Table 2 Demographic data of the study patients

Number of patients	48
Sex, no. (%)	
Male	38 (79)
Female	10 (21)
Age (years)	
Median (range)	72 (47–83)
ECOG PS, no. (%)	
0	32 (67)
1	16 (33)
Child-Pugh score, no. (%)	
5	32 (67)
6	16 (33)
Hepatitis serology, no. (%)	
HBs-Ag positive	5 (10)
HCV-Ab positive	27 (56)
Alcohol	7 (15)
BCLC stage, no. (%)	
B (intermediate)	25 (52)
C (advanced)	23 (48)
Macroscopic vascular invasion, no. (%)	10 (21)
Extrahepatic spread, no. (%)	
Any	18 (38)
Lung	7 (15)
Lymph node	5 (10)
Peritoneum	5 (10)
Bone	2 (4)
Adrenal grand	2 (4)
Prior treatment, no. (%)	
Any	45 (94)
Surgery	13 (27)
Local ablation	18 (38)
Transarterial chemoembolization	41 (85)
Alpha fetoprotein (AFP), ng/ml	
<200, no. (%)	28 (58)
≥200, no. (%)	20 (42)
Median (range)	96.0 (2.7–83,250.0)

ECOG Eastern Cooperative Oncology Group, *BCLC* Barcelona Clinic Liver Cancer

according to RECIST (Fig. 3a); 25.8 months (95 % CI, 12.5–39.0; $n = 7$), 13.8 months (95 % CI, 5.4–22.1; $n = 27$), and 4.1 months (95 % CI, 3.2–5.1; $n = 14$), respectively, according to the EASL criteria (Fig. 3b); and 25.8 months (95 % CI. 12.5–39.0; $n = 7$), 13.8 months (95 % CI, 6.1–21.5; $n = 28$), and 4.1 months (95 % CI, 3.4–4.3; $n = 13$), respectively, according to mRECIST (Fig. 3c). Patients who achieved an OR according to the EASL criteria and mRECIST had a better predicted OS than patients with SD ($p = 0.033$ and 0.028, respectively). Patients who were classified as SD according to RECIST at initial evaluation showed different outcomes according to the EASL criteria and mRECIST (Fig. 4). Of the 34 patients classified as SD by RECIST at initial evaluation, 6 achieved an OR according to the EASL criteria and mRECIST. The patients classified as responders (those who achieved a CR or a PR) according to the EASL criteria and mRECIST had better predicted values for both TTP and OS than did those classified as non-responders (those classified as SD or PD).

Table 3 Tumor lesions included in the efficacy analysis

Eligible lesion for RECIST, no.	
Any	100
Liver	81
Lymph node	5
Bone	4
Lung	4
Peritoneum	4
Adrenal grand	2
Eligible lesion for EASL and mRECIST, no.	
Any	91
Liver	79
Lymph node	4
Peritoneum	4
Bone	2
Lung	1
Adrenal grand	1

Table 4 Radiological response, as determined by RECIST, the EASL criteria, and mRECIST

	First evaluation			Best of overall		
	RECIST	EASL	mRECIST	RECIST	EASL	mRECIST
CR	0	3	3	0	3	3
PR	1	4	4	3	4	4
SD	34	27	28	32	27	28
PD	13	14	13	13	14	13

Correlation between assessment by radiological response and AFP

Thirty-three of the 48 patients were included in the analysis of AFP. Nine patients with a 20 % AFP decrease were classified as responders, while the remaining 24 were non-responders. The one patient who achieved OR, 7 of 23 patients who achieved SD, and 1 of 9 patients who achieved PD were classified as AFP responders according to RECIST (Fig. 5a). All four patients who achieved OR, 4 of 19 patients who achieved SD, and 1 of 10 patients who achieved PD were classified as AFP responders according

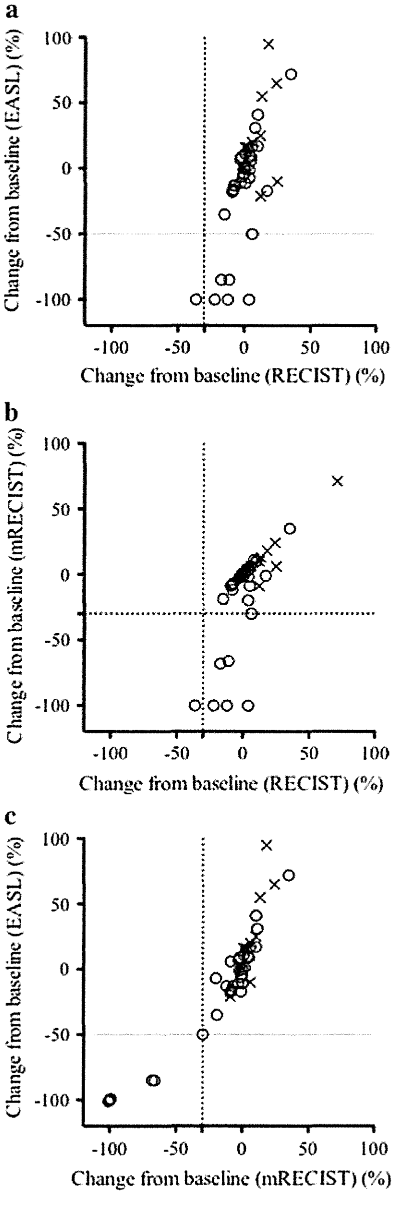

Fig. 2 Scatter plots showing change in size from first evaluation according to **a** RECIST and the EASL criteria, **b** RECIST and mRECIST, and **c** the EASL criteria and mRECIST. *Circles* indicate patients who had no new lesions; *crosses* indicate those who had new lesions

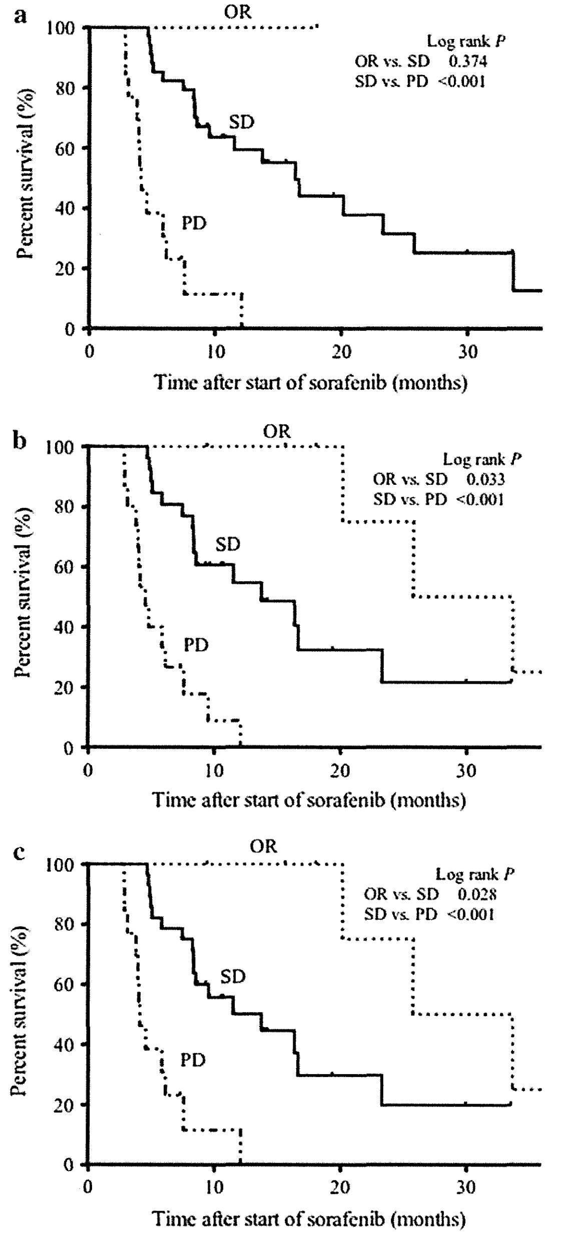

Fig. 3 Kaplan-Meier curve for OS of patients with advanced HCC who were treated with sorafenib. OR (*short dashed line*), SD (*solid line*), and PD (*long dashed line*) at first evaluation according to RECIST (**a**), the EASL criteria (**b**), and mRECIST (**c**) are shown

to EASL (Fig. 5b). All 4 patients who achieved OR, 4 of 20 patients who achieved SD, and 1 of 9 patients who achieved PD were classified as AFP responders according to mRECIST (Fig. 5c).

The median OS for patients classified as AFP responders and non-responders was 20.2 months (95 % CI, 0.0–43.6) and 6.1 months (95 % CI, 2.3–10.0), respectively. The AFP responders had a better predicted value for OS than

