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High cholinesterase predicts tolerance to sorafenib treatment and improved prognosis in patients with transarterial chemoembolization refractory intermediate stage hepatocellular carcinoma

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Abstract. Although sorafenib is the standard treatment for patients with advanced hepatocellular carcinoma (HCC), the predictive factors sorafenib tolerance in intermediate-stage HCC cannot be accurately determined. The aim of the current study was to identify the predictive characteristics for the continuation of sorafenib treatment (≥400 mg) in patients with transarterial chemoembolization (TACE)-refractory intermediate HCC and to identify candidates for second-line sorafenib treatment. A total of 33 TACE-refractory intermediate patients with HCC that were treated with sorafenib, and who had reached progressive disease (PD), were analyzed in the present retrospective study. Of 33 patients, 6 patients (18.1%) were able to continue sorafenib treatment (≥400 mg) until PD, however, a total of 27 patients (71.9%) were unable to continue treatment (<400 mg). The current study compared the baseline characteristics parameters to sorafenib ≥400 mg and <400 mg using a logistic regression model. The overall survival (OS) of patients receiving sorafenib ≥400 mg treatment was significantly increased compared with patients receiving sorafenib treatment <400 mg [554.5 days (228-674) vs. 219 days (134-369); P=0.0315). A univariate analysis was performed and indicated that Age (<75 years; P=0.021), total cholesterol (>180 mg/dl; P=0.026) and cholinesterase (ChE; ≥220 U/l; P=0.024) were significant factors, and a multivariate analysis indicated that ChE (≥220 U/l) was a significant prognostic factor (HR: 11.9; 95% CI: 1.19-118.0; P=0.004). Both progression-free survival [279 (204-403) vs. 117.5 (63-197) days; P=0.0136] and OS [470 (277-679) vs. 171.5 (80-236) days; P=0.0004] were significantly increased in patients with ChE levels ≥220 U/l compared with patients exhibiting ChE levels <220 U/l. Baseline high value of ChE in intermediate-stage HCC predicts the ability to continue sorafenib treatment at ≥400 mg.

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Key words: hepatocellular carcinoma, transarterial chemoembolization refractory, intermediate, sorafenib, second-line

Introduction

Hepatocellular carcinoma (HCC) is the second most common cause of cancer-related death worldwide (1). Several studies have reported the clinical benefits of sorafenib in patients with advanced HCC, and sorafenib is currently the only standard therapy in many countries for patients with advanced HCC (2-4). Furthermore, sorafenib improves survival in intermediate-stage HCC patients not eligible for transarterial chemoembolization (TACE) or who experienced progression after TACE (5-10). Ohki *et al* reported that the therapeutic strategy of early switching to sorafenib with TACE as maintenance therapy prolonged progression-free survival compared with repeated conventional TACE monotherapy in HCC patients unresponsive to TACE (9,10). However, since some patients often become less responsive to sorafenib, other second-line treatments are necessary. Regorafenib has been established as a second-line treatment for patients who experienced disease progression on sorafenib (11). However, administration of regorafenib for HCC is confined to patients with good tolerability of sorafenib ≥400 mg, defined as having received sorafenib ≥400 mg/day for at least 20 of the last 28 days of treatment, and Child-Pugh class A. These criteria apply to only a small proportion of HCC patients in clinical practice (12). If we can predict the subset of HCC patients fulfilling these criteria prior to initiation of sorafenib, it will influence the therapeutic strategy. Although various previous reports about sorafenib showed predictive factors for better prognosis in the advanced HCC with extrahepatic metastasis (EHM) and macroscopic vascular invasion (MVI), there is currently no information on the predictive factors influencing the good tolerability of sorafenib in intermediate-stage HCC patients in other wards advanced HCC without EHM and MVI.

Recently, the indication for treatment HCC with molecular targeting drug is expanding to intermediate stage. We thought that it is necessary to select the patients who will tolerate

sorafenib prior to treatment in intermediate HCC. Therefore, in this study, we reviewed the medical records of TACE-refractory intermediate-stage HCC patients receiving sorafenib at our institution and assessed the predictive baseline characteristics for good tolerability to ≥400 mg sorafenib in order to identify good candidates for second-line treatment with regorafenib.

