In the NLCT study, 77% of patients received the standard dosage regimen of 400 mg twice daily, while 21% were started on a reduced dose.

Comparison of the group started on the standard dose of 800 mg/day and the group started on a reduced dose did not reveal any significant differences in either duration of treatment (117 days vs. 81 days; $P = 0.05$) or number of dosing days (107 days vs. 78 days; $P = 0.10$). Furthermore, dosage was subsequently increased in 22% of the reduced initial dose group. Daily dosage intensity (DI) was 615 mg in the standard-dose group and 387 mg in the reduced-dose group.

It is conceivable to start sorafenib therapy at a reduced dose according to the condition of the patient or prevention of AEs. Because efficacy at reduced doses has not been demonstrated, as long as no AEs are encountered in the course of treatment, consideration should be given to increasing the dose to the standard dosage regimen.

With regard to sorafenib combination therapies, Phase I and Phase II studies on systemic chemotherapy in combination with sorafenib therapy have been published for radiotherapy,[13,14] doxorubicin,[15] tegafur/uracil,[16] and octreotide.[17] Several Japanese clinical trials are also being conducted on combination therapy, specifically low-dose cisplatin/fluorouracil HAIC (UMIN000004315), cisplatin HAIC (UMIN000001496), and S-1 chemotherapy (UMIN000002418, UMIN000002590). Therapies combining sorafenib with other anti-neoplastic agents are therefore still in the research stage, and their efficacy is yet to be demonstrated.

In terms of sorafenib combined with LAT, a Phase III placebo-controlled trial of adjuvant sorafenib chemotherapy following radical treatment (surgical resection or LAT) of HCC (STORM Trial) is presently underway.[12] Meanwhile, sorafenib combined with TACE has been investigated in a Phase III study of post-TACE adjuvant sorafenib chemotherapy versus placebo conducted in Japan and South Korea, but the study failed to demonstrate the usefulness of sorafenib administration.[11] Another Phase II trial on TACE in combination with sorafenib is presently being carried out in Japan (TACTICS; UMIN 000004316).

Discontinuation criteria

CQ1-3 How and when should sorafenib therapy be discontinued?

Recommendation Administration of sorafenib should be discontinued immediately in the event of SAEs.

Discontinuation should also be considered when disease progression is confirmed by radiological imaging or on the basis of patient symptoms.

Scientific statement In the two randomized, placebo-controlled trials demonstrating the usefulness of sorafenib therapy, administration was discontinued upon confirmation of radiologic or symptomatic progression or in the event of SAEs.[1,2]

In the NLCT study, sorafenib therapy was discontinued in 185 patients with 63% due to disease progression and 22% due to AEs. Moreover, 60% of discontinued patients did not undergo post-treatment.

No data are currently available on the efficacy/safety of continued administration of sorafenib after disease progression.

Adverse events

CQ1-4 What are the adverse events associated with sorafenib therapy?

Recommendation Some form of AE has appeared in almost all patients treated with sorafenib.

These AEs vary, and have even included serious adverse events (SAEs) resulting in death. Familiarity with these AEs is therefore essential, to carefully monitor patient progress while taking the necessary precautions, and to respond rapidly when an AE occurs.

The following AEs are known to occur frequently in patients treated with sorafenib.

1 Hand-foot skin reaction (HFSR);
2 Rash/desquamation;
3 Diarrhea;
4 Anorexia;
5 Hypertension;
6 Fatigue;
7 Alopecia;
8 Nausea.

While infrequent, life-threatening SAEs include hepatic failure, interstitial pneumonia, and gastrointestinal hemorrhage.

In addition, the following blood test abnormalities are known to occur frequently in patients treated with sorafenib.

1 Leukopenia;
2 Neutropenia;
3 Anemia;
4 Thrombocytopenia;
5 Hepatic impairment (elevated AST [aspartate aminotransferase], ALT [alanine aminotransferase], ALP [alkaline phosphatase], γ-GTP [γ-glutamyltransferase], T-Bil [total bilirubin]);

6 T-Bil elevation;
7 Amylase elevation;
8 Electrolyte abnormality (hyponatremia, hypokalemia, hypocalcemia, hypophosphatemia);
9 Hypoalbuminemia.

Scientific statement The incidence of sorafenib-related AEs was 80% in the Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) trial and 81.9% in the Asia-Pacific trial. Frequently occurring AEs were HFSR, rash/desquamation, diarrhea, anorexia, hypertension, fatigue, alopecia, and nausea.[1,2]

Sorafenib-related AE incidence in the NLCT study was 87%, of which 36% were ≥grade 3 AEs. While incidences of HFSR, diarrhea and alopecia in the NLCT study were similar to those of the Asia-Pacific trial[2] and SDUS,[6] incidences of rash/desquamation, anorexia, hypertension and fatigue were slightly higher in the present study (Table 2).

Evaluation of changes in clinical laboratory data was achieved by examining the CRFs to find the largest variations during sorafenib therapy, as well as the test date on which variations occurred. Consequently, the frequency of abnormal values in the NLCT study differed from those of the SHARP trial[1] and SDUS[6] (Table 3).

Changes in laboratory values were seen in 96% of the sorafenib group, with 64% showing an AE ≥ grade 3. Incidence of diminished blood cell counts was high compared with previous studies, with thrombocytopenia, leukopenia, neutropenia, and anemia seen in 56%, 43%, 37%, and 34% of the sorafenib group, respectively.

Hepatic impairment was also frequent, with elevated AST and ALT occurring in ≥50% of sorafenib-treated patients (70% and 55%, respectively), of whom a further 25% and 15% had AST and ALT readings ≥grade 3, indicating levels exceeding 200 IU/L after commencement of treatment. Similar results were observed for ALP and γ-GTP. Elevated T-Bil was seen in 53% of the sorafenib group, of whom 11% had readings that were ≥grade 3, which is more than three times the upper limit of normal (ULN).

Increased amylase was seen in 49% of the sorafenib group, of whom 12% had levels ≥grade 3, which is more than twice the ULN. In terms of electrolyte abnormalities, hyponatremia and hypokalemia were observed in 50% and 25% of the sorafenib group, respectively. Hypocalcemia and hypophosphatemia were also seen in ≥50% of the sorafenib group, but the valid response rate was low for these variables.

Hypoalbuminemia was seen in 48% of the sorafenib group, of whom only 5% had readings <2.0 g/dL.

No significant difference was seen in AE incidences for Child–Pugh class A and B patients, at 88% and 83%, respectively ($P = 0.53$). The incidence of AEs ≥grade 3 was also insignificant between Child–Pugh class A and B patients (35% vs. 39%, $P = 0.76$).

Similar comparisons for sorafenib group patients with Child–Pugh class A scoring 5 and 6 also did not reveal any significant differences in either total incidence of AEs at 89% and 88%, respectively ($P > 0.99$), or in the incidence of AEs ≥ grade 3, at 35% each ($P > 0.99$).

Incidence of abnormal laboratory data also did not vary significantly among Child–Pugh class A and B patients, at 96% and 95%, respectively ($P > 0.99$). Similarly, no significant difference was observed in the incidence of abnormal laboratory data ≥grade 3, at 63% and

Table 2 Incidence of drug-related adverse events with sorafenib therapy

AE	NLCT Study ($n = 264$)		SDUS[6] ($n = 777$)		SHARP Trial[1,6] ($n = 267$)		Asia-Pacific Trial[2] ($n = 149$)	
	Total (%)	G3/4 (%)	Total (%)	SAEs (%)	Total (%)	G3/4 (%)	Total (%)	G3/4 (%)
HFSR	44	10	47.9	2.8	21.2	7.7	45.0	10.7
Rash/desquamation	31	5	20.7	3.1	15.8	1.08	21.1	0.7
Diarrhea	32	5	21.9	1.4	39.1	8.4	25.5	6.0
Anorexia	27	4	13.8	1.9	13.8	0.3	12.8	0
Hypertension	26	8	19.2	0.6	5.1	1.7	18.8	2.0
Fatigue	24	2	4.6	0.6	–	–	20.1	3.4
Alopecia	15	0	11.4	–	13.8	–	24.8	–
Nausea	10	1	4.0	0.3	11.1	0.3	11.4	0.7

Common Terminology Criteria for Adverse Events (CTC-AE) v3.0
HFSR, hand-foot skin reaction; NLCT, New Liver Cancer Therapies; SDUS, special drug use surveillance; SHARP, sorafenib hepatocellular carcinoma assessment randomized protocol.