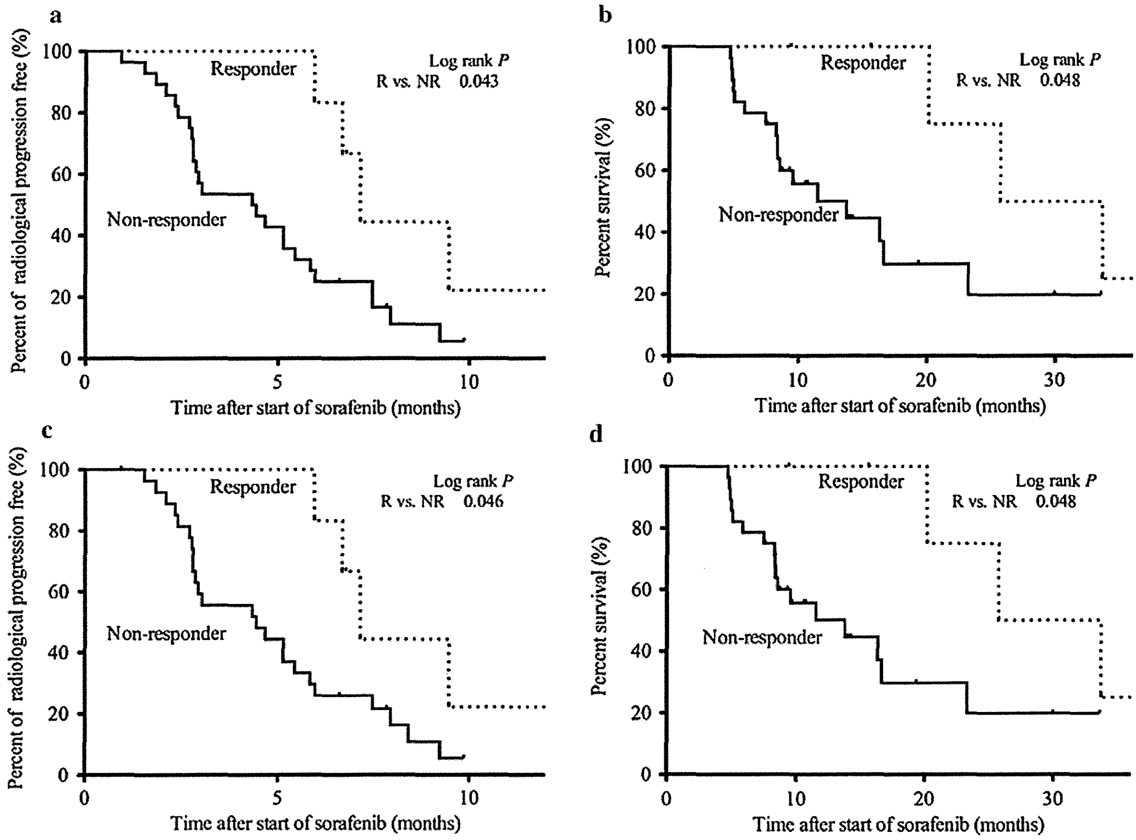

Fig. 4 Kaplan-Meier curve for TTP and OS in patients classified with SD according to RECIST at the first evaluation. Responders (patients who achieved an OR; *short dashed line*) and non-responders (patients who had either SD or PD) at first evaluation according to the EASL criteria (**a**, **b**) and mRECIST (**c**, **d**) are shown. Median TTP of patients who achieved a response (CR or PR) and non-responders (those with SD and PD) were 7.1 months (95 % CI, 6.2–8.0 months, $n = 6$) and 4.3 months (95 % CI, 2.1–6.6 months, $n = 28$), respectively, for the EASL criteria and 7.1 months (95 % CI, 6.2–8.0 months, $n = 6$) and 4.4 months (95 % CI, 1.7–7.2 months, $n = 28$), respectively, for mRECIST. Median OS of responders and non-responders were 25.8 months (95 % CI, 12.5–39.0 months, $n = 6$) and 13.8 months (95 % CI, 6.1–21.5 months, $n = 28$), respectively, for EASL criteria, and 25.8 months (95 % CI, 12.5–39.0 months, $n = 6$) and 13.8 months (95 % CI, 6.1–21.5 months, $n = 28$), respectively, for mRECIST

did the non-responders (Fig. 6a). The patient classified as SD according to RECIST at initial evaluation showed different outcomes according to AFP response and non-response (Fig. 6b). The median OS of patients classified as AFP responders and non-responders was 20.2 months (95 % CI, 3.2–37.1 months, $n = 7$) and 11.5 months (95 % CI, 6.9–16.2 months, $n = 16$), respectively. The patients classified as AFP responders had better predicted OS values than those classified as AFP non-responders.

Discussion

Several studies have reported that sorafenib decreases tumor vascularity in HCC. Kim et al. [17] reported that the mean attenuation value of the hepatic lesions on initial CT was lower than that on baseline CT for both late arterial phase and portal phase images. In a phase II trial of sorafenib plus metromic tegafur/uracil [34], the change in tumor blood flow, as assessed by DCE-MRI after 14 days, could help predict the best tumor response, progression-free survival, and overall survival [35]. Although DCE-MRI is useful for evaluation of the vascular response, it is not yet widely used. A recent study found that JX-594, a granulocyte–macrophage colony stimulating factor (GM-CSF) that expresses oncolytic poxvirus and can selectively replicate in and destroy cancer cells through viral oncolysis and tumor-specific immunity, may sensitize the tumor to subsequent treatment with sorafenib [36]. Although the study included only three patients, treatment with JX-594 followed by sorafenib exhibited antitumoral activity. All patients showed significantly decreased tumor perfusion as

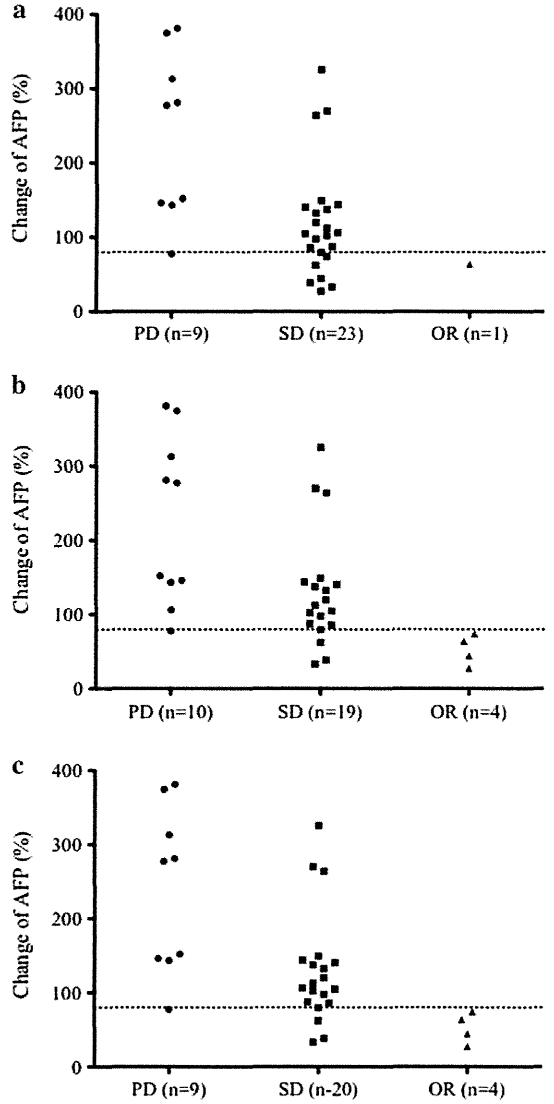

Fig. 5 Comparison of AFP changes between HCC patients who achieved OR, SD, and PD at first evaluation according to RECIST (**a**), the EASL criteria, (**b**) and mRECIST (**c**)

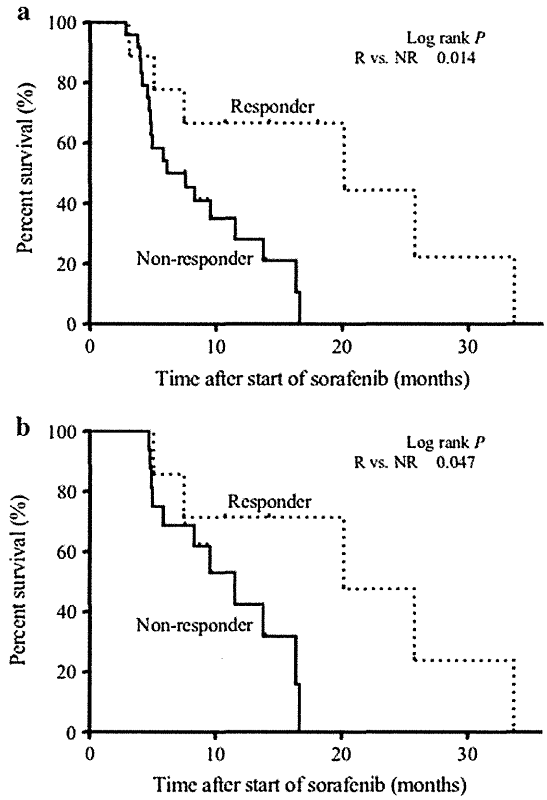

Fig. 6 Kaplan-Meier curve for OS of patients with advanced HCC who were treated with sorafenib (**a**). Kaplan-Meier curve for OS in patients classified as SD according to RECIST at the first evaluation (**b**). AFP responders (*short dashed line*) and non-responders (*solid line*) are shown

assessed by dynamic MRI. During development of molecular target therapy in patients with advanced HCC, it is important to demonstrate the feasibility of using the initial vascular response to predict the clinical outcome of HCC patients who receive sorafenib-based therapy. Early and accurate assessment of the response to sorafenib will facilitate selection of patients with advanced HCC who may benefit from therapy. Furthermore, validation of the early vascular endpoints will aid development of drugs for advanced HCC.

One retrospective study compared the best overall responses, not initial response, according to RECIST and mRECIST for HCC treated with sorafenib [37]. The study showed that the survival of patients who achieved an OR as assessed by mRECIST was significantly better than the survival of non-responding patients. However, the study was limited in that 22.6 % of the subjects were Child-Pugh B patients, which may have skewed the results. On the other hand, in patients treated with molecularly targeted therapies, reductions in tumor vascularity may occur unevenly and discontinuously, unlike in locoregional therapy, and it is not clear whether unidimensional measurements reflect intratumoral enhancement if the tumor is distorted. Raoul et al. reported that mRECIST showed a greater alignment of TTP and a clearer identification of PD than did the modified WHO criteria (bidimensional measurement of tumor size) in a phase II study of brivanib (Bristol-Myers Squibb, Princeton, NJ) in patients with HCC [38]. Nevertheless, the study compared tumor sizes by the product of bidimensional and enhancement approaches used in unidimensional methods. Spira et al. [39] compared RECIST with different enhancement criteria (area, uni- and bidimensional) in HCC patients who received sorafenib and suggested that unidimensional enhancement criteria may be more accurate than RECIST. However, the study included only 25 patients with various radiological periods and did not evaluate the survival predictive value as assessed by enhancement criteria. In the present study we excluded Child-Pugh B patients, compared RECIST and intratumoral enhancement according to the EASL criteria and mRECIST, and evaluated the correlation between the initial response assessed by enhancement criteria and the clinical outcome of HCC patients treated with sorafenib. RECIST version 1.1 reduced the number of lesions required for target selection to a maximum of two per organ and five in total [31]. It has been shown that assessment of five versus ten lesions per patient did not affect the overall response rate and that progression-free survival (PFS) was only minimally affected [40]. In the present study, target lesions were selected as two lesions per organ up to a maximum of five.

In the present study, 3 (6 %) of 48 patients achieved an OR according to RECIST. These results are similar to those of two randomized controlled studies and several retrospective reports from various nations [41–43]. RECIST missed six of seven responders identified based on the EASL criteria and mRECIST at initial evaluation. This may be because sorafenib causes an early reduction in vascularity. The assessments of OR according to the EASL criteria and mRECIST were in agreement. Thus, for the assessment of intratumoral enhancement, including those that are distorted, bidimensional and unidimensional measurements may be equivalent. According to the EASL criteria and mRECIST, patients who achieve an OR have a better prognosis than those classified as SD. Moreover, in patients classified as SD according to RECIST (34 of 48), responders had a better prognosis than did non-responders who were classified according to the EASL criteria and mRECIST. It is feasible that the use of enhancement criteria re-classified patients with SD as responders according to RECIST. The present study shows the utility of enhancement approaches for early prediction of outcome in HCC patients treated with sorafenib. The response to the enhancement approach, measured using the sum of intratumoral enhancement in target lesions by four-phase MDCT, performed prior to therapy and at an early time point after its initiation, was predictive of OS. The objective in the treatment of patients with sorafenib is to prevent disease progression and afford an acceptable quality of life. Sorafenib is associated with a wide range of mild toxicities, which can be troublesome. This would also optimize the clinical outcome and reduce exposure to ineffective treatments.