Patients and methods

Patients. From June 2009 to February 2018, there were 91 consecutive patients with advanced HCC who started sorafenib in our institute. The inclusion criteria for treatment with sorafenib were an Eastern Cooperative Oncology Group performance status (ECOG PS) score of 2 or less, Child-Pugh class A or B, adequate hematologic function (platelet count >50x10^3/μl and hemoglobin >8.5 g/dl), adequate hepatic function (albumin >2.8 g/dl and total bilirubin >3.0 mg/dl), alanine aminotransferase and aspartate aminotransferase ≤5 times the upper limit of the normal range, and adequate renal function (serum creatinine <1.5 times the upper limit of the normal range according to the SHARP study) (2). We targeted the object to only TACE refractory intermediate stage HCC in this retrospective study. The flow chart for this study is showed in Fig. 1. The following patients were excluded: Patients who had EHM or MVI (n=22), Child-Pugh B at the start of sorafenib (n=18), patients who started sorafenib <400 mg for any reason (n=10), patients who discontinued sorafenib due to sorafenib-related adverse events prior to radiological progressive disease (PD) (n=5), patients who dropped out in accordance with patient's wishes after start of treatment (n=2). Remaining Thirty-three patients with TACE-refractory intermediate-stage HCC who had confirmed PD were analyzed in this retrospective study. These subjects were not candidates for surgery or radiofrequency ablation. We compared baseline characteristic parameters between patients who continued sorafenib ≥400 mg (sorafenib ≥400 mg) and patients who could not continue ≥400 mg (sorafenib <400 mg) in order to determine the predictive baseline characteristics parameters for the ability to continue sorafenib ≥400 mg.

Sorafenib treatment. The starting dose of sorafenib was 800 mg/day. Before initiation of sorafenib treatment, patients were informed of adverse events associated with sorafenib, including skin toxicity, hypertension, diarrhea, and gastrointestinal disorders, which have been reported in previous studies (13-15). The efficacy and progression of sorafenib were assessed using the modified Response Evaluation Criteria in Solid Tumors (mRECIST) (13), and adverse effects were assessed according to the Common Terminology Criteria (CTCAE) ver 4.0. All patients began using urea cream to prevent skin toxicity.

Treatment interruptions and dose reductions (400 mg once daily or 400 mg on alternate days) were permitted depending on the severity and type of adverse events. When Grade 3 and more adverse events was occurred sorafenib dose reduction or temporary interruption was maintained until symptoms resolved to Grade 1 or 2 according to the guidelines provided by manufacturer. After dose reduction or interruption, sorafenib dose was increased and maintain ≥400 mg as much as possible. Patients continued sorafenib treatment as long as possible, until adverse events were intolerable or patients reached to PD. We defined sorafenib ≥400 mg for patients could continue sorafenib 400 mg or more at least 80% of the period from initiation of treatment to the final observation, and sorafenib <400 mg for the rest.

The present study was conducted in accordance with the Declaration of Helsinki, and the ethics committee of Hiroshima Red Cross Hospital and Atomic-bomb Survivors Hospital institution approved the study protocol. Written informed consent was obtained from each participating patient. The study was conducted in accordance with the Declaration of Helsinki, and the ethics committee of our institution approved the study protocol.

Statistical analysis. Categorical variables were compared using the Fisher exact test or χ^2-test. Univariate survival analysis of survival was performed using Kaplan-Meier survival curves with log-rank survival comparison and 95% confidence intervals (95% CIs). Cut-off levels for each categorical variable were determined by preliminary testing, including receiver operating characteristic (ROC) analysis. A logistic regression model was used to investigate factors associated with continuation of sorafenib ≥400 mg. $P<0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics (16).

Results

Baseline characteristics. The baseline characteristics of our cohort are summarized in Table I. Tumor staging was based on the Tumor-Node-Metastasis staging system of the Liver Cancer Study Group of Japan (17). All 33 patients were classified as Child-Pugh A at initiation of sorafenib treatment.

Sorafenib treatment. Among the 33 patients, 6 patients (18.1%) continued sorafenib ≥400 mg, but 27 (71.9%) patients continued <400 mg. While all of the patients had some lower than CTCAE Gr2 adverse event during the treatment, they were within tolerable range by supportive treatment. Since all 6 patients with sorafenib ≥400 mg had Child Pugh A liver function and were considered eligible for regorafenib, they switched the treatment sorafenib to regorafenib and survived for a median 8 (6-12) months after initiation of regorafenib.

Comparison of OS based on each dose of sorafenib is shown in Fig. 2. The median survival of patients with sorafenib ≥400 and <400 mg were 554.5 (228-674) days and 219 (134-369) days, respectively. The OS of patients with ≥400 mg sorafenib was significantly longer than that of patients with <400 mg (P=0.0315).

Comparison of baseline characteristics and predictive factors for continuation sorafenib dose ≥400 mg.

The baseline characteristics parameters of patients with sorafenib ≥400 and <400 mg are described in Table II. There were significantly more patients with age <75 years, with total cholesterol (T-Cho) ≥180 mg/dl (P=0.026) and with

Table I. Baseline patient characteristics.

