TNF alone was highly expressed in 10 of the 38 cancer tissues (26.3%), while LTβ alone was not detected in any cases. There may be at least two transcribed states *in vivo*, a TNF-expressing state and a TNF/LTβ-expressing state. We analyzed the correlation between the IHC data and clinical features and found no significant correlations between TNF and/or LTβ expression status and viral status, histological findings (differentiation grade of cancer, presence of chronic hepatitis or cirrhosis), or overall survival of the patients (data not shown). Although it is currently unknown whether the data for HCC tissues are related to higher-order chromatin states of the *TNF/LT* locus (shown in Fig. 6), these results suggest that differential expression of TNF and LTβ occurs *in vivo*.

DISCUSSION

The present study demonstrates the significance of the spatiotemporal regulation of gene activities and higher-order chromatin dynamics in the human *TNF/LT* locus. We identified four CTCF-dependent insulators (TC1, TC2, TC3, and TC4) and an enhancer (TE2) in hepatic cells. The well-known *H19* DMR insulator contains four CTCF binding sites, while each TC site has single CTCF binding sequence with moderate enhancer blocking activities (Fig. 3). The *LTα/TNF* promoters and TE2 were located between TC2 and TC3, while the *LTβ* promoter was between TC3 and TC4, which may play a role in differential regulation of these three genes. The *LTα/TNF* genes were immediately induced by TNF stimulation in a fashion sensitive to inhibition of NF-κB signaling, while the *LTβ* gene was expressed later, as seen in other cell types (1, 39). Our previous report on the human *apolipoprotein* gene locus suggested that CTCF insulators play an essential role in clustered gene control (40). Furthermore, the current study shows that insulator interactions are likely to mediate intrachromosomal association and subsequent dissociation following TNF signaling. The dynamic enhancer-promoter associations and differential expression in the *TNF/LT* locus may be directed by the NF-κB-related regulatory molecules.

From the viewpoint of enhancer-promoter-insulator associations, we propose a spatiotemporal dynamics model in the human *TNF/LT* locus (see Fig. S7 in the supplemental material). In the basal state, CTCF-bound TC sites, the TE2 enhancer, and the *TNF/LT* promoters are located some distance apart in the chromatin structure. After TNF signaling activation, in the *TNF*-expressing state, the TC insulators, TE2, and *TNF/LT* promoters become colocalized and form a compact chromatin structure, resulting in interactions between TE2 and the *TNF* and *LTα* promoters. Because the *LTβ* gene is not fully induced at this stage, the *LTβ* promoter is likely to be sequestered by forming a possible chromatin loop between TC3 and TC4 (see Fig. S5C and D in the supplemental material). In addition, TC sites may be involved in stabilizing the interaction between TE2 and the *TNF* promoter because of the decrease of *TNF* expression in CTCF-depleted cells (Fig. 5C and E). In the *TNF/LTβ*-expressing state, TE2 significantly maintained its interaction with the *LTβ* promoter despite a reduced association with other elements. Thus, sequential chromatin conformation changes may contribute to switching of the enhancer-promoter interaction. Posttranslational modifications of CTCF and changes in the interacting molecules may be involved in the mechanism of intrachromosomal dynamics in the *TNF/LT* locus (47).

Our study revealed that TNF signaling can induce spatiotemporal remodeling of the clustered gene region and that CTCF insulators are likely to mediate higher-order control of transient enhancer-promoter interactions in the *TNF/LT* locus. Previous studies of the *TNF/LT* locus in hematopoietic cells suggested the presence of certain regulatory elements in intron 3 of the *TNF* gene and in the final exon of the *LTβ* gene (5, 66). The sequences, including the TC3 site, showed silencer activity in human T cells, though our study indicated that TC3 had a CTCF-dependent enhancer-blocking function, suggesting that the regulatory elements may differ among cell types. We showed that CTCF-mediated higher-order chromatin is involved in *TNF/LT* gene regulation. Persistent NF-κB activation in chronic inflammation may result in the chromatin conformation of the *TNF/LT* locus being deregulated and maintained in the *TNF/LTβ*-expressing state as an epigenetic memory. Indeed, constitutive NF-κB activation was recently noted to cause *LTβ* expression in inflamed hepatocytes and HCC cells *in vivo* (35), and *LTβ* was demonstrated to be an inducer of HCC (23). The proposed higher-order chromatin conformation of the *TNF/LT* locus may be involved in these *in vivo* situations.

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HEPATOLOGY

Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma according to Child–Pugh classification

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Child–Pugh, hepatic arterial infusion chemotherapy, hepatocellular carcinoma, prognosis, response.

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Abstract

Background and Aim: We compared the treatment response, survival, and safety to hepatic arterial infusion chemotherapy (HAIC) in patients with advanced hepatocellular carcinoma (HCC) according to Child–Pugh (CP) score.

Methods: The study subjects were 249 patients with advanced HCC and CP class A and B who had been treated with HAIC. Patients were grouped according to CP score (5/6, 7 and 8/9) and their tumor response, tolerance, and survival were assessed.

Results: The median survival time (MST) was 8.2, 9.7, 6.3, and 3.9 months for the whole group, patients with CP 5/6, 7 and 8/9, respectively ($P < 0.0001$). Complete response (CR) and partial response (PR) were seen in 11 and 57 patients, respectively, with an overall response rate of 27.3%. The response rate was higher in patients with CP score 5/6 and 7, than CP 8/9 (30.5%, 28.2%, 13.8%). The dropout rate was significantly higher in patients with CP score 8/9 than the other two (8.0%, 12.8%, 33.3%, respectively). The survival rate was significantly better in patients who achieved CR/PR than the others with CP score 5/6, 7. CP score 8/9 was an independent negative factor for response and survival.

Conclusion: Advanced HCC patients with CP score of 5/6 and 7 showed a better response to HAIC and better prognosis than those with CP score 8/9.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers and the third leading cause of cancer-related mortality worldwide.[1,2] Various kinds of treatment options are currently available, such as surgical resection, radiofrequency ablation (RFA), percutaneous ethanol injection (PEI), transcatheter arterial chemoembolization (TACE), radiotherapy and hepatic arterial infusion chemotherapy (HAIC). The application of these treatment modalities has gradually improved the prognosis of HCC patients. However, the survival rates of patients with advanced HCC and complications such as portal vein tumor thrombosis (PVTT), venous tumor thrombosis (VTT), extrahepatic metastasis, and TACE refractory patients remains extremely poor.[3–6] The SHARP trial (Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol) investigated the efficacy of the oral multikinase inhibitor sorafenib in the treatment of advanced HCC. Patients treated with this drug had a statistically significant longer survival (10.7 months) than those who received supportive care only (7.9 months).[7] Sorafenib has become the first-line therapy for advanced HCC worldwide, but it is associated with low tumor response rate, limited safety and benefits in patients with Child–Pugh (CP) class B.[8–10] On the other hand, several studies have reported the survival benefits of HAIC using 5FU for advanced HCC, with a response rate ranging from 12.2 to 52%,[11–19] The median survival times in responders and nonresponders were 18.4–31.6 months and 5.4–6.7 months, respectively.[12,13] The cumulative survival rates of patients stratified by response to therapy after HAIC was significantly higher in responders than others. Therefore, the Japanese evidence-based guidelines for the diagnosis and treatment of HCC and the consensus-based treatment algorithm for HCC proposed by the Japan Society of Hepatology recommend HAIC for HCC with Vp3 or Vp4 and TACE-refractory HCC (the PVTT grade, based on the location of the tumor thrombus, is determined based on the criteria of the Liver Cancer Study Group of Japan: Vp3, tumor thrombus in the first branch of the portal vein, Vp4: tumor thrombus in the trunk of the portal vein).[20,21] However, the prognosis of CP class B patients treated with HAIC is equally poor to that of patients on sorafenib.[13,18] However, there is no information on whether poor survival is related to liver cirrhosis or disease progression. Moreover, the efficacy and toxicity of HAIC for advanced HCC according to CP score have not been rigorously

analyzed. In this regard, the CP scores of patients classified as CP class B cirrhosis range from 7 to 9, and it is possible that the outcome of patients with CP score 7 is similar to that of patients with CP class A. In the present study, we assessed the efficacy and toxicity of HAIC in patients with advanced HCC (divided into three groups of CP score of 5/6, 7, and 8/9) and determined the factors that modulate the response to treatment and survival.

Methods

Patients. Between June 2000 and June 2011, 278 patients with unresectable HCC were treated with HAIC in our hospital. In our hospital, HAIC is used as the treatment option for patients with advanced HCC with PVTT and VTT, as well as those refractory to TACE. Among them, 23 patients treated with sorafenib after HAIC failure and six patients with CP class C were excluded from this study. Accordingly, 249 patients were enrolled in this retrospective cohort study. They included 173 patients (69.4%) with CP score 5/6, 39 (15.6%) with CP score 7, and 37 (14.9%) with CP score 8/9. The study protocol was approved by the Human Ethics Review Committee of Hiroshima University and a signed consent form was obtained from each subject.