EASL criteria were originally developed to assess locoregional therapy [26], and a viable lesion was defined as an intrahepatic lesion that showed enhancement in the arterial phase on CT. With mRECIST, the definition of a viable lesion was enhancement in the arterial phase in CT; the difference between intrahepatic and extrahepatic lesions was not specifically mentioned [28]. On the other hand, the largest overall diameter of extrahepatic lesions was measured in a study based on a phase II brivanib first-line trial that compared modified WHO criteria and mRECIST [38]. The present study was designed such that extrahepatic lesions were included as target lesions for the EASL criteria and mRECIST, if evaluation of their vascularization was possible. Moreover, extrahepatic lesions were evaluated by late-phase MDCT. In the present study, only one patient showed a reduction in vascularity in an extrahepatic lesion. However this patient was classified as SD according to all three methods at initial evaluation and defined as PD at second evaluation because new lesions appeared. Therefore, the results of the present study were not affected by conflicting evaluations of vascularity in extrahepatic lesions. Diagnosis of HCC by the four-phase technique is based on the liver's dual blood supply. Classical hypervascular HCC is fed only by the artery and surrounding liver, whereas non-hypervascular HCC is fed primarily by the portal vein [44]. Generally, HCC is diagnosed by hyperenhancement of the surrounding liver during the arterial phase and hypoattenuation of the surrounding liver during the portal and/or late phase. On the other hand, extrahepatic metastasis of HCC usually occurs through a single blood vessel. Four-phase MDCT images of these lesions showed no clear contrast. Several studies have evaluated tumor response in hypervascular malignant neoplasms such as GIST or RCC by attenuation during the delayed phase of MDCT [23, 25]. Therefore, we considered that evaluation by the enhancement approach using the delayed phase of MDCT was appropriate for extrahepatic lesions in HCC. A recent report demonstrated treatment experience with sorafenib in patients who experienced recurrence after liver transplantation. The report showed that no additional information could be obtained from mRECIST [45]. This was because almost all patients (10 of 11) presented with extrahepatic non-enhancing lesions. Definition of enhancement criteria in cases of reduced extrahepatic lesion vascularity will be necessary for their widespread use in clinical trials of molecularly targeted therapies.

Two studies reported that the early AFP response, which is defined as a decline >20 % from baseline, is a useful surrogate marker for prediction of treatment response and prognosis in advanced HCC patients with baseline AFP levels >20 ng/ml [33, 46]. The results of the present study agreed with those findings. AFP responders were significantly correlated with patients classified as OR according to EASL and mRECIST. Moreover, the AFP response is useful for assessment of prognosis in patients who achieve SD according to RECIST. However, the AFP response was not evaluated in 15 of the 48 patients because the baseline AFP level was <20 ng/ml. The applicability of the AFP response criteria is limited to patients with an increased AFP level (>20 ng/ml). In patients with advanced HCC treated with sorafenib, it may be better to evaluate the response mainly radiologically and for AFP measurements to be auxiliary.

In summary, the enhancement criteria were superior to RECIST for the assessment of response in patients treated with sorafenib. Moreover, mRECIST appears to be simple and convenient. Also, the enhancement approaches had a significantly better predictive value for the clinical benefit of sorafenib than RECIST at the initial evaluation in responders.

Acknowledgments We thank Masaharu Yoshikawa, Shinichiro Okabe, Yusuke Shinozaki, and Natsuko Taguchi for their help with patient care.

References

1. Parlin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108
2. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D. SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359: 378–390
3. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 2009;10: 25–34
4. Faivre S, Raymond E, Boucher E, Douillard J, Lim HY, Kim JS, Zappa M, Lanzalone S, Lin X, Deprimo S, Harmon C, Ruiz-Garcia A, Lechuga MJ, Cheng AL. Safety and efficacy of sunitinib in patients with advanced hepatocellular carcinoma: an open-label, multicentre, phase II study. Lancet Oncol 2009; 10:794–800
5. Park JW, Finn RS, Kim JS, Karwal M, Li RK, Ismail F, Thomas M, Harris R, Baudelet C, Walters I, Raoul JL. Phase II, open-label study of brivanib as first-line therapy in patients with advanced hepatocellular carcinoma. Clin Cancer Res 2011;17: 1973–1983
6. Finn RS, Kang YK, Mulcahy M, Polite BN, Lim HY, Walters I, Baudelet C, Manekas D, Park JW. Phase II, open-label study of brivanib as second-line therapy in patients with advanced hepatocellular carcinoma. Clin Cancer Res 2012;18:2090–2098
7. Kanai F, Yoshida H, Tateishi R, Sato S, Kawabe T, Obi S, Kondo Y, Taniguchi M, Tagawa K, Ikeda M, Morizane C, Okusaka T, Arioka H, Shiina S, Omata M. A phase I/II trial of the oral antiangiogenic agent TSU-68 in patients with advanced hepatocellular carcinoma. Cancer Chemother Pharmacol 2011;67: 315–324
8. Zhu AX, Abrams TA, Miksad R, Blaszkowsky LS, Meyerhardt JA, Zheng H, Muzikansky A, Clark JW, Kwak EL, Schrag D, Jors KR, Fuchs CS, Iafrate AJ, Borger DR, Ryan DP. Phase 1/2 study of everolimus in advanced hepatocellular carcinoma. Cancer 2011;117:5094–5102
9. Miller AB, Hoogstraten B, Staquent M, Winkler A. Reporting results of caner treatment. Cancer 1981;47:207–214
10. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–216
11. Flaherty KT. Sorafenib in renal cell carcinoma. Clin Cancer Res 2007;13:747s–752s
12. Faivre SJ, Bouattour M, Dreyer C, Raymond E. Sunitinib in hepatocellular carcinoma: redefining appropriate dosing, schedule, and activity end points. J Clin Oncol 2009;27:e248–e250
13. Vanel D, Albiter M, Shapeero L, Le Cesne A, Bonvalot S, Le Pechoux C, Terrier P, Petrow P, Caillet H, Dromain C. Role of computed tomography in the follow-up of hepatic and peritoneal metastases of GIST under imatinib mesylate treatment: a prospective study of 54 patients. Eur J Radiol 2005;54:118–123
14. Rixe O, Bukowski RM, Michaelson MD, Wilding G, Hudes GR, Bolte O, Motzer RJ, Bycott P, Liau KF, Freddo J, Trask PC, Kim S, Rini BI. Axitinib treatment in patients with cytokine-refractory metastatic renal-cell cancer: a phase II study. Lancet Oncol 2007; 8:975–984
15. van der Veldt AA, Meijerink MR, van den Eertwegh AJ, Bex A, de Gast G, Haanen JB, Boven E. Sunitinib for treatment of advanced renal cell cancer: primary tumor response. Clin Cancer Res 2008;14:2431–2436
16. Abou-Alfa GK, Schwartz L, Ricci S, Amadori D, Santoro A, Figer A, De Greve J, Douillard JY, Lathia C, Schwartz B, Taylor I, Moscovici M, Saltz LB. Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. J Clin Oncol 2006;24: 4293–4300
17. Kim MJ, Choi JI, Lee JS, Park JW. Computed tomography findings of sorafenib-treated hepatic tumors in patients with advanced hepatocellular carcinoma. J Gastroenterol Hepatol 2011;2:1201–1206
18. Joensuu H, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D, Silberman S, Capdeville R, Dimitrijevic S, Druker B, Demetri GD. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. N Engl J Med 2001;344:1052–1056
19. Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, McArthur G, Judson IR, Heinrich MC, Morgan JA, Desai J, Fletcher CD, George S, Bello CL, Huang X, Baum CM, Casali PG. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 2006;368:1329–1338
20. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, Chen I, Bycott PW, Baum CM, Figlin RA. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 2007;356: 115–124

21. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, Negrier S, Chevreau C, Solska E, Desai AA, Rolland F, Demkow T, Hutson TE, Gore M, Freeman S, Schwartz B, Shan M, Simantov R. TARGET Study Group. Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med 2007;356:125–134
22. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, Grünwald V, Thompson JA, Figlin RA, Hollaender N, Urbanowitz G, Berg WJ, Kay A, Lebwohl D. RECORD-1 Study Group. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 2008;372:449–456
23. Choi H, Charnsangavej C, Faria SC, Macapinlac HA, Burgess MA, Patel SR, Chen LL, Podoloff DA, Benjamin RS. Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. J Clin Oncol 2007;25:1753–1759
24. Benjamin RS, Choi H, Macapinlac HA, Burgess MA, Patel SR, Chen LL, Podoloff DA, Charnsangavej C. We should desist using RECIST, at least in GIST. J Clin Oncol 2007;25:1760–1764
25. van der Veldt AA, Meijerink MR, van den Eertwegh AJ, Haanen JB, Boven E. Choi response criteria for early prediction of clinical outcome in patients with metastatic renal cell cancer treated with sunitinib. Br J Cancer 2010;102:803–809
26. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M. EASL Panel of Experts on HCC. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001;35:421–430
27. Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, Sherman M, Schwartz M, Lotze M, Talwalkar J. Panel of Experts in HCC-Design Clinical Trials. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 2008;100:698–711
28. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Semin Liver Dis 2010;30:52–60
29. James K, Eisenhauer E, Christian M, Terenziani M, Vena D, Muldal A, Therasse P. Measuring response in solid tumors: unidimensional versus bidimensional measurement. J Natl Cancer Inst 1999;91:523–528
30. Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. Hepatology 2011;53:1020–1022
31. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–247
32. Chan SL, Mo FK, Johnson PJ, Hui EP, Ma BB, Ho WM, Lam KC, Chan AT, Mok TS, Yeo W. New utility of an old marker: serial alpha-fetoprotein measurement in predicting radiologic response and survival of patients with hepatocellular carcinoma undergoing systemic chemotherapy. J Clin Oncol 2009;27:446–452
33. Shao YY, Lin ZZ, Hsu C, Shen YC, Hsu CH, Cheng AL. Early alpha-fetoprotein response predicts treatment efficacy of antiangiogenic systemic therapy in patients with advanced hepatocellular carcinoma. Cancer 2010;116:4590–4596
34. Hsu CH, Shen YC, Lin ZZ, Chen PJ, Shao YY, Ding YH, Hsu C, Cheng AL. Phase II study of combining sorafenib with metronomic tegafur/uracil for advanced hepatocellular carcinoma. J Hepatol 2010;53:126–131
35. Hsu CY, Shen YC, Yu CW, Hsu C, Hu FC, Hsu CH, Chen BB, Wei SY, Cheng AL, Shih TT. Dynamic contrast-enhanced magnetic resonance imaging biomarkers predict survival and response in hepatocellular carcinoma patients treated with sorafenib and metronomic tegafur/uracil. J Hepatol 2011;55:858–865
36. Heo J, Breitbach CJ, Moon A, Kim CW, Patt R, Kim MK, Lee YK, Oh SY, Woo HY, Parato K, Rintoul J, Falls T, Hickman T, Rhee BG, Bell JC, Kirn DH, Hwang TH. Sequential therapy with JX-594, a targeted oncolytic poxvirus, followed by sorafenib in hepatocellular carcinoma: preclinical and clinical demonstration of combination efficacy. Mol Ther 2011;19:1170–1179
37. Edeline J, Boucher E, Rolland Y, Vauléon E, Pracht M, Perrin C, Le Roux C, Raoul JL. Comparison of tumor response by Response Evaluation Criteria in Solid Tumors (RECIST) and modified RECIST in patients treated with sorafenib for hepatocellular carcinoma. Cancer 2012;118:147–156
38. Raoul JL, Lencioni R, Park JW, Baudelet C, Walters I. Tumor response in a phase 2 study of first-end second-line brivanib in hepatocellular carcinoma (HCC): comparison of modified WHO and modified RECIST criteria (abstr). Presented at 45th annual meeting of the European Association for the Study of the Liver, Vienna, Austria
39. Spira D, Fenchel M, Lauer UM, Claussen CD, Gregor M, Bitzer M, Horger M. Comparison of different tumor response criteria in patients with hepatocellular carcinoma after systemic therapy with the multikinase inhibitor sorafenib. Acad Radiol 2011;18:89–96
40. Bogaerts J, Ford R, Sargent D, Schwartz LH, Rubinstein L, Lacombe D, Eisenhauer E, Verweij J. RECIST Working Party. Individual patient data analysis to assess modifications to the RECIST criteria. Eur J Cancer 2009;45:248–260
41. Pinter M, Sieghart W, Graziadei I, Vogel W, Maieron A, Königsberg R, Weissmann A, Kornek G, Plank C, Peck-Radosavljevic M. Sorafenib in unresectable hepatocellular carcinoma from mild to advanced stage liver cirrhosis. Oncologist 2009;14:70–76
42. Ogasawara S, Kanai F, Obi S, Sato S, Yamaguchi T, Azemoto R, Mizumoto H, Koushima Y, Morimoto N, Hirata N, Toriyabe T, Shinozaki Y, Ooka Y, Mikata R, Chiba T, Okabe S, Imazeki F, Yoshikawa M, Yokosuka O. Safety and tolerance of sorafenib in Japanese patients with advanced hepatocellular carcinoma. Hepatol Int 2011;5:850–856
43. Iavarone M, Cabibbo G, Piscaglia F, et al. Field-practice study of sorafenib therapy for hepatocellular carcinoma: a prospective multicenter study in Italy. Hepatology 2011;54:2055–2063
44. Matsui O, Kobayashi S, Sanada J, Kouda W, Ryu Y, Kozaka K, Kitao A, Nakamura K, Gabata T. Hepatocellular nodules in liver cirrhosis: hemodynamic evaluation (angiography-assisted CT) with special reference to multi-step hepatocarcinogenesis. Abdom Imaging 2011;36:264–272
45. Weinmann A, Niederle IM, Koch S, Hoppe-Lotichius M, Heise M, Düber C, Schuchmann M, Otto G, Galle PR, Wörns MA. Sorafenib for recurrence of hepatocellular carcinoma after liver transplantation. Dig Liver Dis 2012;44:432–437
46. Yau T, Yao TJ, Chan P, Wong H, Pang R, Fan ST, Poon RT, Yau T, Yao TJ, Chan P, Wong H, Pang R, Fan ST, Poon RT. The significance of early alpha-fetoprotein level changes in predicting clinical and survival benefits in advanced hepatocellular carcinoma patients receiving sorafenib. Oncologist 2011;16:1270–1279

J Gastroenterol
DOI 10.1007/s00535-012-0641-9

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Inhibition of hepatocellular carcinoma by PegIFNα-2a in patients with chronic hepatitis C: a nationwide multicenter cooperative study

Namiki Izumi · Yasuhiro Asahina · Masayuki Kurosaki · Gotaro Yamada · Tsutomu Kawai · Eiji Kajiwara · Yukishige Okamura · Takayuki Takeuchi · Osamu Yokosuka · Kazuya Kariyama · Joji Toyoda · Mie Inao · Eiji Tanaka · Hisataka Moriwaki · Hiroshi Adachi · Shinji Katsushima · Masatoshi Kudo · Kouichi Takaguchi · Yoichi Hiasa · Kazuaki Chayama · Hiroshi Yatsuhashi · Makoto Oketani · Hiromitsu Kumada

Received: 23 April 2012 / Accepted: 25 June 2012

Abstract
Background We investigated whether the administration of maintenance doses of interferon prevented hepatocellular carcinoma (HCC) in patients with chronic hepatitis C.
Methods Study 1: A multicenter, retrospective, cooperative study was carried out to determine whether long-term administration of low-dose peginterferon alpha-2a (PegIFNα-2a) prevented HCC development in patients with chronic hepatitis C. In total, 594 chronic hepatitis C patients without a history of HCC were enrolled and treated with 90 μg PegIFNα-2a administered weekly or bi-weekly for at least 1 year. Study 2: HCC developed in 16 of 99 additional patients without PegIFNα-2a treatment during 3.8 years of observation. A propensity-matched control study was then carried out to compare the incidence of

N. Izumi (✉) · Y. Asahina · M. Kurosaki
Department of Gastroenterology and Hepatology,
Musashino Red-Cross Hospital, Musashino, Japan
e-mail: nizumi@musashino.jrc.or.jp

G. Yamada
Department of Internal Medicine, Kawasaki Hospital of Kawasaki Medical University, Okayama, Japan

T. Kawai
Department of Gastroenterology, Kanbara General Hospital, Fuji, Japan

E. Kajiwara
Department of Gastroenterology, Shinnittetsu Yahata Memorial Hospital, Kitakyushu, Japan

Y. Okamura
Department of Gastroenterology, Sano Kousei Hospital, Kitakyushu, Japan

T. Takeuchi
Department of Gastroenterology, Notogawa Hospital, Higashioumi, Japan

O. Yokosuka
Department of Gastroenterology and Hepatology, Chiba University, Chiba, Japan

K. Kariyama
Department of Hepatology, Okayama Citizens' Hospital, Okayama, Japan

J. Toyoda
Department of Gastroenterology and Hepatology, Sapporo Kousei Hospital, Sapporo, Japan

M. Inao
Department of Gastroenterology and Hepatology, Saitama Medical University, Moroyama, Japan

E. Tanaka
Second Department of Internal Medicine, Shinshu University, Matsumoto, Japan

H. Moriwaki
Department of Gastroenterology and Hepatology, Gifu University, Gifu, Japan

H. Adachi
Department of Hepatology, Tonami General Hospital, Tonami, Japan

S. Katsushima
Department of Gastroenterology, Kyoto Medical Center, Kyoto, Japan

M. Kudo
Department of Gastroenterology and Hepatology, Kinki University, Higashiosaka, Japan

K. Takaguchi
Department of Gastroenterology, Kagawa Central Hospital, Takamatsu, Japan

Published online: 09 August 2012

HCC between the 59 patients who received low-dose PegIFNα-2a (PegIFNα-2a group) and 59 patients who did not receive PegIFNα-2a treatment (control group), matched for sex, age, platelet count, and total bilirubin levels.
Results Study 1: HCC developed in 49 patients. The risk of HCC was lower in patients with undetectable hepatitis C virus RNA, ≤40 IU/L alanine aminotransferase (ALT), or ≤10 ng/L alpha-fetoprotein (AFP) 24 weeks after the start of therapy. Study 2: The incidence of HCC was significantly lower in the PegIFNα-2a group than in the control group.
Conclusions Low-dose and long-term maintenance administration of PegIFNα-2a decreased the incidence of HCC in patients with normalized ALT and AFP levels at 24 weeks compared with patients without normal ALT and AFP levels.

Keywords Chronic hepatitis C · Hepatocellular carcinoma · Peginterferon

Introduction

Hepatocellular carcinoma (HCC), the sixth most common cancer worldwide, often develops because of long-term hepatitis B or C virus infection [1, 2]. In particular, chronic hepatitis C and hepatic cirrhosis increase the risk of HCC; the annual incidence of tumor development in such patients may be as high as 2–4 % [3–5]. The incidence of HCC decreases in patients who achieve a sustained virological response (SVR) to interferon (IFN) treatment, although the incidence remains high in non-SVR patients [6–9]. A detailed analysis of HCC development revealed that chronic hepatitis C patients aged 65 years or more, especially those with advanced fibrosis of the liver, were at an increased risk of developing HCC [10]. For patients 65 years or older with advanced liver fibrosis, the dose of ribavirin is often reduced or the agent is discontinued, resulting in lower SVR rates in those with discontinuation of ribavirin. Establishing an effective treatment strategy for preventing the development of HCC is important for these high-risk patients.

Factors related to the development of HCC have been analyzed in patients who did not achieve an SVR even after IFN treatment; advanced fibrosis of the liver and high levels of serum alanine aminotransferase (ALT), and alpha-fetoprotein (AFP) are risk factors for HCC development [11, 12]. A randomized controlled trial was conducted in Western countries to determine whether combined peginterferon and ribavirin treatment with weekly administration of 90 μg peginterferon alpha-2a (PegIFNα-2a) could prevent HCC in non-responders. A 3.5-year follow up showed that administration of a maintenance dose of PegIFNα-2a did not reduce tumor incidence in these patients [13]. However, after 8.5 years of observation, the incidence of HCC was decreased among those in the PegIFNα-2a group with cirrhosis [14]. Meanwhile, Bruix et al. [15] reported that maintenance therapy with PegIFNα-2b did not prevent HCC in chronic hepatitis C patients with cirrhosis. In Japan, long-term low-dose administration of natural IFN has been reported to decrease the incidence of HCC [16]. In light of these conflicting results, investigations should be carried out in a large number of patients with chronic hepatitis C to resolve the question of whether IFN treatment prevents the development of HCC.

We carried out a multicenter retrospective cooperative study of patients with chronic hepatitis C to determine whether those treated with 90 μg PegIFNα-2a without ribavirin had a reduced incidence of HCC compared with those not treated with IFN.