Items	Value
Sex (male)	26 (78.8)[a]
Age (year)	76 (52-88)
BW (kg)	61.5 (37-93)
BMI	23.88 (14.9-30.1)
HBV/HCV/NBNC [n (%)]	3 (9)/21 (63)/9 (27)[a]
WBC (/μl)	4300 (1700-8600)
RBC (/μl)	406 (275-546)
Hb (g/dl)	13.1 (9.6-16.6)
Ht (%)	38.7 (29.6-46.3)
Plt (x10^3/μl)	98 (44-267)
PT activity (%)	89.8 (54.8-111.4)
T-Bil (mg/dl)	0.7 (0.3-2.6)
AST (IU/l)	33 (16-76)
ALT (IU/l)	31 (12-119)
γGTP (IU/l)	45 (13-238)
BUN (mg/dl)	14.5 (9.9-44)
Cr (mg/dl)	0.84 (0.53-1.73)
ChE (U/l)	204 (105-365)
Alb (g/dl)	3.9 (3.2-5.0)
Tcho (mg/dl)	153 (113-204)
TG (mg/dl)	83 (45-199)
CRP (mg/dl)	0.11 (0.0-0.58)
Child Pugh score 5	23 (70)[a]
ALBI grade 1	15 (46)[a]
MELD SCORE	5.334 (2.851-8.982)
AFP (ng/ml)	89 (2.2-94760)
DCP (mAu/ml)	168 (11-9300)
Tumor number	5 (1-25)
Tumor size (mm)	22 (10-55)
Bilateral lesion [n (%)]	20 (64)[a]

Median (minimum-max) [a]n (%). BW, body weight; BMI, Body mass index; HB, hepatitis B virus; HCV, hepatitis C virus; NBNC, non-HBV and non-HCV; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; Plt, platelet; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGTP, γ-glutamyl transpeptidase; BUN, blood urea nitrogen; Cr, creatinine; ChE, cholinesterase; Alb, albumin; Tcho, total cholesterol; TG, triglyceride; CRP, C reactive protein; ALBI grade, Albumin Bilirubin grade; MELD score, Model for End-stage Liver Disease score; AFP, α-fetoprotein; DCP, des-γ-carboxy protein.

cholinesterase (ChE) ≥220 U/l (P=0.024) in the patients who continued sorafenib ≥400 mg. Among other parameters, BMI ≥24 kg/m^2 (OR 6.871 (0.860-496.9), P=0.062) and AFP levels <10 ng/ml (OR 9.305 (0.641-364.1), P=0.085) had some tendency to influence continuation of sorafenib ≥400 mg, but these tendencies were not significant. Table III shows the univariate and multivariate logistic regression analyses of baseline predictive factors for continuation of sorafenib ≥400 mg in TACE-refractory intermediate-stage HCC. In a univariate analysis for factors influencing continuation of sorafenib ≥400 mg, age <75 years, T-cho ≥180 mg/dl and ChE ≥220 U/l

Table II. The baseline characteristics of patients undergoing sorafenib ≥400 and <400 mg treatment.

Characteristic	≥400 mg (n=6) [n (%)]	<400 mg (n=27) [n (%)]	P-value
Sex			0.38
Male	6 (100)	20 (74.1)	
Female	0 (0)	7 (25.9)	
Age			0.021
<75 year	6 (100)	17 (63.0)	
≥75 year	0 (0)	10 (37.0)	
BW			0.159
≥70 kg	4 (66.7)	8 (29.6)	
<70 kg	2 (33.3)	19 (70.4)	
BMI			0.085
≥24	5 (83.3)	11 (40.7)	
<24	1 (16.7)	16 (59.3)	
HCV			0.643
HCV	3 (50.0)	18 (66.6)	
Non-HCV	3 (50.0)	9 (33.4)	
HBV			0.464
HBV	1 (16.7)	2 (7.4)	
Non-HBV	5 (83.3)	25 (92.6)	
NBNC			1.000
NBNC	2 (33.3)	7 (25.9)	
HCV+HBV	4 (66.7)	20 (74.1)	
WBC			0.616
≥3000/μl	2 (33.3)	6 (22.2)	
<3000/μl	4 (66.7)	21 (77.8)	
Hb			0.153
<13 g/dl	1 (16.7)	15 (55.6)	
≥13 g/dl	5 (83.3)	12 (44.4)	
Ht			0.375
<40%	2 (33.3)	16 (59.3)	
≥40%	4 (66.7)	11 (40.7)	
Plt			0.216
<70x10^3/μl	2 (33.3)	24 (88.9)	
≥70x10^3/μl	4 (66.7)	3 (11.1)	
T-bil			0.309
<1.0 mg/dl	3 (50.0)	21 (77.8)	
≥1.0 mg/dl	3 (50.0)	6 (22.2)	
AST			0.364
<40 IU/l	5 (83.3)	15 (55.6)	
≥40 IU/l	1 (16.7)	12 (44.4)	
ALT			0.216
<50 IU/l	4 (66.7)	24 (88.9)	
≥50 IU/l	2 (33.3)	2 (11.1)	
γGTP			0.658
<50 IU/l	3 (50.0)	17 (63.0)	
≥50 IU/l	3 (50.0)	10 (37.0)	
Alb			0.616
<4.0 g/dl	2 (33.3)	6 (22.2)	
≥4.0 g/dl	4 (66.7)	21 (77.8)	

Table II. Continued.