HAICs. Patients received repeated arterial infusions of anticancer agents via the injection port. Two drug regimens were used for HAIC. Intra-arterial low-dose cisplatin (CDDP, Nihonkayaku, Tokyo, Japan) combined with 5- fluorouracil therapy (FP) (5FU, Kyowa Hakko, Tokyo) or intra-arterial 5FU with subcutaneous interferon (IFN) combination therapy (5FU/IFN). One course of chemotherapy lasted 2 weeks. 5FU (300 mg body weight/day) was administered over 24 h using a mechanical infusion pump from days 1 to 5 of the first and second weeks in both regimens. In addition to 5FU, FP chemotherapy included daily intra-arterial CDDP (6 mg/body on day 1–5 and 8–12). The IFN used in the 5FU/IFN regimen was recombinant IFNα-2b (Intron A, Schering-Plough Pharmaceuticals, Osaka, Japan, 3×10^6 U [3 MU]), or natural IFN-α (OIF, Otsuka Pharmaceuticals, Tokyo, 5×10^6 U [5 MU]) administered intramuscularly on days 1, 3, and 5 of each week (total dose, 36 and 60 MU, respectively). We have reported previously similar effects of recombinant IFNα-2b and natural IFN-α when 5FU/IFN was used for the treatment of advanced HCC.[22] FP was provided to 106 patients between June 2000 and June 2003 and also between July 2008 and April 2010. On the other hand, 143 patients received 5FU/IFN between June 2003 and July 2008 and April 2010 and June 2011. In principle, treatment was repeated several times unless PS changed to 3 or 4 during the treatment. A 2- to 4-week rest period of no treatment was allowed after each treatment course.

Implantation of arterial catheter. A catheter was inserted through the right femoral artery by the Seldinger method. After localization of the HCC, a 3-French heparin-coated catheter was inserted and its tip was advanced to the common hepatic artery or proper hepatic artery. The other end of the catheter was connected to the injection port, which was implanted in a subcutaneous pocket created in the right lower abdominal quadrant. The gastroduodenal artery and right gastric artery were occluded with steel coils to prevent gastroduodenal injury by the chemotherapeutic agents.

Evaluation. The response to the combination therapy was assessed in all patients after two courses of HAIC by contrast-enhanced computed tomography (CT). The response was defined according to the criteria of the New response evaluation criteria in solid tumors (RECIST) guideline version 1.1.[23] We separately assessed the response of intrahepatic HCC and extrahepatic metastases. The prognostic factors were examined in all patients enrolled in this study with intrahepatic HCC. The response to therapy was also assessed separately for patients with intrahepatic HCC and those with extrahepatic metastases. Adverse reactions were assessed with the Common Terminology Criteria for Adverse Events (CTCAE; version 4.0) every week during the treatment.

Additional therapy. According to the response to therapy, we provided various additional therapies such as RFA, TACE, or radiotherapy. These additional therapies were considered for patients with PS of 0–1. Patients assessed with partial response (PR) continued to receive the combination therapy repeatedly. Then, when advanced HCC was downstaged to single tumor $\leq$ 50 mm in diameter or 1–3 tumors $\leq$ 30 mm in diameter by the repeated combination therapy, RFA was considered. In addition to the combination therapy, patients assessed to have stable disease (SD) or progressive disease (PD) received TACE with cisplatin–lipiodol suspension. The catheter tip was advanced superselectively into the feeding artery to deliver sufficient dose of the anticancer agent. Among the patients assessed with SD or PD, radiotherapy was applied for PVTT if present. For patients assessed with complete response (CR), the clinical course was observed without adjuvant chemotherapy or additional therapy.

Statistical analysis. Statistical analysis was performed in September 2011. Differences between groups were examined for statistical significance using the Mann–Whitney *U*-test, logistic regression test, or squared test as appropriate. The cumulative survival rate was calculated from the date of initiation of the combination therapy and assessed by the Kaplan–Meier life-table method, and differences were evaluated by the log rank test. Univariate and multivariate analyses of determinants of the response to HAIC were assessed by the logistic regression test. Univariate analysis of the factors that influenced survival of patients with HCC treated with HAIC was assessed by the Kaplan–Meier life-table method, and differences were evaluated by the log rank test. Multivariate analysis of the factors that influenced survival was assessed by the Cox proportional hazard model. Statistical significance was defined as *P*-value of less than 0.05. All analyses described above were performed with SPSS software (version 11, SPSS, Chicago, IL, USA).

Results

Patient characteristics. Patient characteristics are listed in Table 1. The study subjects were 214 men and 35 women, with the median age of 66 years (range, 26–85 years). The background liver diseases were hepatitis C viral (HCV) infection (n = 132), hepatitis

Table 1 Clinical characteristics of 249 HCC patients treated with HAIC according to Child–Pugh score

	All patients ($n = 249$)	CP score 5,6 ($n = 173$)	CP score 7 ($n = 39$)	CP score 8,9 ($n = 37$)	*P*-value
Age (years)†	66 (26–85)	66 (30–85)	67 (35–77)	63.5 (26–76)	0.23
Gender (M/F)	214/35	151/22	32/7	31/6	0.64
EOCG performance status (0/1/2)	177/64/8	136/34/3	26/10/3	15/20/2	< 0.0001
Etiology (HBV/HCV/others)	66/132/51	46/91/36	6/22/11	14/19/4	0.16
HCC stage (II/III/IVa/IVb)‡	6/43/140/60	5/35/97/36	0/2/27/10	1/6/16/14	0.08
Vp (0/2/3/4)§	65/50/70/64	49/39/50/35	6/9/10/14	10/2/10/15	0.03
Vv (0/2/3)¶	204/17/28	142/11/20	35/2/2	27/4/6	0.43
Rate of tumor occupation in the liver (< 50%/≥ 50%)	157/92	115/58	24/15	18/19	0.12
AFP (ng/mL)†	934 (5–1 895 000)	431 (5–1 895 000)	1 077 (7–593 350)	3 514 (5–708 100)	0.70
DCP (mAU/mL)†	2 359 (10–1 170 900)	2 773 (101–170 900)	1 196 (16–722 140)	2 060 (10–634 690)	0.82
Previous treatment (Yes/No)	124/125	77/96	27/12	20/17	0.02
Additional therapy	162/87	123/50	20/19	19/18	0.01
Regimen (FP) (5FU + IFN)	106/143	69/104	20/19	17/20	0.39

†Data are expressed as median with range values in parentheses, or number of patients.
‡According to the Liver Cancer Group of Japan.
§Portal invasion.
¶Venous invasion.
5FU + IFN, intra-arterial 5-FU with IFN combination therapy; AFP, α-fetoprotein; CP, Child–Pugh stage; DCP, des-γ-carboxy prothrombin; EOCG performance status: Eastern Cooperative Oncology Group performance status; FP, intra-arterial low dose cisplatin and 5FU therapy; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; Vp2, tumor thrombus in a second branch of the portal vein; Vp3, tumor thrombus in a first branch of the portal vein; Vp4, tumor thrombus in the trunk of the portal vein; Vv1, tumor thrombus in peripheral branches of hepatic vein; Vv2, tumor thrombus in the right, middle or left hepatic vein trunk, posterior inferior hepatic vein trunk or short hepatic vein; Vv3, tumor thrombus in inferior vena cava.

Table 2 Response to hepatic arterial infusion chemotherapy (HAIC) treatment

	All patients	CP score 5/6	CP score 7	CP score 8/9	*P*-value		
	$n = 249$	$n = 173$	$n = 39$	$n = 37$	CP score 5/6 *vs* 7	CP score 7 *vs* 8/9	CP score 5/6 *vs* 8/9
CR	11	9	2	0	—	—	—
PR	57	43	9	5	—	—	—
SD	83	63	10	10	—	—	—
PD	67	44	13	10	—	—	—
DO	31	14	5	12	—	—	—
RR	27.3%	30.5%	28.2%	13.8%	1.00	0.16	0.04
DOR	12.4%	8.0%	12.8%	33.3%	0.35	0.05	< 0.0001

CR, complete response; DO, drop-out; DOR, Dropout rate = DO/CR + PR + SD + PD + DO; PD, progressive disease; PR, partial response; RR, response rate = CR + PR/CR + PR + SD + PD + DO; SD, stable disease.

