolisation (TACE) is an important locoregional treatment for patients with unresectable hepatocellular carcinoma (HCC). Sorafenib, a multikinase inhibitor, has been shown effective and safe in patients with advanced HCC. This phase III trial assessed the efficacy and safety of sorafenib in Japanese and Korean patients with unresectable HCC who responded to TACE.

Methods: Patients ($n = 458$) with unresectable HCC, Child-Pugh class A cirrhosis and ⩾25% tumour necrosis/shrinkage 1–3 months after 1 or 2 TACE sessions were randomised 1:1 to

☆ Results from this trial were presented at the American Society of Clinical Oncology Gastrointestinal Cancers Symposium, Orlando, Florida, USA, 22–24 January 2010.

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Randomised Controlled trial

sorafenib 400 mg bid or placebo and treated until progression/recurrence or unacceptable toxicity. Primary end-point was time to progression/recurrence (TTP). Secondary end-point was overall survival (OS).

Findings: Baseline characteristics in the two groups were similar; >50% of patients started sorafenib >9 weeks after TACE. Median TTP in the sorafenib and placebo groups was 5.4 and 3.7 months, respectively (hazard ratio (HR), 0.87; 95% confidence interval (CI), 0.70–1.09; $P = 0.252$). HR (sorafenib/placebo) for OS was 1.06 (95% CI, 0.69–1.64; $P = 0.790$). Median daily dose of sorafenib was 386 mg, with 73% of patients having dose reductions and 91% having dose interruptions. Median administration of sorafenib and placebo was 17.1 and 20.1 weeks, respectively. No unexpected adverse events were observed.

Interpretation: This trial, conducted prior to the reporting of registrational phase III trials, found that sorafenib did not significantly prolong TTP in patients who responded to TACE. This may have been due to delays in starting sorafenib after TACE and/or low daily sorafenib doses.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, the third most common cause of cancer deaths in men and the sixth most common in women.[1] It has been estimated that 650,000 people per year die from HCC, about three-quarters in East Asian countries.[2,3] Aetiologic factors vary by geographic region; ~70% of HCC patients in the Asia-Pacific (AP) region have chronic hepatitis B virus (HBV) infection, except in Japan, where ~75% of HCC patients have chronic hepatitis C virus (HCV) infection.[2,3]

Many patients with HCC are not diagnosed until the disease is unresectable, such that only non-curative treatment options are available.[4,5] The most frequent locoregional treatment for unresectable HCC is transarterial chemoembolisation (TACE), which concentrates chemotherapeutic agents at the tumour site while blocking the primary artery feeding the tumour.[6,7] Compared with symptomatic treatment alone, TACE has been found to enhance survival in patients with unresectable HCC.[8,9] A meta-analysis of seven randomised trials of arterial embolisation in 545 patients showed that chemoembolisation with cisplatin or doxorubicin showed a significant 2-year survival benefit compared with control, whereas embolisation alone showed no benefit.[10] A subsequent meta-analysis of randomised trials showed that TACE improves patient survival compared with untreated patients, but not when compared with patients treated with arterial embolisation alone.[11] Furthermore, no chemotherapeutic agent was found superior to any other, and there was no evidence that lipiodol had any benefit.[11]

Although TACE effectively delays HCC progression or prevents recurrence within 6 months, it is less effective over longer periods,[12] with 2-year survival rates of 24–63%.[13] Recent trials in Asian patients have found that 2-year overall survival (OS) rates following TACE with a suspension of a fine powder formulation of cisplatin in lipiodol, an emulsion of doxorubicin in lipiodol, and epirubicin-loaded superabsorbent polymer microspheres were 76%, 46% and 59%, respectively.[14,15] Although multiple courses of TACE may improve local tumour control,[11] it may also worsen liver function, both because TACE itself damages the hepatic arterial system[16] and because many patients have poor underlying liver function due to cirrhosis.[17] New and effective treatment strategies for patients with unresectable HCC are therefore needed, including the optimisation of TACE and its combination with other treatment modalities.

The high rate of HCC recurrence after TACE may be due to its enhancement of angiogenesis and upregulation of vascular endothelial growth factor (VEGF) expression, resulting in the formation of rich vascular beds in residual tumours.[18–20] Post-TACE treatment with systemic multikinase inhibitors that are both antiproliferative and antiangiogenic may therefore lengthen time to recurrence, improve survival, and target lesions distal to the TACE site.

Sorafenib is a multikinase inhibitor with antiangiogenic and antiproliferative properties, targeting multiple pathways.[21–23] Two large randomised phase III studies, the Sorafenib Health Assessment Randomised Protocol (SHARP)[24] and Sorafenib Asia-Pacific (AP)[25] trials, demonstrated that sorafenib significantly improves OS in patients with advanced HCC, leading to its approval for the treatment of HCC in more than 90 countries. To date, sorafenib remains the only available systemic therapy proven to extend survival in these patients.

In patients with unresectable HCC, sorafenib after TACE may prolong time to recurrence/progression and/or minimise loss of liver function associated with repeated courses of TACE. This double-blind, placebo-controlled, phase III trial, designed before the results of the SHARP and Sorafenib AP trials were reported, assessed the efficacy and safety of sorafenib in patients in Japan and South Korea with unresectable HCC who responded to TACE.

2. Patients and methods

We screened patients ⩾18 years of age with unresectable HCC and Child-Pugh A cirrhosis who sustained a response 1–3 months after TACE, defined using the then-prevailing criteria in Japan as ⩾25% tumour necrosis and/or shrinkage.[26,27] Additional inclusion criteria were life expectancy ⩾12 weeks; maximum target lesion size of 70 mm; ⩽10 target lesions; Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 or 1; and adequate bone marrow (absolute

neutrophil count $\geqslant 1000/mm^3$; platelet count $\geqslant 50 \times 10^9/L$; prothrombin time [PT] – international normalised ratio $\leqslant 2.3$ or PT $\leqslant 6$ s above control), liver (total bilirubin $\leqslant 3$ mg/dL; alanine aminotransferase and aspartate aminotransferase $\leqslant 5 \times$ upper limit of normal [ULN]), and renal (serum creatinine $\leqslant 1.5 \times$ ULN; amylase and lipase $\leqslant 2 \times$ ULN) function.

Patients were excluded if they had macroscopic vascular invasion, renal failure, history of cardiac disease, active clinically serious infection, history of human immunodeficiency virus infection, symptomatic metastatic brain or meningeal tumour, extrahepatic metastasis, seizure disorder requiring medication, prior use of systemic agents for advanced HCC (although prior use of interferon, retinoid and/or vitamin K_2 as adjuvant treatment after curative local treatment was allowed), use of hematopoietic growth factors within 3 weeks before start of study drug, concomitant treatment with cytokines after the last course of TACE, history of organ allograft, documented history of substance abuse, or were pregnant or breast-feeding.

All patients provided written informed consent. The study was approved by the appropriate ethics committees and institutional review boards at each centre, and complied with Good Clinical Practice Guidelines, the Declaration of Helsinki, and local laws and regulations. Ongoing safety and efficacy were assessed independently by the Data Monitoring Committee. This study was registered at Clinicaltrials.gov as trial number NCT00494299.