Table 3 Abnormal clinical laboratory values with sorafenib therapy

Clinical laboratory data	NLCT Study ($n = 264$)		SDUS[6] ($n = 777$)		SHARP Trial[1,6] ($n = 297$)	
	AE incidence					
	Total (%)	G3/4 (%)	Total (%)	SAEs (%)	Total (%)	G3/4 (%)
Leukopenia	43	8	1.9	0.3	0.3	0.3
Neutropenia	37	6	0.9	0.2	–	–
Anemia	34	11	0.8	0.2	4.4	1.3
Thrombocytopenia	56	12	8.5	0.9	1.7	0.7
PT-INR	25	2	–	–	–	–
Elevated AST	70	25	1.4	–	1.7	1.7
Elevated ALT	55	15	0.9	0.2	0.7	0.7
Elevated ALP	35	5	0.3	–	–	–
Elevated γ-GTP	36	19	0.2	–	–	–
Elevated T.Bil	53	11	2.6	0.2	0.7	–
Elevated amylase	49	12	4.2	–	–	–
Elevated lipase	78	37	3.7	–	1.3	–
Elevated Cre	23	2	–	–	–	–
Hyponatremia	50	14	–	–	–	–
Hypokalemia	25	6	–	–	–	–
Hypocalcemia	55	1	–	–	–	–
Hypophosphatemia	66	29	3.6	0.5	34.9	10.5
Hypoalbuminemia	48	5	1.1	–	–	–

Common Terminology Criteria for Adverse Events (CTC-AE) v3.0.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltransferase; NLCT, New Liver Cancer Therapies; SAEs, severe adverse events; SDUS, special drug use surveillance; SHARP, sorafenib hepatocellular carcinoma assessment randomized protocol; T-Bil, total bilirubin.

66% of class A and B patients, respectively. Performing the same comparisons for sorafenib group patients with Child–Pugh class A scoring 5 and 6 also failed to reveal any significant differences either in total incidence of abnormal laboratory values (97% and 95%, respectively; $P > 0.80$) or in the incidence of abnormal laboratory data ≥ grade 3 (58% and 68%, respectively; $P > 0.26$), despite a higher percentage for patients with Child–Pugh score 6.

AE management

CQ1-5 What measures should be taken in management to sorafenib-related AEs?

Recommendation Preventative measures and careful monitoring of the patient are required for frequently occurring AEs such as HFSR, hypertension, and hepatic impairment.

Patients undergoing sorafenib therapy often experience AEs soon after beginning of treatment. Careful monitoring of the patient by carrying out blood test and medical examinations etc. at least once a week for 4 weeks after initiating therapy is therefore preferable.

Scientific statement The NLCT study investigated measures taken in management to sorafenib-related AEs (Table 4). Management to HFSR was common, with topical application of emollients performed most frequently (69%), and followed by topical application of steroids (38%) and consultation to a dermatologist

Table 4 Incidence of drug-related adverse events with sorafenib therapy

Response to AE	Valid responses %	Prevention for AE %
Consultation to dermatologist	89	24
Steroid ointment	89	38
Emollient	91	69
Hypotensive drug dose increased	90	21
Intestinal drug	90	19
Anti-diarrheal drug	89	16
Antiemetic drug	89	5

AE, adverse event.

(24%). An increased dose of hypotensive drugs was prescribed in 21% of patients, while diarrhea was treated with antiflatulent and anti-diarrheal drugs in 19% and 16% of patients, respectively. Antiemetic agents were administered in 5% of patients.

Most AEs observed in the NLCT study, including abnormal laboratory values, occurred early at up to 8 weeks after initiating sorafenib therapy. For this reason, careful, early monitoring of the patient is essential. Bayer Yakuhin's "Nexavar Proper Use Guidelines"[7] recommends that a battery of tests be performed regularly or as required during sorafenib therapy (Table 5). Educating patients to withhold taking the drug and consult their doctors immediately if they begin to feel unwell early in the treatment is another important way to prevent AEs from becoming severe.

Serious adverse events (SAEs) should generally be handled by immediately withholding administration or reducing the dose, and reinstitution of treatment or dose increase can be considered if the patient recovers.

Provided below is a summary of management to prevent and respond to major sorafenib AEs.

- Hand-foot skin reaction (HFSR)

Prevention: HFSR occurs most frequently in areas affected by hyperkeratosis and induration. Risk factors for HSFR include physical stimulation of the skin such as compression, heat or friction, so the patient's hands and feet should always be inspected before treatment. Any thickening of the stratum corneum should be removed and the patient instructed to cover and bathe the affected areas to prevent physical stimulation. An emollient containing urea or salicylic acid should be applied to the hands from 1–2 weeks before commencing therapy.[7]

Management: Minor, painless skin changes such as erythema can be treated with steroid ointment without reducing or discontinuing sorafenib therapy. If further deterioration such as formation of blisters occurs, the dosage should be reduced. If the condition interferes with the patient's activities of daily living due to ulcers, cracking or pain etc., the therapy should be withheld and the patient consulted to a dermatologist as necessary. If the condition improves after withholding the sorafenib, therapy can be resumed at a reduced dose, and can subsequently be increased on the basis of the AE condition.

- Hepatic impairment, hepatic failure and hepatic encephalopathy

Prevention: Sorafenib therapy should be avoided in patients with severe liver impairment; particularly those with AST and ALT levels exceeding 200 IU/L.

Management: The patient should be carefully monitored by performing medical examinations and hepatic function tests once weekly for the first month of treatment, once fortnightly for the next 3 months, and once monthly thereafter. Reducing, withholding, or discontinuing sorafenib therapy should be considered if the patient exhibits symptoms of hepatic failure including hepatic encephalopathy and ascites or a sudden increase in AST and ALT levels. Immediate suspension of therapy and careful in- or outpatient monitoring is recommended if the patient's AST and ALT levels increase beyond 200 IU/L or if T-Bil exceeds 3.0 mg/dL.[7] Treatment can be resumed after the patient recovers and increased on the basis of the AE condition.

- Diarrhea

Prevention: Patients should refrain from eating foods and beverages that contain a lot of spices, fat, or caffeine. Laxatives and dietary fiber supplements should also be avoided.

Management: If frequency of defecation increases to 3 times/day, intestinal drugs such as bifidobacterium powders and albumin tannate, and anti-diarrheal drugs such as loperamide and cholestyramine should be administered.[18] In addition, the patient should be instructed to drink fluids to prevent dehydration. Reducing, withholding, or discontinuing sorafenib therapy should be considered if the frequency of defecation increases to ≥4 times/day and the patient exhibits symptoms of dehydration. Dehydration symptoms should be managed systemically with fluid replacement, etc. Treatment can be resumed after the patient recovers and subsequently increased on the basis of the AE conditions.

- **Hypertension**

Prevention: If hypertension is observed prior to sorafenib therapy, systolic blood pressure (SBP) and diastolic blood pressure (DBP) should be controlled to ≤140 mmHg and ≤90 mmHg, respectively.

Management: Patients should be instructed to measure home blood pressure during the early treatment period. If elevated blood pressure (BP) is observed, hypotensive drugs should be administered or the dosage increased. Calcium antagonists and angiotensin receptor blockers (ARBs) are commonly used as hypotensive agents. A single drug is typically administered to begin with, and other types of hypotensive drugs may be co-administered if the reduction in BP is insufficient. Regardless of therapy, administration of sorafenib should be withheld if SBP is ≥180 mmHg or DBP is ≥110 mmHg. Treatment can be resumed after the patient recovers and then increased on the basis of the AE conditions.

Table 5 Clinical laboratory tests recommended in proper use guidelines for sorafenib therapy[7]

Test/Test variable	Cautionary AEs etc.	Subjects	Frequency/Duration											
			Baseline	1 week	2 weeks	3 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks	16 weeks	20 weeks	– Post-therapy
Hepatic function	Hepatic impairment	All patients	○	○	○	○	○	○	○	○	○	○	○	○
Pancreatic function	Increased pancreatic function, pancreatitis	All patients	○		○		○		○		○	○	○	○
Blood count	Neutropenia, thrombocytopenia, etc.	All patients	○		○		○		○		○	○	○	○
Serum phosphate	Hypophosphatemia	All patients	○		○		○		○		○	○	○	○
Blood pressure	Hypertension, hypertensive crisis, reversible leukoencephalopathy	All patients	At hospital visit (simple HBP measurement once weekly [daily if possible])											
Abdominal imaging	GI perforation, pancreatitis	Patients complaining of abdominal pain	As appropriate											
Coagulation parameters	Hemorrhage	Patients on concomitant vitamin K antagonists	As appropriate											
Thyroid function (thyroid hormone, thyroid-stimulating hormone, etc.)	Reduced thyroid function	Patients with specific symptoms suggestive of reduced thyroid function	As appropriate											
Thoracic imaging (Chest x-ray, chest CT, KL-6)	Interstitial pneumonia	Patients with symptoms suggestive of interstitial pneumonia	As appropriate											

AEs, adverse events; CT, computed tomography; GI, gastrointestinal; HBP, home blood pressure.