B viral (HBV) infection ($n = 66$), and non-HCV-non-HBV ($n = 51$). With regard to PVTT and tumor stage, PVTT grade, based on the location of the tumor thrombus, was determined according to the criteria of the Liver Cancer Study Group of Japan (LCSGJ).[21] Seventy patients were Vp3 with tumor thrombus in the first branch of the portal vein, and 64 patients with Vp4 and tumor thrombus in the trunk of the portal vein. Sixty patients had distant metastases (Stage IVb). Rate of tumor occupation in the liver > 50% of the liver in 92 patients, and 124 patients had previously received other treatments for HCC (surgery, PEI, RFA or TACE). There were no statistically significant differences in the clinical characteristics of the three groups (CP score 5/6, 7 and 8/9), except for EOCG PS, PVTT and previous treatments.

Response to treatment. The number of HAIC courses ranged from 1 to 10 (median: 2 courses). CR, PR, SD, PD, and drop-out were noted in 11 (4.4%), 57 (22.9%), 83 (33.3%), 67 (26.9%), and 31 (12.4%) patients, respectively, with an overall response rate of 27.3%. The response rate was lower in patients with CP score 8/9 than those with CP score 5/6 and 7. The reasons for drop-out were hepatic failure ($n = 14$), device-related ($n = 5$), depression ($n = 2$), refusal after initiation of therapy ($n = 3$), allergic reaction to cisplatin ($n = 3$), and acute renal failure ($n = 1$). The drop-out rate was significantly higher in patients with CP score 8/9 than those with score 5/6 or 7 (Table 2).

Determinants of response to HAIC. Univariate analysis identified HCV antibody positivity ($P = 0.001$), the rate of tumor occupation in the liver ($P = 0.003$), platelet count ($P = 0.004$), and Child–Pugh score ($P = 0.048$) to be significantly associated with the response to HAIC (CR and PR) (Table 3). The above factors were entered in multivariate analysis, which identi-

Table 3 Results of univariate and multivariate analysis for factors that modulated the response to treatment

Parameters	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
Age (< 65/≥ 65)	0.813	0.461–1.434	0.474	—	—	—
Gender (M/F)	1.595	0.662–3.844	0.298	—	—	—
ECOG performance status (0/1,2)	1.514	0.864–2.653	0.147	—	—	—
HCV antibody (positive/negative)	2.771	1.524–5.038	0.001	2.436	1.315–4.513	0.005
Child–Pugh score (5,6,7/8,9)	2.704	1.007–7.256	0.048	2.721	0.993–7.462	0.05
Rate of tumor occupation in the liver (< 50%/≥ 50%)	2.616	1.373–4.983	0.003	—	—	—
Vp (0,2/3,4)	1.050	0.600–1.835	0.865	—	—	—
Vv (0/2,3)	1.927	0.847–4.381	0.118	—	—	—
Extrahepatic metastasis (Yes/No)	1.502	0.780–2.894	0.224	—	—	—
Platelet (< 15×10^4/≥ 15×10^4/μL)	2.607	1.349–5.036	0.004	2.170	1.098–4.289	0.026
AFP (< 1000/≥ 1000 ng/mL)	0.673	0.384–1.181	0.168	—	—	—
DCP (< 1000/≥ 1000 mAU/mL)	0.988	0.561–1.741	0.967	—	—	—
Previous treatment (Yes/No)	1.261	0.721–2.206	0.416	—	—	—
Regimen (FP/5FU + IFN)	1.514	0.864–2.653	0.147	—	—	—

5FU + IFN, intra-arterial 5-FU with IFN combination therapy; AFP, α-fetoprotein; CI, confidence interval; DCP, des-γ-carboxy prothrombin; EOCG performance status, Eastern Cooperative Oncology Group performance status; Vp2, tumor thrombus in a second branch of the portal vein; Vp3, tumor thrombus in a first branch of the portal vein; Vp4, tumor thrombus in the trunk of the portal vein; Vv1, tumor thrombus in peripheral branches of hepatic vein; Vv2, tumor thrombus in the right, middle or left hepatic vein trunk, posterior inferior hepatic vein trunk or short hepatic vein; Vv3, tumor thrombus in inferior vena cava.

fied HCV antibody positivity ($P = 0.005$, odds ratio [OR] 2.436, 95% confidence interval [CI] 1.315–4.513) and platelet count ($P = 0.026$, OR 2.170, 95%CI 1.098–4.289), as significant and independent determinants of the response to HAIC (Table 3). The CP score tended to influence the response with border line statistical significance ($P = 0.052$, OR 2.721, 95%CI 0.993–7.462).

Overall survival. The median survival time (MST) for all patients was 8.2 months, and the cumulative survival rates at 6, 12, 24, and 36 months were 62, 37, 19 and 13%, respectively (Fig. 1a). The overall survival (OS) rate was significantly longer in patients with CP score 5/6 than those with scores of 7/8 and 9. The MST for patients with CP 5/6, 7 and 8/9 was 9.7, 6.3 and 3.9 months, respectively ($P < 0.0001$, Fig. 1b). The survival rate was significantly higher in patients who achieved CR/PR (responders) than in other patients with CP score 5/6 (MST: 25.4 *vs* 7.3 months, respectively, $P < 0.0001$), 7 (MST 15.2 *vs* 4.4 months, respectively, $P = 0.0001$). However, there was no difference between patients with CP score 8 and 9 (MST 9.2 *vs* 2.5 months, respectively, $P = 0.19$, Fig. 2a–c).

Prognostic factors affecting overall survival. We investigated the prognostic factors that affected overall survival. Univariate analysis demonstrated that treatment response ($P < 0.0001$), CP score ($P < 0.0001$), ECOG performance status ($P < 0.0001$), the proportion of tumor size (relative to that of the liver) ($P < 0.0001$), systemic metastases ($P < 0.0001$), platelet count ($P = 0.0009$), α-fetoprotein (AFP) ($P = 0.0002$), HCV antibody positivity ($P = 0.002$), additional therapy ($P = 0.0035$) and venous tumor thrombosis ($P = 0.05$) were significantly related to OS (Table 4). Multivariate analysis using these factors identified treatment response ($P < 0.0001$, HR 3.408, 95%CI 2.418–4.802), AFP ($P < 0.0001$, HR 1.948, 95%CI 1.463–2.593), EOCG performance status ($P < 0.0001$, HR 1.854, 95%CI 1.332–2.580), systemic metastasis ($P < 0.0001$, HR 1.776, 95%CI 1.287–2.451), CP score ($P = 0.036$, HR 1.540, 95%CI 1.029–2.304), additional therapy ($P < 0.033$, HR = 1.382, 95%CI 1.026–1.862) as significant and independent determinants of OS (Table 4).

Adverse events. The most common side effects of HAIC were fever, nausea, and loss of appetite, but these were mostly CTCAE grade 1/2. The only CTCAE grade 3/4 side-effect was hepatic failure, and its incidence was dependent on the CP score (Table 5). Other complications were device-related complications in 15 patients (6.0%), including subcutaneous hematoma (three patients), lymphorrhoea (two patients), infection (four patients), port device trouble (one patient), catheter dislocation (one patient), and artery occlusion (one patient). The devices-related complications tended to be less common in patients with CP 5/6 than CP 7 or 8/9, (4.6% *vs* 10.2% *vs* 8.1%).

Additional therapy. Among the 249 patients, 162 patients received additional therapies. Specifically, three (1.2%), three (1.2%), three (1.2%), 75 (30.1%), 51 (20.4%), 36 (14.4%), and nine (3.6%) patients received RFA, PEI, resection, TACE, RT for PVTT, S1-based chemotherapy, and radiotherapy for extrahepatic metastasis, respectively. Patients with CP score 5/6 were more likely to receive additional therapies than those with score 7 and 8/9 (71.0% *vs* 51.2% *vs* 51.5%, respectively, $P = 0.01$). HAIC responders were more likely to receive additional therapies than non-responders (80.8% *vs* 59.1%, retrospectively, $P = 0.002$).