Patients and methods

Study 1: analysis of risk factors for HCC in patients treated with long-term low-dose-PegIFNα-2a

In total, at 21 hepatitis centers throughout Japan, 743 patients with hepatitis C who had received 90 μg of PegIFNα-2a therapy weekly or bi-weekly for 1 year or more without having received the full dose (180 μg) since December 2003 were examined retrospectively for the development of HCC. The end of enrollment in this study was the end of December 2008 and the end of follow up was the end of December 2010. Patients with a history of HCC before the start of therapy and those with a therapy period of less than 48 weeks were excluded, leaving 594 patients who had undergone long-term administration of PegIFNα-2a for analysis. At the 21 centers involved in this

Y. Hiasa
Department of Gastroenterology and Hepatology, Ehime University, Matsuyama, Japan

K. Chayama
Department of Gastroenterology and Hepatology, Hiroshima University, Hiroshima, Japan

H. Yatsuhashi
Department of Gastroenterology and Hepatology, Nagasaki Medical Center, Nagasaki, Japan

M. Oketani
Department of Gastroenterology and Hepatology, Kagoshima University, Kagoshima, Japan

H. Kumada
Department of Hepatology, Toranomon Hospital, Tokyo, Japan

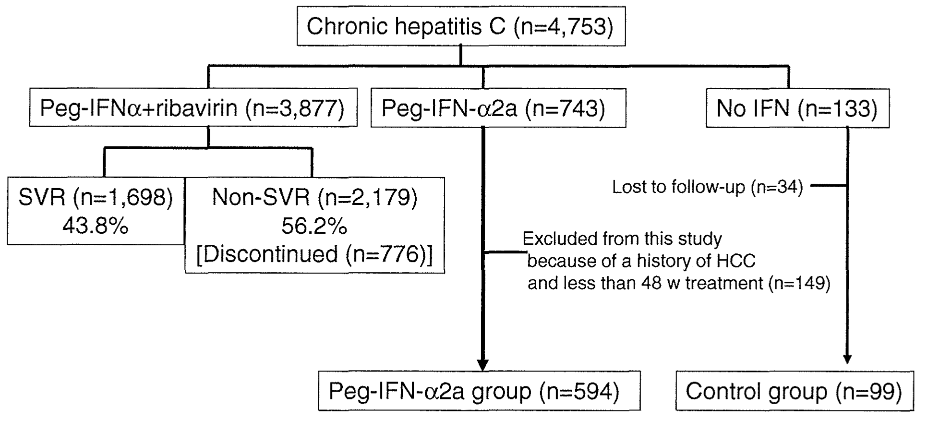

Fig. 1 Flow diagram of the patients' enrollment in the study. *Peg-IFNα* pegylated interferon α, *SVR* sustained viral response, *HCC* hepatocellular carcinoma, *w* week

study, 4,753 patients with chronic hepatitis C had been treated; Peg-IFN and ribavirin combination treatment had been administered to 3,877 patients, 743 patients had received Peg-IFN alone, and 133 patients had not agreed to receive IFN (a flow diagram of the enrollment of patients in this study is shown in Fig. 1). In the patients with Peg-IFN and ribavirin combination treatment, the SVR rate was 43.8 %; SVR was not achieved in 2,179 patients, and in 776 of these patients, the combination therapy was discontinued owing to adverse events or the patient's choice. Patients who failed to achieve an SVR were not included in this study, because the incidence of HCC is known to be reduced even in non-responders to IFN [17].

The backgrounds of the 594 patients studied are shown in Table 1. Findings from the liver biopsies of the patients were classified according to international standards [18]. Long-term PegIFNα-2a treatment is approved by the Japanese Medical Insurance system. Written informed consent was obtained from all patients prior to participation in this study. The study design was approved by the regional ethics committees of the 21 centers involved in this study, including the Musashino Red Cross Hospital, in accordance with the Helsinki Declaration. The 743 patients treated with PegIFNα-2a alone were not indicated for PegIFNα and ribavirin combination therapy because of anemia or heart disease. The 133 patients who did not agree to receive IFN served as the control group (see Fig. 1). A large proportion of the 594 study patients had advanced fibrosis of the liver and active inflammation. A dose of 90 μg PegIFNα-2a was administered to 512 and 82 patients weekly and biweekly, respectively, according to the patients' wishes. There were no significant differences between the weekly and biweekly groups in the patients' background data (data not shown).

The median duration of follow up in the PegIFNα-2a group was 1,273 days (range 228–2,768 days) and HCC was observed in 49 of the 594 patients (Table 1). Pretreatment and on-treatment factors associated with the development of HCC were analyzed by Student's *t*-test, the Mann–Whitney *U*-test, and the χ^2 test (Table 2). Independent factors for the development of HCC were assessed by multivariate analysis using logistic regression. The

Table 1 Background data of patients treated with PegIFNα-2a ($n = 594$)

	$n = 594$
Age (years)	61.7 ± 11.7
Sex (male/female)	258/336
BMI	23.2 ± 3.3
Genotype (1/2)	443/151
Diagnosis (ASC/CH/LC)	4/460/130
History of excess alcohol consumption (≥60 g/day; yes/no)	118/376
Fibrosis (F0, 1, 2/F3, 4)	443/151
Inflammatory activity (A0, 1/A2, 3)	469/125
Diabetes mellitus (no/yes)	499/95
LDL cholesterol (mg/dL)	94.2 ± 31.1
Fasting blood sugar (mg/dL)	106.3 ± 28.5
White blood cell count (/mm^3)	4,360 ± 1,470
Red blood cell count (×10^6/μL)	423.8 ± 56.4
Hemoglobin (g/dL)	13.3 ± 1.8
Platelet count (×10^3/μL)	137 ± 56
Albumin (g/dL)	4.0 ± 0.5
Total bilirubin (mg/dL)	0.8 ± 0.6
AST (IU/L)	65.8 ± 47.8
ALT (IU/L)	72.1 ± 68.0
Gamma-GTP (IU/L)	55.2 ± 51.3
Esophageal varices (no/yes)	344/31
Alpha fetoprotein (ng/L)	6.9 (4.2–13.8)
Once weekly or biweekly PegIFNα-2a	512:82
Baseline HCV RNA (KIU/mL)	1,024 (73–2,130)
Development of HCC (no/yes)	545/49

PegIFN pegylated interferon, *BMI* body mass index, *ASC* asymtotomatic carrier, *CH* chronic hepatitis, *LC* liver cirrhosis, *LDL* low-density lipoprotein, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GTP* guanosine triphosphate, *HCV* hepatitis C virus, *HCC* hepatocellular carcinoma

Values are means ± SD, with ranges in parentheses incidence of HCC was analyzed according to the ALT, AFP, and hepatitis C virus (HCV) RNA levels 24 weeks after the start of PegIFNα-2a administration by using the Kaplan–Meier method. The risk of HCC was analyzed, using the Kaplan–Meier method, only in the non-responders with detectable HCV RNA during PegIFNα-2a administration by dividing them according to the ALT and AFP levels 24 weeks after the start of therapy. The incidence of HCC was compared between the patients with ALT levels of <41 IU/L and those with levels of ≥41 IU/L, and between patients with serum AFP levels of <10 ng/L and those with levels of ≥10 ng/mL at 24 weeks after starting treatment, because at most of the centers participating in the this study, the upper normal range of serum ALT is set at 40 IU/L, and the most significant difference in the incidence of HCC was observed between the PegIFNα-2a and control group with the cut-off serum ALT set at 41 IU/L and cutoff serum AFP set at 10 ng/mL, 24 weeks after starting treatment. The HCV RNA level was measured using the Amplicor Monitor method with a lower detection limit of 50 IU/L (Roche Diagnostics, Tokyo, Japan). A history of excess alcohol consumption was determined as >60 g alcohol per day in order to exclude alcoholic liver disease.

An asymptomatic carrier was defined as a patient with a serum ALT level within the normal range and minimal inflammation or fibrosis in the biopsied tissues of the liver. Chronic hepatitis was defined as mild-to-severe fibrosis of the liver according to liver biopsy [18]. The diagnosis of liver cirrhosis was based on the results of histological examination of the biopsied liver tissues.

Study 2: incidence of HCC in the PegIFNα-2a therapy and non-administration (control) groups in comparison with propensity-matched controls

Ninety-nine of the 133 chronic hepatitis C patients who had not received IFN were examined as controls; patients in this group received liver-protective agents such as glycyrrhizin or were untreated, and the group was observed for more than 1 year. None of the individuals in the control groups had received IFN alone or PegIFNα and ribavirin combination treatment. They were treated for a median of 1,395 days (range 75–6,556 days). Fifty-nine of these patients underwent liver biopsy before the treatment and were considered the control group for the propensity-matched study. For the propensity-matched study, 59 patients were selected from the PegIFNα-2a group according to their age, sex, platelet count, and total bilirubin levels, which had been identified as independent pretreatment risk factors for the development of HCC in Study 1. The rates of HCC were analyzed using the Kaplan–Meier method, and the risk of HCC was analyzed particularly in patients with advanced fibrosis of the liver (F3 and F4).

Table 2 Comparison of HCC and non-HCC patients with long-term PegIFNα-2a administration ($n = 594$)

	Patients with or without development of HCC		*p* value
	With HCC ($n = 49$)	Without HCC ($n = 545$)	
Pretreatment parameters			
Age (years)	63.8 ± 1.7	61.3 ± 0.5	<0.05
Sex (male/female)	32/17	226/319	<0.01
BMI	24.0 ± 0.5	23.1 ± 0.2	n.s.
Genotype (1/2)	47/6	397/148	n.s.
History of excess alcohol consumption (≥60 g/day; yes/no)	11/38	107/338	n.s.
Fibrosis (F0, 1, 2/F3, 4)	25/24	418/127	<0.001
Inflammatory activity (A0, 1/A2, 3)	7/42	462/83	<0.001
Diabetes mellitus (no/yes)	38/11	461/84	n.s.
LDL cholesterol (mg/dL)	88.2 ± 9.0	94.7 ± 2.6	n.s.
White blood cell count (/mm^3)	4,355 ± 210	4,360 ± 64	n.s.
Red blood cell count (×10^6/μL)	420.8 ± 8.1	424.1 ± 2.6	n.s.
Hemoglobin (g/dL)	13.6 ± 0.3	13.3 ± 0.1	n.s.
Platelet count (×10^3/μL)	106 ± 8	140 ± 2	<0.001
Albumin (g/dL)	3.8 ± 0.1	4.0 ± 0.1	<0.001
Total bilirubin (mg/dL)	1.2 ± 0.1	0.8 ± 0.1	<0.001
AST (IU/L)	78.1 ± 6.8	64.6 ± 2.1	n.s.
ALT (IU/L)	72.8 ± 9.7	72.0 ± 2.9	n.s.
Gamma-GTP (IU/L)	68.7 ± 7.5	53.9 ± 2.3	n.s.
Alpha fetoprotein (ng/L)	17.1 (4.4–36.8)	16.7 (4.1–23.1)	n.s.
Esophageal varices	29.0 % (9/31)	6.4 % (22/344)	<0.01
On-treatment parameters			
ALT (IU/L)	59.4 ± 5.7	44.6 ± 1.8	<0.05
Alpha fetoprotein (ng/L)	9.8 (4.6–17.4)	5.5 (3.7–11.1)	<0.01
HCV RNA level (KIU/mL)	236 (<0.5–2,210)	21 (<0.5–1,780)	<0.05

n.s. not significant

Statistical analysis

Categorical data were compared using the χ^2 test or Fisher's exact test. The distributions of continuous variables were analyzed using Student's *t*-test and the Mann–Whitney *U*-test for two groups. Multivariate analysis was conducted using logistic regression. The cumulative incidence curve was determined using the Kaplan–Meier method and differences between groups were assessed by the log-rank test. For all methods, the level of significance was set at $p < 0.05$. Multivariate analysis of the risk of HCC was carried out using the Cox proportional hazard model. Statistical analyses were performed using the Statistical Package for the Social Sciences software version 11.0 (SPSS, Chicago, IL, USA). In Study 1, age, sex, platelet count, and total bilirubin levels were identified as independent factors for the development of HCC; therefore, these factors were selected for the propensity-matched control study (Study 2) in which 59 patients from the PegIFNα-2a group were included.