- **Amylase elevation**

Management: Increases in amylase are usually transient and gradually subside even when sorafenib therapy is continued. However, some cases of pancreatitis has previously been reported in patients treated with sorafenib, so if the patient has abdominal pain or other symptoms suggestive of pancreatitis, or elevated amylase levels are sustained, sorafenib therapy should be withheld and imaging procedures such as dynamic CT performed to determine whether pancreatitis is present.[7]

- **Interstitial pneumonia**

Management: Interstitial pneumonia should be suspected and sorafenib therapy discontinued immediately in patients exhibiting clinical symptoms such as dyspnea, dry cough and fever, and lung crepitation or reduced SpO_2 (percutaneous oxygen saturation) on physical examination. In addition, diagnosis and proper treatment should be carried out based on prompt diagnostic imaging such as chest X-ray or high-resolution chest CT (HRCT) and blood tests such as KL-6 after consulting with a respiratory specialist.[7]

Evaluation of therapeutic response

CQ1-6 How and when should therapeutic response of sorafenib be evaluated?

Recommendation The antitumor effects of sorafenib therapy are normally evaluated by diagnostic imaging with dynamic CT or dynamic magnetic resonance imaging (MRI) and subsequent measurement of tumor size based on a single cycle of 4–6 weeks of sorafenib administration.

Changes in intra-tumoral blood flow are often seen following sorafenib therapy, so evaluation can also be performed by measuring the area of tumor staining in addition to tumor size.

α-fetoprotein (AFP) and PIVKA-II (DCP) (protein induced by vitamin K absence or abnormality, des-γ-carboxyprothrombin) tumor markers are also typically evaluated in conjunction with tumor images at cycles of 4–6 weeks.

Elevated PIVKA-II (DCP) concentrations during sorafenib therapy may not always be due to disease progression. Consideration should also be given to evaluation of tumors in patients for whom treatment was interrupted due to AEs.

Scientific statement In the two randomized, placebo-controlled trials demonstrating the usefulness of sorafenib therapy,[1,2] therapeutic response to sorafenib was evaluated every 6 weeks on the basis of diagnostic imaging.

In the NLCT study, median overall survival (OS) was 10.8 months, 6-month survival rate was 65%, 1-year survival rate was 45%, and median progression-free survival (PFS) was 2.1 months (Fig. 1). Comparison of efficacy evaluation findings with those of previous clinical trials[1,2,5] are presented in Table 6.

Reductions in intra-tumoral blood flow are often observed with sorafenib therapy, so instead of simply evaluating tumor size based on the conventional Response Evaluation Criteria in Solid Tumors (RECIST), the use of therapeutic response criteria for evaluating intra-tumoral necrotic regions such as modified RECIST[19] or the Response Evaluation Criteria in Cancer of the Liver (RECICL)[20] has recently been advocated.[21,22] Even if the size of the tumor has slightly increased, therapy may be deemed effective and subsequently continued if the area of reduced intra-tumoral blood flow has increased.

Previous studies have reported that PIVKA-II (DCP) expression is induced in hypoxic HCC cells following sorafenib therapy[23] and that elevated PIVKA-II (DCP) concentrations may act as surrogate markers for HCC tissue ischemia.[24] However, elevated PIVKA-II levels are also seen in disease progression, so care should be taken during assessment of therapeutic response.

According to the NLCT study data, therapeutic response was not evaluated in 20% of sorafenib group patients. However, short-term administration of sorafenib was found to inhibit tumors in some patients on whom therapy was interrupted due to AEs, suggesting that regular tumor assessment should also be considered for patients with interrupted treatment.

Continuation of therapy

CQ1-7 How long should sorafenib therapy be continued?

Recommendation Sorafenib therapy should preferably be maintained until clear disease progression is determined on evaluation of therapeutic response.

If clear disease progression is not identified in diagnostic imaging, therapy may be continued after considering the risks and benefits.

No data are currently available on the efficacy/safety of continued sorafenib administration after disease progression has been confirmed.

Scientific statement In the NLCT study, 31% of patients in the sorafenib group underwent some form of additional treatment after completion of the therapy. Specifically, 12% underwent TACE, 8% underwent systemic chemotherapy, 7% underwent HAIC, 4% underwent radiotherapy, and 2% underwent hepatectomy/LAT.

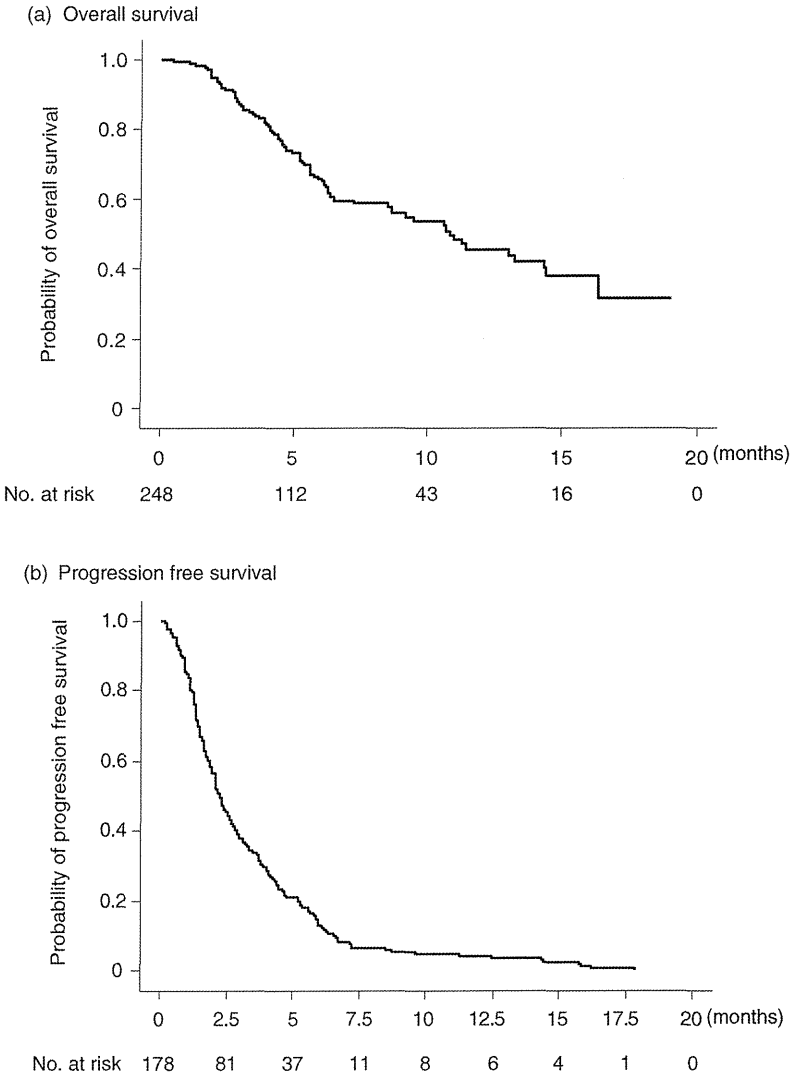

Figure 1 Therapeutic efficacy of sorafenib. (a) Overall survival. (b) Progression free survival.

Progressive disease (PD) was confirmed in 165 patients in the sorafenib group during the study's observation period, of whom a further 23 patients (14%) underwent continued oral administration of sorafenib for ≥1 month after PD confirmation. Comparison of these 23 patients with those in whom therapy was discontinued did not reveal any significant differences in OS, and no data are currently available regarding the efficacy/safety of continued sorafenib administration after confirmation of PD.

Predictors of therapeutic efficacy

CQ1-8 What are the predictors of therapeutic efficacy for sorafenib therapy?

Recommendation Clear predictors of therapeutic efficacy for sorafenib have yet to be established, but the number of intrahepatic lesions and pretreatment levels of tumor markers (AFP, PIVKA-II [DCP]) may be predictors of efficacy.

Scientific statement A study of biomarkers in patients treated with sorafenib has suggested the efficacy of sorafenib is associated with low serum HGF and high c-KIT levels at baseline.[25] Efficacy of sorafenib has also been linked to high levels of ERK expression in tumor tissue.[25,26] However, these reported associations cannot yet be described as established predictors of efficacy, and biomarkers are currently being sought in some prospective clinical trials using sorafenib.

Table 6 Summary of efficacy measures for sorafenib therapy

	NLCT Study ($n = 250$)	SHARP Trial[1] ($n = 299$)	Asia-Pacific Trial[2] ($n = 150$)	Sorafenib phase II[5] ($n = 137$)
OS (months)				
Median	11.0	10.7	6.5	9.2
1-year SR (%)	45	44	–	59
6-month SR (%)	65	–	53	–
PFS (months)	†			
Median	2.1	5.5	3.5	4.2/5.5
Antitumor effect (%)	‡			
Complete remission	0	0	0	0
Partial remission	4	2	5	2
Stable	45	71	46	34
Tumor control rate	49	43	53	–

†Patients who died without confirmation of disease progression were excluded.
‡Patients not evaluated for therapeutic response were excluded.
NLCT, New Liver Cancer Therapies; OS, overall survival; PFS, progression-free survival; SHARP, sorafenib hepatocellular carcinoma assessment randomized protocol.