2.1. Procedures

TACE was performed by injecting gelatin foam plus lipiodol in all cases. The chemotherapeutic agents used concurrently were epirubicin, cisplatin, doxorubicin and mitomycin. Eligible patients were stratified by response to TACE (complete response [CR], defined as 100% tumour necrosis or shrinkage versus non-complete response [non-CR], defined as $\geqslant 25\%$ but <100% tumour necrosis or shrinkage),[26] by ECOG PS (0 versus 1), and by number of courses of TACE (one versus two). Patients were blindly randomised 1:1 to 400 mg (two 200-mg tablets) sorafenib (Bayer Schering Pharma; Leverkusen, Germany) or matching placebo twice daily.

Treatment interruptions and dose reductions (first 400 mg qd, then 400 mg qod) were allowed for drug-related toxicity. Patients were monitored for adverse events (AEs) using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0, except that the hand-foot skin reaction (HFSR) was classified and managed by a protocol-defined scale. Treatment continued until radiologic progression or recurrence of HCC, unacceptable toxicity associated with study drug, or withdrawal of consent.

The trial was divided into 28-day cycles. Patients were evaluated for safety and compliance every 2 weeks during cycles 1–3, and every 4 weeks thereafter. Tumours were evaluated, centrally at an image registration centre, $\leqslant 28$ days before the first dose of study drug and every 8 weeks thereafter, or when evaluating recurrence or progression. Throughout treatment, lesions were evaluated by dynamic computed tomography (CT), preferably by the same investigator or radiologist as at screening.

The primary study end-point was time to progression (TTP) by central review, defined as time to recurrence in patients with CR and TTP in those with non-CR at study entry. Progression was defined as a $\geqslant 25\%$ increase in tumour size or development of a new lesion. The secondary end-point was OS, defined as time from randomisation to death from any cause. Exploratory analyses included TTP by investigator assessment and subgroup analyses of TTP by central review, based on aetiology (HBV versus HCV), response to TACE (CR versus non-CR), number of lesions ($\leqslant 3$ versus >3), number of prior courses of TACE (1 versus 2), age (<65 versus $\geqslant 65$ years), sex, treatment lag ($\leqslant 9$ versus >9 weeks), country of enrolment (Japan versus South Korea), and ECOG PS (0 versus 1).

2.2. Statistical analysis

Patient sample size was estimated based on TTP. If 30% and 70% of patients achieved CR and non-CR, respectively, in response to TACE, the median TTP for the placebo group in the mixed population would be 5.7 months. Clinically meaningful improvement was defined as median TTP 50% higher in the sorafenib than in the placebo group. Assuming one formal interim and one final analysis performed using an O'Brien-Fleming-type alpha spending function with a two-sided alpha of 0.05, 318 events would be required to achieve a statistical power of 95%. Accrual of 372 patients (186 in each group) within 18 months would be expected to result in 318 events after 30 months; if 10% of patients were lost to follow-up, 414 patients would have to be randomised to observe 318 events.

Efficacy was assessed in the intention-to-treat (ITT) population, defined as all randomised patients. The safety population included all patients who received at least one dose of study medication. TTP and OS in the two treatment arms were calculated by the Kaplan–Meier method and compared by the log-rank test, as were subgroups stratified by response to TACE (CR versus non-CR), ECOG PS (0 versus 1) and number of prior courses of TACE (1 versus 2). Hazard ratios (HRs) for sorafenib versus placebo and 95% confidence intervals (CI) were estimated by Cox proportional hazards models.

2.3. Role of the funding source

The study sponsors were involved in the design of the study; the collection, analysis and interpretation of data; the writing of the report; and the decision to submit the paper for publication.

3. Results

3.1. Patients

From 27th April 2006 to 10th July 2009, 552 patients were screened at 69 centres in Japan and seven centres in South Korea. Of these, 458 patients (387 at 67 centres in Japan and 71 at six centres in South Korea) met the eligibility criteria and were randomised, 229 each to the sorafenib and placebo groups. All were included in the ITT analysis (Fig. 1), whereas

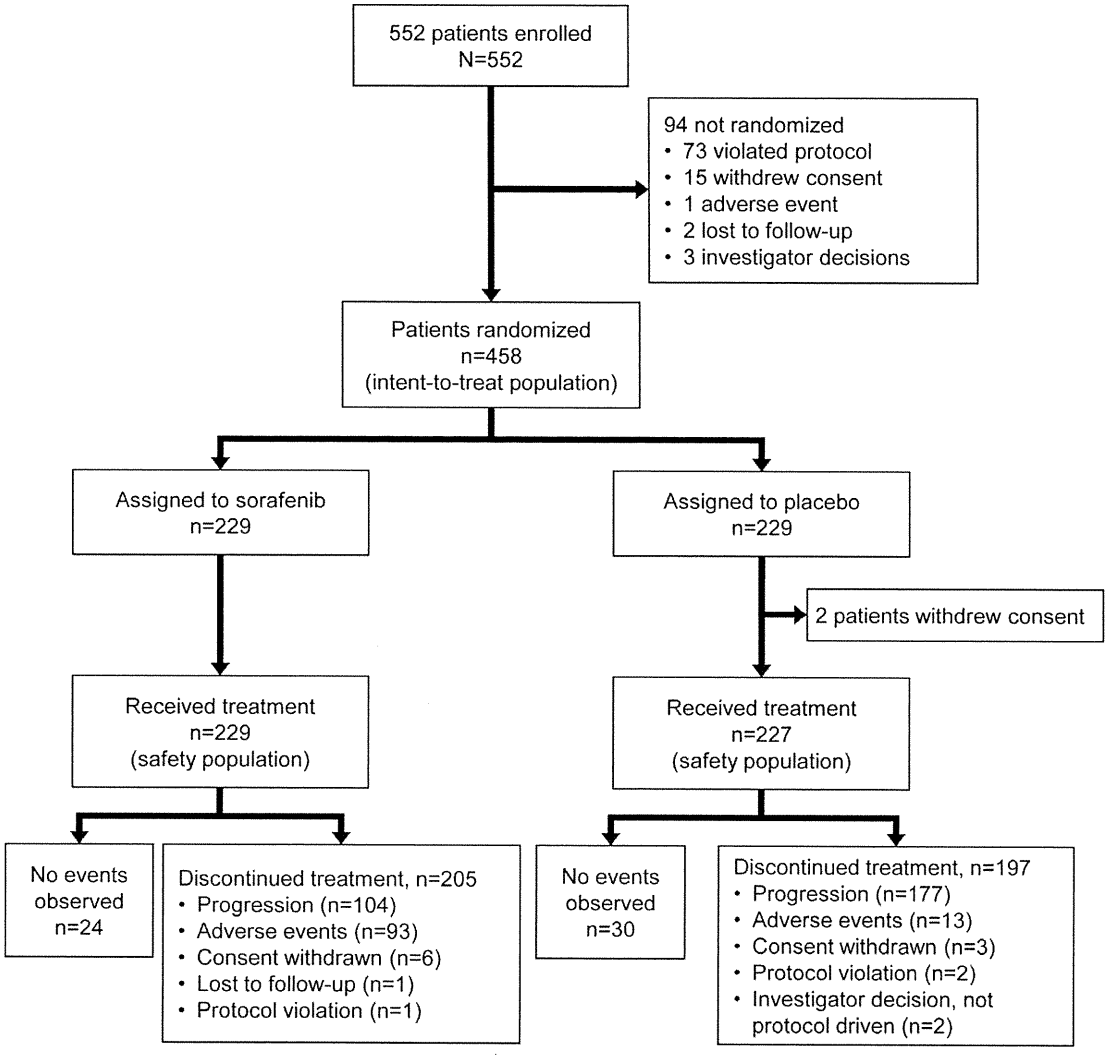


Fig. 1 – Enrolment and outcomes.

the 456 who received at least one dose of study drug were included in the safety analysis.