Results

Study 1

We analyzed the factors involved in the development of HCC in patients who received 90 μg PegIFNα-2a weekly or biweekly for more than a year. The incidence of HCC did not differ significantly between the groups treated with PegIFNα-2a weekly and biweekly (34 of 512 vs. 15 of 82, respectively). As shown in Table 2, univariate analysis revealed statistically significant differences in the pretreatment parameters including age, sex, fibrosis of the liver, platelet count, albumin level, and total bilirubin, between patients who developed HCC and those who did not. Endoscopy was carried out in 375 patients, and esophageal varices were noted in 31 of them. The incidence of HCC was higher in patients with esophageal varices than in those without varices [29.0 % (9 of 31) vs. 6.4 % (22 of 344)]. Assessment of on-treatment factors by univariate analysis revealed statistically significant differences in serum ALT, AFP, and HCV RNA levels 24 weeks after the start of PegIFNα-2a maintenance treatment (Table 2).

Multivariate analysis including pretreatment parameters revealed that age, sex, fibrosis of the liver, platelet count, and total bilirubin were independent risk factors for HCC development (Table 3). Multivariate analysis including on-treatment parameters identified ALT levels of ≥41 IU/L and AFP levels of ≥10 ng/L 24 weeks after the start of the PegIFNα-2a therapy as independent risk factors for HCC development (Table 3).

The incidence of HCC was significantly lower in patients with ALT levels of ≤40 IU/L than in those with ALT levels of ≥41 IU/L 24 weeks after the start of observation (Fig. 2). The incidence of HCC was also significantly lower in patients with AFP concentrations of <10 ng/mL at 24 weeks after the start of observation than in those with AFP concentrations of ≥10 ng/mL (Fig. 3). The dose of PegIFNα-2a was reduced to 45 μg in 16 patients because of neutropenia and thrombocytopenia. In addition, PegIFNα-2a was discontinued in 18 patients because of adverse events, including depression (7 patients), interstitial pneumonitis (3 patients), thrombocytopenia (3 patients), neutropenia (1 patient), itching (1 patient), and ascites (3 patients). No statistically significant differences were found between the patients with reduced dosage or treatment interruption and those without treatment modifications with respect to overall survival, HCC incidence, ascites formation, variceal bleeding, hepatic encephalopathy, and 2-point increases in the Child-Pugh score. No patients underwent liver transplantation.

Table 3 Independent risk factors for HCC development in patients treated with 90 μg PegIFNα-2a weekly or bi-weekly, evaluated by multivariate analysis (logistic regression analysis)

	Multivariate analysis		
	Odds ratio	95 % Confidence interval (CI)	p
Age (years) (every 5 years)	2.24	1.76–9.33	<0.005
Sex (male/female)	3.16	1.56–10.7	<0.005
Fibrosis (F3, 4/F0, 1, 2)	1.69	1.18–5.2	<0.01
Platelet count (<120 × 10^3/μL vs. ≥120 × 10^3/μL)	3.24	1.44–27.6	<0.01
Total bilirubin (mg/dL)	1.59	1.09–2.58	<0.05
ALT (at 24 weeks) (≥41 vs. <40 IU/L)	2.49	1.51–8.28	<0.05
AFP (at 24 weeks) (≥10 vs. <10 ng/L)	3.78	1.92–11.8	<0.01

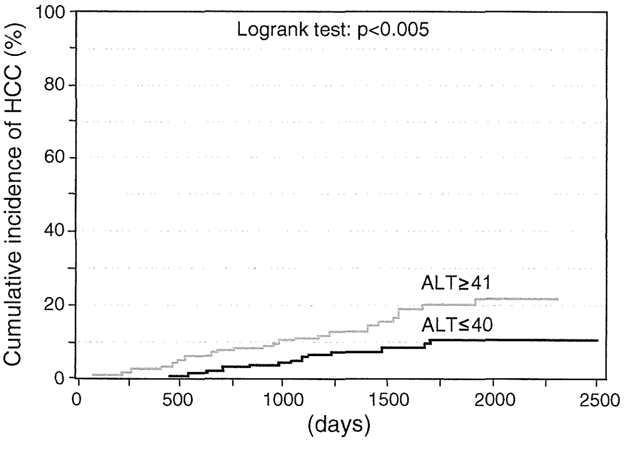

Fig. 2 Comparison of HCC rates in patients administered with PegIFNα-2a ($n = 594$) with respect to alanine aminotransferase (*ALT*) levels 24 weeks after the start of therapy. *Black line* patients with ALT ≥41 IU/L in the first 24 weeks, *gray line* patients with ALT ≤40 IU/L in the first 24 weeks

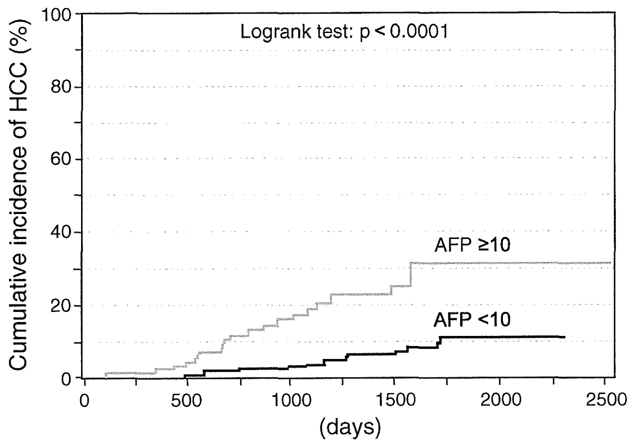

Fig. 3 Comparison of HCC rates in patients administered PegIFNα-2a ($n = 594$) with respect to alpha-fetoprotein (*AFP*) levels in the first 24 weeks after the start of therapy. *Black line* patients with AFP ≥10 ng/mL at 24 weeks, *gray line* patients with AFP <10 ng/mL at 24 weeks

Study 2

We compared the incidence of HCC between 59 patients in the control group and the same number of patients in the PegIFNα-2a group using the matched-pair test. The backgrounds of the patients are shown in Table 4. The PegIFNα-2a group had higher rates of advanced fibrosis (F3 and F4) and active inflammation (A2 and A3). No other differences were found between the two groups, except for the white blood cell count (Table 4).

Development of HCC was observed in 2 patients in the PegIFNα-2a group and 8 in the control group. The incidence of HCC was compared between the two groups, using the Kaplan–Meier method. The incidence of HCC in the PegIFNα-2a group was significantly lower than that in the control group (log-rank test, $p = 0.0187$; Fig. 4). Among the patients with advanced fibrosis of the liver (F3 and F4), those in the PegIFNα-2a group had a lower incidence of HCC than those in the control group. The independent risk factors for the development of HCC were analyzed using the stepwise Cox proportional hazard model. Only PegIFNα-2a administration and age were identified as independent risk factors for the development of HCC (Table 5).

Table 4 Backgrounds of the patients in the propensity-matched control study (PegIFNα-2a group, $n = 59$; control group, $n = 59$)

	PegIFNα-2a group ($n = 59$)	Control group ($n = 59$)	p value
Age (years)	60.5 ± 13.0	63.3 ± 10.5	n.s.
Gender (male/female)	24/35	25/34	n.s.
BMI	22.9 ± 3.6	22.9 ± 3.4	n.s.
Genotype (1/2)	49/10	46/13	n.s.
History of excess alcohol consumption (60 g/day; yes/no)	10/49	4/55	n.s.
Fibrosis (F0, 1, 2/F3, 4)	37/22	43/16	<0.05
Development of HCC (F0–2/F3, 4)	1/1	1/7	n.s.
Inflammatory activity (A0,1/A2, 3)	19/40	30/29	<0.05
Diabetes mellitus (no/yes)	57/2	56/3	n.s.
LDL cholesterol (mg/dL)	95.3 ± 23.8	117.0 ± 4.2	n.s.
White blood cell count (/mm^3)	4,260 ± 1,239	5,193 ± 2,078	<0.05
Red blood cell count ($\times 10^{-4}$/μL)	430 ± 57.8	441 ± 44.9	n.s.
Hemoglobin (g/dL)	13.6 ± 1.5	13.6 ± 1.9	n.s.
Platelet count ($\times 10^{-3}$/μL)	14.5 ± 5.7	15.8 ± 5.7	n.s.
Albumin (g/dL)	4.1 ± 0.5	4.1 ± 0.4	n.s.
Total bilirubin (mg/dL)	0.7 ± 0.5	0.9 ± 0.7	n.s.
AST (IU/L)	58.3 ± 47.7	49.7 ± 26.6	n.s.
ALT (IU/L)	63.6 ± 68.7	58.0 ± 39.2	n.s.
Gamma-GTP (IU/L)	78.3 ± 81.3	55.3 ± 75.1	n.s.
Baseline alpha-fetoprotein (AFP) (ng/L)	7.2 (4.3–14.2)	7.7 (3.9–13.8)	n.s.
Baseline HCV RNA level (KIU/mL)	1,230 (24–3,870)	1,024 (38–3,110)	n.s.

Discussion

The number of HCC cases resulting from HCV infection continues to increase worldwide [19]. To date, IFN therapy is the most effective preventive measure against HCC in patients with chronic hepatitis C; furthermore, the incidence of HCC is reduced in patients who achieve an SVR to IFN [6–9] Therefore, achieving an SVR is the most effective approach for reducing the risk of developing HCC. In Japan, the incidence of HCC is elevated in older patients with hepatitis C. Corroborating this finding, the results of a Japanese study show a higher risk of HCC in patients aged 65 years and more [10]. Therefore, prevention of HCC in aged patients is an important challenge.