The current results indicate that early AFP response is a useful surrogate marker to predict treatment response and prognosis in patients with advanced HCC who receive anti-angiogenic therapy.[27]

In an attempt to identify predictors of therapeutic efficacy for sorafenib, the NLCT study examined baseline patient characteristics (age, sex, BMI [body mass index], ECOG-PS [Eastern Cooperative Oncology Group – performance status], hepatic functional reserve, prior treatment, cause of hepatic impairment, clinical laboratory values) and tumor factors (presence or absence of intrahepatic/extrahepatic lesions, maximum tumor size, vascular invasion, stage), and consequently found that tumor control rates tended to be higher in patients with <5 intrahepatic lesions compared to those with ≥5 lesions (54% vs. 40%, respectively; $P = 0.058$). In addition, the tumor control rate was significantly higher in patients with a baseline AFP value <10 ng/mL compared with those with values ≥10 ng/mL (68% vs. 43%, respectively; $P = 0.021$). The tumor control rate also tended to be higher in patients with baseline PIVKA-II (DCP) value <40 mAU/mL than in those with a value of ≥40 mAU/mL (60% vs. 42%, respectively; $P = 0.051$) (Table 7).

Hepatic arterial infusion with miriplatin

Indications

CQ2-1 Is miriplatin a platinum preparation that can be used on renal disorder patients?

Recommendation Renal disorder patients can be treated using miriplatin as long as they are capable of undergoing angiography (serum Cre [creatinine] level <2.0 mg/dL) and as long as administration is performed carefully so as to avoid elevation in serum Cre levels after treatment.

Scientific statement Miriplatin remains in the tumor together with Lipiodol, where it slowly releases platinum compounds. This agent is thus believed to gradually increase serum platinum concentration with minimal adverse effect on renal function.

In a randomized phase II trial comparing miriplatin and zinostatin stimalamer (SMANCS) in patients with normal serum Cre levels, renal dysfunction indicated by serum Cre level >1.5 mg/dL was observed in only 2.4% of patients in the miriplatin treatment group (Table 8).[28]

In the NLCT study, median serum Cre prior to miriplatin therapy was 0.8 mg/dL (range, 0.4–10.5 mg/dL), of which patients with a serum Cre level >1.0 mg/dL accounted for 17.7%. Median serum Cre after treatment was 0.8 mg/dL (range, 0.1–12.6 mg/dL), which was unchanged from baseline, and 94.7% of patients experienced an increase of ≤0.5 mg/dL (Table 9). Only 1.8% of patients exhibited renal dysfunction ≥grade 3 as indicated by serum Cre level >3 mg/dL.

Analysis of patients with baseline serum Cre <2.0 mg/dL shows that just 2.5% of patients increased serum Cre >0.5 mg/dL, and no more than 0.6% of patients experienced renal dysfunction ≥grade 3 (Table 9).

In addition, no serious renal dysfunction was observed after miriplatin administration in patients with serum Cre levels around 2.0 mg/dL.

Table 7 Factor analysis of tumor control with sorafenib therapy

	n	Tumor control rate (%)	*P**
Age (years)			
≥65	137	49	0.75
<65	56	46	
Gender			
Male	147	50	0.72
Female	43	47	
ECOG-PS			
0	163	50	0.24
1–3	29	38	
Child–Pugh score			
5	65	48	0.82
6	70	44	
7	23	48	
≥8	10	60	
Child–Pugh class			
A	135	46	0.52
B–C	33	56	
Prior treatment			
Yes	173	48	0.87
None	18	50	
HBs antigens			
Positive	36	50	0.91
Negative	149	49	
HCV antibodies			
Positive	112	50	0.66
Negative	77	47	
Intrahepatic lesions			
Yes	174	47	0.26
None	18	61	
Intrahepatic nodules			
≥5	95	40	0.058
<5	83	54	
Advanced vascular invasion			
Yes	36	50	0.68
None	141	46	
Extrapulmonary lesion(s)			
Yes	105	47	0.64
None	88	50	
Maximum tumor size (mm)			
≥30	108	47	0.79
<30	67	49	
Stage (Japanese Classification of Lung Cancer)			
I–II	15	53	0.41
III	53	57	
IV A	31	39	
IV B	84	46	
Initial dose			
Normal dose	153	48	0.91
Reduction	39	49	
Baseline AFP			
≥10	151	43	0.021
<10	25	68	
Baseline PIVKA-II			
≥40	132	42	0.051
<40	40	60	

*Fisher's exact test.
AFP, α fetoprotein; ECOG-PS, Eastern Cooperative Oncology Group Performance status; HBs, Hepatitis B surface antigen; HCV, hepatitis C virus.

Based on these findings, the Study Group considers that miriplatin therapy can be administered without instigating renal dysfunction in patients with serum Cre <2.0 mg/dL who are capable of undergoing angiography.

However, transcatheter arterial infusion (TAI)/TACE with miriplatin simultaneously uses an iodinated contrast medium with drugs that can cause renal dysfunction such as anti-inflammatory analgesics to treat postoperative fever. Sufficient consideration should therefore be given to the risk of drug-induced renal dysfunction, and monitoring of urine volume and fluid replacement should be implemented as necessary.

CQ2-2 Can miriplatin be used safely in patients with Child–Pugh class B?

Recommendation Miriplatin can be used to treat these patients without causing serious complications.

Furthermore, no demonstrable difference in the antitumor effects of miriplatin has been observed between Child–Pugh class A and B patients.

Scientific statement The NLCT study included 281 Child–Pugh class A and 144 Child–Pugh class B patients. In Child–Pugh class B patients, the only SAEs ≥grade 3 were fever and anorexia, at incidences of 0.7% each, with no cases of ascites or hepatic failure ≥grade 3 (Table 10). In a study of TAI with miriplatin, in 17 Child–Pugh class B patients, no significant differences were seen in pre- or posttreatment 15-min retention rates of indocyanine green (ICG_{15}), and no SAEs or increased ascites or hepatic failure necessitating additional therapy or prolonged hospitalization were observed.[30]

Although the retrospective analysis of the NLCT study coupled with differences in characteristics of Child–Pugh class A and B patient effectively precludes simple comparisons of these patients, no significant differences in respective AE incidences were seen, apart from a higher frequency of fever and thrombocytopenia ≥grade 3 among Child–Pugh class B patients (Tables 10 and 11).

In terms of evaluation of antitumor effects according to the RECICL proposed by the Liver Cancer Study Group of Japan, the present study did not reveal any significant differences in therapeutic responses of Child–Pugh class A and B patients (Table 12), while 50% of Child–Pugh class B patients in the aforementioned study of TACE with miriplatin achieved a treatment effect (TE) of "TE3" or "TE4", in which tumor was controlled.[30]

CQ2-3 Is miriplatin effective against cisplatin-resistant HCCs?

Table 8 Abnormal clinical laboratory values with miriplatin therapy

	NLCT Study ($n = 535$)		Phase III Trial[29] ($n = 16$)		Randomized Phase II Trial[28] ($n = 83$)	
	Total (%)	G3/4 (%)	Total (%)	G3/4 (%)	Total (%)	G3/4 (%)
Leukopenia	38.2	5.1	51	0	41.0	1.2
Neutropenia	20.1	5.1	63	19	53.0	8.4
Eosinophilia	14.6	–	100	0	84.3	0
Monocytosis	–	–	–	–	57.8	0
Lymphocytopoenia	–	–	51	0	79.5	0
Thrombocytopenia	32.1	9.3	44	0	50.6	1.2
Increased AST	49.9	12.4	56	44	62.7	26.5
Increased ALT	78.4	26.6	44	19	59	24.1
Increased bilirubin	31.6	3.2	31	19	57.8	12.0
Increased γGTP	16.1	2.0	–	–	49.4	0
Increased ALP	12.3	0.2	44	0	30.1	1.2
Elevated Cre	11.5	1.8	25	0	–	2.4†

CTC-AE v3.0 Japan Society of Clinical Oncology Adverse Drug Reaction Criteria.
†Increased Cre data includes G2 patients.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltransferase.

Table 9 Incidence of drug-related adverse events with miriplatin therapy (Renal dysfunction)

Elevated Cre	all ($n = 513$)	Baseline Cre <2.0 mg/dL	Baseline Cre ≥2.0 mg/dL
≤0.5 mg/dL	94.7%	97.5%	13.3%
0.6–1.0 mg/dL	2.4%	1.7%	20.0%
1.1–2.0 mg/dL	1.2%	0.2%	33.3%
2.1–3.0 mg/dL	0.6%	0.0%	20.0%
>3.0 mg/dL	1.0%	0.6%	13.3%

Recommendation The clinical usefulness of miriplatin against cisplatin-resistant HCC is not currently known.