Characteristic	≥400 mg (n=6) [n (%)]	<400 mg (n=27) [n (%)]	P-value
ChE			0.024
≥220 U/l	5 (83.3)	8 (29.6)	
<220 IU/l	1 (16.7)	19 (70.4)	
BUN			0.308
≥21 mg/dl	0 (0.0)	7 (25.9)	
<21 mg/dl	6 (100)	20 (74.1)	
Cr			0.375
<0.8 mg/dl	4 (66.7)	11 (40.7)	
≥0.8 mg/dl	2 (33.3)	16 (59.3)	
CRP			0.309
<0.2 mg/dl	3 (50.0)	21 (77.8)	
≥0.2 mg/dl	3 (50.0)	6 (22.2)	
T-cho			0.026
≥180 mg/dl	4 (66.7)	4 (14.8)	
<180 mg/dl	2 (33.3)	23 (85.2)	
Child Pugh score			0.640
5	5(83.3)	18(66.6)	
>5	1 (16.7)	9 (33.3)	
ALBI			0.375
1	4(66.7)	11(40.7)	
>1	2 (33.3)	16 (59.3)	
MELD score			1
<4	1 (16.7)	10 (37.0)	
≥4	5 (83.3)	17 (63.0)	
AFP			0.062
<10 ng/ml	1 (16.7)	18 (66.7)	
≥10 ng/ml	5 (83.3)	9 (33.3)	
DCP			0.373
<50 mAu/ml	2 (33.3)	6 (22.2)	
≥50 mAu/ml	4 (66.7)	21 (77.8)	
Tumor number			0.159
<5	2 (33.3)	19 (70.4)	
≥5	4 (66.7)	8 (29.6)	
Tumor size			0.64
>30 mm	1 (16.7)	9 (33.3)	
≤30 mm	5 (83.3)	18 (66.7)	
Bilateral lesion			
Bilateral	3 (50.0)	17 (63.0)	0.659
One-side	3 (50.0)	10 (37.0)	
Prior TACE			1
<3 times	5 (83.3)	21 (77.8)	
≥3 times	1 (16.7)	6 (22.2)	

BW, body weight; BMI, Body mass index; HB, hepatitis B virus; HCV, hepatitis C virus; NBNC, non-HBV and non-HCV; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; Plt, platelet; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGTP, γ-glutamyl transpeptidase; BUN, blood urea nitrogen; Cr, creatinine; ChE, cholinesterase; Alb, albumin; Tcho, total cholesterol; TG, triglyceride; CRP, C reactive protein; ALBI grade, Albumin Bilirubin grade; MELD score, Model for End-stage Liver Disease score; AFP, α-fetoprotein; DCP, des-γ-carboxy protein.

were significant factors, and a multivariate analysis of these factors confirmed ChE ≥220 U/l as a prognostic factor significantly predicting continuation of sorafenib ≥400 mg [hazard ratio: 11.9, 95% CI: 1.190-118.0; P=0.004]. The Kaplan-Meier survival curves for progression-free survival (PFS) and OS with each parameter are shown in Figs. 3 and 4. Median PFS in patients with ChE levels ≥220 U/l and those with ChE levels <220 U/l were 279 (204-403) days and 117.5 (63-197) days, respectively. Furthermore, PFS was significantly longer in patients with ChE levels ≥220 U/l than in patients with ChE levels <220 U/l (P=0.0136; Fig. 3). Furthermore, median OS of patients with ChE levels ≥220 U/l and those with ChE levels <220 U/l were 470 (277-679) days and 171.5 (80-236) days, respectively. The OS of patients with ChE ≥220 U/l was significantly longer than that of patients with ChE <220 U/l (P=0.0004; Fig. 4).

Discussion

Recently, the indication for treatment of HCC with molecular targeting drug is expanding to intermediate stage. We thought that it is necessary to select the patients who will tolerate the molecular targeting drug prior to treatment. We revealed that patients with TACE-refractory HCC who had high ChE levels prior initiation of sorafenib would be more likely to be able to continue sorafenib ≥400 mg, which means that they would be eligible for second-line treatment with regorafenib. Takeda *et al* reported that lower serum ChE level is a significant predictor of poor prognosis and severe liver damage in advanced HCC patients with sorafenib (18). These findings showed our result prove our result was reasonable evidence. However, the object of their study included advanced stage HCC such as extra hepatic metastasis and vascular invasion case. Therefore, we focused on only intermediate stage. To the best of our knowledge, this is the first study to investigate the factors associated with continuation of second-line sorafenib focused to only intermediate-stage TACE-refractory HCC in other wards without advanced HCC without EHM and MVI.