Discussion

Previous retrospective studies reported poor response of patients with CP B cirrhosis to HAIC, with a response rate of 16.7%[19] and

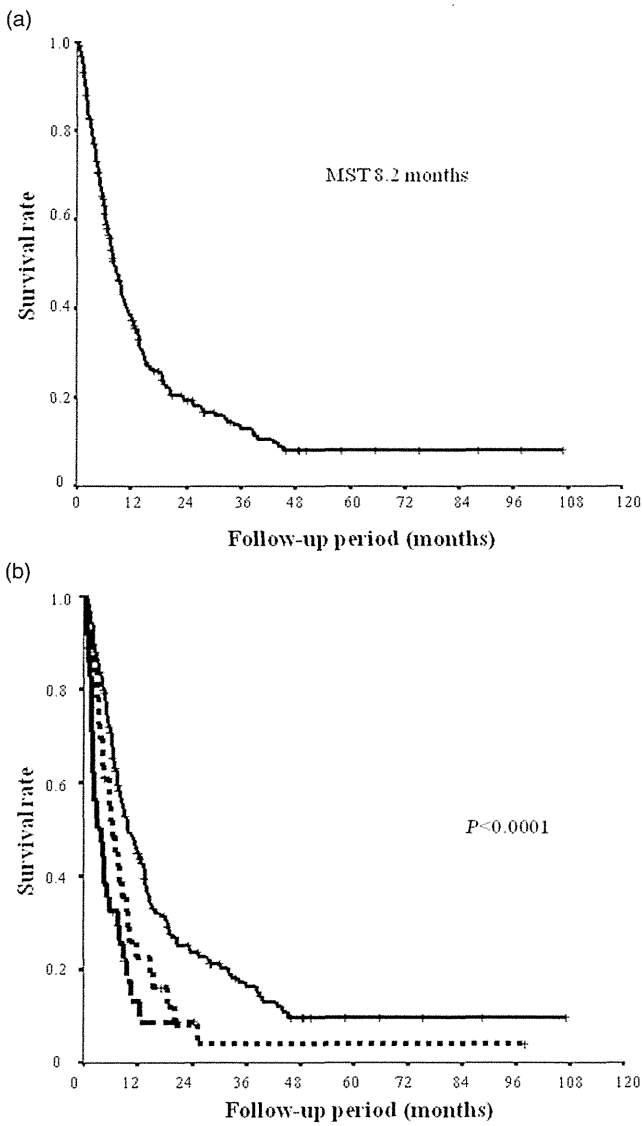

Figure 1 (a) Overall survival rate for all patients. Cumulative survival rate of 249 patients with advanced hepatocellular carcinoma (HCC) treated with hepatic arterial infusion chemotherapy (HAIC). The 6, 12, 24, and 36 months were 62, 37, 19, and 13%, respectively. The median survival time was 8.2 months. MST, median survival time. (b) Overall survival according to CP score. Cumulative survival rates of patients according to the CP score. The 6, 12, 24, and 36 months cumulative survival rates were 70, 44, 22, and 15%, respectively, for patients with CP score 5/6. The MST was 9.7 months. The 6, 12 and 24 months cumulative survival rates for patients with CP score 7 were 51, 22, and 4%, respectively. The MST was 6.3 months. The respective cumulative survival rates for patients with CP score 8/9 were 32, 8, and 8%. The MST was 3.9 months. There were significant difference between the three groups ($P < 0.0001$). ——, Child–Pugh (CP) 5/6; ·····, CP 7; ----, CP 8/9.

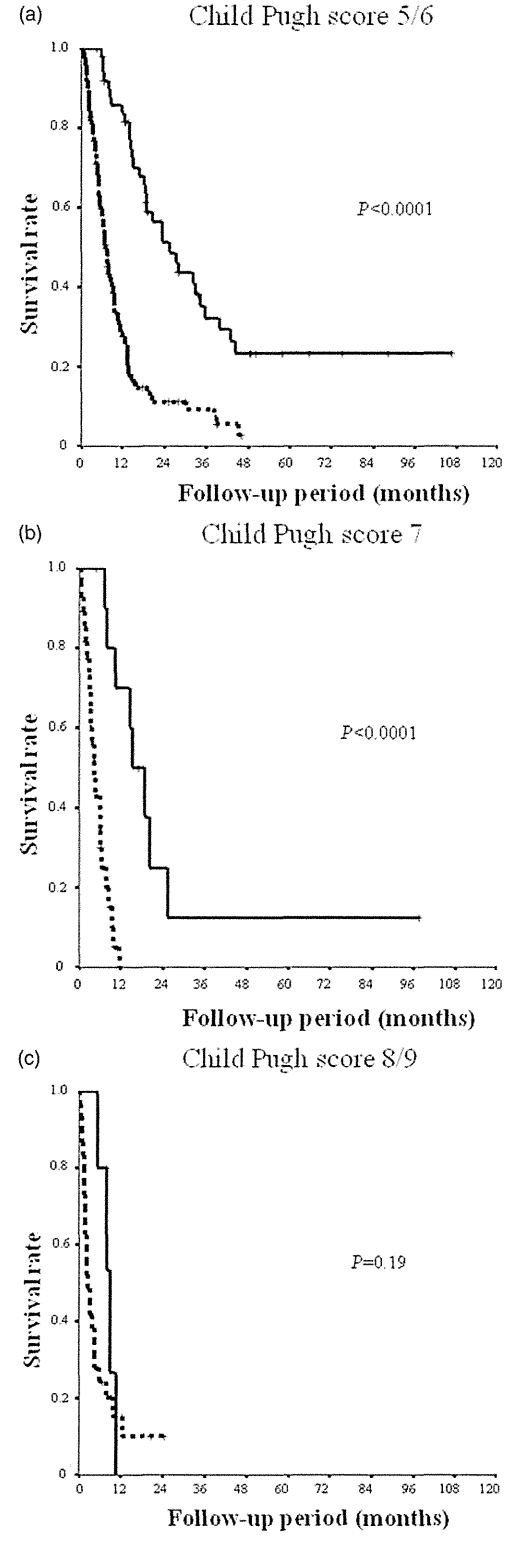

Figure 2 Comparison of the cumulative survival rates among patients with complete/partial responders (CR/PR) and stable disease (SD)/progressive disease (PD)/and dropout (DO) according to Child–Pugh score. The survival rate was significantly higher in CP 5/6 and 7 patients who achieved CR/PR than those who showed SD/PD/DO ($P < 0.0001$). This was not true for those with CP score 8/9 ($P = 0.19$). ——, CR, PR; ----, SD, PD, DO.

Table 4 Univariate and multivariate analysis of factors that influenced survival

Parameters	Univariate analysis		Multivariate analysis		
		P-value	Hazard ratio	95% CI	*P*-value
Age (< 65/≥ 65)	141/108	0.23	—	—	—
Gender (M/F)	214/35	0.35	—	—	—
ECOG performance status (0/1,2)	177/72	< 0.0001	1.854	1.332–2.580	< 0.0001
HCV antibody (positive/negative)	183/66	0.002	—	—	—
Child–Pugh score (5/6/7 and 8/9)	212/37	< 0.0001	1.540	1.029–2.304	0.036
Rate of tumor occupation in the liver (< 50%/≥ 50%)	157/92	< 0.0001	—	—	—
Vp (0,2/3,4)	115/134	0.07	—	—	—
Vv (0/2,3)	200/43	0.05	—	—	—
Extrahepatic metastasis (Yes/No)	69/180	< 0.0001	1.776	1.287–2.451	< 0.001
Platelet (< 15×10^4/≥ 15×10^4/μL)	162/87	0.0009	—	—	—
AFP (< 1000/≥ 1000 ng/mL)	124/125	0.0002	1.948	1.463–2.593	< 0.0001
DCP (< 1000/≥ 1000 mAU/mL)	102/147	0.47	—	—	—
Previous treatment (Yes/no)	124/125	0.23	—	—	—
Regimen (FP/5FU + IFN)	106/143	0.80	—	—	—
Additional therapy (Yes/no)	162/87	0.0035	1.382	1.026–1.862	0.033
Treatment effect (CR,PR/SD,PD,DO)	68/181	< 0.0001	3.408	2.418–4.802	< 0.0001

5FU + IFN, intra-arterial 5-FU with IFN combination therapy; AFP, α-fetoprotein; CI, confidence interval; CR, complete response; DCP, des-γ-carboxy prothrombin; DO, drop-out; EOCG performance status: Eastern Cooperative Oncology Group performance status; PD, progressive disease; PR, partial response; SD, stable disease; Vp2, tumor thrombus in a second branch of the portal vein; Vp3, tumor thrombus in a first branch of the portal vein; Vp4, tumor thrombus in the trunk of the portal vein; Vv1, tumor thrombus in peripheral branches of hepatic vein; Vv2, tumor thrombus in the right, middle or left hepatic vein trunk, posterior inferior hepatic vein trunk or short hepatic vein; Vv3, tumor thrombus in inferior vena cava.

