を行った。その結果，13例(32.5%)が切除可能となり，平均観察期間19か月における無再発生存期間中央値は14.3か月であったと報告している[7]。また，oxaliplatinおよびirinotecanを併用するFOLFOXIRIをレジメンに用いた報告もされており，Masiらは奏効率72%，切除率26%，4年生存率37%を得たと報告している[8]。

これらの良好な治療成績が報告される一方で，化学療法に関連した肝障害や周術期合併症に関する報告も散見される[9-11]。Rubbia-Brandtらは化学療法により，steatohepatitisを合併した症例(14.7%)では正常肝の症例(1.6%)に比べ，有意に高い手術死亡率であったと報告している[9]。Karouiらはmajor hepatectomyを施行した67例を対象とした検討を行い，化学療法施行群45例と対照群22例を比較し，周術期合併症はそれぞれ37.8%，13.6%と前者で有意に高く，化学療法の施行コース数と合併症発生率にも有意に相関があったと報告している[10]。

今後はさらに，bevacizumabを中心とした分子標的薬を併用するレジメンに関する報告が増加すると予想される。分子標的薬に特有な合併症が懸念される一方で，bevacizumabを併用した術前化学療法施行後の肝切除は安全に施行可能であったと報告されており[12-14]，今後の動向に注意する必要がある。

本症例においては，全身化学療法の投与期間は合計4クールと比較的短期間であった。前述したように，全身化学療法に伴う肝障害は諸家によって報告されており[9-11]，正常肝の脂肪症もしくはsteatohepatitisやsinusoidal dilatationが惹起された結果，肝実質は脆弱で易出血性となり，周術期合併症へ影響を及ぼすと考えられている。特に長期の化学療法は，より重度の肝障害を引き起こすと考えられている。本症例において肝実質はやや脆弱で，化学療法による軽度の脂肪症によるものと考えられた。ただし，全身化学療法も比較的短期間であったため，肝障害も軽度であり，周術期合併症を引き起こすことなく経過した。長期予後の観点からみると，本症例は術前化学療法により根治切除が得られており，切除可能病変に対する肝切除と同等の予後が見込めると思われる。

近年では，切除可能肝転移に対する術前化学療法に関する議論も盛んに行われている。術前化学療法に関する唯一の無作為化臨床試験(randomized clinical trial : RCT)はNordlingerらによって2007年のASCOで報告されている。この研究は切除可能大腸癌肝転移 364例を対象にデザインされ，FOLFOXを用いた術前および術後化学療法施行群が手術単独群に比して，3年無増悪生存期間の有意な延長を認めたと報告されている[15]。ただし，このRCTは術前化学療法単独の有効性に関するevidenceではない点に注意する必要がある。切除可能肝転移に対する術前化学療法はmicro metastasisを根絶させ，根治切除率および切除成績の向上が主目的である。しかし，化学療法期間中の病変増悪による切除率および治療成績の低下[16]や先述したように，化学療法による肝障害や周術期合併症に関する報告[9-11]も散見されるため，切除可能肝転移に対する術前化学療法のコンセンサスは未だ得られていない。こうした現状を踏まえ，長期生存の唯一の手段である根治切除を施行する最善のタイミングを検討することが重要であると思われる。

おわりに

下大静脈浸潤を伴う高度進行大腸癌肝転移に対し，化学療法が奏効し切除可能となった症例を経験した。化学療法の有効性を示した1例であり，文献的考察を加え報告した。

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［質疑応答］

病理コメント

化学療法により高度に壊死に陥った大腸癌の肝転移巣が認められる。化学療法が非常に効いているが，少量ながらviableな癌細胞が残っており切除しないと再発する状況と思われる。

治療前に切除不能と判断された癌と下大静脈，右肝静脈との関係について，癌の圧迫だけだったのか浸潤していた癌が治療で消失したのかは切除標本からはわからなかった。

（望月　眞）

［質疑応答］

有井(座長)：内科と外科がうまくコラボレーションして2年8か月生存中という症例です。われわれはこういう症例の紹介を受けると，化学療法によって肝機能が見掛け上はよくても，拡大切除をした時に少しそういうストレスに弱いのではないかと非常に気になることがありますが，それはいかがでしたか。

演者(高橋)：非常にもろくて，脂肪症の影響を受けている状況もあります。この症例に関してはそういう非常にもろい実質ではなく，比較的正常肝に近い形で，ややダメージを受けているかなといった程度の肝実質だったと思います。

有井：少し割り引いて考えないと怖いかなという場合もあります。肝機能はよいけれども，脂肪肝を呈しており何か不安だという場合は術前にびっちり化学療法を行った症例に多いように思います。

たとえば今思いだしましたが，*Ann. Surg.* に化学療法によりsinusoidal dilatationや肝壊死が生じ，術後の合併症率が上昇したという報告がありました。転移性肝癌なので拡大切除を非常にやりやすいということでどんとやると危険な場合があるかもしれません。ですから少し気をつけなければいけないという気がします。

この症例は非常によく，大体はこういう症例だと思いますが，たまに気をつけないといけないのではないかと思います。

講　評

FOLFOX，FOLFIRIというレジメンに代表されるように最近の大腸癌に対する化学療法の進歩は著しく，大腸癌肝転移に対しても従来のレジメンに比べて非常に良好な生存率が欧米から報告されている。このレジメンを用いて，切除不能肝癌がダウンステージングし，切除可能となる確率も高くなったようである。本症例報告は正にこの例であり，見事に腫瘍が縮小し安全な肝切除が行われたというものである。また著者も考察で述べているように，切除可能例に対しても術前後に投与する試みもなされている。本症例も術後補助化学療法としてFOLFOX6が行われている。ただ最近，このようなレジメンによる化学療法後の肝切除において術後の合併症が増加し，肝臓における類洞拡張，肝細胞の萎縮や壊死が認められたとの報告や脂肪症，steatohepatitisが高率に生じ，肝切除後90日以内の死亡率が化学療法群で有意に高いという論文が発表された。また，アバスチンとの併用はこれらの肝障害を軽減させたという論文も発表されている。いずれにしても，今後本論文のような症例の増加が予想されることから，化学療法による肝障害，肝予備力低下を念頭において切除範囲を決定すべきであろう。ただ，残念ながらこれらの論文はすべて欧米のものであり，抗癌剤感受性や忍容性については人種差も無視できないことから，わが国独自のデータが望まれるところである。さらにこのレジメンの意味するところは，肝転移に対しても全身化学療法が主体であるということであろう。従来，肝転移に対してはわが国では肝動注による化学療法が主体であったが，今後肝動注の意義あるいは役割についても再考されなければならないと考える。

（有井　滋樹）

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Original Paper

Hepatectomy for Hepatocellular Carcinoma Patients Who Meet the Milan Criteria

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ABSTRACT

Background/Aims: Although hepatocellular carcinoma patients who meet the Milan criteria are optimal candidates for liver transplantation, most such patients in Japan have been treated without liver transplantation.

Methodology: In this retrospective analysis, the patient selection criteria were (1) admission between 1992 and 2005, (2) fulfillment of the Milan criteria, (3) classification within the Barcelona Clinic Liver Cancer stages A1-A4, and (4) no previous anticancer treatment.

Results: Of 451 patients who met the selection criteria, 162 underwent hepatectomy. The proportion of patients who underwent hepatectomy was 58% of 106 with stage A1 and 29% of 345 with stages A2-A4. For patients with stages A2-A4, the survival probability after hepatectomy at 3, 5, and 7 years was 89%, 70%, and 61%, respectively. There were no significant differences in survival time between stages A1 and stages A2-A4 after hepatectomy. Among patients with Child-Pugh scores of 5 and 6 in stages A2-A4, 51% and 29% underwent hepatectomy, respectively.

Conclusions: Hepatectomy may be an appropriate first-line treatment option for patients with stages A2-A4 who meet the Milan criteria, when they have a good hepatic reserve and a long waiting time for liver transplantation.

KEY WORDS: Liver neoplasm; Prognosis; Liver transplantation

ABBREVIATIONS: Hepatocellular Carcinoma (HCC); Barcelona Clinic Liver Cancer (BCLC);

INTRODUCTION

Clinical cancer staging defines the prognosis and provides an outline for appropriate treatment decisions. Although there have been many staging or scoring systems proposed for hepatocellular carcinoma (HCC) (1-6), the Barcelona Clinic Liver Cancer (BCLC) staging **(Table 1)** (7) is notable for linking staging with treatment modalities (8-10). Patients classified as stage A under the BCLC system are those with early HCC who may benefit from curative therapies, such as hepatectomy, liver transplantation, or percutaneous ablation. According to the BCLC proposal (10), the treatment assignment for stage A1 is hepatectomy and for stages A2-A4 is liver transplantation. Among the 3 curative modalities, liver transplantation is the best curative option for patients who meet the Milan criteria (11), i.e., a solitary tumor up to 5cm in diameter, or no more than 3 nodules, each 3cm or less in diameter. When the criteria were instituted, 5-year survival rates of 70% and low recurrence rates were expected (12). In Japan, however, only 24 deceased donor liver transplantations have been performed for noncancer recipients in the past 6 years because the supply of liver from deceased donors is extremely limited (13). Accordingly, few Japanese HCC patients who meet the Milan criteria have received liver transplants.

TABLE 1 Definition of the Barcelona Clinic Liver Cancer (BCLC) Staging

Stage	PST[1]	Tumor stage	Okuda stage	Liver function status
A1	0	Single	I	No portal hypertension and normal bilirubin
A2	0	Single	I	Portal hypertension and normal bilirubin
A3	0	Single	I	Portal hypertension and abnormal bilirubin
A4	0	3 tumors <3 cm	I-II	Child-Pugh A-B
B	0	Large multinodular	I-II	Child-Pugh A-B
C	1-2[2]	Vascular invasion or extrahepatic spread	I-II	Child-Pugh A-B
D	3-4[3]	Any		Child-Pugh C

[1]: Performance status test; Stage A and B: all criteria should be met; Stage C: at least one of the criteria should be met, [2]: PST 1-2 or vascular invasion/extrahepatic spread; Stage D: at least one of the criteria should be met, [3]: PST 3-4 or Okuda stage III/Child-Pugh C

The aim of this study was to evaluate the outcomes for liver transplantation candidates who did not receive transplants, in a Japanese high-volume center. The study was performed with special reference to the BCLC staging, and with a focus on the effects of hepatectomy on survival.