Demographic and baseline disease characteristics were similar in the sorafenib and placebo groups (Table 1). Of the 458 patients, 342 (74.7%) were male and 306 (66.8%) were ⩾65 years. Median age was 69 years (range, 29–86 years). At baseline, 403 patients (88.0%) had an ECOG PS of 0, 287 (62.7%) had HCV infection, and 336 (73.4%) had ⩽3 tumours. TACE consisted of gelatin foam plus lipiodol in all 458 patients, 60 for palliative intent and 398 for curative intent. Of these 458 patients, 355 received TACE monotherapy, including epirubicin ($n = 219$), cisplatin ($n = 89$), doxorubicin ($n = 49$) and mitomycin ($n = 1$); and 103 received combination treatments, including epirubicin + mitomycin ($n = 57$), cisplatin + epirubicin ($n = 16$), cisplatin + doxorubicin + mitomycin ($n = 13$), mitomycin + mitoxantrone ($n = 8$), doxorubicin + mitomycin ($n = 5$) and doxorubicin + iodixanol ($n = 4$). The median time from last TACE to randomisation was 9.3 weeks (range, 5.6–13.3 weeks), and the median time from initial diagnosis to study entry was 9.8 months (range, 1.6–144.3 months). Ten patients (2.2%) had received prior systemic anticancer therapy, consisting of prior adjuvant treatment with interferon, retinoid and/or vitamin K2 treatment after curative local treatment, and 219 (47.8%) had previously undergone some type of locoregional treatment, including radiofrequency ablation alone (10.7%), surgery alone (9.6%), percutaneous ethanol injection alone (5.9%), microwave coagulation therapy alone (0.2%) and other procedures (0.2%), with 21.2% having undergone multiple procedures (Table 1).

3.2. *Primary efficacy analysis*

By the cutoff date of 10th July 2009, 324 progression events (137 in the sorafenib and 187 in the placebo group) were confirmed by the Response Evaluation Committee. Median TTP by central review was 5.4 months (95% CI, 3.8–7.2 months) in the sorafenib group and 3.7 months (95% CI, 3.5–4.0 months) in the placebo group (HR [sorafenib/placebo], 0.87; 95% CI, 0.70–1.09; $P = 0.252$; Fig. 2). The 3-month progression-free rates in the sorafenib and placebo groups were 65.0% and 58.7%, respectively, and their 6-month progression-free rates were 45.7% and 33.5%, respectively.

Table 1 – Demographic and baseline characteristics of randomised patients (ITT population).

Variable	All patients			Japanese patients			Korean patients		
	Sorafenib + placebo (n = 458)	Sorafenib (n = 229)	Placebo (n = 229)	Sorafenib + placebo (n = 387)	Sorafenib (n = 196)	Placebo (n = 191)	Sorafenib + placebo (n = 71)	Sorafenib (n = 33)	Placebo (n = 38)
Median age (years)	69	69	70	71	70	71	60	61	59
Male (%)	74.7	76.0	73.4	72.9	74.0	71.7	84.5	87.9	81.6
ECOG PS[a] (%)									
0	88.0	87.8	88.2	91.5	91.3	91.6	69.0	66.7	71.1
1	12.0	12.2	11.8	8.5	8.7	8.4	31.0	33.3	28.9
Number of lesions (%)									
⩽3	73.4	72.9	73.8	70.8	69.9	71.7	87.3	90.9	84.2
>3	26.6	27.1	26.2	29.2	30.1	28.3	12.7	9.1	15.8
Aetiology (%)									
Alcohol	6.8	8.3	5.2	6.5	7.7	5.2	8.5	12.1	5.3
HBV	21.1	20.5	22.7	12.7	12.2	13.1	70.4	69.7	71.1
HCV	62.7	60.7	64.6	71.3	68.4	74.3	15.5	15.2	15.8
Other	5.9	7.0	4.8	7.0	8.2	5.8	0	0	0
Liver cirrhosis[b] (%)	68.3	69.4	67.2	66.7	67.3	66.0	77.5	81.8	73.7
Number of prior TACE[a] (%)									
1	64.4	64.2	64.6	66.7	66.3	67.0	52.1	51.5	52.6
2	35.6	35.8	35.4	33.3	33.7	33.0	47.9	48.5	47.4
Response to prior TACE[a,c] (%)									
CR	62.0	62.0	62.0	58.1	58.7	57.6	83.1	81.8	84.2
Non-CR	38.0	38.0	38.0	41.9	41.3	42.4	16.9	18.2	15.8
Prior local therapy (%)									
RFA	10.7	11.8	9.6	10.3	11.7	8.9	12.7	12.1	13.2
Surgery	9.6	7.0	12.2	10.3	8.2	12.6	5.6	0	10.5
PEI	5.9	4.8	7.0	6.5	5.1	7.9	2.8	3.0	2.6
MCT	0.2	0.4	0	0.3	0.5	0	0	0	0
Others	0.2	0.4	0	0	0	0	1.4	3.0	0
Multiple	21.2	20.5	21.8	24.0	23.0	25.1	5.6	6.1	5.3
Prior systemic therapy (%)	2.2	3.1	1.3	2.6	3.6	1.6	0	0	0

ITT = intention-to-treat; ECOG PS = Eastern Cooperative Oncology Group performance status; HBV = hepatitis B virus; HCV = hepatitis C virus; TACE = transarterial chemoembolisation; CR = complete response; non-CR = non-complete response; RFA = radiofrequency ablation; PEI = percutaneous ethanol injection; MCT = microwave coagulation therapy.

[a] Protocol-defined stratification factor.

[b] Clinically and/or histologically confirmed liver cirrhosis.

[c] Complete response was defined in the study protocol as 100% tumour shrinkage or necrosis.