Scientific statement Miriplatin is classified as a third-generation platinum drug and a basic research on the drug suggested potential activity in cisplatin-resistant HCCs because cisplatin-resistant HCC cell lines did not show cross-resistance to miriplatin.[31]

A Japanese Phase I trial combining miriplatin and TAI using Lipiodol (Lip-TAI) on HCC refractory to cisplatin/Lip-TAI has reported a treatment success rate of 18.2%.[32]

Table 10 Comparison of adverse events with miriplatin therapy according to Child–Pugh classification

	All ($n = 535$)		Child–Pugh class A ($n = 281$)		Child–Pugh class B ($n = 144$)	
	Total (%)	G3/4 (%)	Total (%)	G3/4 (%)	Total (%)	G3/4 (%)
Fever	81.3	0.2	75.5	0	86.1	0.7*
Biphasic fever	2.8	–	2.5	–	5.1	–
Anorexia	29.7	0.2	31.7	0	34.0	0.7
Administration site pain	21.2	0	25.6	0	15.3	0
Nausea	18.8	0	21.4	0	12.5	0*
Vomiting	13.5	0	11.6	0	6.1	0
Fatigue	9.3	0	12.2	0	10.3	0
Diarrhea	2.0	0	1.8	0	1.0	0
Ascites	1.2	0	0	0	3.0	0
Hepatic failure	0.3	0.3	0.3	0.3	0	0

CTC-AE v3.0.
*$P < 0.05$ (A vs. B).

Table 11 Comparison of clinical laboratory value anomalies with miriplatin therapy according to Child–Pugh classification

	All ($n = 535$)		Child–Pugh class A ($n = 281$)		Child–Pugh class B ($n = 144$)	
	Total (%)	G3/4 (%)	Total (%)	G3/4 (%)	Total (%)	G3/4 (%)
Leukopenia	38.2	5.1	18.2	3.3	25.2	5.8
Neutropenia	20.1	5.1	17.3	3.6	23.4	5.8
Eosinophilia	14.6	–	17.9	–	11.5	–
Thrombocytopenia	32.1	9.3	30.9	5.8	30.2	13.7*(G3)
Increased AST	49.9	12.4	45.2	13.5	50.7	19.4
Increased ALT	78.4	26.6	81.0	28.8	70.3	28.3*
Increased bilirubin	31.6	3.2	26.1	0	46.0	5.8*
Increased γGTP	16.1	2.0	15.8	2.6	14.5	0
Increased ALP	12.3	0.2	12.7	0	10.1	0.7
Elevated Cre	11.5	1.8	11.6	2.2	10.8	1.4

CTC-AE v3.0.
*$P < 0.05$ (A vs. B).
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltransferase.

However, the study was conducted on a small patient population, so the usefulness of this therapy is yet to be established and future studies are awaited.

Furthermore, no data are currently available regarding the efficacy of miriplatin therapy in patients who are unresponsive to TAI/HAIC using cisplatin.

Method of administration

CQ2-4 What are the effects and AEs of combining embolic materials with miriplatin?

Recommendation Combination therapy of embolic materials and miriplatin is expected to improve antitumor effects compared with miriplatin alone, but there is currently insufficient evidence to support this.

Adverse events associated with combination therapy of embolic materials and miriplatin may not differ noticeably from those of conventional TACE therapy using epirubicin.

Scientific statement Compared with stand-alone therapy, the combination of embolic materials in the hepatic arterial catheterization treatment is generally considered to deliver enhanced antitumor effects based on its blood flow blockage effect,[33] so treatment combined with embolic materials are mostly selected for the treatment of HCC. However, Phase I and II trials using miriplatin have opted not to use embolic materials in combination with miriplatin.[29,32]

Meanwhile, two studies on miriplatin used in combination with embolic materials on a small number of patients have reported high rates of treatment success, with TE3 and TE4 scores obtained in 60.0–77.7% of patients.[30,34]

Table 12 Summary of efficacy measures with miriplatin therapy

	NLCT Study			Phase II Trial[29] ($n = 16$)	Randomized Phase II Trial[28] ($n = 83$)
	All ($n = 535$)	Child–Pugh class A ($n = 281$)	Child–Pugh class B ($n = 144$)		
Anti-neoplastic effect (%)					
TE4	22.8	25.3	23.6	56	26.5
TE3	24.3	26.7	20.8	6	25.3
TE2	26.0	26.0	29.9	19	22.9
TE1	16.6	12.5	17.4	19	20.5
Not evaluated	10.3	9.6	8.3	0	4.8
TE3 + TE4	47.1	52.0	44.4	61	51.8

Response Evaluation Criteria in Cancer of the Liver' (RECICL).

Table 13 Independent factors contributing to effective (TE3/4) achievement with miriplatin therapy

Factor	Category	Risk ratio	95% CI	*P*-value
Embolic material	None	1		<0.001
	Yes	3.66	2.13–6.29	
No. tumors	Single	1		0.017
	2–3	1.01		
	4–9	0.66		
	≥10	0.3	0.13–0.67	
Past history of TAE	None	1		0.018
	Yes	0.48	0.26–0.88	

Cox proportional hazards model.
CI, confidence interval; TAE, transcather arterial embolization.

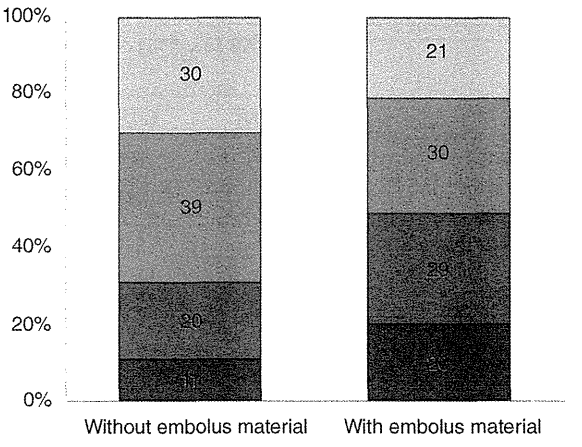

Figure 2 Therapeutic efficacy of miriplatin with or without embolus material.

In the NLCT study, embolic material was used in combination with miriplatin on 473 patients (88.4%). Simple comparison of patients undergoing miriplatin/embolic material combination therapy and those who underwent miriplatin alone therapy was not possible due to the retrospective nature of this study, as well as the different patient characteristics of the respective treatment groups. However, antitumor effects were higher in the miriplatin/embolic material therapy group than in the miriplatin therapy group, at 49% and 31%, respectively (Fig. 2). Analysis of independent factors contributing to the achievement of TE3/4 scores in TAI/TACE therapy using miriplatin showed that the use of embolic material had a higher risk ratio of 3.66 ($P < 0.001$) (Table 13).

A Phase III trial of TACE using miriplatin is currently underway, and the results will likely be useful in investigating the efficacy of using miriplatin in combination with embolic materials.

In the NLCT study, patients who underwent combination therapy with embolic material showed a high incidence of fever, suspected to be due to post-embolization syndrome. Although high incidences of hematological AEs neutropenia and elevated AST were seen, no significant differences were identified in the incidences of most AEs, and no serious complications such as hepatic failure or ascites were observed (Tables 14 and 15).

Table 14 Comparison of adverse events with or without embolic material during miriplatin therapy

	All ($n = 535$)		TACE patients ($n = 425$)		TAI patients ($n = 54$)	
	Total (%)	G3/4 (%)	Total (%)	G3/4 (%)	Total (%)	G3/4 (%)
Fever	81.3	0.2	84.4	0.2	56.1	0*
Biphasic fever	2.8	–	3.0	–	0	–
Anorexia	29.7	0.2	30.4	0.2	22.4	0
Administration site pain	21.2	0	22.2	0	13.8	0
Nausea	18.8	0	20.1	0	4.0	0
Vomiting	13.5	0	14.2	0	0	0
Fatigue	9.3	0	9.2	0	–	–
Diarrhea	2.0	0	2.1	0	0	0
Ascites	1.2	0	0.9	0	5.6	0
Hepatic failure	0.3	0.3	0.3	0.3	0	0

CTC-AE v3.0.
*$P < 0.05$ (TACE vs. TAI).
TACE, transcatheter arterial chemoembolization; TAI, transcatheter arterial infusion.

Table 15 Comparison of abnormal clinical laboratory values with or without embolic material during miriplatin therapy

	All ($n = 535$)		TACE patients ($n = 425$)		TAI patients ($n = 54$)	
	Total (%)	G3/4 (%)	Total (%)	G3/4 (%)	Total (%)	G3/4 (%)
Leukopenia	38.2	5.1	22.8	5.5	20.4	1.9
Neutropenia	20.1	5.1	21.4	5.5	3.7	0*
Eosinophilia	14.6	–	14.8	–	11.8	–
Thrombocytopenia	32.1	9.3	33.2	10.4	24.1	0
Increased AST	49.9	12.4	52.8	19.3	25.9	8.6*
Increased ALT	78.4	26.6	78	24.5	81.5	44.4*
Increased bilirubin	31.6	3.2	32.1	3.3	27.8	0
Increased γ-GTP	16.1	2.0	16.1	1.8	14.8	3.7
Increased ALP	12.3	0.2	12.6	0.2	9.3	0
Elevated Cre	11.5	1.8	10.7	1.8	18.5	1.9

CTC-AE v3.0.
*$P < 0.05$ (TACE vs. TAI).
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltransferase; TACE, transcatheter arterial chemoembolization; TAI, transcatheter arterial infusion.