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METHODOLOGY

Patients

The patient selection criteria were: admission between July 1992 and May 2005; fulfillment of the Milan criteria (11); diagnosis of stages A1-A4 according to the BCLC staging (7); and no previous anticancer treatment. A list of all the patients who met the above criteria was compiled from a database that was approved by the Hospital Information Committee.

In the National Cancer Center Hospital East, diagnosis and staging of HCC were usually based on a characteristic arterial vascularization observed on dynamic computed tomography and/or magnetic resonance imaging. For the tumors that did not show arterial vascularization, fine-needle biopsy was mandatory before percutaneous ablation. For BCLC staging in this study **(Table 1)**, the normal bilirubin level was set at 1.2mg/dL or less, which was our institutional upper normal limit. The status of portal hypertension was clinically estimated by the presence of splenomegaly, esophageal varices, or a platelet count of less than 100,000/mm^3.

TABLE 2 Clinical Data for the 451 Patients

Sex	Male/female	339 (75%)/112
Age	Median (range)	66 (30-86)
Modality of diagnosis	Histology	376 (83%)
	Imaging with AFP2>400	14 (3%)
	Imaging without AFP>400	61 (14%)
HBsAg/HCVAb[1]	Negative/negative	55 (12%)
	Positive/negative	38 (8%)
	Negative/positive	349 (77%)
	Positive/positive	9 (2%)
Total bilirubin (mg/dL)	Median (range)	1.0 (0.3-2.9)
Albumin (g/dL)	Median (range)	3.5 (2.2-4.6)
ICG test (%)[2]	Median (range)	21.6 (1.5-98)
Platelet (10^4/mm^3)	Median (range)	9.9 (2.4-39.8)
Prothrombin time (%)	Median (range)	76 (38-155)
Portal hypertension	Present/absent	308 (68%)/143
Child-Pugh stage	A/B	333 (74%)/118
Tumor number	Solitary/2/3	346 (77%)/75/30
Tumor size	<2cm	130 (29%)
	2-3cm	188 (42%)
	3-5cm	133 (29%)
AFP (ng/ml)[3]	Median (range)	27.3 (1.3-25046)
Barcelona Clinic Liver	A1	106 (24%)
Cancer staging	A2	153 (34%)
	A3	87 (19%)
	A4	105 (23%)
Treatment	Hepatectomy	162 (36%)
	Percutaneous ethanol injection	107 (24%)
	Percutaneous microwave coagulation	20 (4%)
	Percutaneous radiofrequency	59 (13%)
	Transcatheter arterial chemoembolization	77 (17%)
	Radiotherapy	11 (2%)
	Others	15 (3%)

[1]: Hepatitis B virus surface antigen/hepatitis C virus antibody; [2]: Indocyanine green retention test at 15 minutes; The ICG test was not available in 21 patients. [3]: Alpha-fetoprotein: AFP level was not available in 2 patients.

Treatment choice for each patient was determined at a conference held every Tuesday evening that was attended by surgeons, medical oncologists, and radiologists. The strategy for treatment assignment was as follows. All patients were first assessed for hepatectomy. Patients who met the Milan criteria and wanted to receive living donor liver transplantation were referred to other university hospitals where transplantation was available. Patients for whom hepatectomy was not feasible received percutaneous ablation if they had 3 or fewer HCC nodules without vascular invasion, of which the largest diameter was 3cm or smaller. This treatment included ethanol injection, microwave coagulation, and radiofrequency ablation therapy. Microwave coagulation was used between April 1998 and December 1999, and radiofrequency ablation has been used since June 1999. Even in technically resectable cases, percutaneous ablation was selected as the treatment modality for some elderly patients and for those with cardiopulmonary complications or deeply located small tumors that did not show the arterial vascularization, based on the consensus at the conference. External beam radiotherapy, including proton beam radiotherapy (14), was also an option for those unresectable cases with a solitary tumor. Transcatheter arterial chemoembolization was indicated for patients who were not candidates for hepatectomy, percutaneous ablation, or definitive radiotherapy.

Follow-up examination was performed 1 month after the treatment and then every 3-4 months using dynamic computed tomography or magnetic resonance imaging, and diagnosis of recurrence was made by typical arterial vascularization. The treatment modality for recurrence was assigned the same way as for the initial treatment.

Analysis and Statistics

Overall survival was measured from the first day of the initial treatment to the time of the final follow-up evaluation or death by any cause. Progression-free survival was measured from the first day of the initial treatment to the first day when recurrence was diagnosed by follow-up imaging or hepatic failure (development of clinical jaundice or irreversible ascites). All the patients were censored on November 30, 2005; 5 months after treatment of the last patient. Survival curves were calculated using the Kaplan-Meier method (15). Differences in survival among subgroups were evaluated with log-rank tests. Continuous variables were grouped by the median value. The Cox proportional hazards model (16) was used to determine the most significant variables related to survival. Forward and backward stepwise regression procedures based on the partial likelihood ratio were used to determine which factors were the major independent predictors of survival. The variables entered into the Cox regression included those found to be associated with survival based on the log-rank tests. For comparison of patients' clinical data, differences in continuous variables between the sub-

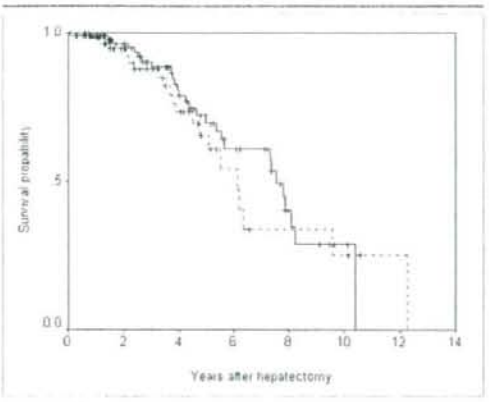

FIGURE 1 Overall survival curves after hepatectomy according to the BCLC staging. The dotted line indicates stage A1 (N=62) and the solid line indicates stages A2-A4 (N=100). There are no significant differences between them ($p=0.50$).

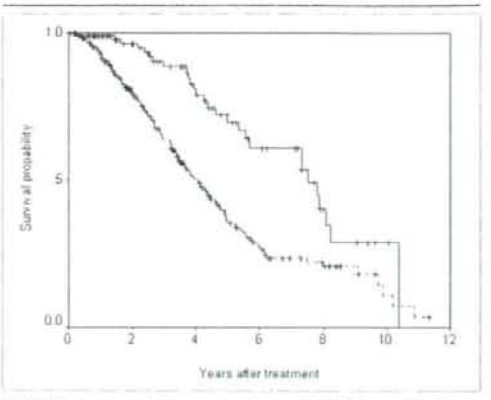

FIGURE 2 Overall survival curves in patients with BCLC stages A2-A4. The solid line indicates the hepatectomy group (N=100) and the dotted line indicates the nonhepatectomy group (N=245). The data for the 2 groups are significantly different ($p<0.001$).

groups were compared using the Mann-Whitney test. Frequency analysis was performed using the Fisher exact test for 2 x 2 tables. All the analyses were performed using the statistical software package SPSS 11.0J for Windows (SPSS Inc, Chicago, IL). Statistical significance was defined as a 2-sided p value of .05 or less.

RESULTS

Four hundred fifty-one patients met the selection criteria. The patients' clinical data are shown in **Table 2**. Of the 451 patients, it was established at the time of analysis that 192 (43%) had died and 195 (43%) were still alive, while 64 (14%) were not available for follow-up evaluation. The proportion of patients who were lost to follow-up was 22 (14%) of 162 hepatectomy patients, 42 (15%) of 289 nonhepatectomy patients, 15 (14%) of 106 patients with stage A1 and 49 (14%) of 345 patients with stages A2-A4. The median observation period (25%-75%) was 34 (18-56) months. Of the 192 patients who died, 179 (93%) died from cancer-related or hepatic failure deaths and 13 died from other causes.

Stage A1 *vs.* Stages A2-A4

The survival probability at 3, 5, 7, and 10 years was 84%, 65%, 40%, and 32%, respectively, for patients in stage A1 (N=106), and 71%, 45%, 33%, and 15%, respectively, for those in stages A2-A4 (N=345). Survival for stage A1 was significantly better than for stages A2-A4 ($p=0.005$). The proportion of patients who underwent hepatectomy with stages A1, A2, A3 and A4 was 58% (62/106), 39% (60/153), 16% (14/87) and 25% (26/105), respectively. Of 345 patients with stages A2-A4, 100 (29%) underwent hepatectomy.

TABLE 3 Comparison of Clinical Data between the Hepatectomy and Nonhepatectomy Groups for the 345 Patients with BCLC Stages A2-A4

		Hepatectomy (N=100)	Nonhepatectomy (N=245)	p-value
Sex	Male/female	76/24	175/70	0.426
Age	Median (range)	64 (38-78)	65 (33-86)	0.237
HBsAg[1]	Positive/negative	19/81	22/223	0.016
HCVAb[2]	Positive/negative	69/31	212/33	<0.001
Total bilirubin (mg/dL)	Median (range)	0.9 (0.4-2.1)	1.1 (0.4-2.9)	< 0.001
Albumin (g/dL)	Median (range)	3.7 (2.9-4.4)	3.4 (2.2-4.5)	< 0.001
ICG test (%)[3]	Median (range)	16.6 (1.5-93.7)	31.2 (2.9-98.0)	< 0.001
Platelet (10^4/mm^3)	Median (range)	9.6 (4.5-30.8)	7.9 (2.4-32.4)	< 0.001
Prothrombin time (%)	Median (range)	77 (54-155)	72 (40-134)	0.001
Portal hypertension	Present/absent	63/37	174/71	0.160
Tumor number	Solitary/2/3	74/22/4	166/53/26	0.137
Tumor size (mm)	Median (range)	27 (11-50)	22 (7-50)	< 0.001
AFP (ng/ml) ¶	Median (range)	43 (1.3-25046)	40 (2.4-8466)	0.467

[1]: Hepatitis B virus surface antigen ; [2]: Hepatitis C virus antibody; [3]: Indocyanine green retention test at 15 minutes. The ICG test was not available for 20 patients in the nonhepatectomy group. ¶: Alpha-fetoprotein. The AFP was not available for 2 patients in the hepatectomy group.