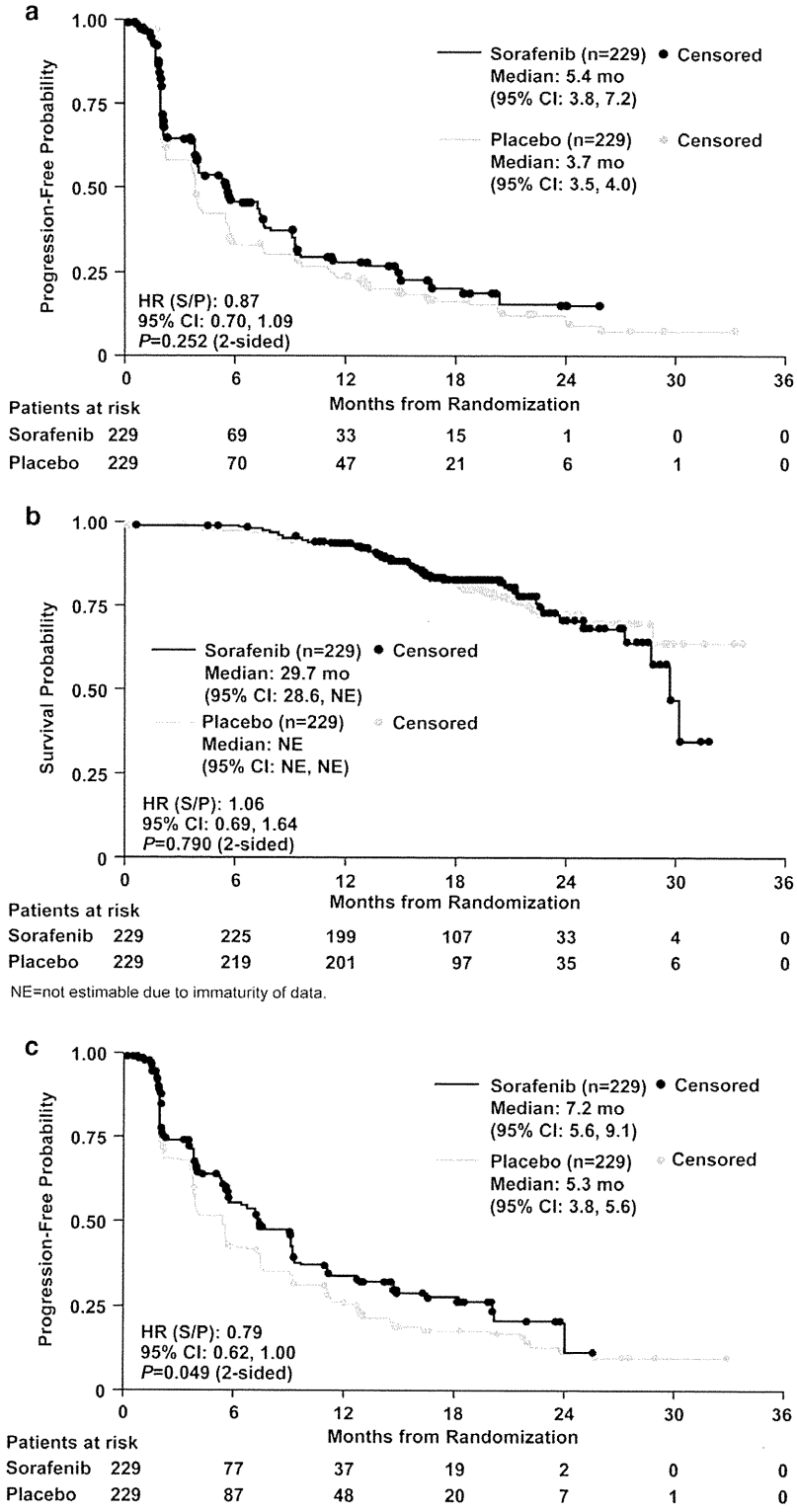


Fig. 2 – Kaplan–Meier analysis of time to progression (TTP) and overall survival (OS). (a) TTP by central review (primary intention-to-treat (ITT) analysis); (b) OS (secondary ITT analysis) and (c) TTP by investigator assessment (exploratory ITT analysis).

3.3. *Secondary efficacy analysis*

At the same cutoff date, there were 84 deaths, 43 in the sorafenib and 41 in the placebo group; the remaining patients were censored on that date. Median OS was 29.7 months in the sorafenib group (95% CI, 28.6 months – not yet reached) but had not yet been reached in the placebo group (HR [sorafenib/placebo], 1.06; 95% CI, 0.69–1.64; P = 0.790). The

Table 2 – Exploratory subgroup analyses of TTP by central review based on demographic, baseline and prognostic characteristics (ITT population; subgroups that included at least 10% of patients).

Variable	Subgroup	*n*	Number of events	Number of patients censored	Median TTP (95% confidence interval [CI]) (months)		Hazard ratio [HR] (95% CI) for Sorafenib/placebo
					Sorafenib	Placebo	
Aetiology	HBV	99	56	43	9.1 (5.6–20.3)	5.6 (3.7–10.9)	0.84 (0.49–1.44)
	HCV	287	217	70	5.3 (3.7–7.1)	3.6 (2.0–3.7)	0.81 (0.62–1.07)
Response to TACE	CR	284	179	105	7.4 (5.6–9.2)	5.3 (3.7–7.4)	0.84 (0.63–1.14)
	Non-CR	174	145	29	2.1 (1.8–3.9)	1.9 (1.8–3.6)	0.85 (0.61–1.18)
Number of lesions	⩽3	336	219	117	7.1 (5.3–7.8)	3.8 (3.7–5.5)	0.83 (0.64–1.09)
	>3	122	105	17	3.7 (2.0–5.3)	2.0 (1.9–3.7)	0.87 (0.59–1.29)
Number of prior TACE	1	295	212	83	5.4 (3.8–7.4)	3.7 (3.5–5.5)	0.91 (0.70–1.20)
	2	163	112	51	5.3 (3.7–7.8)	3.7 (2.1–3.8)	0.76 (0.52–1.11)
Age group	<65 years	152	90	62	9.1 (5.6–18.2)	3.7 (3.5–7.2)	0.68 (0.44–1.03)
	⩾65 years	306	234	72	3.8 (3.5–5.4)	3.7 (2.1–3.9)	0.99 (0.76–1.28)
Sex	Male	342	241	101	5.4 (3.8–7.4)	3.7 (3.5–5.3)	0.78 (0.60–1.00)
	Female	116	83	33	5.3 (3.6–7.4)	3.7 (2.1–5.3)	1.16 (0.75–1.79)
Treatment lag[a]	⩽9 weeks	205	150	55	5.5 (3.9–9.1)	3.7 (3.5–5.3)	0.74 (0.53–1.03)
	>9 weeks	253	174	79	5.1 (3.7–7.2)	3.7 (2.0–5.3)	0.95 (0.71–1.29)
Country of enrolment	Japan	387	289	98	3.9 (3.7–5.5)	3.7 (2.1–3.8)	0.94 (0.75–1.19)
	South Korea	71	35	36	NE[b] (9.0–NE)	5.5 (3.7–11.0)	0.38 (0.18–0.81)
ECOG PS	0	403	286	117	5.4 (3.8–7.2)	3.7 (3.6–5.3)	0.88 (0.69–1.11)
	1	55	38	17	5.4 (1.8–16.6)	3.5 (1.8–5.5)	0.78 (0.40–1.51)