Similarly, a small pilot study (Phase II clinical trial) on miriplatin combined with an embolic material found some mild complications, but none of a serious nature.[34] Another study on the small number of patients did not reveal any serious complications.[30]

CQ2-5 Is standard hydration required prior to administration of miriplatin?

Recommendation Standard hydration is not required except in the case of renal failure.

Scientific statement Sufficient hydration before and after administration of cisplatin (IA-call, Nippon Kayaku, Tokyo, Japan) used in HAIC is necessary to prevent nephrotoxicity.

Miriplatin is highly soluble in Lipiodol and remains in tumor with Lipiodol, where it continuously releases platinum compounds.[35] So only a small amount enters systemic circulation expecting to reduce systemic AEs, including renal dysfunction.

As stated in CQ1, the effect of miriplatin on renal function is considered to be mild. Two of the aforementioned Phase II trials did not perform pretreatment hydration to prevent renal impairment.[28,30] In the NLCT study, patients with advanced renal insufficiency were excluded and no serious renal impairment occurred in patients treated with miriplatin without prior hydration.

Adverse events

CQ2-6 What are the adverse events associated with miriplatin therapy?

Recommendation Post-embolization syndrome characterized mainly by fever is often seen, and biphasic fever is relatively infrequent. Incidences of nausea and vomiting are also low compared with other platinum agents. Complications such as ascites, liver abscess, biloma, and dyspnea have incidences of about 1%.

Scientific statement In the NLCT study, post-embolization syndrome was observed in ≥90% of patients treated with miriplatin. However, the incidence of biphasic fever, which is said to be a characteristic AE associated with miriplatin, was low at 2.8% (Tables 16, 17).

Incidences of nausea and vomiting were low compared with other platinum agents, at 18.8% and 13.5%, respectively.

Hematological AEs were leukopenia at 38.2%, thrombocytopenia at 32.1%, and neutropenia at 20.1%. Incidence of eosinophilia, which is also reported as a characteristic AE of miriplatin, was relatively low at 14.6% (Table 8).[28,29]

Abnormal hepatic function was frequent, with elevated AST and ALT occurring in 49.9% and 78.4% of patients, respectively, of whom a further 12.4% and 26.6% had respective AST and ALT values ≥grade 3. Elevated T-Bil was seen in 31.6% of patients, of whom 3.2% had value ≥grade 3, more than three times the upper limits of normal (ULN).

CQ2-7 What is the extent of deterioration in hepatic function caused by TAI/TACE using miriplatin?

Table 16 Incidence of drug-related adverse events with miriplatin therapy (1)

	NLCT Study ($n = 535$)		Phase II Trial[29] ($n = 16$)		Randomized Phase II Trial[28] ($n = 83$)	
	Total (%)	G3/4 (%)	Total (%)	G3/4 (%)	Total (%)	G3/4 (%)
Fever	81.3	0.2	94	0	96.4	3.6
Biphasic fever	2.8	–	–	–	–	–
Anorexia	29.7	–	–	–	–	–
Abdominal pain	21.2	0	50	0	–	–
Nausea	18.8	0	25	0	–	–
Vomiting	13.5	0	–	–	55.4	1.2
Fatigue	9.3	0	–	–	39.8	0
Chills	–	0	–	–	39.8	0
Administration site pain	21.2	0	50	0	43.4	0
Diarrhea	2.0	0	31	0	–	–
Ascites	1.2	0	–	–	–	–
Hepatic failure	0.3	0.3	–	–	–	–
Vascular injury	–	–	–	–	0	0

CTC-AE v3.0 Japan Society of Clinical Oncology Adverse Drug Reaction Criteria

Recommendation Typically, no deterioration is seen in postoperative ICG_{15}, but prothrobmin time (PT) ratio (%) may display a transient decline.

Scientific statement Hepatic impairment after miriplatin administration has been reported to peak within 2 weeks in 46% of patients, at 3–5 weeks in 23% of patients, and at 9–11 weeks in 31% of patients.[29]

The NLCT study also found that in evaluable patients, ICG_{15} values had not deteriorated at 1–2 weeks after therapy and that PT ratio (%) exhibited a transient decline, but subsequently recovered in the majority of patients.

Child–Pugh class B patients did not find any significant differences in pre- or post-treatment ICG_{15}, and did not find any SAEs or increased ascites or hepatic failure necessitating additional therapy and prolonged hospitalization.[32]

However, the safety of miriplatin used in combination with embolic materials has yet to be established, and a Phase III study on concomitant use of miriplatin and embolizing agents is currently underway.[34]

Table 17 Incidence of drug-related adverse events with miriplatin therapy (2)

	Incidence (%)
Ascites	1.2
Liver abscess	0.6
Biloma	0.3
Dyspnea	0.3

CQ2-8 Does vascular injury occur after intra-arterial administration of miriplatin?

Recommendation Vascular injuries such as hepatic artery occlusion, arterial stenosis and arterioportal shunts, and hepatic lobar atrophy caused by vascular damage are rare.

Scientific statement No reports have described vascular injuries from non-hematological toxicity in previous Japanese Phase I and II trials on miriplatin therapy.[29,32] Likewise, no vascular injuries have been reported in the NLCT study (Table 16). In TAI without the use of embolic materials, the aforementioned randomized phase II trial comparing miriplatin and zinostatin stimalamer (SMANCS) found that vascular injuries occurred in 48.4% of the SMANCS treatment group ($n = 31$), but that no vascular injuries occurred in the miriplatin treatment group ($n = 73$).[28] In a limiting study performing follow-up angiography on nine patients at 2–6 months after treatment, no arterial stenoses, arterial occlusions, or arterioportal shunts were observed.[30]

Evaluation of therapeutic response

CQ2-9 After how many weeks should therapeutic response to miriplatin be evaluated?

Recommendation Non-specific accumulation of Lipiodol appears on dynamic CT at 1 week after administration of miriplatin, so evaluation of therapeutic response should preferably be performed at 4–8 weeks after administration.

Scientific statement Evaluation of therapeutic response performed at 1 day or 1 week after starting miriplatin therapy may result in overestimation of response due to the appearance of non-specific Lipiodol deposits. Evaluation of therapeutic response using dynamic CT at 4–8 weeks after therapy is therefore preferable, to allow these non-specific deposits to disappear. In the above-mentioned Phase I clinical trial, therapeutic response to miriplatin was evaluated with dynamic CT at 1 week, 5 weeks, and 3 months after therapy,[32] while the Phase II trial evaluated the antitumor effects of miriplatin using dynamic CT every 3 months.[29]

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Cancer Prevention Research

Research Article

Possible Role of Visfatin in Hepatoma Progression and the Effects of Branched-Chain Amino Acids on Visfatin-Induced Proliferation in Human Hepatoma Cells

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Abstract

Obesity and related metabolic abnormalities, including adipocytokine dysbalance, are risk factors for hepatocellular carcinoma (HCC). Visfatin, an adipocytokine that is highly expressed in visceral fat, is suggested to play a role in the progression of human malignancies. Branched-chain amino acids (BCAA) reduce the incidence of HCC in obese patients with liver cirrhosis and prevent obesity-related liver carcinogenesis in mice. In this study, we investigated the possible role of visfatin on HCC progression and the effects of BCAA on visfatin-induced proliferation of HCC cells. In patients with HCCs, serum visfatin levels were significantly correlated with stage progression and tumor enlargement. Visfatin preferentially stimulated the proliferation of HepG2, Hep3B, and HuH7 human HCC cells compared with Hc normal hepatocytes. Visfatin phosphorylated extracellular signal–regulated kinase (ERK), Akt, and GSK-3β proteins in HepG2 cells. LY294002 [a phosphoinositide-3-kinase (PI3K) inhibitor], PD98059 [a MAP/ERK 1 kinase (MEK1) inhibitor], CHIR99021 (a GSK-3β inhibitor), and BCAA significantly inhibited visfatin-induced proliferation in HepG2 cells. BCAA also inhibited phosphorylation of GSK-3β, increased cellular levels of $p21^{CIP1}$, caused cell-cycle arrest in G_0/G_1 phase, and induced apoptosis in HCC cells in the presence of visfatin. These findings suggest that visfatin plays a critical role in the proliferation of HCC cells and may be associated with the progression of this malignancy. In addition, BCAA might inhibit obesity-related liver carcinogenesis by targeting and, possibly, by overcoming the stimulatory effects of visfatin. *Cancer Prev Res; 4(12); 2092–100. ©2011 AACR.*

Introduction

In addition to established risk factors such as hepatitis and alcohol consumption, obesity and its related metabolic abnormalities raise the risk of hepatocellular carcinoma (HCC; refs. 1–4). Several pathophysiologic mechanisms linking obesity and liver carcinogenesis have been shown, including the emergence of insulin resistance and the subsequent inflammatory cascade (5). In obese individuals, increased adipose tissue leads to the expression of a variety of adipocytokines. Recently, the role of obesity-associated dysfunctional adipose tissue and subsequent adipocytokine dysbalance in carcinogenesis has attracted attention (6). Clinical trials have shown that adipocytokine disorders, including increased levels of leptin and decreased levels of adiponectin in the serum, are implicated in hepatocarcinogenesis (7, 8). Leptin induces proliferation and inhibits apoptosis in human HCC cells (9). These findings suggest that adipocytokine dysbalance may play an important role in the development and progression of HCCs.