Currently, most treatment guidelines for advanced HCC, which use BCLC staging, indicate sorafenib as the standard systemic therapy (16-22). Furthermore, the European guidelines also recommend sorafenib for patients with intermediate-stage HCC (BCLC stage B) who have failed at least two cycles of TACE or with progression following TACE (2-10). The Japanese guidelines base their treatment recommendations on other factors, recommend sorafenib as the first-line treatment for patients with EHM and/or MVI and for TACE-refractory patients classified as Child-Pugh A (23). Since sorafenib is recommended for TACE-refractory patients, we also treat TACE-refractory patients with advanced HCC, including intermediate-stage, with sorafenib. However, many patients in this study could not continue sorafenib treatment because of adverse events. Some previous studies have reported that sorafenib discontinuation may cause HCC flares, and patients who could continue sorafenib had better prognosis than patients who discontinued sorafenib (14,15,24-26). Whereas our study also showed that patients who continued sorafenib ≥400 mg had good liver function and were candidates for regorafenib or other treatment; therefore, we analyzed predictive factors for continuation of sorafenib ≥400 mg.

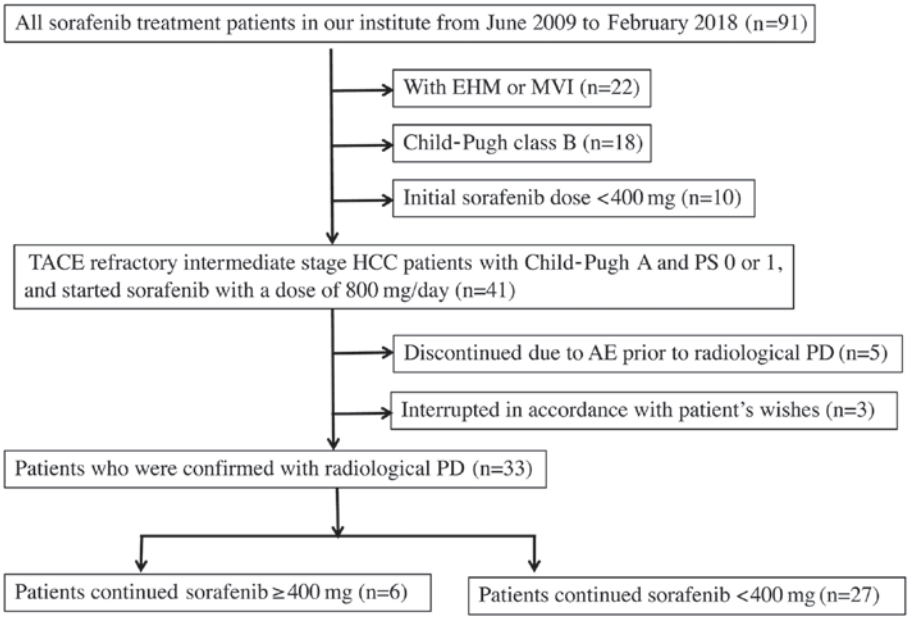

Figure 1. Flow chart of study design. EHM, extrahepatic metastasis; MVI, macroscopic vascular invasion; TACE, transarterial chemoembolization; HCC, hepatocellular carcinoma; PS, performance status; AE, adverse effects; PD, progressive disease.