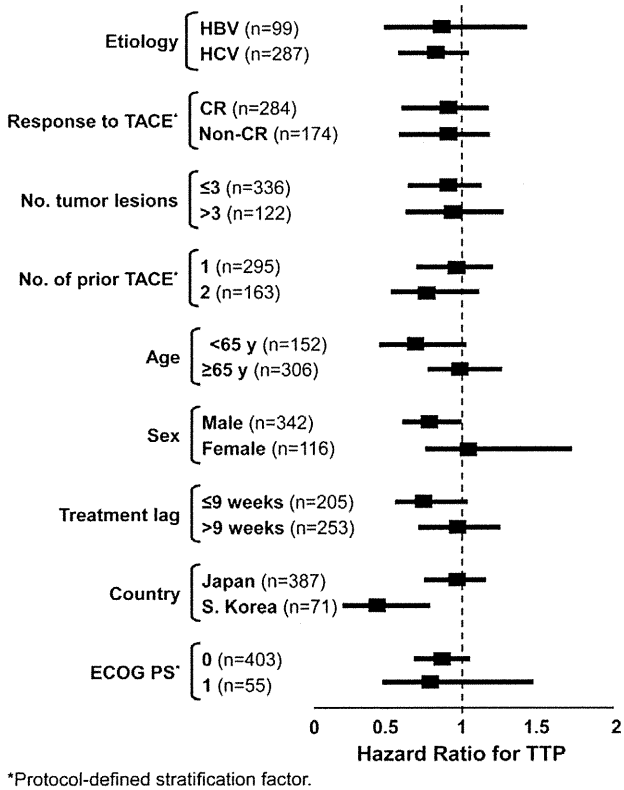
[a] Treatment lag was defined as time from the most recent TACE to randomisation.
[b] NE = not estimable due to censored data.

1-year survival rates in the sorafenib and placebo groups were 94.6% and 94.1%, respectively, and their 2-year survival rates were 72.1% and 73.8%, respectively.

3.4. *Exploratory analyses*

At the cutoff date, investigators had reported 304 progression events, 120 in the sorafenib and 184 in the placebo group. Median TTP by investigator assessment in the sorafenib and placebo groups were 7.2 months (95% CI, 5.6–9.1 months) and 5.3 months (95% CI, 3.8–5.6 months), respectively (HR [sorafenib/placebo], 0.79; 95% CI, 0.62–1.00; $P = 0.049$). Their 3-month progression-free rates were 74.1% and 67.9%, respectively, and their 6-month progression-free rates were 54.9% and 41.4%, respectively.

Exploratory analyses of TTP by central review were performed in subgroups containing ⩾10% of patients, including by aetiology (HBV versus HCV), response to TACE (CR versus non-CR), number of lesions (⩽3 versus >3), number of prior courses of TACE (1 versus 2), age (<65 versus ⩾65 years), sex, treatment lag (⩽9 versus >9 weeks), ECOG PS (0 versus 1) and country of enrolment. These analyses were performed to provide descriptive information only; the study was not powered to compare subgroup response to treatment, and no adjustments were made for multiple comparisons. Median TTP and the HR for TTP (sorafenib/placebo) in each subgroup are shown in Table 2, and Forest plots of HRs for TTP are shown in Fig. 3. Most HRs favored sorafenib. Differences were observed, however, between Japanese and Korean patients. The HR for TTP was 0.94 (95% CI, 0.75–1.19) for Japanese patients and 0.38 (95% CI, 0.18–0.81) for Korean patients (Fig. 4). Median TTP in sorafenib-treated patients in the



*Protocol-defined stratification factor.

Fig. 3 – Subgroup analyses of TTP by central review (exploratory ITT analyses in subgroups that include at least 10% of patients): forest plot depicting hazard ratio (HR) for TTP (sorafenib over placebo) for each subgroup.

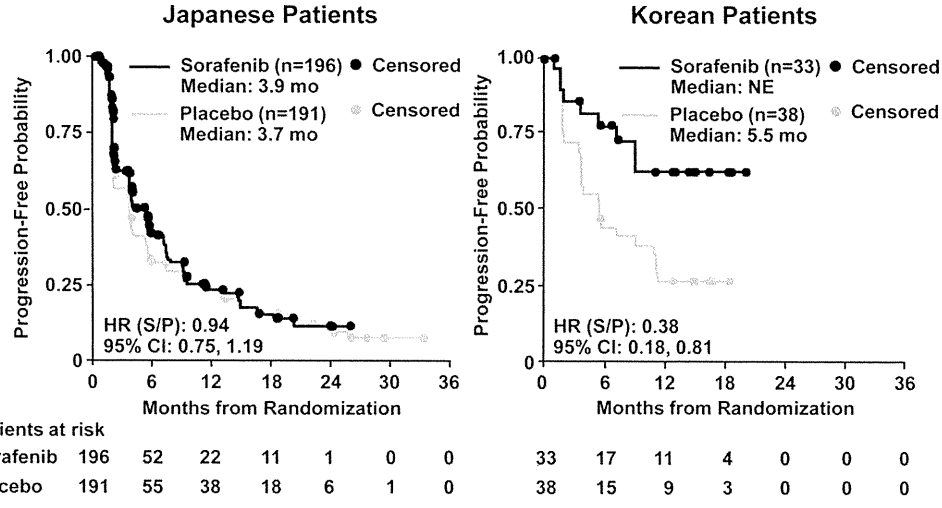


Fig. 4 – Kaplan–Meier analysis of TTP by central review, by country of enrolment (exploratory ITT analysis).

Korean subgroup could not be estimated since it was not attained by the study cutoff date.

3.5. *Safety*

The safety analysis included 229 sorafenib-treated and 227 placebo-treated patients; their incidence of drug-related AEs (DRAEs) were 100% and 61%, respectively. Most DRAEs were mild to moderate (Table 3), with the most frequent in the sorafenib and placebo groups being HFSR (82% versus 7%), elevated lipase (44% versus 8%), alopecia (41% versus 3%) and rash/desquamation (40% versus 11%). In the sorafenib group, 24% and 4% of patients experienced grades 3 and 4 elevated lipase, respectively, compared with 3% and <1%, respectively, in the placebo group. There was no radiographic or clinical evidence of pancreatitis in either group. The overall incidences of grade 3 HFSR (protocol-defined scale) in the sorafenib and placebo groups were 35% and 0%, respectively, and the overall incidence of serious DRAEs was 18% and 9%, respectively. There were no drug-related deaths.

The median durations of treatment in the sorafenib and placebo groups were 17.1 weeks (range, 1.0–112.1 weeks) and 20.1 weeks (range, 2.1–144.1 weeks), respectively (Table 4), and the median daily doses of sorafenib and placebo were 386.0 mg (range, 112.0–794.5 mg) and 785.8 mg (range, 276.1–810.3 mg), respectively. In the sorafenib group, 40 patients (17.5%) received >80% of the planned dose, compared with 206 (90.7%) in the placebo group. The most common reasons for discontinuing treatment in the sorafenib and placebo groups were disease progression (104/229 [45%] versus 177/229 [77%]) and adverse events (93/229 [41%] versus13/229 [6%]).