Table 5 Proportion of patients who developed Common Terminology Criteria for Adverse Events (CTCAE) grade 3/4 adverse events according to Child–Pugh (CP) score

	All patients $n = 249$	CP score 5/6 $n = 173$	CP score 7 $n = 39$	CP score 8/9 $n = 37$	*P*-value CP score 5/6 *vs* 7	CP score 7 *vs* 8/9	CP score 5/6 *vs* 8/9
Leukopenia	9.2% ($n = 23$)	8.0% ($n = 14$)	12.8% ($n = 5$)	11.1% ($n = 4$)	0.35	1.00	0.53
Neutropenia	6.4% ($n = 16$)	5.7% ($n = 10$)	7.6% ($n = 3$)	8.3% ($n = 3$)	0.71	1.00	0.70
Anemia	2.4% ($n = 6$)	2.3% ($n = 4$)	2.5% ($n = 1$)	2.7% ($n = 1$)	1.00	1.00	1.00
Thrombocytopenia	9.2% ($n = 23$)	7.5% ($n = 13$)	15.3% ($n = 6$)	11.1% ($n = 4$)	0.12	0.73	0.51
Fever	0.4% ($n = 1$)	0% (n = 0)	2.5% ($n = 1$)	0% ($n = 0$)	0.18	1.00	–
Anoxia	3.6% ($n = 9$)	3.4% ($n = 6$)	2.5% ($n = 1$)	5.5% ($n = 2$)	1.00	0.61	0.63
Nausea	0.4% ($n = 1$)	0% ($n = 0$)	0% ($n = 0$)	2.7% ($n = 1$)	–	0.48	0.17
Fatigue	0.8% ($n = 2$)	1.1% ($n = 2$)	0% ($n = 0$)	0% ($n = 0$)	1.00	–	1.00
Hepatic failure	7.2% ($n = 18$)	2.8% ($n = 5$)	7.6% ($n = 3$)	27.7% ($n = 10$)	0.16	0.03	< 0.0001

median survival time of 6.7–7.4 months.[13,18] However, there is no specific information on the efficacy and toxicity of HAIC in patients with CP B scores ranging from 7 to 9, and whether the prognosis of patients with CP score 7 is similar or different to that of patients with CP score 5/6. For this reason, patients were divided in this study into three groups according to the CP score (5/6, 7 and 8/9).

In this retrospective cohort study, we assessed the treatment response, survival and safety of HAIC in patients with advanced HCC and PVTT, VTT, systemic metastases and those refractory to TACE. The MST was 8.2 months for all patients, and the cumulative survival rates at 6, 12, 24, and 36 months were 62%, 37%, 19% and 13%, respectively. The MST for patients with CP 5/6, 7 and 8/9 was 9.7, 6.3 and 3.9 months, respectively ($P < 0.0001$). These results indicated that worsening liver function was associated with poor prognosis. The survival rate was significantly higher in patients who achieved CR/PR (responders) than other patients (MST 20.2 *vs* 5.9 months, respectively, $P < 0.0001$). Further analysis according to CP score showed that the prognosis correlated with CP score 5/6 and 7 ($P < 0.0001$, each). However, the responders did not survive longer than those with CP score 8/9 ($P = 0.19$). Furthermore, the drop-out and hepatic failure rates were significantly higher in patients with CP score 8/9 than those with score 5/6 and 7. The results of multivariate analysis demonstrated that the CP score 8/9 was an independent factor for no response to treatment and poor survival. Previous analyses have shown poor response in patients with CP B, These findings indicate that patients with CP scores 5/6 and 7 are better suited for HAIC than those with scores 8/9, and that the use of this treatment modality is limited in patients with CP score 8/9. HAIC is based on the delivery of 5FU via the hepatic artery. However, 5FU is known to exacerbate liver damage by inducing liver fibrosis.[19] Further-

more, there is no adequate hepatic reserve in patients with CP score 8/9, thus limiting the use of HAIC in such patients.

Sorafenib is an approved therapeutic agent for advanced HCC worldwide based on double-blind, placebo-controlled trials,[7,24] although it should be used with caution in patients with CP class B due to poor survival and hepatic failure. Previous retrospective analyses demonstrated that the efficacy of sorafenib was worse in patients with CP B cirrhosis, with OS rate of less than 5 months.[9,25] Kim *et al.*[8] reported that the efficacy of sorafenib and survival outcome were poorer in patients with CP class B, while the rate of adverse events in patients with CP score 7 were similar to cirrhosis-related complications seen in those with CP A liver function. Furthermore, many of their patients with CP score 8/9 (26.3%) had to stop sorafenib due to cirrhosis-related complications. These results point to the suitability of inclusion of patients with CP score of 7 in clinical trials of new agents such as sorafenib. Sorafenib is primarily metabolized in the liver through oxidation by cytochrome P-450 3A 4 (CYP3A4), and through glucuronidation by uridine diphosphate-glucuronosyl-transferase (UGT)1A9. Impaired metabolism of sorafenib in patients with liver dysfunction is believed to lead to higher drug level and high frequency of dose-related toxicities.[10]

Hepatic arterial infusion chemotherapy is currently widely used in Asia, especially Japan. Several studies have reported the survival benefits of HAIC for advanced HCC, with a response rate ranging from 12.2% to 52%,[11–17] In all such reports, survival was significantly better in responders than others. CP class B has been identified as an independent prognostic factor,[13,18] although the efficacy and toxicity of HAIC according to CP score was not analyzed thoroughly. Our study demonstrated that the CP score 8/9 was an independent factor for lack of response to treatment and poor survival. Sorafenib is an oral multikinase inhibitor and known to block tumor cell proliferation by targeting Raf/MEK/ERK signaling at the level of Raf kinase, and to have an anti-angiogenic effect by targeting vascular endothelial growth factor receptor beta (VEGFR-β, PDGF-β) tyrosine kinase. Sorafenib is a cytostatic agent known to improve survival rate, though it is associated with a low tumor response rate. 5FU is a cytotoxic agent known to inhibit deoxyribonucleic acid (DNA) synthases, with a response rate of 27.3% in this study. Thus, HAIC is associated with a higher response rate and the survival time and rate of responders may exceed those of sorafenib, although there were no survival benefits for non-responders. We consider that HAIC could be a potentially suitable treatment modality for advanced HCC and be tried before sorafenib.

In this study, univariate analysis identified four factors that influenced the response to HAIC; including HCV antibody positivity, the rate of tumor occupation in the liver < 50%, platelet count < $15 \times 10^4/\mu L$, and CP score 5/6 or 7. It might be meaningful to identify patients with advanced HCC in whom HAIC can potentially improve prognosis before the use of sorafenib treatment. Univariate analysis of determinants of survival identified CP score 8/9, ECOG performance status 1/2, the rate of tumor occupation in the liver > 50%, systemic metastasis, platelet count > $15 \times 10/\mu L$, AFP > 1000 ng/mL, HCV antibody negativity and venous tumor thrombosis, additional therapy, treatment response to be associated with short survival. These results are in agreement with those of other investigators who showed that systemic metastasis was a poor prognostic sign in HAIC-treated patients. On the other hand, patients who responded to HAIC with CP score 5/6 and 7 were more likely to receive additional therapies that could provide better survival.

Hepatic arterial infusion of anticancer drugs allows the delivery of high doses of drugs directly to the hypervascular intrahepatic HCC. We reported previously the limited benefits of HAIC for advanced HCC with Vp3/4 and extrahepatic metastases,[26] and that such limitation is mostly due to the frequent development of extrahepatic metastasis. The benefits of sorafenib have been reported to be consistent in patients with extrahepatic spread.[7,24] Patients with advanced HCC, extrahepatic metastases and the abovementioned factors may have to be treated with sorafenib from the start instead of HAIC.

This study has several limitations. These include data generated from a single institution, and retrospective study design. There was no control group in our study; there is no ability to demonstrate survival benefit.

Our study showed the best response to HAIC in patients with CP score 5/6 or 7, and that the same treatment was not suitable for those with CP score 8/9. Thus, patients with CP score 5/6 or 7 are suitable candidates for HAIC, in addition to sorafenib. Unfortunately, large randomized trials are still lacking, in contrast to the worldwide double-blind, placebo-controlled SHARP trial for sorafenib. HAIC is not yet a well-established treatment modality for advanced HCC. Further randomized clinical studies with a larger sample size should be conducted to compare the separate response to HAIC and sorafenib.

In conclusion, our results demonstrated that advanced HCC patients with CP score 5/6 and 7 showed a better treatment response to HAIC and better prognosis than those with CP score 8/9.