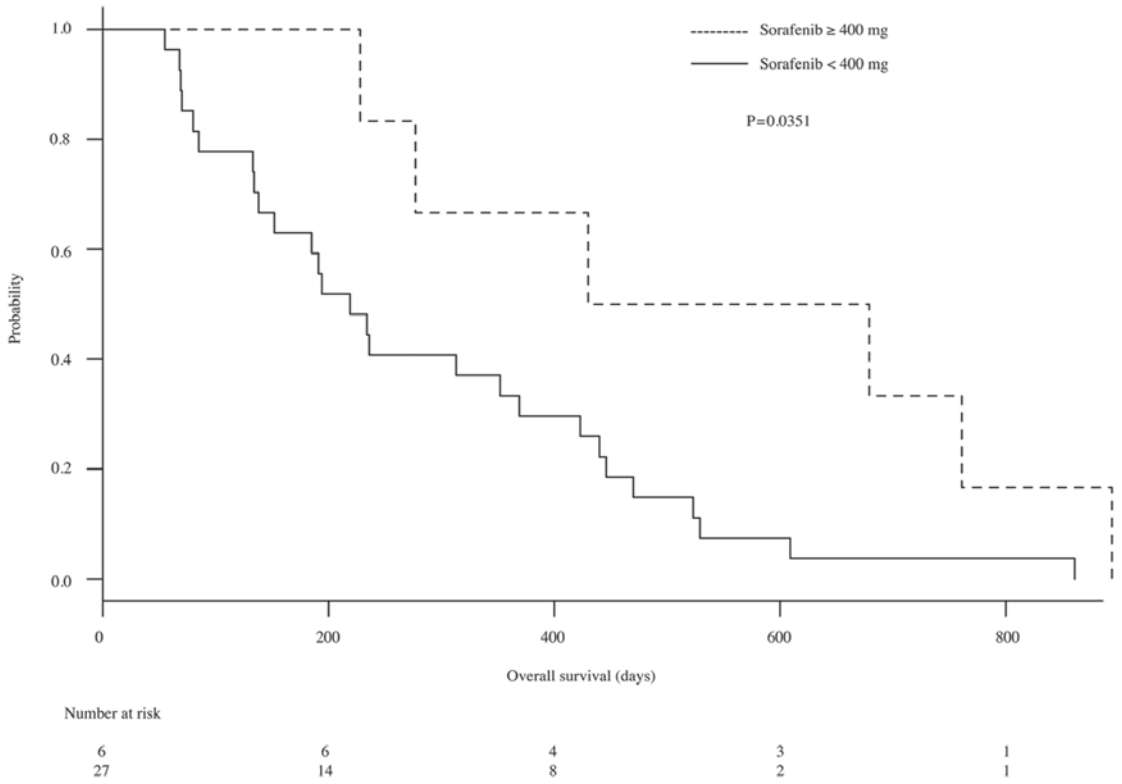

Figure 2. Kaplan-Meier curves indicating overall survival in patients who continued sorafenib ≥400 and <400 mg treatment.

Most of the previous studies, objects for these studies were included EHM and MVI, found that candidates for second-line sorafenib who failed sorafenib had better prognosis than non-candidates. This can be explained by previous studies. Uchikawa *et al* revealed that absence of MVI and serum albumin >3.5 g were predictive factors for candidates (14). Ogasawara *et al* showed that a lower Child-Pugh score and a better ECOG PS were predictors of eligibility for second-line therapy, such as sorafenib ≥400 mg (12). However, these studies included patients with advanced-stage HCC, and therefore these predictive factors may be specific to that patient cohort. In contrast, most TACE-refractory patients in whom sorafenib treatment is indicated in the real world have intermediate-stage disease without MVI and EHM, and the predictive factors of candidates for second-line sorafenib treatment in these patients have not been defined. Therefore,

Table III. Factors influencing continuation of sorafenib ≥400 mg.

	Univariate analysis			Multivariate analysis		
Factor	OR	95% CI	P-value	OR	95% CI	P-value
Sex (male/female)	inf	0.313-inf	0.301			
Age <75/≥75 (years)	0	0.000-0.643	0.021			
BW ≥70/<70 (kg)	4.502	0.524-59.44	0.159			
BMI ≥24/<24	6.871	0.641-364.1	0.085			
HCV/non-HCV	0.643	0.056-4.609	0.511			
WBC ≥3000/<3000 (μl)	0.582	0.063-7.916	0.616			
Hb <13/≥13 (g/dl)	5.937	0.555-314.2	0.175			
Ht <40/≥40(%)	2.43	0.238-19.30	0.418			
Plt <70x10^3/≥70x10^3 (μl)	2.815	0.022- 4.09	0.216			
T-bil <1.0/≥1.0 (mg/dl)	3.345	0.355-32.27	0.309			
AST <40/≥ 40 (IU/l)	0.259	0.005- 2.79	0.364			
ALT <50/≥50 (IU/l)	3.783	0.245-46.43	0.216			
γGTP <50≥ 50 (IU/l)	1.672	0.187-15.05	0.658			
Alb <4.0/≥4.0 (g/dl)	1.717	0.126-15.94	0.616			
ChE ≥220/<220 (U/l)	38	1.003-588.0	0.024	11.9	1.190-118.0	0.004
BUN ≥21<21 (mg/dl)	0	0.000-3.189	0.301			
Cr <0.8/≥0.8 (mg/dl)	0.355	0.027-2.986	0.374			
CRP <0.2/≥0.2 (mg/dl)	3.345	0.354-32.28	0.309			
T-cho ≥180/<180 (mg/dl)	10.31	1.085-151.5	0.026			
Child Pugh score 5/>5	0.409	0.007-4.535	0.640			
ALBI 1/>1	0.355	0.027-2.986	0.375			
MELD score <4/≥4	0.635	0.039-39.29	1			
AFP <10/≥10 (ng/ml)	9.305	0.860-496.9	0.062			
DCP <50/≥50 (mAu/ml)	0.582	0.063-7.916	0.616			
Tumor number <5/≥5	1.5	0.524-59.45	0.159			
Tumor size >30/≤30 (mm)	0.714	0.008-4.536	0.64			
Bilateral lesion/non-bilateral lesion	0.598	0.067-5.353	0.659			
Prior TACE <3 times/≥3 times	0.707	0.013-8.437	1			