Doses were reduced in 166 of the 229 sorafenib-treated (72.5%) and in 33 of the 227 placebo-treated (14.5%) patients,

Table 3 – Treatment-emergent, drug-related adverse events occurring in ⩾20% of patients in either group.[a]

Adverse event	Sorafenib ($n = 229$) Grade (%)			Placebo ($n = 227$) Grade (%)		
	Any	3	4	Any	3	4
HFSR	82	35	–	7	0	–
Elevated lipase[b]	44	24	4	8	3	<1
Alopecia	41	–	–	3	–	–
Rash/desquamation	40	4	0	11	0	0
Other metabolic abnormality	32	8	1	4	2	<1
Diarrhoea	31	6	0	5	1	0
Hypertension	31	15	0	7	1	0
Hypophosphatemia	28	16	0	6	3	0
Thrombocytopenia	25	11	1	2	<1	0
Elevated AST	25	12	<1	5	3	0
Elevated ALT	21	8	<1	5	2	0
Elevated amylase	21	6	1	8	2	<1

HFSR = hand–foot skin reaction; AST = aspartate aminotransferase; ALT = alanine aminotransferase; NCI-CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events.

[a] Patients were monitored for adverse events using NCI-CTCAE v3.0, except for HFSR, which was classified according to a 3-grade, protocol-defined scale (grade 1, HFSR does not disrupt normal activities; grade 2, HFSR affects the activities of the patient; and grade 3, patient is unable to work or perform activities of daily living because of HFSR).

[b] There was no radiographic or clinical evidence of pancreatitis in either arm.

Table 4 – Summary of study drug administration.

Assessment	All patients		Japan		South Korea	
	Sorafenib (*n* = 229)	Placebo (*n* = 227)	Sorafenib (*n* = 196)	Placebo (*n* = 190)	Sorafenib (*n* = 33)	Placebo (*n* = 37)
Median duration of treatment (weeks)	17	20	16	20	31	33
Median daily dose (mg)	386	786	382	786	403	766
Patients with dose reduction (%)	73	14	71	11	82	32
Patients with dose interruption (%)	91	18	92	17	85	24
Patients with discontinuation (%)	90	87	93	88	70	78
Due to progression (%)	51	90	52	90	39	90
Due to adverse events (%)	45	7	44	7	57	3
HFSR	11	0	10	0	18	0
Thrombocytopenia	4	0	5	0	3	0
Hypophosphatemia	4	<1	4	1	3	0
Hypertension	4	0	5	0	0	0
Neutropenia	4	<1	4	1	0	0
Elevated AST	2	<1	2	1	3	0
Rash/desquamation	2	0	2	0	3	0
Elevated ALT	2	1	1	1	6	0
Diarrhoea	1	0	1	0	3	0
Other	11	4	19	3	18	3

HFSR = hand–foot skin reaction; AST = aspartate aminotransferase; and ALT = alanine aminotransferase.

due primarily to AEs (163 versus 27). Forest doses were interrupted temporarily in 208 of the 229 sorafenib-treated (90.8%) and 41 of the 227 placebo-treated (18.1%) patients, again due primarily to AEs (206 versus 38).

A total of 107 patients – 94 of the 229 (41.0%) in the sorafenib group and 13 of the 227 (5.7%) in the placebo group – permanently discontinued study drug due to AEs. The most common AEs leading to discontinuation of sorafenib were HFSR (11.4%), thrombocytopenia (4.4%), hypertension (3.9%), hypophosphatemia (3.9%) and neutropenia (3.5%); the most common AE leading to discontinuation of placebo was increased ALT (0.9%).

Death within 30 days of receiving study drug occurred in one patient (0.4%) in each group; neither was deemed drug-related.

4. Discussion

This phase III randomised, controlled trial, assessing the efficacy and safety of sorafenib after response to TACE in Japanese and Korean patients with unresectable HCC, employed a protocol consistent with the practice of TACE in these countries at that time.[28,29] Moreover, the protocol was designed before the combination or sequential use of TACE and sorafenib or their optimal timing had been adequately studied, and before the effect of TACE on susceptibility to sorafenib had been characterised. In this setting, sorafenib did not significantly prolong TTP or OS by central review in patients with unresectable HCC who responded to TACE. Exploratory secondary and subgroup analyses suggested, however, that post-TACE sorafenib had a positive impact on these patients. Median TTP by investigator review was approximately 2 months longer in the sorafenib than in the placebo group, and exploratory subgroup analyses suggested that TTP may have been affected by several factors, including age, number of prior TACE courses, treatment lag, treatment duration, total exposed dose and nationality.

Several factors may have contributed to these results. For example, unusually high percentages of sorafenib-treated patients required dose reductions (73%) and/or interruptions (91%), resulting in a much lower than planned median daily dose of sorafenib (386 mg). In comparison, 26% and 44% of sorafenib-treated patients in the SHARP trial, and 31% and 43% of those in the Sorafenib AP trial, required dose reductions and interruptions, respectively, due to AEs,[24,25] and median daily doses of sorafenib were higher in the SHARP (797 mg) and Sorafenib AP (795 mg) trials.

The better outcomes observed in Korean patients may have been due to their substantially longer median treatment duration (31 versus 16 weeks), resulting in a favourable HR in Koreans (0.38; 95% CI, 0.18–0.81). Moreover, the Korean and Japanese subgroups differed in baseline characteristics. Japanese patients were older and a higher percentage had $\geqslant 3$ lesions on enrolment. Moreover, Japanese patients were less likely to have received >1 TACE to achieve CR prior to sorafenib. Finally, these subgroups differed in principal aetiology of HCC, in that ~70% of Japanese patients had HCV and ~70% of Korean patients had HBV.

We found that the incidence of treatment-emergent adverse events in the sorafenib-treated patients in this trial was generally higher than that observed in previous trials of sorafenib in patients with HCC. We found that the rates of all grade HFSR, Grade 3 HFSR and discontinuation due to HFSR were higher in this trial than in the SHARP[24] and Sorafenib AP[25] trials. We also found that the rates of all grade alopecia; rash/desquamation; hypertension, including grade 3 hypertension; thrombocytopenia and elevated liver function enzymes were higher in this trial than in the two previous phase III trials of sorafenib in patients with HCC. These results were unexpected and may have been due to the combination of TACE with sorafenib treatment in this trial. These findings suggest that adjustments in sorafenib dose (e.g. starting at a lower dose after TACE) or the timing of sorafenib treatment with respect to TACE may be required for these two

modalities to be tolerated in combination and also have synergistic effects.

The timing of post-TACE sorafenib may also have contributed to the absence of a positive effect of sorafenib observed in this study. Local hypoxia resulting from TACE can induce angiogenesis[18] and enhance serum concentrations of VEGF,[19,20] suggesting that sorafenib may exert its greatest antiangiogenic effects when administered immediately after or even before TACE. Serum VEGF concentrations have also been found to correlate with impaired liver function, tumour size, tumour number, macroscopic vascular invasion,[30] and poor OS.[31] Of our sorafenib-treatment patients, 60% had a treatment lag >9 weeks prior to randomisation, due primarily to the need for central review of CT scans, and shorter lag time has been found associated with better outcomes.