In the present multicenter, cooperative, retrospective study conducted in Japan, the incidence of HCC was reduced in patients who received 90 μg PegIFNα-2a weekly or biweekly and had AFP values of <10 ng/mL and ALT values of ≤40 IU/L 24 weeks after the start of the treatment. The results of the matched case–control study of the PegIFNα-2a group and the non-IFN control group show that the incidence of HCC was significantly lower in the PegIFNα-2a group than in the control group, especially in patients with advanced fibrosis of the liver (F3 and F4). However, there could have been a selection bias between

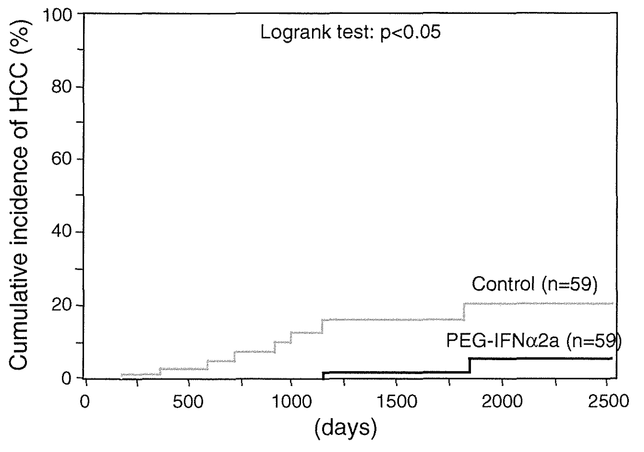

Fig. 4 Comparison of HCC rates between the long-term PegIFNα-2a administration group ($n = 59$) and non-administration group ($n = 59$) in the propensity-matched control study (Kaplan–Meier log-rank test, $p = 0.019$)

Table 5 Risk factors for HCC in the propensity-matched control study (Cox proportional hazard model)

Variables	Risk ratio	95 % CI	p value
PegIFN versus control	0.17	0.03–0.75	<0.05
Age (every 1 year)	1.12	1.02–1.25	<0.05
Fibrosis (F3, 4 vs. F0, 1, 2)	1.70	0.75–4.16	n.s.
Platelet count (every 10 × 10^3/μL)	0.89	0.73–1.09	n.s.
Albumin (every 1.0 g/dL)	0.80	0.10–6.68	n.s.
On-treatment AFP (<10 vs. ≥10 ng/L)	4.07	0.59–40.12	n.s.

the PegIFNα-2a group and the control group (patients who did not agree to receive IFN treatment), because this was a retrospective and non-randomized study. However, concordant with the findings of the HALT-C study [14], the present results show that PegIFNα-2a inhibits the development of HCC in patients with advanced fibrosis of the liver.

Recent studies show that polymorphisms in the host *IL28B* gene are important factors in the response to PegIFNα and ribavirin combination therapy [20, 21]. However, the mechanism of IL28B involvement in the response to PegIFNα and ribavirin has not been elucidated completely. A recent report has shown that IL28B is a significant factor in the development of HCC as well as in the response to IFN therapy [22]. Further studies are warranted to analyze the relationship between IL28B and inhibition of the development of HCC by PegIFNα in chronic hepatitis C.

Risk factors for the development of HCC have been discussed previously. Increased intrahepatic fat is involved in the development of HCC in chronic hepatitis C patients [23, 24]. In addition, diabetes-associated fat disorder [25, 26], hepatic iron overload [27], advanced fibrosis, older age, and fatty deposits in the liver are risk factors for HCC development [4]. Therefore, it is important to establish strategies to mitigate these risk factors to prevent the development of HCC and thus improve the outcomes of hepatitis C patients.

IFN therapy after HCC treatment is reported to inhibit the recurrence of tumors [28, 29], and a meta-analysis has revealed a trend toward inhibition of the recurrence of HCC [30, 31]. The prevention of HCC is an important issue that needs to be addressed to improve the survival of chronic hepatitis C patients. The findings of the present study and the HALT-C trial [14] indicate the effectiveness of long-term administration of maintenance IFN for preventing the development of HCC in chronic hepatitis C patients without an SVR. Improvement in ALT levels is also known to be an important predictor for the prevention of HCC [32]. A low AFP value during IFN administration is also recognized as a significant indicator of a lower risk of HCC [33, 34]. Recently, Osaki et al. [35] reported that a decrease of serum AFP during treatment with IFN was associated with a reduced incidence of HCC. Taking these findings and our own together, we conclude that maintenance administration of low-dose PegIFNα-2a weekly or biweekly to non-SVR patients with chronic hepatitis C decreases the incidence of HCC, especially in patients whose serum ALT and AFP levels are within the normal range 24 weeks after the start of treatment. The preventive effects of IFN against the development of HCC without elimination of the virus may be associated with its anticarcinogenic effects [16, 35]; however, the precise mechanism should be investigated.

The limitations of the present study are that it is retrospective and multicentric; therefore, potentially there may have been a selection bias. However, the reduction of the rate of development of HCC by maintenance administration of PegIFNα-2a in the patients in whom serum ALT and AFP levels were within the normal ranges 24 weeks after the start of treatment may be attributable to the anticarcinogenic effects of IFN without elimination of the virus.

Conclusion

The incidence of HCC was lower in non-SVR patients with chronic hepatitis C who were administered with maintenance low-dose PegIFNα-2a; especially in those whose serum ALT and AFP levels were within the normal ranges 24 weeks after the start of treatment.

Acknowledgments This study was supported by a Grant-in-Aid from the Japanese Ministry of Health, Welfare, and Labor.

Conflict of interest Namiki Izumi received lecture fees from Chugai Co. and MSD Co. in 2011.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005;55:74–108. doi:10.3322/canjclin.55.2.74.
2. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet. 2003;362:1907–17. doi:10.1016/S0140-6736(03)14964-1.
3. Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano K, et al. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. Hepatology. 1990;12:671–5. doi:10.1002/hep.1840120409.
4. Namiki I, Nishiguchi S, Hino K, Suzuki F, Kumada H, Itoh T, et al. Management of hepatitis C; Report of the consensus meeting at the 45th annual meeting of the Japan Society of Hepatology (2009). Hepatol Res. 2010;40:347–68. doi:10.1111/j.1872-034X.2010.00642.x.
5. Tanaka Y, Hanada K, Mizokami M, Yeo AE, Shin JW, Gojobori T, et al. A comparison of the molecular clock of hepatitis C virus in the United States and Japan predicts that hepatocellular carcinoma incidence in the United States will increase over the next two decades. Proc Natl Acad Sci USA. 2002;99:11584–9. doi:10.1073/pnas.242608099.
6. Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, et al. Effect of interferon therapy on hepatocellular carcinoma in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology. 1999;29:1124–30.
7. Imai Y, Kawata S, Tamura S, Yabuuchi I, Noda S, Inada M, et al. Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Ann Intern Med. 1998;129:94–9.
8. Bruno S, Stroffolini T, Colombo M, Bollani S, Benveguu L, Mazzella G, et al. Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. Hepatology. 2007;45:579–87. doi:10.1002/hep.21492.
9. Veldt BJ, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, Zeuzem S, et al. Sustained virological response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. Ann Intern Med. 2007;147:677–84.
10. Asahina Y, Tsuchiya K, Tamaki N, Hirayama I, Tanaka T, Sato M, et al. Effect of aging on risk for hepatocellular carcinoma in chronic hepatitis C virus infection. Hepatology. 2010;52:518–27. doi:10.1002/hep.23691.
11. Amarapurkar D, Han KH, Chan HL, Ueno Y, Asia-Pacific working party on prevention of hepatocellular carcinoma. Application of surveillance programs for hepatocellular carcinoma in the Asia-Pacific Region. J Gastroenterol Hepatol. 2009;24:955–61. doi:10.1111/j.1440-1746.2009.05805.x.
12. Tamura Y, Yamagiwa S, Aoki Y, Kurita S, Suda T, Ohkoshi S, et al. Serum alpha-fetoprotein levels during and after interferon therapy and the development of hepatocellular carcinoma in patients with chronic hepatitis C. Dig Dis Sci. 2009;54:2530–7.
13. Di Bisceglie AM, Shiffman ML, Everson GT, Lindsay KL, Everhart JE, Wright EC, et al. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. N Engl J Med. 2008;359:2429–41. doi:10.1056/NEJMoa0707615.
14. Lok AS, Everhart JE, Wright EC, Di Bischeglie AM, Kim HY, Stering RK, et al. Maintenance peginterferon therapy and other factors associated with hepatocellular carcinoma in patients with advanced hepatitis C. Gastroenterology. 2011;140:840–9. doi:10.1053/j.gastro.2010.11.050.
15. Bruix J, Poynard T, Colombo M, Schiff E, Burak K, Heathcote EJ, et al. Maintenance therapy with peginterferon alfa-2b does not prevent hepatocellular carcinoma in cirrhotic patients with chronic hepatitis C. Gastroenterology. 2011;140:1990–9. doi:10.1053/j.gastro.2010.11.050.
16. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, et al. Prolonged-interferon therapy reduces hepatocarcinogenesis in aged-patients with chronic hepatitis C. J Med Virol. 2007;79:1095–102. doi:10.1002/jmv.20866.
17. Poynard T, Moussali J, Ratziu V, Regimberu C, Opolan P. Effects of interferon therapy in "non-responder" patients with chronic hepatitis C. J Hepatol. 1999;31S:178–83. doi:10.1016/S0168-8278(99)80397-3.
18. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer P. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology. 1994;19:1513–20. doi:10.1016/0270-9139(94)90250-X, doi:10.1002/hep.1840190629.
19. Kanwal F, Hoang T, Kramer JR, Asch SM, Goetz MB, Zeringue A, et al. Increasing prevalence of HCC and cirrhosis in patients with chronic hepatitis C virus infection. Gastroenterology. 2011;140:1182–8. doi:10.1053/j.gastro.2010.12.032.
20. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009;461:399–401. doi:10.1038/nature08309.
21. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nature. 2009;41:1105–9.
22. Fabris C, Falleti E, Cussigh A, Bitetto D, Fontanini E, Bignulin S, et al. IL-28B rs 12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. J Hepatol. 2011;54:716–22. doi:10.1016/j.jhep.2010.07.019.
23. Kurosaki M, Hosokawa T, Matsunaga K, Hirayama I, Tanaka T, Sato M, et al. Hepatic steatosis in chronic hepatitis C is a significant risk factor for developing hepatocellular carcinoma independent of age, sex, obesity, fibrosis stage and response to interferon therapy. Hepatol Res. 2010;40:870–7. doi:10.1111/j.1872-034X.2010.00692.x.
24. Koike K. Steatosis, liver injury, and hepatocarcinogenesis in hepatitis C viral infection. J Gastroenterol. 2009;44(Suppl 19):82–8. doi:10.1007/s00535-008-2276-4.
25. Veldt BJ, Chen W, Heathcote EJ, Wedemeyer H, Reichen J, Hofman WP, et al. Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. Hepatology. 2008;47:1856–62. doi:10.1002/hep.22251.
26. Lai MS, Hsieh MS, Chiu YH, Chen TH. Type 2 diabetes and hepatocellular carcinoma: a cohort study in high prevalence area of hepatitis virus infection. Hepatology. 2006;43:1295–302. doi:10.1002/hep.21208.
27. Furutani T, Hino K, Okuda M, Gondo T, Nishina S, Kitase A, et al. Hepatic iron overload induces hepatocellular carcinoma in transgenic mice expressing the hepatitis C virus polyprotein. Gastroenterology. 2006;130:2087–98. doi:10.1053/j.gastro.2006.02.060.
28. Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Kinoshita H. Randomized clinical trial of long-term outcome after resection of hepatitis C virus-related hepatocellular carcinoma by

postoperative interferon therapy. Br J Surg. 2002;89:418–22. doi:10.1046/j.0007-1323.2001.02054.x.