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In daily practice, the concept of fiberoptic intubation in the awake patient is not clearly defined. In most cases, the choice of technique is dependent on institutional and personal preferences. Ultimately, such a choice is a compromise between safety, practicability, and acceptance. The technique as shown in the video is a thoroughly documented, well-tested method that has not been changed for many years.[5]

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Deferoxamine for Advanced Hepatocellular Carcinoma

TO THE EDITOR: We have previously reported that the iron chelator deferoxamine can prevent liver injury as well as the development of preneoplastic lesions in rats,[1,2] and we have proposed the use of deferoxamine as an anticancer drug. The antiproliferative effect of deferoxamine arrests the cell cycle and induces apoptosis.[3] To our knowledge, no clinical study has been performed to evaluate deferoxamine therapy in patients with hepatocellular carcinoma.[4]

Our study involved 10 patients (6 men and 4 women) who had advanced hepatocellular carcinoma and did not have a response to hepatic arterial infusion chemotherapy with anticancer drugs. The average age of the patients was 64 years (range, 43 to 77). Written informed consent was obtained before the study, which was approved by the institutional review board of Yamaguchi University Hospital. Seven patients had hepatitis C virus infection, 2 patients had hepatitis B virus infection, and 1 patient did not have either type of infection. The tumor stages were classified as II, IVA, and IVB (according to the Liver Cancer Study Group of Japan criteria) for 1, 2, and 7 patients, respectively. The Child–Pugh class was A, B, and C for 3, 5, and 2 patients, respectively. (In the Child–Pugh classification of liver disease, class A indicates the least severe disease, class B moderately severe disease, and class C the most severe disease.) The patients received an arterial infusion of deferoxamine (at a dose of 10 to 80 mg per kilogram of body weight) over 24 hours on alternate days, through the injection port.

Deferoxamine was administered an average of 27 times (range, 9 to 78). Two, three, and five patients had a partial response, stable disease, and progressive disease, respectively (according to the Eastern Cooperative Oncology Group criteria). The overall response rate was 20%.

Tumor-marker levels (alpha-fetoprotein, des-γ-carboxyprothrombin, alpha-fetoprotein L3, or all of these levels) decreased in patients with a partial response. In one patient, a massive hepatocellular tumor with lung metastases disappeared with deferoxamine treatment (Fig. 1). The 1-year cumulative survival rate was 20%. Four patients had grade 2 or 3 interstitial pneumonia (according to the Common Terminology Criteria for Adverse Events, version 4.0), and one patient had grade 2 renal dysfunction. However, no grade 4 adverse events were observed.

Sorafenib, a multikinase inhibitor, has recently been established as the standard of care for patients with advanced hepatocellular carcinoma and preserved liver function (Child–Pugh class A) because it increases survival.[5] However, its safety and efficacy for patients with Child–Pugh class B or C disease is still unknown. Deferoxamine may warrant testing in patients with Child–Pugh class B or C hepatocellular carcinoma.

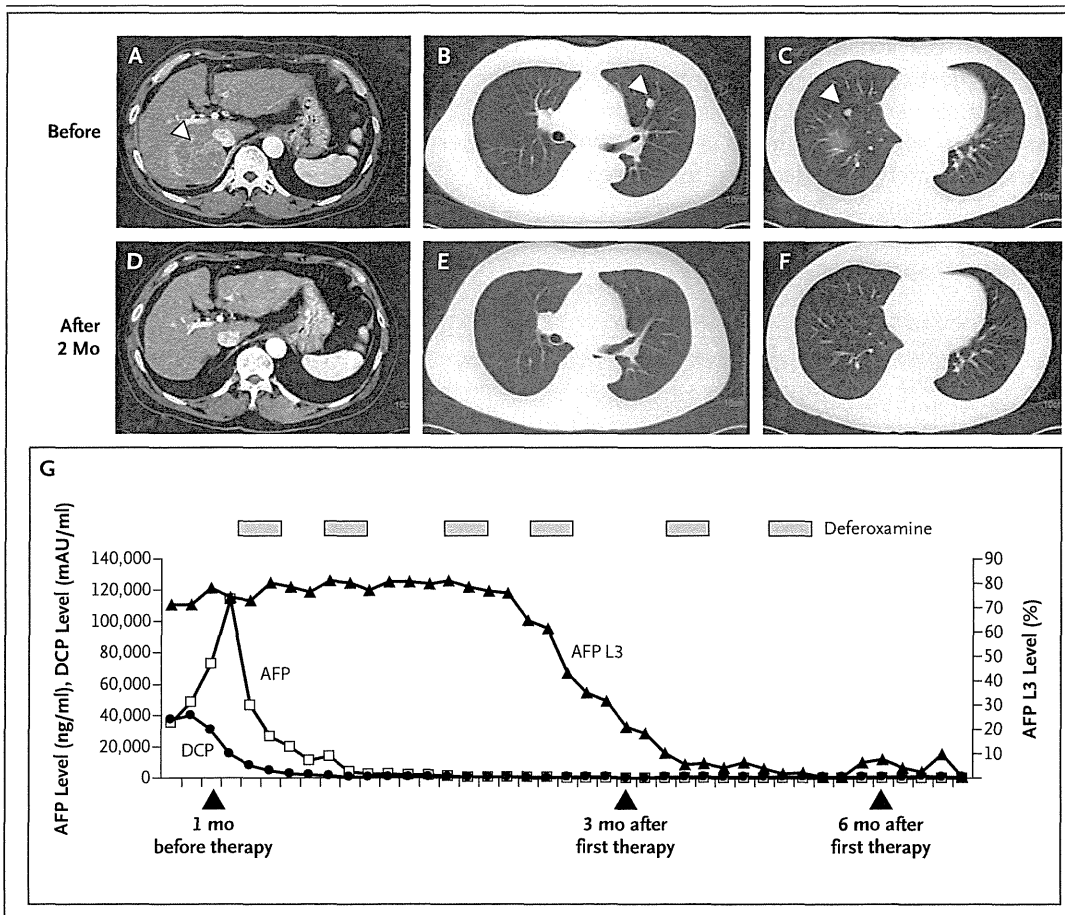

Figure 1. **Deferoxamine Treatment in One Patient.**

A 62-year-old man presented with a massive hepatocellular tumor with multiple small lung metastases (stage IVB). The hepatic reserve function was Child–Pugh class B (a score of 8) before treatment (in the Child–Pugh classification of liver disease, class A [a score of 5 or 6] indicates the least severe disease, class B [7 to 9] moderately severe disease, and class C [10 to 15] the most severe disease). In Panel A, abdominal dynamic computed tomography (CT) before treatment shows a tumor (arrowhead). In Panels B and C, plain chest CT scans obtained before treatment show multiple lung metastases (arrowheads). Two months after arterial infusion of deferoxamine (at a dose of 50 mg per kilogram of body weight per day), the liver tumor and multiple lung metastases were not observed on abdominal dynamic CT (Panel D) and plain chest CT (Panels E and F). As shown in Panel G, after arterial infusion of deferoxamine, the levels of three tumor markers (alpha-fetoprotein [AFP], des-γ-carboxyprothrombin [DCP], and AFP L3) decreased. The patient was considered to have a partial response according to the Eastern Cooperative Oncology Group criteria. The patient survived 20.9 months with deferoxamine treatment.

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Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

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Contact Tamara Rios, Rios Associates, 3729 N. Bay Horse Loop, Tucson, AZ 85719; or call (520) 907-3318; or see http://www.medspanish.org.

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Initial response to sorafenib by using enhancement criteria in patients with hepatocellular carcinoma

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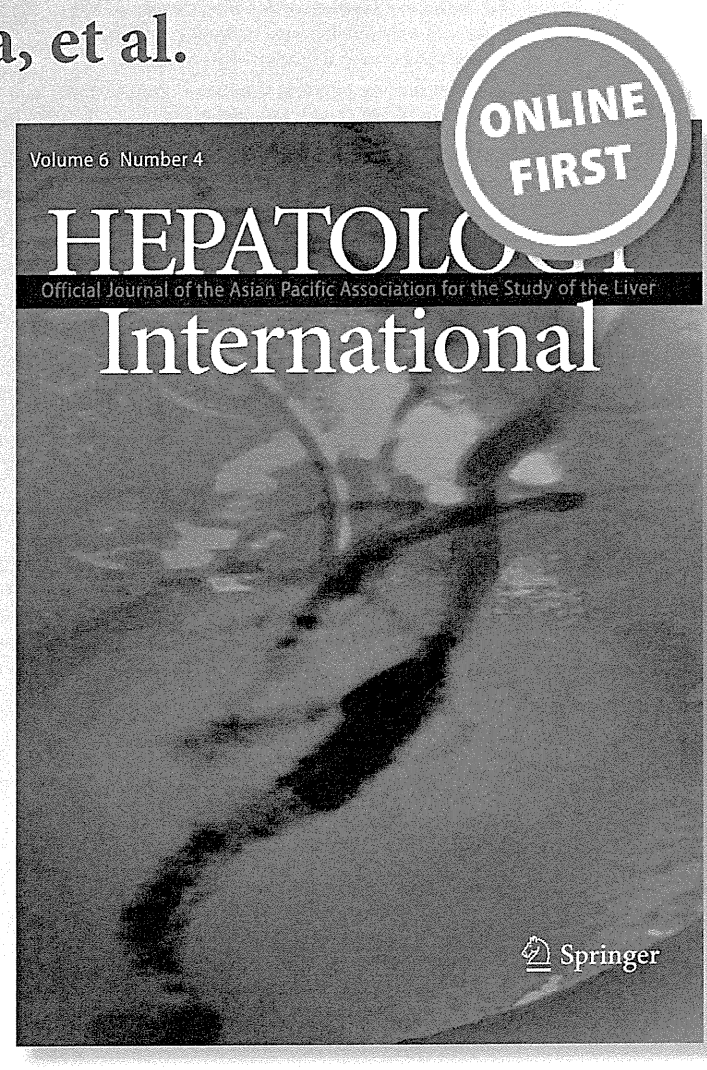