Several ongoing phase II/III trials in patients with unresectable HCC may provide insight into the optimal combination treatment and the optimal timing of sorafenib relative to TACE. These include trials testing TACE with doxorubicin-eluting beads and sorafenib or placebo and alterations in timing of conventional TACE relative to sorafenib or placebo.[32–35]

5. Conclusion

Sorafenib did not significantly improve median TTP by central review in Japanese and Korean patients with unresectable HCC who responded to TACE, although exploratory analyses suggested that sorafenib may have clinical benefits in certain patient subsets, including males, patients <65 years of age, and those with a shorter treatment lag between TACE and sorafenib; and that longer treatment duration and greater total daily dose may be associated with clinical improvements. No new or unexpected AEs were observed. The results of these and other clinical investigations may help refine the use of sorafenib and TACE, and define their optimal combination, in patients with unresectable HCC.

Author contributions

Drs. Masatoshi Kudo and Kiwamu Okita were involved with the study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; and study supervision.

Drs. Kazuho Imanaka, Nobuyuki Chida, Kohei Nakachi, Won-Young Tak, Tadatoshi Takayama, Jung-Hwan Yoon, Takeshi Hori, Hiromitsu Kumada, Norio Hayashi, Shuichi Kaneko, Hirohito Tsubouchi, Dong Jin Suh, Junji Furuse, Takuji Okusaka, Katsuaki Tanaka and Osamu Matsui were involved with the acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; and study supervision.

Drs. Michihiko Wada, Iku Yamaguchi, Toshio Ohya and Gerold Meinhardt were involved with the study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; administrative and technical support; and study supervision.

Clinical trials

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Conflict of interest statement

Masatoshi Kudo received advisory and speaker fees and research and travel grants from Bayer. Won-Young Tak received advisory and speaker fees from Bayer, Junji Furuse received advisory fees from Bayer, Takuji Okusaka received advisory and speaker fees, research and travel grants from Bayer. Osamu Matsui received consulting and advisory fees and research grants from Bayer. Michihiko Wada, Iku Yamaguchi, Toshio Ohya and Gerold Meinhardt are employees of Bayer. Kiwamu Okita received consulting fees from Bayer. All other authors declared no conflicts of interest.

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SUPPLEMENT

Tissue Biomarkers as Predictors of Outcome and Selection of Transplant Candidates With Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is a common cause of cancer deaths worldwide, and its annual incidence is rising. Liver transplantation (LT) is an accepted curative treatment for patients with tumors satisfying the Milan criteria (a single tumor $\leq$ 5 cm in diameter or up to 3 tumors with individual diameters $\leq$ 3 cm and no macrovascular invasion). These criteria predict an overall 5-year survival rate of 70% after LT.[1] Since the introduction of the Milan criteria, subsequent studies have explored the expansion of transplant recipient selection to include individuals with tumors exceeding the Milan criteria.[2] A recent study demonstrated an acceptable overall 5-year survival rate (71.2%) for patients who underwent transplantation for tumors that were beyond the Milan criteria but satisfied the up-to-7 rule (7 is the sum of the size of the largest tumor in centimeters and the number of tumors) in the absence of microvascular invasion.[3] This approach is based on the best data available for understanding tumor behavior after LT, but it is still based on pathological data. The tumor size and the tumor number cannot be used to define subclasses of patients with better biology and better outcomes, so biomarkers are expected to be a major step forward in this setting during the next decade.

Numerous molecular pathways involved in the pathogenesis of HCC have been identified. These include activation pathways that are involved in angiogenesis [vascular endothelial growth factor (VEGF)], in cell proliferation and survival (epidermal growth factor, insulin-like growth factor, and hepatocyte growth factor/Met), and in cell differentiation and proliferation (Wnt/β-catenin and hedgehog signaling). The activation of

Abbreviations: AFP, alpha-fetoprotein; Ang2, angiopoietin 2; HCC, hepatocellular carcinoma; LT, liver transplantation; miRNA, microRNA; mRNA, messenger RNA; REMARK, Reporting Recommendations for Tumor Marker Prognostic Studies; VEGF, vascular endothelial growth factor.

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VEGF,[4] Serine/threonine protein kinase Akt (Bombyx mori) [AKT],[5] and met proto-oncogene (hepatocyte growth factor receptor) [MET][6] has been correlated with an aggressive phenotype and a poor prognosis after liver resection. Similarly, several gene signatures have been used to predict the outcomes of patients with HCC.[7] Gene expression profiling with formalin-fixed, paraffin-embedded tissue samples from HCC resection specimens has been described and validated for the prediction of survival outcomes for patients after resection for HCC.[8] This profiling technique offers the ability to perform retrospective studies with stored histological specimens. In addition, it potentially offers a practical clinical application through the ability to perform gene profiling with common formalin-fixed biopsy specimens rather than frozen tissue.

This article summarizes 3 areas in which molecular tissue biomarkers should be considered for the management of HCC in LT patients:

1. Role of tissue biomarkers in the diagnosis of HCC.
2. Role of biomarkers in the prediction of prognosis (ie, the use of gene signatures or tissue biomarkers to predict a patient's prognosis and thus aid in the extension of the Milan criteria for HCC).
3. Role of biomarkers in the prediction of the response to molecular-targeted therapies.

Serum markers such as alpha-fetoprotein (AFP), angiopoietin 2 (Ang2), and des-gamma-carboxyprothrombin are not analyzed here.

ROLE OF TISSUE BIOMARKERS IN THE DIAGNOSIS OF HCC

The diagnosis of HCC is based on pathological or noninvasive criteria.[9] The pathological differentiation of dysplastic nodules (particularly high-grade nodules) from very early HCC is sometimes difficult, especially with a cirrhotic background. Few studies have tested the accuracy of the molecular diagnosis of early HCC in this setting. For instance, gene signatures have allowed molecular demarcations between low-grade dysplastic nodules, high-grade dysplastic nodules, and early HCC in both Asian[10] and Western patients.[11] More specifically, a 3-gene signature (including glypican 3, lymphatic vessel endothelial hyaluronan receptor 1, and survivin) has been reported to distinguish early HCC (<2 cm) from dysplastic nodules with an accuracy of approximately 90%.[12] Nonetheless, this signature has not yet been externally validated. More recently, an immunohistochemistry study found the expression of glypican 3, heat shock protein 70, and glutamine synthetase to be useful in detecting well-differentiated HCC in biopsy samples,[13] and this is currently being considered for HCC management guidelines.[9]

ROLE OF BIOMARKERS IN THE PREDICTION OF PROGNOSIS

Patients who develop HCC with cirrhosis and undergo resection have a high rate of recurrence (approximately 70% at 5 years).[2,14] A molecular assessment of the prognosis could determine which patients with HCC would benefit from adjuvant therapy after resection or radio frequency ablation (2 curative treatments with a high risk of relapse). Moreover, it could be used to refine the group of patients who should undergo transplantation for HCC beyond the Milan criteria. Whether the risk of tumor seeding counterbalances the advantages of tissue-based molecular profiling is still an area of discussion. In a recent meta-analysis, the risk of tumor seeding after liver biopsy was 2.7% with a median time of 17 months between biopsy and seeding.[15] These data also include large tumors, so the risk of complications with small, early tumors is expected to be significantly lower and thus acceptable.