TABLE 4 The Relationship between Clinical Data and Survival Times in Patients with BCLC Stages A2-A4

		Median survival years (95% confidence interval)	*p*-value
Sex	Male	4.94 (4.30-5.57)	0.038
	Female	3.94 (3.42-4.45)	
Age	Younger than 66	4.93 (3.75-6.11)	0.131
	66 or older	4.45 (3.70-5.21)	
HBsAg[1]	Negative	4.63 (4.10-5.17)	0.119
	Positive	7.47 (1.21-13.7)	
HCVAb[2]	Negative	5.58 (3.20-7.96)	0.088
	Positive	4.59 (3.95-5.24)	
Total bilirubin (mg/dL)	≤1.2	5.34 (4.74-5.94)	<0.001
	>1.2	3.22 (2.29-4.16)	
Albumin (g/dL)	≤3.5	4.05 (3.54-4.56)	<0.001
	>3.5	7.47 (5.48-9.46)	
ICG test (%)[3]	≤22	5.74 (3.54-7.93)	<0.001
	>22	4.27 (3.55-4.99)	
Platelet (10^4/mm^3)	≤10	4.40 (3.70-5.10)	0.044
	>10	5.35 (4.08-6.63)	
Prothrombin time (%)	≤70	3.75 (2.79-4.71)	0.038
	>70	4.96 (4.29-5.64)	
Portal hypertension	Absent	6.14 (3.36-8.92)	0.264
	Present	4.63 (4.10-5.16)	
Tumor number	Solitary	4.76 (3.83-5.68)	0.138
	2 or 3	4.36 (3.35-5.37)	
Tumor size (mm)	≤30	4.63 (4.11-5.14)	0.783
	>30	5.35 (3.19-7.51)	
AFP (ng/mL)[4]	≤27	5.55 (5.12-5.97)	0.001
	>27	3.99 (3.41-4.56)	
Treatment	Hepatectomy	7.51 (6.86-8.16)	<0.001
	Nonhepatectomy	4.05 (3.51-4.59)	

[1]: Hepatitis B virus surface antigen ; [2]: Hepatitis C virus antibody; [3]: Indocyanine green retention test at 15 minutes. The ICG test was not available for 20 patients in the nonhepatectomy group. [4]: Alpha-fetoprotein. The AFP was not available for 2 patients in the hepatectomy group.

Stage A1 *vs.* Stages A2-A4 in Hepatectomy Cases

The survival probability at 3, 5, 7, and 10 years was 88%, 66%, 34%, and 25%, respectively, for hepatectomy patients with stage A1 (N=62), and 89%, 70%, 61%, and 29%, respectively, for those with stages A2-A4 (N=100). There were no significant differences between these groups (**Figure 1**, p=0.50).

Hepatectomy *vs.* Nonhepatectomy in Stages A2-A4

Of 345 patients with stages A2-A4, 245 (71%) received nonsurgical treatments, including percutaneous ablation (N=152) and transcatheter arterial chemoembolization (N=78). The survival probability at 3, 5, 7 and 10 years was 64%, 37%, 24% and 11%, respectively, for the 245 patients. Survival for the hepatectomy group was significantly better than for the nonhepatectomy group in stages A2-A4 (**Figure 2**, p<0.001). **Table 3** shows the patients' clinical data for the hepatectomy and nonhepatectomy groups. In the hepatectomy group, patients negative for hepatitis C virus antibody, with good hepatic reserve, and with larger tumors were seen significantly more frequently than in the nonhepatectomy group.

Prognostic Factors in Stages A2-A4

The univariate analysis of the 345 patients with stages A2-A4 is shown in **Table 4**. Gender, total bilirubin, albumin, indocyanine green retention test at 15 minutes, platelet count, prothrombin time, alpha-fetoprotein and treatment (hepatectomy or not) were associated with survival by log-rank tests. Among these, hepatectomy, higher albumin level, lower total bilirubin level and lower alpha-fetoprotein level were identified as favorable independent prognostic factors by Cox regression. The same result was obtained with forward and backward analysis. **Table 5** shows the relative risk of each factor calculated by backward analysis.

Progression-Free Survival for Hepatectomy in Stages A2-A4

The progression-free survival probability at 1, 2, 3, 5 and 7 years was 79%, 51%, 32%, 21% and 15%, respectively, for 100 patients with stages A2-A4 who underwent hepatectomy. Of 64 patients with documented progression, 63 had cancer recurrence in the remnant liver and the remaining individual had hepatic failure.

DISCUSSION

Hepatocellular carcinoma patients with BCLC stages A2-A4 who meet the Milan criteria are believed to be the most favorable candidates for liver

TABLE 5 Significant Prognostic Factors as Determined by Multivariate Analysis in Patients with BCLC Stages A2-A4

		Hazard ratio (95% confidence interval)	Standard error	*p*-value
Treatment	Hepatectomy	0.487 (0.318-0.745)	0.217	0.001
	Nonhepatectomy	1		
Albumin (g/dL)	>3.5	0.584 (0.405-0.843)	0.187	0.004
	≤3.5	1		
Total bilirubin (mg/dL)	≤1.2	0.613 (0.437-0.862)	0.173	0.005
	>1.2	1		
AFP (ng/mL)[1]	≤27	0.709 (0.503-0.997)	0.175	0.048
	>27	1		

[1]: Alpha-fetoprotein

transplantation (8-12). However, hepatectomy is the main practical treatment modality for such patients in Japan because of the limited donor resource (13). The current study aimed to evaluate the treatment outcome for hepatectomy in these patients.

In this series, the 5-year survival rate after hepatectomy was 70% for patients with stages A2-A4 who met the Milan criteria, which might be comparable with liver transplantation for the same population (11,12). Although favorable factors for survival time such as lower total bilirubin and higher albumin levels were more frequently seen in the hepatectomy group than in the nonhepatectomy group with stages A2-A4, multivariate analysis showed that the treatment choice of hepatectomy was one of the independent favorable prognostic factors. Accordingly, it is likely that the good outcome after hepatectomy results from both proper patient selection and the efficacy of hepatectomy as a treatment for patients with stages A2-A4.

One limitation of this study was the difficulty in defining the criteria for hepatectomy. The feasibility of hepatectomy depends mainly on the correlation between hepatic reserve and anatomical location of the tumor(s), but these factors are difficult to characterize precisely. Regarding hepatic reserve, total bilirubin and albumin are not only prognostic factors for patients with stages A2-A4 **(Table 5)** but are also factors included in the Child-Pugh score. Of 100 patients who underwent hepatectomy, 92 were classified as Child-Pugh class A **(Table 6)**. Among patients with Child-Pugh scores of 5 and 6, 51% (53/104) and 29% (39/134) underwent hepatectomy, respectively. Hepatectomy candidates had therefore been selected mainly from those with Child-Pugh class A. However, tumor location is another factor that determines the feasibility of hepatectomy. A deeply located tumor can be difficult to resect even in patients with Child-Pugh class A, whereas for a tumor located near the liver surface it is easy to perform a partial resection. In our opinion, when considering criteria for hepatectomy, terms describing tumor location such as "deeply located (central)" or "near the liver surface (peripheral)" are inadequate. Furthermore, it can be difficult to define whether a tumor is central or peripheral in an individual patient.

Since the report of Bruix *et al.* in 1996 (17), the presence of portal hypertension has been an important factor used for identifying optimal candidates for hepatectomy. In their report, portal hypertension status was defined using the hepatic venous pressure gradient obtained from a hemodynamic study. Such

TABLE 6 The Relationship between Treatment and the Child-Pugh Classification in Patients with BCLC Stages A2-A4

Child-Pugh Class	Score	Hepatectomy	Nonhepatectomy	Total
A	5	53	51	104
	6	39	95	134
B	7	8	75	83
	8	0	15	15
	9	0	9	9
Total	100	245	354	

hemodynamic studies are not popular in Japan and have not been carried out for hepatectomy candidates at our hospital, so portal hypertension was estimated using clinical parameters and the platelet count. We cannot rule out the possibility that portal hypertension was not a strong prognostic factor in this study because the means for estimating it did not accurately reflect the real portal pressure.

The outcome for the nonhepatectomy group with stages A2-A4 was not as good as for the hepatectomy group with the same stages **(Figure 2)**. There may have been some patients among the hepatectomy group who would have survived even longer if they had been treated with percutaneous ablation at the time they underwent hepatectomy (18,19). However, we suggest that of all the patients with stages A2-A4, the nonhepatectomy group would receive the maximum benefit from liver transplantation.

Liver transplantation appears to give the best chance for recurrence-free survival, given that in this series the recurrence rate after hepatectomy reached 70% within 3 years. However, the supply of donated liver for the treatment of HCC is limited worldwide. Given this situation, and based on the findings of this study, we propose that patients with stages A2-A4 should not be uniformly assigned to liver transplantation as a first-line surgical intervention. Even in stages A2-A4, there are a considerable number of patients for whom hepatectomy is a reasonable first-line treatment, particularly among patients with Child-Pugh class A. In this population, salvage transplantation for recurrence after primary hepatectomy might be a reasonable strategy (20,21).

In conclusion, hepatectomy may be an appropriate treatment option for patients with stages A2-A4 who meet the Milan criteria, especially when they have a good hepatic reserve and when there is a long waiting time for liver transplantation.