OR, odds ratio; inf, infinity; BW, body weight; BMI, Body mass index; HB, hepatitis B virus; HCV, hepatitis C virus; NBNC, non-HBV and non-HCV; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; Plt, platelet; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGTP, γ-glutamyl transpeptidase; BUN, blood urea nitrogen; Cr, creatinine; ChE, cholinesterase; Alb, albumin; Tcho, total cholesterol; TG, triglyceride; CRP, C reactive protein; ALBI grade, Albumin Bilirubin grade; MELD score, Model for End-stage Liver Disease score; AFP, α-fetoprotein; DCP, des-γ-carboxy protein.

we limited our subjects in this study to patients with intermediate-stage HCC. In this study, in the univariate analysis for factors influencing continuation of sorafenib ≥400 mg found only age <75 years, T-cho levels ≥180 mg/dL, and ChE levels ≥220 U/l to be significant factors. Among other parameters, BMI ≥24 kg/m^2 and AFP levels <10 ng/ml had some tendency to influence continuation of sorafenib ≥400 mg, but these tendencies were not significant. No significant differences were observed in other parameters, including Child-Pugh score, MELD score, and ALBI grade. Eventually, ChE levels ≥220 U/l was only significant factor in multivariate analysis.

The patients with high levels of ChE did not have severe obesity or fatty liver disease; therefore, we thought that high ChE indicated lack of cirrhosis and good remnant liver function. This idea is similar to those proposed in previous studies (12,14). Takeda *et al* reported that pretreatment ChE level is a reliable prognostic marker for advanced HCC in the sorafenib era (18). The cut-off level in their study was 140 U/l, whereas the cut-off level in our study was 220 U/l. We performed preliminary testing for various cut-off levels, including 140 U/l, 205 U/l (median of our cohorts) by refereeing to Takeda's report, similar results were obtained in each parameter. Of these cut-off levels, the ChE level of 220 U/l had most significant odds ratio in our study cohort. Additionally, on performing ROC analysis, the cut-off level of ChE was 220 IU/ml (specificity: 0.692, sensitivity: 0.714, area under the curve, 0.6264). Therefore, we decided to use a ChE cut-off level of 220 U/l. We considered that since patients with baseline ChE levels ≥220 U/l had better remnant liver function, they could continue receiving sorafenib ≥400 mg.

The present study has some limitations. First, this study was performed retrospectively and was a single institute

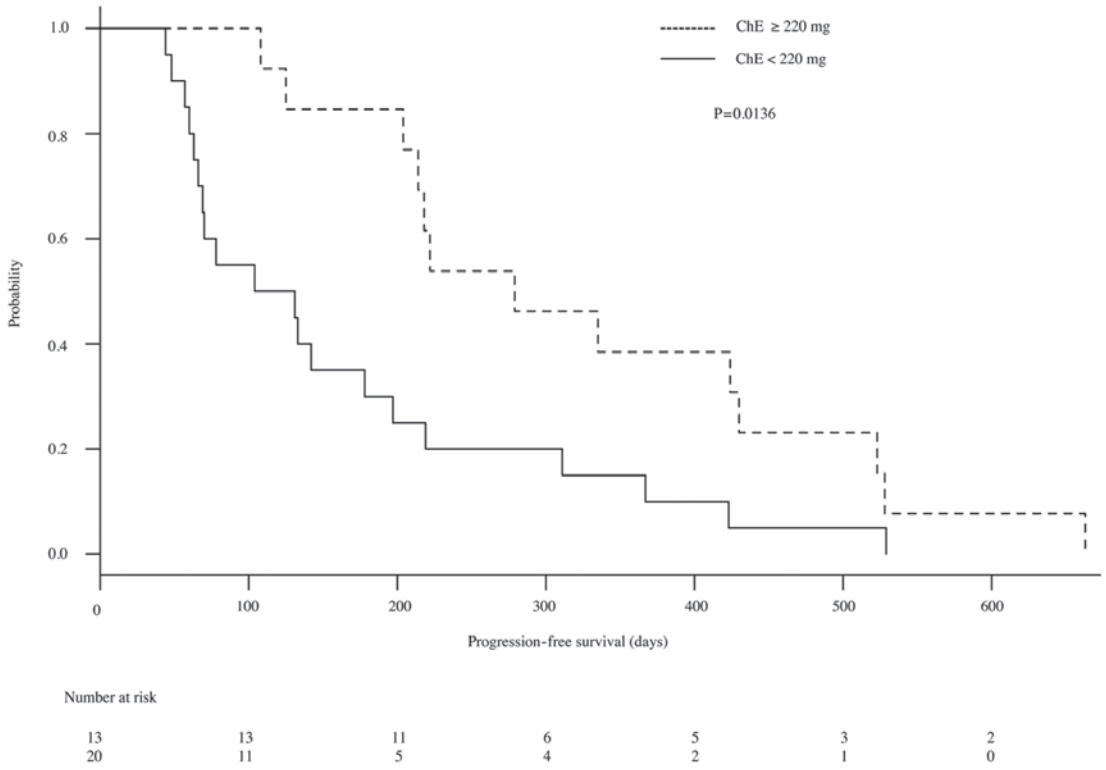

Figure 3. Kaplan-Meier curves indicating PFS in patients who had high baseline ChE levels (≥220 U/l) and low baseline ChE levels (<220 U/l). PFS, progression-free survival; ChE, cholinesterase.