Biomarkers predicting a patient's prognosis or response to therapy are crucial in modern oncology. Novel prognostic biomarkers enabling tumor classification, disease state monitoring, or both could advance our efforts to realize the potential of personalized medicine in cancer.[16] Besides reports on AFP levels and outcomes,[17-19] recent studies have correlated various types of markers, such as gene expression, microRNAs (miRNAs), and methylation changes, with the survival of HCC patients; this topic has been reviewed elsewhere[20] (see Table 1). Five markers or signatures (epithelial cell adhesion molecule [EPCAM signature], which is a hepatic stem cell marker in tumor tissue[21,22]; the G3 proliferation subclass[23]; the expression status of the miR-26 miRNA precursor[24]; and 2 prognostic gene signatures in nontumor hepatic tissue[8,25]) have emerged as more consistent ones. Finally, both VEGF and Ang2 were shown to have independent prognostic value in a large cohort of patients with advanced HCC.[26] Although these results support the possibility of using these genetic and molecular markers as prognostic biomarkers for patients with HCC, they require external validation before they can be included in staging systems and/or incorporated into clinical management guidelines. The fractional allelic imbalance, which is used to measure chromosomal instability, has been associated with outcomes for patients with HCC and with recurrence after LT; this observation requires attention in future studies.[27,28] Similarly, data about $CD90^+$ circulating cells may lead to a tractable supply of tissue for molecular characterization, but this is still under investigation.[29]

In this era of limited organ availability, better predictors of HCC recurrence are needed for selecting appropriate LT candidates whose tumors exceed the Milan criteria. The identification of a subgroup of patients whose tumors are beyond the Milan criteria but who have a favorably low risk of recurrence after transplantation offers a potential cure to those who would otherwise be excluded according to current organ allocation policies. Whether any of the aforementioned biomarkers or gene signatures can be used to identify those patients with better biological profiles needs to be elucidated in molecular studies addressing this point. Only a small study has addressed this

TABLE 1. Main mRNA-Based, miRNA-Based, Epigenetic, and Structural Alterations Whose Prognostic Impact for HCC Patients Needs to Be Tested or Confirmed

Molecular Alteration	Clinical Significance	REMARK Recommendation	Current Status*
mRNA-based (gene signatures)†			
Poor survival signature	Poor survival	Okay	Lacks external validation
Epithelial cell adhesion molecule signature	Poor survival	Okay	Lacks external validation
Venous metastasis signature	Hepatic metastasis	Okay	Lacks external validation
Class A/hepatoblast signature	Poor survival	Okay	Lacks internal and external validation
G3 subclass	Poor survival	—	Lacks internal and external validation
AFP and Ang2	Poor survival	Okay (unclear cutoff)	Lacks external validation
miRNA-based			
Down-regulation of miR-26a	Poor survival	Okay	Lacks external validation
20-miRNA signature	Venous metastasis, overall survival	Okay	Lacks external validation
Down-regulation of miR-122	Poor survival	—	Lacks internal and external validation
Down-regulation of Drosophila melanogaster members	Early recurrence	—	Lacks internal and external validation
Up-regulation of miR-125a	Better survival	—	Lacks internal and external validation
19-miRNA signature	Poor survival	—	Lacks internal and external validation
Epigenetic			
Genome-wide hypomethylation	Tumor progression, survival	—	Lacks internal and external validation
Hypermethylation of E-cadherin or glutathione S-transferase π1	Poor survival	—	Lacks internal and external validation
Structural			
Fractional allelic imbalance/ chromosomal instability	Recurrence/survival	Okay	Lacks external validation

NOTE: Adapted with permission from *Clinical Cancer Research*.[20]
*In terms of clinical implementation.
†Molecular classifications (mRNA-based) with a prognostic impact have been thoroughly discussed elsewhere.[5,6,20]

question in a specific manner, and it found that chromosomal instability (measured with the fractional allelic imbalance) independently predicted which patients beyond the Milan criteria had a low risk of recurrence.[27] Similarly, preliminary reports describing surrogates of microvascular invasion (the main predictor of HCC recurrence after LT) require independent validation in the setting of transplantation.[30]

ROLE OF BIOMARKERS IN THE PREDICTION OF THE RESPONSE TO MOLECULAR-TARGETED THERAPIES

Biomarkers for treatment responses are still rarities in oncology; only a few have made their way into routine clinical use. Well-defined biomarkers are believed to characterize an oncogenic addiction loop (the proposed mechanism by which a tumor cell becomes largely reliant on a single activated oncogene[31]) and define particular tumor subtypes that respond to specific molecular-targeted therapies. Examples of oncogenic addiction include an amplification of human epidermal growth factor receptor 2 in patients with breast cancer responding to trastuzumab,[32] mutations in epidermal growth factor receptor that distinguish patients with non–small cell lung cancer responding to erlotinib,[33] and v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (c-KIT)–positive gastrointestinal stromal tumors responding to the multikinase inhibitor imatinib.[34] In addition, wild-type v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) has recently emerged as a marker of a response to cetuximab and panitumumab in patients with colorectal cancer, although the mechanism is entirely different and involves the downstream regulation of epidermal growth factor receptor signaling.[35] Moreover, a new step in personalized medicine has been achieved recently with the development of a specific inhibitor of mutated V600E v-raf murine

sarcoma viral oncogene homolog B1 (BRAF); this inhibitor has shown impressive clinical efficiency with few adverse events in a recent phase 2 study of melanoma.[36] In the future, therefore, mapping the genetic alterations of tumors before the treatment or after treatment failure will improve the clinical care of patients with cancer.[37]

The use of biomarkers for HCC is somewhat more complex because HCC is a very heterogeneous disease for which oncogenic addiction loops have yet to be characterized. Initial approaches for defining a molecular classification have not yet been linked to specific treatment responses.[38,39] So far, only 1 small molecule, sorafenib, has been shown to improve the survival of HCC patients.[40] Sorafenib is a multikinase inhibitor that targets a number of kinases; these kinases include VEGF receptors 2 and 3, platelet-derived growth factor receptor β, c-KIT, Ret proto-oncogene (RET), fms-related tyrosine kinase 3, and Raf kinase, efector of Ras (RAF).[41] Isolated reports have described the use of sorafenib in the adjuvant setting after LT. In a companion biomarker study of the pivotal Sorafenib HCC Assessment Randomized Protocol trial, 10 serum markers and 1 tissue marker were tested, but none of them succeeded in identifying subclasses of responders.[26] Nonetheless, the fast development of new biotherapies and the growing number of clinical trials for HCC are expected to lead to the use of the molecular features of tumors in defining types of treatment. In this setting, we have to reevaluate the utility of tumor biopsy for easy access to tissue and its frequency.

FUTURE PROSPECTS

Novel molecular data may change our approach to the diagnosis, staging, and prognosis of HCC in this decade. For prognosis assessments, recently reported prognostic gene signatures and miRNAs may be added to staging systems to complement clinical variables once they have been externally validated by independent studies. These advances in our understanding of HCC ultimately need to be transferred to clinical practice as daily tools for selecting management and treatment methods. Moreover, treatment response predictors will emerge along with novel drugs for the treatment of HCC. Positive results with sorafenib[40] have opened a new era in HCC research. Future trends in drug development will pivot on the accurate assessment of genetic traits associated with human diseases on an individual basis (ie, personalized medicine). For HCC, the identification of these singularities will allow maximization of the therapeutic response through the selection of the best drug for the ideal candidate.

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