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SNP Communication

Twenty Novel Genetic Variations and Haplotype Structures of the *DCK* Gene Encoding Human Deoxycytidine Kinase (dCK)

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Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: Deoxycytidine kinase (dCK) is a rate-limiting enzyme in the activation of nucleoside anticancer drugs, such as gemcitabine and cytarabine (Ara-C), to their active metabolites. In this study, the 5′-flanking region, 7 exons and their flanking introns of DCK were comprehensively screened for genetic variations in 256 Japanese cancer patients administered gemcitabine. Twenty-nine genetic variations, including twenty novel ones, were found: 11 in the 5′-flanking region, 1 in the 5′-untranslated region (UTR), 1 in the coding exon, 9 in the 3′-UTR, and 7 in the introns. The novel variations included −1110C>T, −757G>A, −639C>T, −465G>A, −402T>C, −224C>A, −199C>G, IVS1+38G>T, IVS2+78_+83delTTTTTC, IVS3−9C>T, IVS4+12T>C, IVS5+39T>C, 1357A>G, 1545A>T, 1572delA, 1736G>A, 1749G>A, 1838T>C, 1889G>A, and 2048A>T. The frequencies were 0.01 for IVS2+78_+83delTTTTTC, 0.008 for −402T>C, 0.006 for −639C>T and IVS4+12T>C, 0.004 for −757G>A and 1572delA, and 0.002 for the other 14 variations. A known nonsynonymous SNP 364C>T (Pro122Ser) was detected at a 0.061 frequency. Using the detected polymorphisms, linkage disequilibrium analysis was performed, and 24 haplotypes were identified or inferred. Our findings suggest considerable ethnic differences in genetic variations of *DCK* and provide fundamental and useful information for genotyping *DCK* in the Japanese and probably other Asian populations.

Keywords: *DCK*, genetic variation, haplotype, gemcitabine, ethnic differences

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On February 15, 2008, these variations were not found in "A database of Japanese Single Nucleotide Polymorphisms (http://snp.ims.u-tokyo.ac.jp/)", "dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/)", or "PharmGKB (http://www.pharmgkb.org/do/)".

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Introduction

Deoxycytidine kinase (EC 2.7.1.74, dCK) is a key enzyme in the salvage pathway of deoxyribonucleotide biosynthesis and is responsible for phosphorylation of both pyrimidine and purine deoxyribonucleosides to the corresponding deoxyribonucleotides.[1] dCK also catalyzes the rate-limiting step in the phosphorylation of pharmacologically important anticancer and antiviral drugs such as 2′,2′-difluorodeoxycytidine (gemcitabine), cytosine arabinoside (ara-C), 2-chlorodeoxyadenosine (cladribine), and 2′,3′-dideoxycytidine.[2]

The *DCK* gene consists of 7 exons and spans over 34-kb on chromosome 4 (4q13.3-q21.1).[3] The transcription regulatory region of *DCK* is GC-rich and lacks a TATA-box but harbors a number of binding sites for transcription factors such as Sp1 and E2F.[4-6] Human dCK protein (260 amino acid residues) is constitutively expressed throughout the cell cycle and is present at low levels in most tissues. Therefore, this enzyme could be involved in the activation of chemotherapeutic nucleoside analogues in tumor cells as well as normal cells.[7-9] Expression levels of dCK are critical determinants of gemcitabine and ara-C antitumor activities, and dCK-deficient cells are highly resistant to nucleoside analogues.[10] Furthermore, the introduction of *DCK* cDNA into dCK-deficient tumor cell lines restores *in vitro* sensitivities to ara-C,[11,12] and the decreased dCK expression is known to be associated with *in vitro* acquired resistance to gemcitabine.[13]

Recently, several single nucleotide polymorphisms (SNPs) and haplotypes of *DCK*, including four nonsynonymous SNPs, 70A>G (Ile24Val), 356C>G (Ala119Gly), 364C>T (Pro122Ser) and 727A>C (Lys243Gln), have been identified in Africans, Europeans, and Chinese[14-16] and published in the PharmGKB database. *In vitro* functional characterization showed that three nonsynonymous variations, 70A>G (Ile24Val), 356C>G (Ala119Gly) and 364C>T (Pro122Ser), were associated with dCK activity and expression.[16] In Chinese, several SNPs were detected in the promoter region, and the haplotype with two regulatory SNPs, −360C>G and −201C>T, was associated with increased transcriptional activity.[14] However, there has been no report of a *DCK* SNP survey and haplotype analysis for a Japanese population. In this study, all 7 exons and their surrounding introns were resequenced for comprehensive screening of *DCK* genetic variations. Sequence analysis detected 29 variations from 256 Japanese cancer patients administered gemcitabine. Frequencies of haplotypes with both regulatory SNPs, −360C>G and −201C>T, and nonsynonymous SNP 364C>T (Pro122Ser) were estimated in a Japanese population, and ethnic differences among Japanese, Chinese, Europeans and Africans were shown.

Materials and Methods

Human genomic DNA samples: All 256 Japanese cancer patients were administered gemcitabine at the National Cancer Center Hospital and National Cancer Center Hospital East. Total DNA was extracted from blood leukocytes and used as template in the polymerase chain reaction (PCR). The ethical review board of the National Cancer Center and National Institute of Health Sciences approved this study. Written informed consent was obtained from all participants.

PCR conditions for DNA sequencing: The Genbank reference sequence NT_006216.14 was used for primer design and SNP detection. First, the entire *DCK* gene was divided into two regions (exons 1 and 2 and exons 3 to 7), and each region was amplified from 100 ng of genomic DNA using 1.25 units of *Z-Taq* (Takara Bio. Inc., Shiga, Japan) with 0.2 μM primers listed in **Table 1** (1st PCR). The first PCR conditions consisted of 30 cycles of 98°C for 5 sec, 60°C for 10 sec, and 72°C for 150 sec. Next, each exon except for exon 1 was amplified by *Ex Taq* (1.25 units) (Takara Bio. Inc.) with appropriate primers (0.5 μM) designed in the introns (**Table 1**, 2nd PCR). Conditions of the second round PCR with *Ex Taq* were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. For amplification of the region from 1.5-kb upstream of the translation initiation site to exon 1, *LA Taq* (2.5 units) (Takara Bio. Inc.) with GC buffer I and exon 1 specific primers (0.5 μM) were used. PCR with *LA Taq* was carried out under the following conditions: 94°C for 1 min followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 3 min, and then a final extension at 72°C for 5 min. Following the PCR, products were treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the sequencing primers listed in **Table 1** (Sequencing). Excess dye was removed by a DyeEx96 kit (Qiagen, Hilden, Germany), and the eluates were analyzed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). All variations were confirmed by sequence analysis of PCR products generated by a new amplification of the original genomic DNA templates. Furthermore, the rare SNPs found in only one sample as heterozygotes were confirmed by re-sequencing the PCR fragments produced by amplification with a high fidelity DNA polymerase KOD-Plus- (TOYOBO, Tokyo, Japan).

Haplotype analysis: Hardy-Weinberg equilibrium and linkage disequilibrium (LD) analyses were performed by SNPAlyze software (Ver 3.1, Dynacom Co., Yokohama, Japan). All allele frequencies were in Hardy-Weinberg equilibrium. Some haplotypes were unambiguously

Table 1. Primer sequences used in this study

	Amplified or sequenced region	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Amplified region*	Length (bp)
1st PCR	Exons 1 to 2	CGGTTTATTAGTGTTACTGGATGGG	AGTCACCCCTCAGTAACCTCAAGAA	364945_371347	6403
	Exons 3 to 7	GGCATGGTACTGCTTGGTTTTTTCA	TCTAGGTGGCTCGGTATAAGTTTCA	394431_403862	9432
2nd PCR	Exon 1	ACCAAGTGCTTCAAGAGTCC	AAAGAGGGAGCAGAGGTTCA	365110_366917	1808
	Exon 2	GCAGGGAGCCTTTTCATTTT	GATATGGAGAGCCAACTGTA	370620_371078	459
	Exon 3	AGGATTTTCCAGACCTCAGA	ACCAGACTGCTGAGGGATTT	394902_395443	542
	Exon 4	TCTGCTTTCCACGGCACTAT	ATTGAGGAAGCACAAAGAGC	396162_396663	502
	Exon 5	CAAGTGGCTGAAAAGCTCAT	ACGCTATCAATACCACCAAG	398368_398883	516
	Exon 6	GACAACATTTTGATTTTCCAAG	GGATCTTTATTTTAGCTCAGGC	399289_399681	393
	Exon 7 fragment 1	TGGCATTGTGGTAGTTACTT	ATACACAGGAAAAACACTGC	401952_403074	1123
	Exon 7 fragment 2	AGATGGTTCCAGTATCAGCA	TCCAGACCACCATTAGGCTC	402353_403692	1340
Sequencing	Exon 1	AGTGCTTCAAGAGTCCCAAT	GATGGGACAAATGCAGTGTA		
		TGCTGTTCTTTTGCTTATGC	CTGAGAGGCTGCTTGTTCCA		
		TCTCAGTGCCTGTTTTCCCA	AAAACCCGCCTCTCTAGTGG		
		CACTAGAGAGGCGGGTTTTC	GGTGTCGGGGTTTGACTTTG		
		GCAGGTCAGGATCTGGCTTA	AGGTAAGGGAAGGATGCTCT		
	Exon 2	GCAGGGAGCCTTTTCATTTT	GATATGGAGAGCCAACTGTA		
	Exon 3	AGGATTTTCCAGACCTCAGA	AAGTCCAGTTCTAAGATAAAAAC		
	Exon 4	TGAAATGATACATGTGTTGATG	ATTGAGGAAGCACAAAGAGC		
	Exon 5	CAAGTGGCTGAAAAGCTCAT	GAAGATACCAATAAGCAAAACG		
	Exon 6	TTGTTGAATTTCTGATTATTTTA	GGATCTTTATTTTAGCTCAGGC		
	Exon 7 fragment 1	TGGCATTGTGGTAGTTACTT	AAAACGATTAAAAACTTGGGTT		
		AGATGGTTCCAGTATCAGCA	GACTTTAACTTTATAGCAGGCT		
	Exon 7 fragment 2	GCTTTCTCTACTGTCTGGAT	ATACACAGGAAAAACACTGC		
		TTTGTTTAGTTAAGGTGTGC	ATTATGACCACCACACTGAG		

*The reference sequence is NT_006216.14.

assigned in subjects with homozygous variations at all sites or a heterozygous variation at only one site. Separately, diplotype configurations (combinations of haplotypes) were inferred by LDSUPPORT software, which determines the posterior probability distribution of the diplotype configuration for each subject based on estimated haplotype frequencies.[17] The haplotypes are described as numbers plus small alphabetical letters.