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ORIGINAL ARTICLE

Initial response to sorafenib by using enhancement criteria in patients with hepatocellular carcinoma

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Abstract
Purpose Sorafenib induces early vascularity reduction in patients with hepatocellular carcinoma (HCC). We sought to identify differences in radiological assessment approaches and to evaluate their usefulness for the prediction of the initial response to sorafenib.
Methods Forty-eight patients with advanced HCC treated with sorafenib were evaluated by four-phase contrast-enhanced computed tomography. All target lesions were analyzed using the Response Evaluation Criteria in Solid Tumors (RECIST), the EASL criteria, and modified RECIST (mRECIST).
Results At the initial evaluation at 4–6 weeks, rates of objective response (OR) (including both complete and partial responses), stable disease (SD), and progressive disease (PD) were 2, 71, and 27 %, respectively, according to RECIST; 15, 56, and 29 %, respectively, according to the EASL criteria; and 15, 58, and 27 %, respectively, according to mRECIST. Patients who achieved an OR according to the EASL criteria also achieved an OR according to mRECIST. Patients who achieved an OR according to the EASL criteria or mRECIST had better predicted overall survival (OS) than did patients who achieved SD ($p = 0.033$ and 0.028, respectively). Patients with SD according to RECIST had different outcomes depending on the response according to enhancement criteria. Patients classified as responders (complete and partial) had better predicted OS than those classified as non-responders (those classified as SD and PD) ($p = 0.048$).
Conclusions The enhancement criteria could be useful for prediction of the initial response to sorafenib in patients with HCC. Moreover, mRECIST appears to be simple and convenient.

Keywords Sorafenib · Hepatocellular carcinoma · RECIST · The EASL criteria · mRECIST · Initial response

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and ranks as the third leading cause of neoplasm-related death globally [1]. Although patients with advanced HCC have a poor prognosis, the oral multikinase inhibitor sorafenib has been shown to improve survival in two phase III studies [2, 3]. However, more than half of patients show tumor progression within a few months after initiating sorafenib therapy: the median time to progression (TTP) in the SHARP study and Asia-Pacific trial were 5.5 and 2.8 months, respectively. Furthermore, progression after sorafenib therapy is generally associated with a poor outcome: the median survival times in the above-mentioned phase III trials were 10.7 and 6.5 months. There is a need for treatments to be replaced with sorafenib, or after sorafenib, in patients with advanced HCC. Although several agents with known molecular targets have been developed, survival benefits have not been confirmed in phase III studies [4–8].

In solid tumors, evaluation of the tumor response to any systemic regime relies heavily on objective radiological assessment as outlined by the World Health Organization

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(WHO) criteria [9] and the Response Evaluation Criteria in Solid Tumors (RECIST) [10]. However, despite insubstantial 2 and 5 % objective response (OR) rates in the SHARP and Asia-Pacific studies as assessed by RECIST, sorafenib produced a significant improvement in overall survival (OS) [2, 3]. The molecularly targeted agents sorafenib, sunitinib, imatinib, and axitinib cause an early and extensive reduction in tumor vascularity [11–15]. There have been a number of cases in which sorafenib induced tumor shrinkage or complete disappearance of the tumor stain in HCC [16, 17]. The development of molecularly targeted therapies for gastrointestinal stromal tumor (GIST) and renal cell carcinoma (RCC) preceded that for HCC [18–22]. In these cancers, several approaches were used to simultaneously evaluate tumor size and tumor attenuation by computed tomography (CT) [23–25]. The predictive value of early reduction of tumor vascularity in patients who undergo treatment with sorafenib has not been clarified, in spite of the early changes in vascularity following molecularly targeted therapy.

In patients with early and intermediate-stage HCC, locoregional therapies such as local ablation and transarterial chemoembolization are the standard care options, the treatment efficacy of which is assessed in terms of tumor necrosis. The guidelines for evaluating anatomical tumor diameter do not translate to locoregional therapies. Thus, the European Association for Study of the Liver (EASL) consensus conference on HCC in 2000 recommended that assessment of tumor response should incorporate vascularization of the lesion [26]. The use of EASL guidelines in assessing the response to systemic therapy with cytostatic agents has been proposed [27]. With the EASL criteria, bidimensional measurement is used to estimate intratumoral enhancement on the basis of the WHO criteria. The calculation process is complicated. The use of mRECIST, which, like RECIST, evaluates tumors according to measurement of only the longest axis, to assess HCC—including tumors treated with molecularly targeted therapies—was proposed by AASLD-JNCI (Journal of the National Cancer Institute) [28]. In anatomical assessments, the unidimensional measurement of tumors according to RECIST is sufficient compared to bidimensional measurements and cross products in the WHO criteria [29]. However, it is not clear whether uni- or bidimensional measurements are preferable for the assessment of tumor response for viable lesions. Additionally, there is little or no information on the correlation between prognosis and the initial response to enhancement criteria. The aims of the present study were to evaluate the correlation between the initial response assessed by RECIST, the EASL criteria, and mRECIST, and to study the clinical outcomes in advanced HCC patients treated with sorafenib.

Materials and methods

Patients and treatment

The medical records of patients treated with sorafenib (Nexavar; Bayer Yakuhin, Ltd., Osaka, Japan) for advanced HCC at a single institution in Japan (Chiba University Hospital) between May 2009 and January 2012 were retrieved. The following inclusion criteria were applied: HCC proven histologically or diagnosed according to the current AASLD practice guidelines [30]; Eastern Cooperative Oncology Group Performance Status (ECG-PS) 0 or 1; Child-Pugh A; adequate hematological, hepatic, and renal function (platelet count $\geq 50 \times 10^9$/l, hemoglobin concentration ≥ 8.5 g/l, albumin concentration ≥ 2.8 g/l, total bilirubin concentration ≤ 3.0 mg/dl, alanine and aspartate aminotransferase concentrations ≤ 5 times the upper limit of the normal range (ULN), and a serum creatinine concentration ≤ 1.5 times the ULN); no previous systemic chemotherapy; interval of at least 4 weeks since prior treatment; at least one target lesion in the baseline CT scan with a diameter of not <10 mm. The CT scan was performed within 3 weeks before and at 4–6 weeks after administration of sorafenib for the baseline and initial evaluation, respectively, and was performed after initial evaluation every 2–3 months. α-Fetoprotein (AFP) serum values were determined at baseline and monthly after beginning treatment. In the response assessment by AFP measurement, only patients with baseline AFP values of >20 ng/ml were included.

Sorafenib was administered orally at a dose of 400 mg, twice daily. A dose reduction (to 400 mg once daily or 400 mg every other day) and interruption of sorafenib were allowed, depending on the type and severity of adverse events. We continued administration of sorafenib until intolerable toxicity or disease progression, as defined by RECIST.

Imaging analysis

CT examinations were performed by a four-phase (pre-contrast, arterial, portal, delayed) technique using MDCT system (Aquillion One or Aquillion 64; Toshiba Medical, Tokyo, Japan). The slice thickness was 5 mm, and the table feed was 5 mm. Each patient was injected with 100 ml of a nonionic contrast material (iopamidol, Iopamiron 370; Bayer Yakuhin, Ltd, Osaka, Japan) at a rate of 3.5 ml/s using a power injector (Nemoto Kyorindo Co, Ltd, Tokyo, Japan). The bolus tracking method was used to scan each patient. The hepatic arterial phase, portal phase, and delayed phase spiral scans were automatically started at 18, 45, and 180 s, respectively, after descending aortic enhancement reached a threshold of 100 HU above the pre-contrast aortic attenuation value. The pre-contrast phase,

the early phase, and the portal phase were carried out from the level of the diaphragm to the inferior border of the kidneys. The late phase was carried out from the superior border of the thorax to the pubic symphysis.