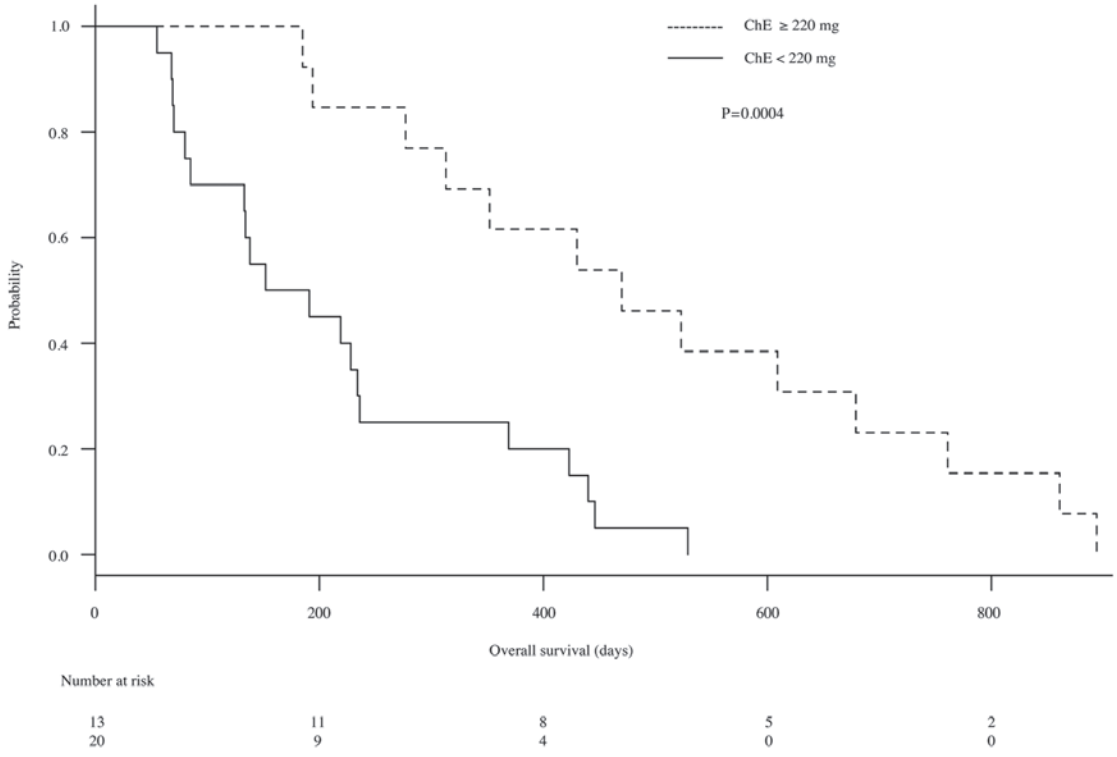

Figure 4. Kaplan-Meier curves indicating overall survival in patients who had higher baseline ChE (ChE; ≥220 U/l) and lower baseline ChE (<220 U/l). ChE, cholinesterase.

study. Second, its sample size was small, since there are few in intermediate stage HCC treated with only sorafenib at that time. Third, all of the included patients were Japanese, who are well known to experience adverse events due to sorafenib. Finally, the subject of this study was limited only PD case to analyze the indication of second line treatment. However, good

response case such as CR should be occurred in real clinical setting, while this study was not included CR case. Whereas we presented baseline high value of ChE in intermediate-stage HCC predicts the ability to continue sorafenib ≥400 mg, it is uncertain that high value of ChE patients will be the better prognosis of intermediate HCC in real clinical setting. Therefore, we consider that larger sample size and prospective additional analyses are needed in order to clarify whether intermediate HCC patients with high value of ChE levels will show a good prognosis.

In conclusion, we are the first to determine that continuation of sorafenib is associated with better prognosis in patients with TACE-refractory intermediate-stage HCC. We suggest that it is important to select appropriate therapy beyond second-line treatment in this population. This is particularly important since patients with high baseline levels of ChE could continue sorafenib ≥400 mg, leading them to be candidates for second-line treatment and to have a better prognosis.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

ST was responsible for the concept and design of the study and for data interpretation and wrote this manuscript. TF and NM assisted with data interpretation and article preparation. KT assisted with data interpretation and article preparation and supervised the project.

Ethics approval and consent to participate

The current study was approved by the Ethics Committee of Hiroshima Red Cross Hospital and Atomic-bomb Survivors' Hospital, as per the Declaration of Helsinki. Written informed consent was obtained from the patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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