Results and Discussion

The *DCK* 5′-flanking region (up to 1.5-kb upstream of the translational start site), all 7 exons and their flanking introns were sequenced in 256 Japanese cancer patients administered gemcitabine, and 29 variations, including 20 novel ones were found (see **Table 2**). The novel variations were −1110C>T, −757G>A, −639C>T, −465G>A, −402T>C, −224C>A and −199C>G in the 5′-flanking region (A of the translational start codon is numbered +1), IVS1+38G>T in intron 1, IVS2+78_+83delTTTTTC in intron 2, IVS3−9C>T in intron 3, IVS4+12T>C in intron 4, IVS5+39T>C in intron 5, and 1357A>G, 1545A>T, 1572delA, 1736G>A, 1749G>A, 1838T>C, 1889G>A, and 2048A>T in the 3′-noncoding region of exon 7. The frequencies were 0.01 for IVS2+78_+83delTTTTTC, 0.008 for −402T>C, 0.006 for −639C>T and IVS4+12T>C, 0.004 for −757G>A and 1572delA and 0.002 for the other 14 variations.

Nine SNPs detected in this study were previously reported[14-16] and/or found in the dbSNP and PharmGKB databases. Among them, two regulatory SNPs, −360C>G and −201C>T, were found at allele frequencies of 0.131, which are comparable to those in a Chinese population (0.156),[14] but higher than those in Europeans (0.01–0.025) and Africans (not detected).[15,16] The frequency of nonsynonymous SNP 364C>T (Pro122Ser) in Japanese (0.061) is slightly higher than that in Europeans (0.015–0.025) and Africans (0.017).[15,16] Other nonsynonymous SNPs, 70A>G (Ile24Val), 356C>G (Ala119Gly) and 727A>C (Lys243Gln), found in Europeans and Africans were not detected in a Japanese population.

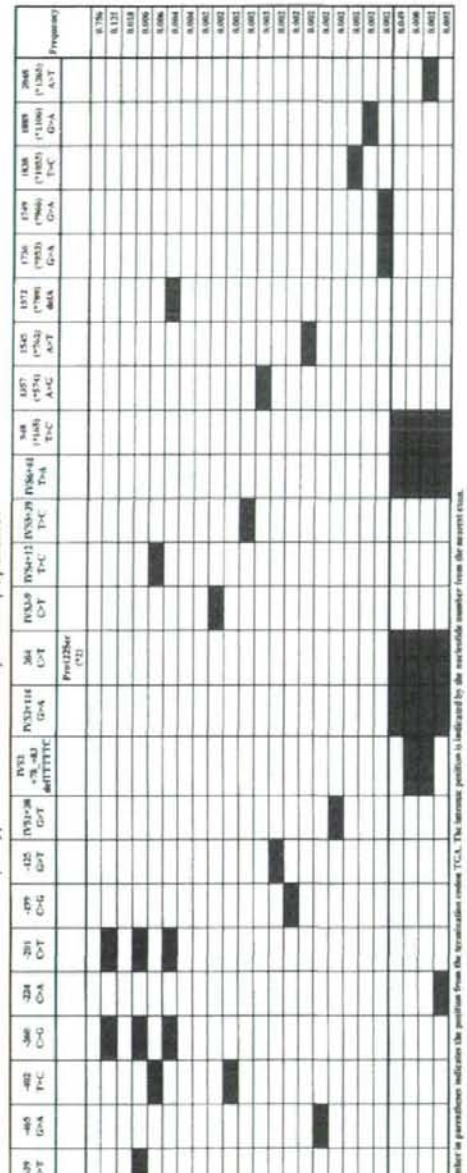
The 5′-flanking region of the *DCK* gene contains binding sites for several transcription factors which regulate *DCK* expression.[4-6] In this study, 11 variations were found in the *DCK* 5′-flanking region. Among them, two associated SNPs, −360C>G and −201C>T, were reported to increase ara-C efficacy: −360C>G results in

Table 2. Genetic variations of *DCK* found in a Japanese population

SNP ID		Reference	Location	Position		Nucleotide change and flanking sequences (5′ to 3′)	Amino acid change	Allele frequency
This study	NCBI (dbSNP)			NT_006216.14	From the translational initiation site or from the nearest exon			
MPJ6_DCK001	rs1906021		5′-Flanking	365234	−1329[c]	AGGATTGGCCTG**C/T**GACCAATCAGAG		0.018
MPJ6_DCK002[a]			5′-Flanking	365453	−1110[c]	ACTAAAAATGCA**C/T**TCATCTAGCTGG		0.002
MPJ6_DCK003[a]			5′-Flanking	365806	−757[c]	TCCCACTGGCAG**G/A**TAAATGGGCTAA		0.004
MPJ6_DCK004			5′-Flanking	365865_365866	−698_−697[c]	ATGAAAAGCACA**TA/-**GAAGAAAACAGC		0.131
MPJ6_DCK005[a]			5′-Flanking	365924	−639[c]	GACGGCACTTCG**C/T**CTGATAGTCTTC		0.006
MPJ6_DCK006[a]			5′-Flanking	366098	−465[c]	AAAGCCTGGCAC**G/A**GCCCACTGCAGG		0.002
MPJ6_DCK007[a]			5′-Flanking	366161	−402[c]	GTCCACCCTTCC**T/C**CCCCACCCGACT		0.008
MPJ6_DCK008		13, 14, 15	5′-Flanking	366203	−360[c]	GCCCTGCCCGGG**C/G**GCCTGGCTGCTT		0.131
MPJ6_DCK009[a]			5′-Flanking	366339	−224[c]	AGCTAGGGAGCG**C/A**GGCTTGAGGAGG		0.002
MPJ6_DCK010	rs2306744	13, 14, 15	5′-Flanking	366362	−201[c]	GGGCGGGGCCGC**C/T**CCGCAGGCCCGC		0.131
MPJ6_DCK011[a]			5′-Flanking	366364	−199[c]	GCGGGGCCGCCC**C/G**GCAGGCCCGCCA		0.002
MPJ6_DCK012		13	Exon 1 (5′-UTR)	366438	−125[c]	GCGGGCCGGTGA**G/T**CTCACTAGCTGA		0.002
MPJ6_DCK013[a]			Intron 1	366691	IVS1 + 38	CGCAAGGCTGGG**G/T**TGTCGCGGCAGT		0.002
MPJ6_DCK014[a]			Intron 2	370987_370992	IVS2 + 78_ + 83	TTTTTCTTTTTC**TTTTTC**/-AATAAAACTTTC		0.010
MPJ6_DCK015	rs6446988	15	Intron 2	371023[b]	IVS2 + 114	CGCTTTAGGTAT**G/A**TATCTTCATCTA		0.061
MPJ6_DCK016		14, 15	Exon 3	395250	364[c]	GATGCAGAGAAA**C/T**CTGTATTATTTT	Pro122Ser	0.061
MPJ6_DCK017[a]			Intron 3	396277	IVS3−9	TTGATGAAGACT**C/T**TCTTTTAGGTAT		0.002
MPJ6_DCK018[a]			Intron 4	396445	IVS4 + 12	GGTAAAACCCAA**T/C**AAAAATGTGTTT		0.006
MPJ6_DCK019[a]			Intron 5	398697	IVS5 + 39	ATTTTAAATACC**T/C**TTGTTACCTTTG		0.002
MPJ6_DCK020	rs1486271	14	Intron 6	399523[b]	IVS6 + 41	TTTGTTTTTTCTT**/A**AAAAAGTGTACT		0.061
MPJ6_DCK021	rs4643786	15	Exon 7 (3′-UTR)	402270[b]	948(*165)[c]	AAAACTTTTTGA**T/C**CAGTTTCTTTTC		0.061
MPJ6_DCK022[a]			Exon 7 (3′-UTR)	402679	1357(*574)[c]	TCTGCTTTCTCT**A/G**CTGTCTGGATTA		0.002
MPJ6_DCK023[a]			Exon 7 (3′-UTR)	402867	1545(*762)[c]	TATCCTGAAAGC**A/T**TTATTTTTTGTT		0.002
MPJ6_DCK024[a]			Exon 7 (3′-UTR)	402894	1572(*789)[c]	ATAGGAAATAAAA**/-**TTAATGAAGACA		0.004
MPJ6_DCK025[a]			Exon 7 (3′-UTR)	403058	1736(*953)[c]	TTAAGGTGTGCA**G/A**TGTTTTTCCTGT		0.002
MPJ6_DCK026[a]			Exon 7 (3′-UTR)	403071	1749(*966)[c]	TGTTTTTCCTGT**G/A**TATTAAACCTTT		0.002
MPJ6_DCK027[a]			Exon 7 (3′-UTR)	403160	1838(*1055)[c]	AACTACTATTTTT**A/C**CTTTCCAAGTCA		0.002
MPJ6_DCK028[a]			Exon 7 (3′-UTR)	403211	1889(*1106)[c]	GATGATAATTTA**G/A**TGGATTAACCAG		0.002
MPJ6_DCK029[a]			Exon 7 (3′-UTR)	403370	2048(*1265)[c]	TCTTAAGTATAA**A/T**CCTTATGAACTA		0.002

[a]Novel variations detected in this study.
[b]The reference sequence NT_006216.14 has the minor allele.
[c]A of the translation initiation codon ATG is numbered +1 and the number in the parentheses indicates the position from the termination codon TGA.

Table 3. Haplotypes of *DCK* in a Japanese population



a novel AP2 site, and −201C>T leads to loss of Sp1 and AP2 sites.[14] Biological significance of the other 9 variations in the 5′-flanking region should be further determined.