Assessment of response

All CT scans were evaluated by two medical oncologists who specialized in HCC. For each patient, a maximum of five delineated tumor target lesions were identified (not more than two lesions per organ). The longest diameter of the tumor lesions was ≥10 mm. Lymph node minor axes had to be ≥15 mm. Radiological response was assessed using RECIST, the EASL criteria, and mRECIST (Table 1) [26, 27, 31]. The EASL criteria are based on the sum of the product of the bidimensional diameters of the enhancing areas of measurable lesions, RECIST criteria are based on the sum of the unidimensional measurements of lesions, and mRECIST criteria are based on the sum of the unidimensional measurements of the arterially enhancing lesions. In cases of discontinuation enhancement or residual multinodular enhancement, the major and minor axes of the largest zone of continuous enhancement were measured (Fig. 1). Lymph node minor axes were measured according to RECIST and mRECIST. Extrahepatic lesions, if present, could also be considered target lesions for the EASL criteria and mRECIST, but without taking into account vascularization. In the event of ascites, a cytologic examination was performed, and progression was retained only after cytologic confirmation of neoplasia.

Tumor response was defined as either a complete response (CR), as defined by complete disappearance of measureable lesions, or a partial response (PR), defined as a 50 % decrease from the baseline sum of the EASL criteria, and a 30 % decrease from the baseline sum of RECIST and mRECIST. For EASL criteria, progressive disease (PD) was defined as a 25 % increase from the smallest sum recorded after treatment was started; for RECIST and mRECIST, PD was defined as a 20 % increase from the smallest sum recorded after treatment was started. The appearance of new lesions denoted PD in all three methods. Stable disease (SD) was defined as being between PD and PR. OR was defined as CR or PR.

The initial AFP response was defined as a decline >20 % from the baseline AFP level within the first 6 weeks of treatment. If more than one AFP level was available during the first 6 weeks after treatment, then the higher level was chosen [32, 33].

Statistical analysis

All analyses were carried out using PASW Statistics, version 18.0. TTP and OS were estimated using the Kaplan-Meier method, and comparisons were made by log-rank test. A conventional two-sided p value of <0.05 was considered statistically significant.

Results

Patient characteristics and follow-up

Of the 99 patients who were treated with sorafenib at our institution, 48 were eligible for inclusion in the study. Fifty-one patients were excluded, of whom 10 had received other systemic therapy before administration of sorafenib, 14 were classified with Child-Pugh B disease, 8 had not undergone a CT scan during the appropriate time period, 9 were evaluated by another modality, such as magnetic resonance imaging (MRI), and 10 had no suitable target lesion.

The characteristics of the 48 eligible patients are summarized in Table 2. Most patients were male (79 %), and the median age was 72 years (range: 47–83). At baseline,

Table 1 Definitions of target lesion radiological response

	RECIST	EASL	mRECIST
CR	Disappearance of all target lesions	Disappearance of any intratumoral arterial enhancement in all target lesions	Disappearance of any intratumoral arterial enhancement in all target lesions
PR	At least 30 % decrease in the sum of the unidimensional diameter of target lesions (reference: baseline sum)	At least 50 % decrease in the sum of the product of bidimensional diameters of viable (enhancement) target lesions (reference: baseline sum)	At least 30 % decrease in the sum of the product of bidimensional diameters of viable (enhancement) target lesions (reference: baseline sum)
SD	Any cases that do not qualify for either PR or PD	Any cases that do not qualify for either PR or PD	Any cases that do not qualify for either PR or PD
PD	An increase of at least 20 % in the sum of the unidimensional diameter of target lesions (reference: smallest sum recorded since treatment started), or new lesions	An increase of at least 25 % in the sum of the product of bidimensional diameters of viable (enhancement) target lesions (reference: smallest sum recorded since treatment started), or new lesions	An increase of at least 20 % in the sum of the product of unidimensional diameters of viable (enhancement) target lesions (reference: smallest sum recorded since treatment started), or new lesions

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

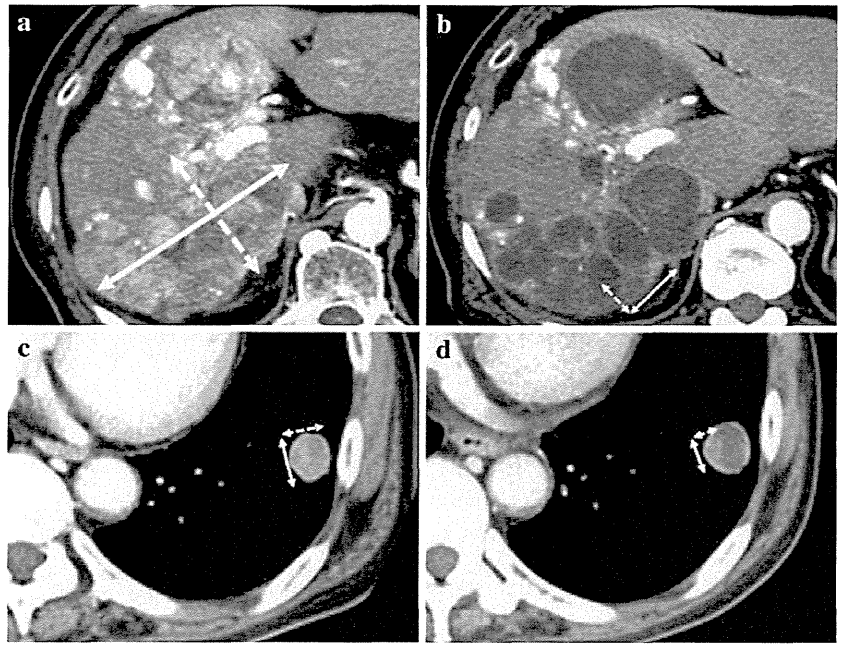

Fig. 1 Two examples of target lesions with enhancement disappearance. Target intrahepatic lesion at baseline (**a**) and the first evaluation (early phase CT) (**b**). Target extrahepatic lesion at baseline (**c**) and the first evaluation (late-phase CT) (**d**). The *solid arrow* indicates the major axis of the enhancement target lesion and the *dotted arrow* the minor axis. EASL criteria were estimated using the sum of the product of the major and minor axes. mRECIST were estimated using the sum of the major axis

32 patients (67 %) had an ECOG PS of 0, 32 (67 %) had a Child-Pugh score of 5, and 27 (56 %) were seropositive for hepatitis C antibody (HCV-Ab). Ten (21 %) had macrovascular invasion, 18 (38 %) had extrahepatic metastasis, and 23 (48 %) were classified as BCLC stage C. The median AFP value was 96.0 ng/ml (range: 2.7–83,250). Forty-five patients had received prior treatments. Thirteen (27 %) had undergone surgery, 18 (38 %) local ablation, and 41 (85 %) transarterial chemoembolization. Data collection ended at the end of August 2012. The median follow-up was 8.7 months (range: 2.9–38.7 months).

Radiological response

For RECIST assessment, 100 tumor lesions were identified as target lesions and 81 as intrahepatic lesions. For the EASL criteria and mRECIST assessment, 91 tumor lesions were identified as target lesions and 79 as intrahepatic lesions (Table 3). The time point response at initial evaluation and the best radiological response, as assessed by RECIST, the EASL criteria, and mRECIST, are shown in Table 4. At initial evaluation according to RECIST, only one patient achieved an OR. Two additional patients achieved an OR at a later time point. At initial evaluation according to the EASL criteria and mRECIST, seven patients achieved an OR; no additional patients achieved an OR at a later time point. Figure 2 shows the change in the sum of target lesion sizes between baseline and initial evaluation according to RECIST, the EASL criteria, and mRECIST. RECIST missed six of seven patients who achieved an OR according to the EASL criteria and mRECIST at initial evaluation. The seven patients who achieved OR according to the EASL criteria at initial evaluation also achieved OR according to mRECIST.

During the follow-up period, 41 patients showed disease progression according to RECIST and mRECIST, and 42 patients according to the EASL criteria. Of these, 13 had PD at initial evaluation according to RECIST and mRECIST, while 14 had PD according to the EASL criteria. Thirteen patients had new lesions at initial evaluation. Although the same patients were classified as PD according to RECIST and mRECIST, one patient classified as SD according to RECIST and mRECIST was classified as PD according to the EASL criteria. Median TTP was 2.9 months (95 % CI, 1.2–4.7) according to RECIST, 2.9 months (95 % CI, 1.1–4.6) according to the EASL criteria, and 2.9 months (95 % CI, 1.2–4.7) according to mRECIST. There were no remarkable differences in TTP according to assessment approach.

Survival analysis

By the end of the study period, 32 patients had died. The overall median OS was 11.5 months (95 % CI, 6.4–16.7). Median OS for patients who achieved an OR and were classified as SD or PD at initial evaluation was undefined (one patient), 16.4 months (95 % CI, 11.3–21.4; $n = 34$), and 4.1 months (95 % CI, 3.4–4.8; $n = 13$), respectively,