Visfatin/pre-B-cell–enhancing factor, which was originally isolated from peripheral lymphocytes, has been described as a secreted growth factor for early B-cell proliferation (10). More recently, visfatin has also been characterized as an adipocytokine that is highly expressed in the visceral fat of humans and rodents. Increased levels of visfatin, which are positively correlated with the size of visceral fat deposits, are observed in various clinical conditions such as obesity and diabetes mellitus (11, 12). Abnormalities in serum levels of visfatin have also been reported in nonalcoholic fatty liver disease, which is a hepatic manifestation of metabolic syndrome (13). These results are somewhat conflicting, however, as both increased and decreased serum levels of this adipocytokine have been found in patients with nonalcoholic fatty liver disease (14, 15).

Furthermore, previous studies have shown that visfatin may play a role in the development and progression of certain types of human malignancies (16). For instance,

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colorectal cancer, the development of which is associated with metabolic abnormalities (17), is accompanied by the overexpression of visfatin (18). Serum visfatin level is a good biomarker of colorectal malignant potential and stage progression (19). Visfatin stimulation increases cell proliferation in prostate and breast cancer cells (20, 21), whereas the use of visfatin inhibitor exerts an antitumor effect by inducing apoptosis (22). These findings suggest that visfatin is one of the key adipocytokines that links obesity and tumorigenesis and thus may be an effective target for the inhibition of obesity-related carcinogenesis. However, no detailed studies of the relationship between visfatin and HCCs have yet been conducted.

Branched-chain amino acids (BCAA; leucine, isoleucine, and valine) are used in patients with liver cirrhosis to improve protein malnutrition (23). Recent clinical trials have shown that oral supplementation with BCAA prevents progressive hepatic failure, improves event-free survival in patients with chronic liver diseases, and reduces the risk of HCCs in these patients who are obese (body mass index $\geq$ 25; refs. 4, 24). BCAA supplementation also prevents obesity-related carcinogenesis in both the liver and the colorectum of diabetic mice (25, 26). In the present study, we measured serum visfatin concentration in patients with HCCs and examined whether it was correlated with stage progression and tumor enlargement. We also examined in detail the effects of visfatin on the acceleration of HCC cell proliferation, focusing on the activation of signaling pathways, and investigated whether BCAA suppresses visfatin-induced growth of HCC cells.

Materials and Methods

Patients and measurement of serum visfatin concentration

Eighty-five primary HCC patients who underwent initial treatment at our hospital from January 2006 to December 2008 were enrolled in this study. Tumor stage was defined according to the staging system of the Liver Cancer Study Group of Japan (27). The greatest diameter of HCC was determined with dynamic computed tomography or magnetic resonance imaging. Fasting serum samples were collected at the time of diagnosis, and serum levels of visfatin were determined by ELISA (AdipoGen). The study protocol was approved by the Institutional Review Board for human research, and all patients gave written informed consents to enter the study.

Materials

Recombinant human visfatin was purchased from PeproTech Inc. BCAA (total amino acid content, 12.28 mmol/L), ΔBCAA (10.28 mmol/L), and neutral amino acid media (12.28 mmol/L) were obtained from Ajinomoto Pharmaceuticals Co. ΔBCAA serves as basal medium and contains 17 amino acids except BCAA. The concentrations of amino acids in the medium are as follows (in mmol/L): glycine, 0.40; alanine, 0.40; serine, 0.40; threonine, 0.80; cystine, 0.20; methionine, 0.20; glutamine, 4.00; asparagine, 0.40; glutamic acid, 0.40; aspartic acid, 0.40; phenylalanine, 0.40; tyrosine, 0.40; tryptophan, 0.08; lysine, 0.80; arginine, 0.40; histidine, 0.20; and proline, 0.40. BCAA medium was prepared by adding 2 mmol/L BCAAs (0.952 mmol/L leucine, 0.476 mmol/L isoleucine, and 0.572 mmol/L valine) to ΔBCAA medium. The composition of BCAA (2:1:1.2 = leucine:isoleucine:valine) was set at the clinical dosage used for the treatment of decompensated liver cirrhosis in Japan (4, 24). The neutral amino acid medium was prepared by adding 2 mmol/L neutral amino acids (0.667 mmol/L each of alanine, serine, and glycine) to the ΔBCAA medium and served as an amino acid content-matched control for BCAA medium. LY294002 was purchased from Cell Signaling Technology; PD98059, from Sigma; and CHIR99021, from Stemgent.

Cell lines and cultures

HepG2, Hep3B, and HuH7 human HCC cell lines were obtained from the Japanese Cancer Research Resources Bank and maintained in RPMI-1640 medium (Sigma) supplemented with 10% fetal calf serum. Hc human normal hepatocyte cell line was purchased from Cell Systems and maintained in a CS-S complete medium (Cell Systems). The cell lines have been characterized by each source, and any further authentication was not done in our laboratory. These cells were cultured in an incubator with humidified air with 5% CO_2 at 37°C.

Cell proliferation assay

Cell proliferation assays were conducted by a cell proliferation kit [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT); Roche)] according to the manufacturer's instructions. To examine the effects of visfatin on the proliferation of the HepG2, Hep3B, HuH7, and Hc cells, these cells were seeded on 96-well plates (1×10^4 cells per well). After 16 hours of serum starvation, the cells were treated with the indicated concentrations (0–400 ng/mL) of exogenous visfatin for 48 hours in the absence of serum. To investigate the effect of LY294002, PD98059, CHIR99021, and BCAA, HepG2 cells were treated with these agents in the absence and presence of visfatin (100 or 400 ng/mL) for 48 hours in serum-free medium. All assays were conducted in triplicate.

Protein extraction and Western blot analysis

Total cellular protein was extracted and equivalent amounts of protein were examined by Western blot analysis (28). The primary antibodies used to detect the respective protein bands have been described previously (28). An antibody to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control. The intensities of the blots were quantified with NIH Image software, version 1.62.

Cell-cycle assays

Cell-cycle assays were conducted by a cell-cycle detection kit (Cayman) according to the manufacturer's instructions. HepG2 cells were treated with BCAA for 48 hours in the

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absence and presence of 100 ng/mL visfatin. After the harvested cells were fixed and stained, they were analyzed for DNA histograms and cell-cycle phase distribution with a FACScan flow cytometer (BD). The data were analyzed with the CellQuest computer program (BD) as described previously (28).

Apoptosis assays

The Annexin V-binding capacity of treated cells was examined with flow cytometry by the Annexin V-FITC Apoptosis Detection Kit I (BD) to evaluate the induction of apoptosis. HepG2 cells were treated with BCAA for 48 hours in the absence and presence of 100 ng/mL visfatin. After the cultured cells were washed with cold PBS, they were incubated in Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) for 15 minutes on ice. Stained cells were analyzed within 1 hour. Annexin V-FITC–positive and PI-negative cells were counted as apoptotic cells as described previously (29).

Statistical analysis

The data are expressed as mean ± SD. The statistical significance of the difference in mean values was assessed with one-way ANOVA, followed by the Scheffe *t* test. Values of $P < 0.05$ were considered significant.

Results

Association of serum visfatin concentration with HCC clinical stage and tumor size

We initially analyzed the possible association of serum visfatin concentration with the clinical stage and tumor size (greatest diameter) of HCCs in 85 patients (54 men and 31 women, median age 73 years). The median serum visfatin concentration was 5.8 ng/mL (range: 1.2–42.0). We found that the progression of clinical stage was correlated with serum visfatin concentration; the level of this adipocytokine was significantly increased in stage IV patients compared with levels in those with stage I and II disease ($P < 0.05$; Fig. 1A). In 85 patients, the mean Pearson product-moment correlation coefficient (r) and the P value (P) of tumor size with serum visfatin concentration were 0.315 and 0.003, respectively (Fig. 1B). Moreover, similar results ($r = 0.326$ and $P = 0.01$) were obtained when patients with diabetes mellitus (HbA1c ≥ 6%) and/or obesity were excluded ($n = 53$, Fig. 1C), indicating a positive correlation between HCC tumor size and serum visfatin levels regardless of complications with obesity and diabetes.