Using the detected variations, linkage disequilibrium (LD) analysis was performed. A perfect linkage ($r^2 = 1$) was observed among −698_−697delTA, −360C>G and −201C>T, and among IVS2+114G>A, 364C>T, IVS6+41T>A and 948T>C. Therefore, the entire region was analyzed as one LD block for haplotype estimation. The determined/inferred haplotypes are shown as numbers plus small alphabetical letters (**Table 3**). In this study, the haplotypes without amino acid changes were defined as the **1* group, and the haplotype harboring the nonsynonymous SNP (Pro122Ser) was assigned as the **2* group. Several haplotypes were first unambiguously assigned by homozygous variations at all sites (**1a*, **1b* and **2a*) or heterozygous variation at only one site (**1c* and **1g* to **1s*). Separately, the diplotype configurations (combinations of haplotypes) were inferred by LDSUPPORT software. The additionally inferred haplotypes were three **1* subtypes (**1d* to **1f*), and **2b*. The most frequent haplotype was **1a* (frequency, 0.756), followed by **1b* (0.121), **2a* (0.049), and **1c* (0.018). The frequencies of the other minor haplotypes were less than 0.01. Some haplotypes (**1t*, **2c* and **2d*) were inferred in only one subject and ambiguous (**Table 3**).

Previously, Shi *et al.*[14] reported that the two SNPs, −360C>G and −201C>T, were in perfect LD. It was reported that these SNPs were associated with the clinical outcome for Chinese AML patients treated with ara-C, which was explained by increased transcriptional activity.[14] These SNPs were also found in Europeans at a low frequency (0.025), but their association with *DCK* mRNA expression levels was not confirmed. In our study, haplotypes harboring these SNPs were **1b* (frequency, 0.121), **1d* (0.006) and **1f* (0.004). It must be noted that −698_−697delTA is completely associated with these haplotypes.

Lamba *et al.*[16] reported that the recombinant 122Ser dCK protein showed reduced enzyme activity (43±4% of the wild-type), and lymphoblast cell lines from subjects carrying heterozygous 364C>T had reduced dCK activity compared with those from homozygous wild-type subjects.[16] In our study, the haplotype frequency of the **2* group harboring SNP 364C>T was 0.061. The **2* group also harbors the known SNPs, IVS2+114G>A, IVS6+41T>A and 948T>C. These three SNPs were also found in Europeans and Africans at different frequencies (0.05 in Europeans and 0.767 in Africans) and constitute the common haplotype group, Group 1/Block 1.[16] However, Group 1 haplotype did not harbor 364C>T in both populations. The LD profile of Japanese was similar to that of Europeans except for the linkage of 364C>T, but different from that of Africans. In a Chinese popula-

tion (n=48), 364C>T (Pro122Ser) and three SNPs, IVS2+114G>A, IVS6+41T>A and 948T>C, were not found.[14] Thus, these findings indicate considerable ethnic differences in *DCK* SNPs and haplotypes.

In conclusion, 29 variations including 20 novel ones were identified in *DCK* from 256 Japanese cancer patients administered gemcitabine. Using the detected polymorphisms, 24 haplotypes were determined or inferred. Our findings suggest considerable ethnic differences in genetic variations of *DCK* and provide fundamental and useful information for genotyping *DCK* in the Japanese and probably other Asian populations.

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Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma

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Sorafenib is an orally active multikinase inhibitor that targets serine and threonine, and tyrosine kinases that are involved in tumor-cell signal transduction and tumor angiogenesis. This phase I trial was conducted to evaluate the pharmacokinetics (PK), safety, and preliminary efficacy of sorafenib in Japanese patients with hepatocellular carcinoma (HCC) with underlying liver dysfunction. Patients with unresectable HCC, Child–Pugh status A or B, and adequate organ functions were treated. A single dose of sorafenib was administered, followed by a 7-day wash-out period, after which patients received either sorafenib 200 mg (cohort 1) or 400 mg (cohort 2) twice daily. The PK were investigated after a single dose and during steady state. The efficacy was evaluated using the Response Evaluation Criteria in Solid Tumors. A total of 27 patients were evaluated for PK, safety, and efficacy. Although both area under the concentration–time curve for 0–12 h and maximal concentration at steady state were slightly lower in Child–Pugh B patients than in Child–Pugh A patients, the difference was not considered to be clinically relevant. Common adverse drug events included elevated lipase, amylase, rash or desquamation, diarrhea, and hand–foot skin reaction. A dose-limiting toxicity of hand–foot skin reaction was observed in one patient (cohort 2). Among the 24 patients evaluable for tumor response, one patient (4%) achieved a partial response, 20 (83%) had stable disease, and three (13%) had progressive disease. Sorafenib demonstrated a favorable tolerability and safety profile in Japanese HCC patients. Moreover, promising preliminary antitumor activity has been observed. Finally, there were no clinically relevant differences in PK between Child–Pugh A and B patients. (*Cancer Sci* 2008; 99: 159–165)

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. Surgery and local ablation therapy, including radiofrequency, are considered curative treatment for HCC.[1–3] Transcatheter arterial chemoembolization (TACE) has been applied to patients with advanced incurable HCC.[3–5] The majority of patients, however, have recurrence or metastasis after these treatments. Although systemic therapy, including chemotherapeutic agents, is available for metastatic or TACE-refractory advanced HCC, the prognosis remains poor. No standard systemic therapy that prolongs survival has been identified.

Sorafenib (BAY 43-9006; Bayer HealthCare Pharmaceuticals, West Haven, CT, USA) was discovered based on its potent activity against Raf kinase in a battery of biochemical, cellular, and *in vivo* assays.[6,7] Extensive mechanism of action studies have shown that sorafenib may inhibit tumor growth through multiple mechanisms: by inhibiting tumor-cell proliferation that is dependent on activation of the mitogen-activated protein kinase (MAPK) pathway, and by inhibiting tumor angiogenesis through inhibition of vascular endothelial growth factor receptor (VEGFR)-2 and platelet-derived growth factor receptor (PDGFR)-β. Some evidence points to the MAPK signal-transduction pathway as playing an important role in tumor growth and progression in HCC.[8] Published data suggest that vascular endothelial growth factor (VEGF) also plays a critical role in angiogenesis of HCC, which is important for the growth and progression of HCC.[9] Sorafenib has been investigated in various solid tumors in clinical studies[10–15] and has been approved in many countries for the treatment of renal cell carcinoma. Promising results with sorafenib were recently observed in a phase II study in HCC patients.[15]

Various factors, such as liver function or disease extension, influence treatment selection and prognosis for HCC.[2,3,16] Etiology, underlying condition, and treatment for HCC vary across countries or regions.[2,3,17] Most HCC patients in Japan have hepatitis or cirrhosis due to hepatitis B or C virus[2] and suffer from complications of liver dysfunction, with potential changes in the activity of metabolic enzymes, a reduction in blood flow in the liver, or protein-binding ability due to low serum albumin. However, the degree of influence of these factors on the pharmacokinetics (PK) and tolerability of sorafenib in Japanese patients with HCC is unknown. A phase I study in Japanese patients with advanced solid tumors was conducted before the present study,[18] and found that sorafenib at 400 mg b.i.d. was well tolerable and recommended for phase II studies based on safety and efficacy data. To investigate the effect of liver dysfunction and its complications on the PK, safety, and tolerability of sorafenib in Japanese patients with HCC, a phase I study was conducted. The primary objective of the present study was to evaluate the PK of sorafenib, and the secondary objectives were to evaluate the safety and tolerability of sorafenib, tumor response, time to progression (TTP), and overall survival in Japanese patients with HCC.

Materials and Methods

Patient eligibility. The eligibility criteria for enrolment in the study were: (1) histologically confirmed HCC; (2) unresectable and incurable with ablation therapy or TACE; (3) age ≥ 20 years; (4) Eastern Cooperative Oncology Group performance status of 0 or 1; (5) adequate bone marrow (absolute neutrophil count ≥ 1500 cells/mm^3, platelet count ≥ 75 000 cells/mm^3, and hemoglobin ≥ 8.0 g/dL), coagulation (prothrombin time ≤ 1.5 × upper limit of normal [ULN] and activated partial thromboplastin time ≤ 1.5 × ULN), renal function (serum creatinine concentration ≤ 1.5 × ULN), and hepatic function (serum total bilirubin level ≤ 3.0 mg/dL, serum aspartate and alanine transaminase levels ≤ 5.0 × ULN); (6) cirrhotic status of Child–Pugh A or B; (7) life expectancy of at least 12 weeks; and (8) written informed consent from the patient.

Exclusion criteria included clinically evident congestive heart failure, serious cardiac arrhythmias, active or symptomatic coronary artery disease or ischemia, active clinically serious infections, seizure disorder requiring medication, history of organ allograft, prior malignancy (any cancer treated curatively

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>3 years prior to entry was not excluded), metastatic brain or meningeal tumors, anticancer therapy within 3 months of study entry, and pregnancy or lactation for women. This protocol was approved by the National Cancer Center's institutional review board for clinical investigation with the provisions of the Declaration of Helsinki, Good Clinical Practice guidelines, and local laws and regulations.

Treatment methods. The dose for the first cohort was 200 mg bid sorafenib, and the dose for the second cohort was escalated to 400 mg bid. To investigate the PK profile of sorafenib, including its elimination phase, a single dose was given as one-time administration followed by a 7-day wash-out period. Subsequently, the drug was given twice daily for 28 days without a resting period (cycle 1). Either 200 mg or 400 mg sorafenib was given to all patients orally twice daily, in the morning and in the evening (every 12 h as far as possible). Patients were allowed to continue on sorafenib after cycle 1 if they consented to continue, and no intolerable adverse event was experienced, as assessed by investigators. Treatment was continued until disease progression, intolerable adverse event, or consent of withdrawal.

Examination and observation for safety was conducted every 2 weeks, and administration of the drug was to be terminated immediately when the patient met the criteria for removal from the study, described in this protocol with due consideration for the patient's safety.

Study design. The present study was a non-randomized, uncontrolled, non-blinded, single-center phase I study to investigate the PK, safety, and tolerability of sorafenib in Japanese patients with HCC. The dose level investigated in this study was 200 mg bid for the first cohort and 400 mg bid for the second cohort. Twelve patients, including six with Child–Pugh A and six with Child–Pugh B, were to be enrolled in each cohort. Tolerability was evaluated at the end of cycle 1 by Child–Pugh classification. If less than two out of six patients experienced dose-limiting toxicity (DLT) in the 200-mg bid cohort, the study would proceed to the 400-mg bid cohort. DLT that needed dose modification was defined as: (1) grade 3 and grade 4 non-hematological toxicity, except for pancreatic enzyme abnormality and hand–foot skin reaction; (2) grade 4 pancreatic enzyme elevation with values that persisted on two consecutive determinations with a 3-day interval, or clinical and/or imaging findings of pancreatitis, or pancreatic adverse event considered to be life threatening, or having a high risk of serious or chronic disorders; (3) severe hand–foot skin reaction, moist desquamation, ulceration, blistering, or severe pain of the hands or feet, or severe discomfort that caused the patient to be unable to work or carry out the activities of daily living; (4) grade 4 neutropenia (absolute neutrophil count less than 500/μL) for 7 days duration; (5) grade 4 neutropenia of any duration with fever of 38.5°C and above; and (6) platelet count < 25 000 cells/mm^3. Toxicity was graded according to the National Cancer Institute common toxicity criteria version 2.0. The independent safety committee for this study gave advice on the evaluation of tolerability of the dose level and the cohort transition.

Pharmacokinetics. All patients who received at least one dose of study medication were included in the PK analysis. Blood samples for the determination of plasma concentrations of sorafenib (and its metabolites) were collected prior to drug administration, as well as 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, and 120 h after single-dose administration. For the first cycle, blood was sampled prior to the first dosing on days 1, 4, 7, 10, 14, 21, and 28, along with 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h after the first dose on days 14 and 28. Urine voided up to 48 h after single administration was collected.

Concentrations of sorafenib and its metabolites in plasma and urine were determined using validated liquid chromatography and tandem mass spectrometry methods. Plasma PK parameters were calculated by non-compartment analysis by the KINCALC program (Bayer HealthCare Pharmaceuticals).[10] Primary plasma PK parameters were area under the concentration–time curve (AUC), AUC for 0–12 h (AUC_{0-12}), and maximal concentration (C_{max}). Plasma concentrations and PK parameters were analyzed by dose and Child–Pugh classification.

Clinical assessments. Physical examination, complete blood cell counts, serum chemistries, and urinalysis were carried out at baseline and at least twice monthly after initiating treatment with sorafenib. Patients underwent dynamic computed tomography (CT) to evaluate tumor response at baseline, the end of cycle 1, and every two cycles thereafter. CT was carried out by obtaining contiguous transverse sections with the helical scanning method at a section thickness of 5 mm. Tumor evaluation was assessed using the Response Evaluation Criteria in Solid Tumours (RECIST).[19]

Statistical analysis. The data were analyzed using SAS (SAS Institute, Cary, NC, USA). The safety and efficacy were evaluated on an intention-to-treat basis. Progression-free survival was calculated from the first day of treatment until evidence of tumor progression, clinical progression, or death due to any cause. Overall survival was calculated from the first day of treatment until death due to any cause. Survival data were analyzed using the Kaplan–Meier method.

Results

Patient characteristics and treatments. From April 2004 through January 2005, a total of 27 patients were enrolled in the present study. Thirteen patients were enrolled at the treatment level of 200 mg (cohort 1) and 14 at the treatment level of 400 mg (cohort 2) twice daily (b.i.d.) for 28 days (cycle 1). One out of 13 patients in cohort 1 discontinued the study due to consent withdrawal after single-dose administration. One out of 14 patients in cohort 2 dropped out of this study due to adverse events during cycle 1. Patient characteristics are shown in Table 1. The median number of cycles administered per patient was five (range, 1–13 cycles). None of the patients from the 200-mg group reduced the dose of sorafenib, whereas two patients required dose reduction in the 400-mg group.

Evaluation of PK. Plasma drug concentrations were analyzed in 27 patients in the PK analysis. Plasma PK parameters of patients in the 200 and 400 mg bid groups are shown in Tables 2 and 3. There was a large interpatient variability in the PK of sorafenib. Geometric means of AUC, AUC_{0-12}, and C_{max} on day 1 of single-dose administration were not statistically different between 200 and 400 mg bid or between Child–Pugh A and B. Dose-dependent increases in AUC_{0-12} and C_{max} were observed at steady state (day 14) in the 200-mg bid and 400-mg bid patients; however, these increases were not dose proportional. Geometric means of AUC_{0-12} and C_{max} were slightly lower in the Child–Pugh B patients compared with the Child–Pugh A patients at steady state. The $t_{1/2}$ after single dose was similar between the Child–Pugh A and B groups for both dose levels.

Dose-dependent increases in the AUC_{0-12} and C_{max} of metabolites M-2 (*N*-oxide), M-4 (*N*-demethyl), and M-5 (*N*-oxide, desmethyl derivative) were observed. M-2 was the main metabolite in plasma. Ratios of each metabolite to the sum of all analytes were similar between the 200-mg bid and 400-mg bid patients and for baseline Child–Pugh class (Tables 2,3). M-7 (glucuronide of sorafenib) and M-8 (glucuronide of M-2) were detected in urine though no unchanged substance or M-2 was detected. There was no difference between the Child–Pugh A (1.21% for M-7 and 0.02% for M-8 at 400 mg) and B (1.18% and 0.02%, respectively, at 400 mg) groups in the urinary excretion rate of compounds at steady state. Interestingly, these PK results were similar to those obtained from the Japanese phase I study in non-HCC tumors.[18]

Adverse events. Adverse events of all 27 patients are shown in Table 4. Twenty-six out of 27 patients (96.3%) experienced an adverse event: 12 out of 13 patients (92.3%) in the 200-mg

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Table 1. Patient characteristics

Characteristic	200 mg bid (n = 13)	400 mg bid (n = 14)	Total (n = 27)
Sex (n)			
Male	12	13	25
Female	1	1	2
Median age (years)	69 (range 48–77)	70 (range 63–79)	70 (range 48–79)
Eastern Cooperative Oncology Group performance status			
0	13	14	27
Child–Pugh classification			
A	7	6	13
B	6	8	14
Viral markers			
HB antigen$^+$, HCV antibody$^-$	3	1	4
HB antigen$^-$, HCV antibody$^+$	9	11	20
HB antigen$^-$, HCV antibody$^-$	1	2	3
Previous treatment			
–	1	3	4
+	12	11	23
Tumor stage			
II	1	2	3
III	7	8	15
IVa	1	1	2
IVb	4	3	7
Portal vein tumor thrombus			
–	12	13	25
+	1	1	2
Metastasis			
–	9	11	20
+	4	3	7
Lung	3	1	4
Lung + lymph node	1	1	2
Lymph node	0	1	1

HB, hepatitis B; HCV, hepatitis C virus.

Table 2. Pharmacokinetic parameters of sorafenib and metabolites M-2, M-4, and M-5: sorafenib following single dose and multiple dose of 200 mg and 400 mg geometric mean (coefficient of variation)

Sorafenib	Parameter	Unit	200 mg bid				400 mg bid			
			Child–Pugh A		Child–Pugh B		Child–Pugh A		Child–Pugh B	
Single	n		7		6		6		8	
Dose	AUC	mg*h/L	28.29	190.29[†]	18.64	74.1	20.33	90.31	26.87	96.97
Day 1	$AUC_{0\text{-}12}$	mg*h/L	5.02	190.36	2.75	61.06	3.82	86.06	3.11	88.16
	C_{max}	mg/L	0.81	195.96[†]	0.49	67.85	0.55	83.75	0.53	86.68
	T_{max}	h[†]	7	3–12[†]	18	4–24	8	6–24	24	4–24
	$T_{1/2}$	H	25.14	30.13[†]	30.44	35.67	22.28	12.49	27.2	45.19
Cycle 1	N		6		6		6		6	
Day 14	$AUC_{0\text{-}12}$	mg*h/L	25.52	75.04	15.28	55.26	33.47	60.13	29.45	59.44[§]
	C_{max}	mg/L	3.36	87.29	1.89	62.14	4.66	66.12	3.04	94.39
Cycle 1	N		6		6		6		5	
Day 28	$AUC_{0\text{-}12}$	mg*h/L	31.63	101.64	20	73.4	28.91	86.79	20.71	72.06
	C_{max}	mg/L	4.22	92.32	3.32	78.65	3.32	113.47	4.01	79.12

[†]Median (range), [§]n = 6, [§]n = 5. $AUC_{0\text{-}12}$, area under the concentration–time curve for 0–12 h.

group and 14 out of 14 patients (100%) in the 400-mg group. The most common drug-related adverse events were elevated lipase or amylase (88.9%), dermatological events (81.5%), and gastrointestinal events (70.4%). Common dermatological events were rash or desquamation (55.6%), and hand–foot skin reaction (44.4%). The incidence of adverse events in the 400-mg dose level was higher than that in the 200-mg dose level by ≥20%. These events fell under the categories of dermatology/skin (100.0 *vs* 61.5%), general cardiovascular (35.7 *vs* 7.7%), and renal/genitourinary (21.4 *vs* 0%).

Elevation of lipase and amylase was transient in most of the cases, and decreased gradually in all patients without treatment. One patient on 400 mg bid experienced acute pancreatitis that necessitated sorafenib withdrawal. The patient experienced abdominal pain 6 months after beginning treatment (cycle 6). Moreover, high lipase and amylase, as assessed by blood test,

Table 3. Pharmacokinetic parameters of sorafenib and metabolites M-2, M-4, and M-5: metabolites following multiple dose of 200 mg and 400 mg, measured at steady state (cycle 1, day 14) geometric mean (% coefficient of variation)

Parameter	200 mg bid			400 mg bid		
	M-2	M-4	M-5	M-2	M-4	M-5
Child–Pugh A						
n	6	6	6	6	6	6
AUC_{0-12} (mg × h/L)	4.18 (126)	0.92 (158)	0.79 (167)	6.18 (127)	1.68 (159)	1.22 (193)
Ratio[1] (%)	13.08 (30)	2.89 (60)	2.48 (81)	14.16 (39)	3.85 (55)	2.79 (85)
Child–Pugh B						
n	6	5	4	5	5	5
AUC_{0-12} (mg × h/L)	1.62 (173)	0.36 (131)	0.44 (351)	5.67 (90)	2.13 (142)	1.25 (117)
Ratio[1] (%)	9.05 (67)	1.85 (42)	1.95 (157)	14.46 (36)	5.44 (56)	3.19 (47)

[1]Median ratio of each metabolite to sum of all analytes. BAY 43-9006: M-2, BAY 67-3472; M-4, BAY 43-9007; and M-5, BAY 68-7769. AUC_{0-12}, area under the concentration–time curve for 0–12 h.