Effects of visfatin on cell proliferation and phosphorylation of extracellular signal–regulated kinase, Akt, and GSK-3β proteins in human HCC cells

We next examined whether visfatin stimulates the proliferation of HCC cells by XTT assay. When series of HCC cells (i.e., HepG2, Hep3B, and HuH7 cells) were treated with visfatin (25–400 ng/mL) for 48 hours, cell proliferation was significantly stimulated in a dose-dependent

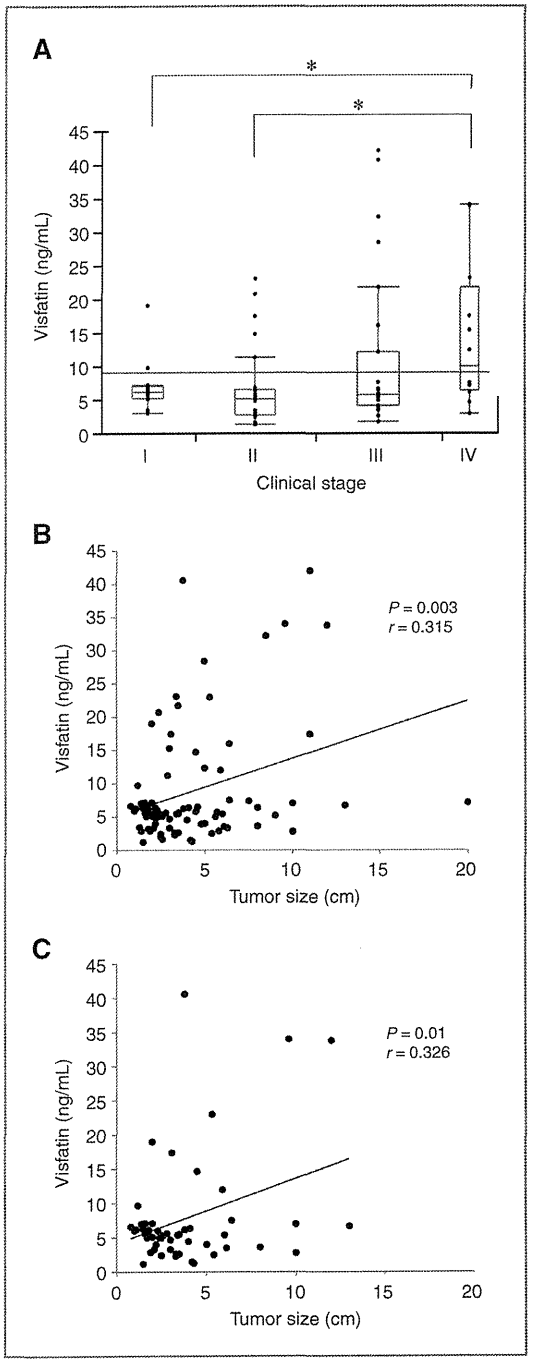

Figure 1. Correlation between serum visfatin concentrations and the clinical stage (A) and tumor size (B, C) of HCCs. A and B, the correlations were determined by analyzing 85 patients with primary HCCs. C, the correlation was determined by analyzing 53 HCC patients who are not obese and did not have diabetes mellitus. *, $P < 0.05$.

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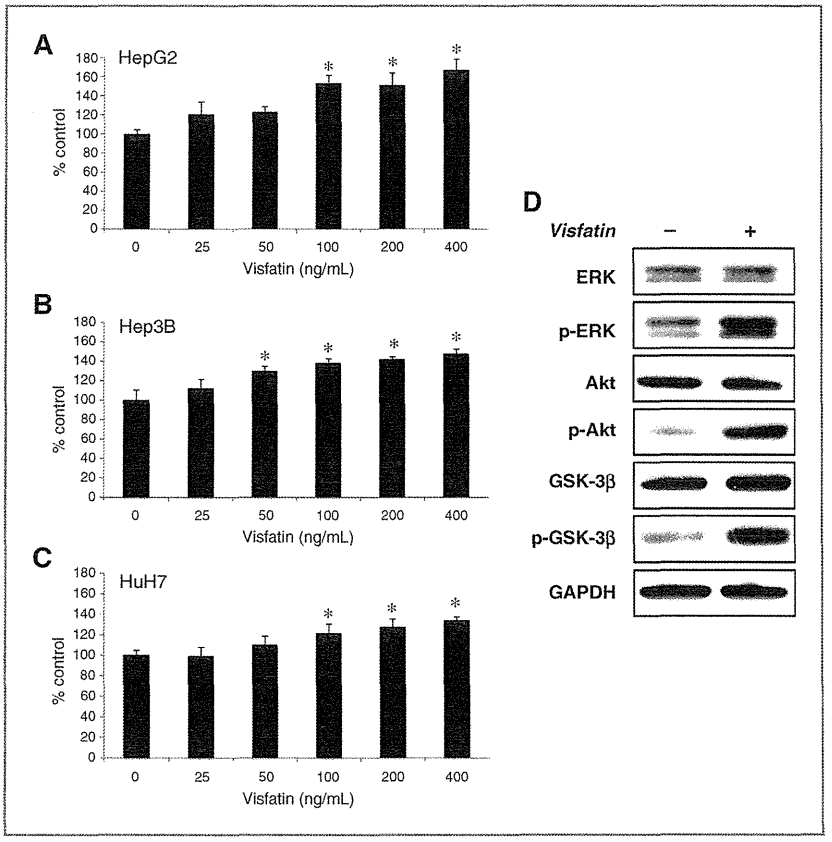

Figure 2. Effects of visfatin on the cell proliferation and phosphorylation of ERK, Akt, and GSK-3β proteins in HCC cells. HepG2 (A), Hep3B (B), and HuH7 (C) cells were treated with the indicated concentration of visfatin for 48 hours in serum-free medium. Cell proliferation was evaluated by an XTT assay. Results were expressed as a percentage of the control value. Bars, SD of triplicate assays. *, $P < 0.05$. D, HepG2 cells were treated with and without 100 ng/mL visfatin for 30 minutes, and cell lysates were prepared. The cell lysates were then analyzed with a Western blot using respective antibodies. Equal protein loading was verified by the detection of GAPDH. Repeated Western blotting yielded similar results. p-ERK, phosphorylated ERK; p-Akt, phosphorylated Akt; p-GSK-3β, phosphorylated GSK-3β.

manner ($P < 0.05$; Fig. 2A–C). In addition, treatment of HepG2 cells with 100 ng/mL of visfatin for 30 minutes caused a marked phosphorylation of extracellular signal-regulated kinase (ERK), Akt, and GSK-3β proteins (Fig. 2D), suggesting that visfatin might induce cell proliferation in HCC cells by activating PI3K/Akt and MAPK/ERK signaling pathways.

Effects of phosphoinositide-3-kinase, MAP/ERK 1 kinase, and GSK-3β inhibitors on visfatin-induced proliferation of HepG2 cells

We next examined whether pharmacologic inhibitors of phosphoinositide-3-kinase (PI3K; LY294002), MAP/ERK 1 kinase (MEK1; PD98059), and GSK-3β (CHIR99021) suppress visfatin-induced proliferation in HepG2 cells because the activation of PI3K/Akt and MAPK/ERK pathways might be involved in this proliferation (Fig. 2). As shown in Fig. 3, treatment with LY294002 (Fig. 3A), PD98059 (Fig. 3B), and CHIR99021 (Fig. 3C) significantly inhibited HepG2 cell proliferation both in the absence and presence of visfatin stimulation (100 and 400 ng/mL; $P < 0.05$). These findings suggest that PI3K and MAPK pathways could be effective targets for the inhibition of visfatin-induced proliferation in HepG2 cells.

Effects of BCAA on visfatin-induced proliferation of HepG2 cells

BCAA is reported to suppress obesity-related liver carcinogenesis (4, 25). Therefore, we next examined whether BCAA inhibits visfatin-stimulated proliferation of HepG2 cells because this adipocytokine, which is increased in obese individuals (11, 12), might play a role in the progression of HCCs (Fig. 1). As shown in Fig. 4A, the proliferation of HepG2 cells was significantly inhibited when the cells were treated in BCAA medium; meanwhile, this inhibition did not occur in neutral amino acid medium, which was served as an amino acid content-matched control for BCAA medium ($P < 0.05$). This finding possibly indicates that BCAA itself is specific in inhibiting the growth of HCC cells. In addition, a marked potentiation in the proliferative activity of HepG2 cells occurred after stimulation with 100 and 400 ng/mL visfatin, whereas BCAA treatment inhibited such proliferation in a dose-dependent manner regardless of visfatin stimulation ($P < 0.05$). The inhibition of proliferation with 2 mmol/L BCAA was greater (65% reduction) when the cells were cultured at higher concentration of visfatin (400 ng/mL) than that in the absence of the adipocytokine (41% reduction; Fig. 4B). In contrast, cell proliferation was not induced when Hc normal hepatocytes