Table 4. Adverse events

Child–Pugh	Grade 3/4				All grades			
	200 mg bid		400 mg bid		200 mg bid		400 mg bid	
	A (*n* = 7)	B (*n* = 6)	A (*n* = 6)	B (*n* = 8)	A (*n* = 7)	B (*n* = 6)	A (*n* = 6)	B (*n* = 8)
Hematological								
Leukocytopenia	0	0	0	0	2 (29%)	0	1 (17%)	0
Lymphopenia	2 (29%)	1 (17%)	1 (17%)	1 (13%)	2 (29%)	1 (17%)	1 (17%)	2 (25%)
Platelets	0	0	1 (17%)	1 (13%)	0	1 (17%)	2 (33%)	3 (38%)
Non-hematological								
Hypertension	0	1 (17%)	1 (17%)	3 (38%)	0	1 (17%)	1 (17%)	3 (38%)
Fatigue	0	0	0	0	0	1 (17%)	0	0
Fever	0	0	0	0	1 (14%)	2 (33%)	0	1 (13%)
Weight loss	0	0	0	0	2 (29%)	1 (17%)	1 (17%)	4 (50%)
Hand–foot skin reaction	0	0	0	2 (27%)	2 (29%)	2 (33%)	5 (83%)	3 (38%)
Rash	0	0	0	2 (27%)	2 (29%)	3 (50%)	4 (67%)	6 (75%)
Alopecia	0	0	0	0	2 (29%)	1 (17%)	2 (33%)	0
Dry skin	0	0	0	0	0	0	0	3 (38%)
Pruritus	0	0	0	0	0	1 (17%)	4 (67%)	3 (38%)
Anorexia	0	0	0	0	2 (29%)	1 (17%)	1 (17%)	2 (25%)
Diarrhea	0	0	1 (17%)	0	4 (57%)	4 (67%)	2 (33%)	5 (63%)
Stomatitis	0	0	0	0	0	0	1 (17%)	2 (25%)
Lipase	3 (43%)	4 (67%)	4 (67%)	6 (75%)	6 (86%)	6 (100%)	6 (100%)	6 (75%)
Amylase	1 (14%)	1 (17%)	1 (17%)	1 (13%)	4 (57%)	3 (50%)	4 (67%)	5 (63%)

and swelling of the pancreas were observed. The patient's abdominal pain resolved 1 day after stopping sorafenib, and lipase and amylase normalized 2 days later. Sorafenib was restarted 20 days after resolution and continued over 122 days, without recurrence of pancreatitis.

Grade 3 or worse drug-related adverse events were observed in 23 patients (85.2%), the majority of which were related to laboratory abnormalities: 10 patients in the 200-mg group and 13 in the 400-mg group. One patient with Child–Pugh B in the 400-mg bid group experienced DLT of hand–foot skin reaction at the end of cycle 1. There were no drug-related deaths in either of the groups.

There was no major difference in the incidence and grade of drug-related adverse events between the Child–Pugh A and B groups. At the dose level of 200 mg, the drug-related adverse event whose incidence was at least 20% higher in the Child–Pugh B group than in the Child–Pugh A group was rash or desquamation (50.0 *vs* 28.6%). The differences at the 400-mg dose level were diarrhea (62.5 *vs* 33.3%), weight loss (50.0 *vs* 16.7%), hypertension (37.5 *vs* 16.7%), dry skin (37.5 *vs* 0%), and fatigue (25.0 *vs* 0%).

Table 5. Tumor response

Response	200 mg bid (*n* = 13)	400 mg bid (*n* = 14)	Total (*n* = 27)
Partial response	1	0	1 (3.7%)
Stable disease	10	11	21 (77.8%)
Progressive disease	1	2	3 (11.1%)
NA	1	1	2 (7.4%)

NA, not assessed because these patients did not complete cycle 1.

Tumor response and survival. Partial response was achieved in one of the 27 patients. No complete response was observed (Table 5; Fig. 1). The overall response rate was 3.7% (95% confidence interval, 0.1–14.0%). Stable disease was noted in 21 patients (77.8%) and the disease control rate (partial response + stable disease rate) was 81.5% in 27 patients. Progressive disease was noted in three patients (11.1%).

Disease progression or death was observed in all patients. Sixteen of the 27 patients died of disease progression, and two

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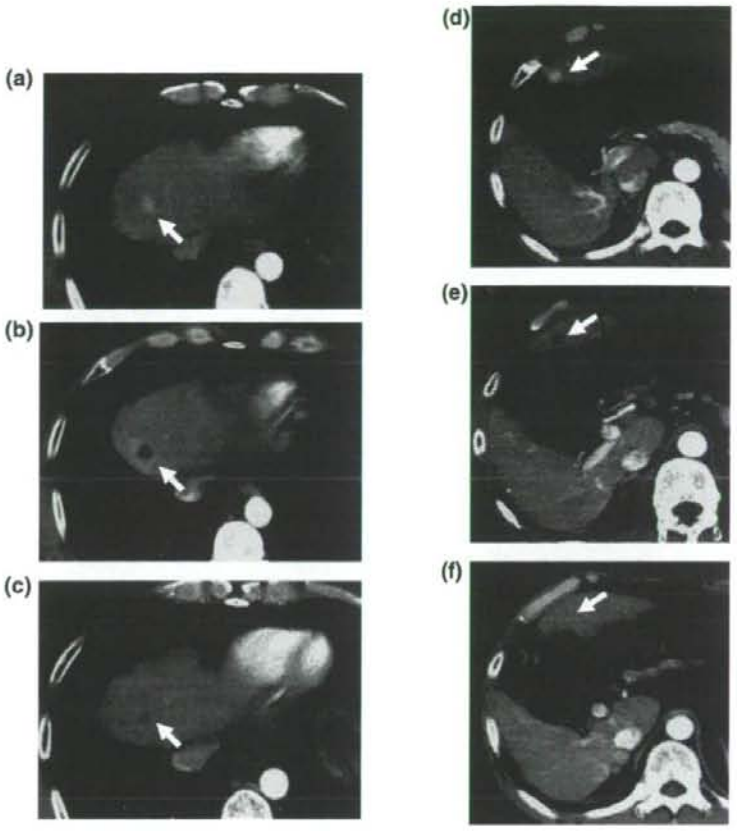

Fig. 1. A 48-year-old man with multiple tumors of hepatocellular carcinoma (HCC) after hepatectomy, percutaneous ethanol injection, and transcatheter arterial embolization. (a) Hypervascular HCC lesion, 1 cm in diameter, was revealed at the early phase of dynamic computed tomography (CT) before administration of sorafenib at the anterior superior segment of the liver (arrow). (b) The vascularity of this tumor disappeared 1 month after the administration of sorafenib. (c) The tumor was reduced 3 months after the administration of sorafenib. (d) Another hypervascular HCC lesion, 1 cm in diameter, was revealed at the early phase of dynamic CT before administration of sorafenib in the left lobe of the liver (arrow). (e) The vascularity of this tumor disappeared 8 months after the administration of sorafenib. (f) The tumor almost completely disappeared 10 months after the administration of sorafenib.

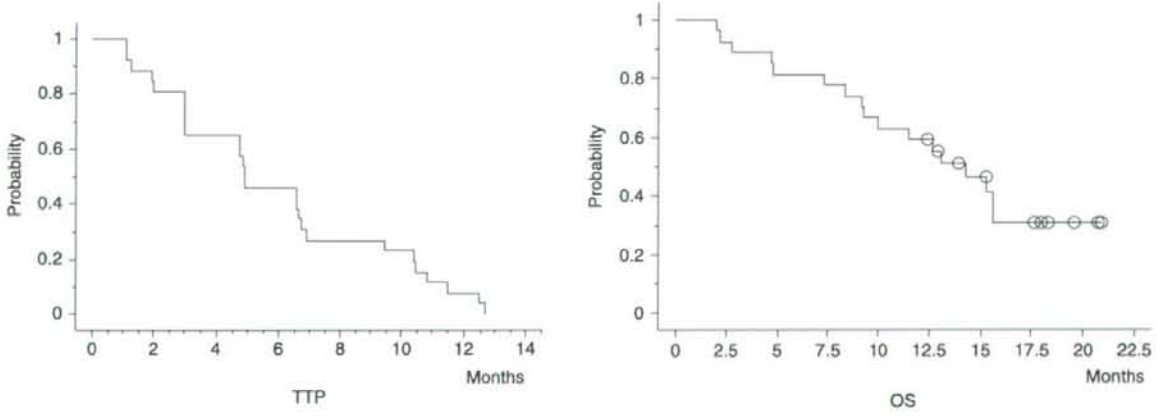

Fig. 2. Time to progression (TTP) in all 27 patients treated with sorafenib. The median TTP was 4.9 months, and the 6-month survival rate was 46.2%. Overall survival (OS) in the 27 patients treated with sorafenib. The median OS period was 15.6 months, and the 1-year survival rate was 59.3%.

died of cerebral infarction or myocardial infarction. Of the 27 patients, the median TTP was 4.9 months, and the median overall survival (OS) was 15.6 months (Fig. 2). The 6-month progression-free rate based on TTP was 46.2%, and 1- and 2-year OS were 59.3 and 30.9%, respectively.

Discussion

The PK, safety, and tolerability of sorafenib were investigated in Japanese patients with HCC treated with doses of 200 mg bid or 400 mg bid.