29. Kudo M, Sakaguchi Y, Chung H, Hatanaka K, Hagiwara S, Ishikawa E, et al. Long-term interferon maintenance therapy improves survival in patients with HCV-related hepatocellular carcinoma after curative radiofrequency ablation. A matched case–control study. Oncology. 2007;72(Suppl 1):132–8. doi:10.1159/000111719.
30. Singal AK, Freeman DH Jr, Anand BS. Meta-analysis: interferon improves outcomes following ablation or resection of hepatocellular carcinoma. Aliment Pharmacol Ther. 2010;32:851–8. doi:10.1111/j.1365-2036.2010.04414.x.
31. Miyake Y, Takaki A, Iwasaki Y, Yamamoto K. Meta-analysis: interferon-alpha prevents the recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. J Viral Hepat. 2010;17:287–92. doi:10.1111/j.1365-2893.2009.01181.x.
32. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, et al. Interferon-induced prolonged biochemical response reduces hepatocarcinogenesis in hepatitis C virus infection. J Med Virol. 2007;79:1485–90. doi:10.1002/jmv.20925.
33. Nomura H, Kashiwagi Y, Hirano R, Tanimoto H, Tsutsumi N, Higashi M, et al. Efficacy of low dose long-term interferon monotherapy in aged patients with chronic hepatitis C genotype 1 and its relation to alpha-fetoprotein: a pilot study. Hepatol Res. 2007;37:490–7. doi:10.1111/j.1872-034X.2007.00073.x.
34. Chen TM, Huang PT, Tsai MH, Lin LF, Liu CC, Ho KS, et al. Predictors of alpha-fetoprotein elevation in patients with chronic hepatitis C, but not hepatocellular carcinoma, and its normalization after pegylated interferon alfa 2a-ribavirin combination therapy. J Gastroenterol Hepatol. 2007;22:669–75. doi:10.1111/j.1440-1746.2007.04898.x.
35. Osaki Y, Ueda Y, Marusawa H, Nakajima J, Kimura T, Kita R, et al. Decrease in alpha-fetoprotein levels predicts reduced incidence of hepatocellular carcinoma in patients with hepatitis C virus infection receiving interferon therapy: a single center study. J Gastroenterol. 2012;47:444–51.

J Gastroenterol (2012) 47:1152–1159
DOI 10.1007/s00535-012-0574-3

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Systemic combination therapy of intravenous continuous 5-fluorouracil and subcutaneous pegylated interferon alfa-2a for advanced hepatocellular carcinoma

Koji Uchino · Shuntaro Obi · Ryosuke Tateishi · Shinpei Sato · Miho Kanda · Takahisa Sato · Toru Arano · Kenichiro Enooku · Eriko Goto · Ryota Masuzaki · Hayato Nakagawa · Yoshinari Asaoka · Yuji Kondo · Noriyo Yamashiki · Tadashi Goto · Shuichiro Shiina · Masao Omata · Haruhiko Yoshida · Kazuhiko Koike

Received: 15 November 2011 / Accepted: 27 February 2012 / Published online: 22 March 2012

Abstract

Background In Japan, sorafenib is now the first-line therapy for individuals with advanced hepatocellular carcinoma (HCC), but no other treatment is available for such patients. The aim of this study was to assess the efficacy and safety of combination therapy with systemic continuous intravenous infusion of 5-fluorouracil (5-FU) and subcutaneous peginterferon alfa-2a, which was used before sorafenib was introduced to Japan.

Methods Two hundred and twenty-three HCC patients, who were not amenable to curative surgery, percutaneous ablation, or transarterial chemoembolization (TACE), and for whom intraarterial chemotherapy was not indicated because of the presence of extrahepatic metastasis or stenosis of the common hepatic artery, received peginterferon alfa-2a (90 μg subcutaneously on days 1, 8, 15, and 22) and 5-FU (500 mg/day intravenously given continuously on days 1–5 and 8–12). We assessed their response to treatment and survival, and treatment safety.

Results The response rate was 9.4 % (including six patients with complete response) and the disease-control rate was 32.7 %. The median time to progression was 2.0 months. The overall median survival time was 6.5 months (Child–Pugh class A: 9.2 months vs. Child–Pugh class B: 2.8 months). In a multivariate analysis, Eastern Cooperative Oncology Group (ECOG) performance status >0, Child–Pugh class B, and the presence of macroscopic vascular invasion were independent predictors of poor prognosis. The major grade 3–4 adverse events were leucopenia (13.9 %) and thrombocytopenia (5.8 %). No treatment-related deaths occurred.

Conclusions This combination therapy was well tolerated and showed promising efficacy. Further studies are needed to establish the usefulness of this treatment.

Keywords Hepatocellular carcinoma · Systemic chemotherapy · Survival analysis · Time to progression

Abbreviations

AIC	Akaike information criterion
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CR	Complete response
CT	Computed tomography
DCP	Des-gamma-carboxy prothrombin
ECOG	Eastern Cooperative Oncology Group
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
MRI	Magnetic resonance imaging
MST	Median survival time
NA	Not assessable
PD	Progressive disease
PR	Partial response

K. Uchino and S. Obi contributed equally to this work.

K. Uchino · R. Tateishi (✉) · T. Arano · K. Enooku · E. Goto · R. Masuzaki · H. Nakagawa · Y. Asaoka · Y. Kondo · N. Yamashiki · T. Goto · S. Shiina · H. Yoshida · K. Koike
Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
e-mail: tateishi-tky@umin.ac.jp

S. Obi · S. Sato · M. Kanda · T. Sato
Department of Gastroenterology and Hepatology, Kyoundo Hospital, Tokyo, Japan

M. Omata
Yamanashi Prefectural Hospital Organization, Yamanashi, Japan

RECIST	Response to treatment in solid tumors
SD	Stable disease
TACE	Transcatheter arterial chemoembolization
TTP	Time to progression
5-FU	5-Fluorouracil

Introduction

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death, with a particularly high incidence in Asian countries, including Japan [1, 2]. HCC usually develops in a liver already suffering from chronic disease, most notably due to hepatitis B virus (HBV) or hepatitis C virus (HCV) infection [3]. In the past, HCC was diagnosed often only at a very advanced stage, which was associated with a very poor prognosis [4]. Close surveillance of designated high-risk patients, using advanced diagnostic modalities, has now facilitated HCC detection at a much earlier stage. Together with the considerable advances in HCC treatment, such as surgical resection, percutaneous ablation, transcatheter arterial chemoembolization (TACE), and liver transplantation, the survival time of HCC patients has been much prolonged in recent years [5–10].

However, the potentially curative treatment modalities described above are not indicated for patients with advanced HCC with extrahepatic metastasis or macroscopic vascular invasion , and their prognosis remains poor. In two recent large randomized controlled trials, sorafenib, a multi-kinase inhibitor, significantly prolonged survival in patients with advanced HCC, even when the primary lesion was associated with vascular invasion or extrahepatic metastases, and this agent is now widely regarded as the standard treatment for such patients [11, 12]. However, even with sorafenib, the median survival time (MST) of such patients is rather short, ranging from 6.5 to 10.7 months. Thus, the development of new drugs or new regimens that include cytotoxic and molecular-targeted agents still remains necessary.

Previously, we reported the efficacy of therapy using a combination of intrahepatic arterial 5-fluorouracil (5-FU) and subcutaneous interferon alfa for patients with advanced HCC with portal venous invasion [13]. Because most intraarterially administered 5-FU is taken up by the liver during the first pass, this combination chemotherapy would not be effective against extrahepatic metastasis. Nevertheless, the mechanism underlying the chemotherapy with intraarterial 5-FU would function if 5-FU could reach extrahepatic lesions via systemic administration. Therefore, we expected that a combination of systemic intravenous 5-FU and subcutaneous interferon would be effective against extrahepatic metastasis of HCC. We report the efficacy and safety of this treatment for advanced HCC, which we performed before sorafenib was introduced to Japan.

Patients, materials, and methods

Patients

The present study was conducted as a retrospective cohort study. We analyzed 223 consecutive patients who received combination therapy comprised of continuous intravenous infusion of 5-FU and subcutaneous pegylated interferon-alfa for advanced HCC at Kyoundo Hospital from January 1, 2004, to May 31, 2009, when sorafenib was licensed in Japan. The study population consisted of patients with advanced HCC who were not amenable to curative surgery, percutaneous ablation, or TACE, and for whom intraarterial chemotherapy was not indicated because of the presence of extrahepatic metastasis or stenosis of the common hepatic artery. Patients with a previous history of treatment, including systemic chemotherapy, were included. The eligibility criteria also included an Eastern Cooperative Oncology Group (ECOG) performance status score of 2 or less [14], Child–Pugh liver function class A or B, adequate hematologic function (white blood cell count, $\geq$3000/μL; hemoglobin, $\geq$8.5 g/dL; platelet count >30000/μL; and prothrombin time international normalized ratio, $\leq$2.3), adequate hepatic function (albumin, $\geq$2.8 g/dL; total bilirubin, $\leq$3 mg/dL; and alanine aminotransferase [ALT] and aspartate aminotransferase [AST], $\leq$5 times the upper limit of the normal range), and adequate renal function (serum creatinine, $\leq$1.5 times the upper limit of the normal range). Patients were required to have at least one measurable target lesion according to the response to treatment in solid tumors (RECIST) guidelines ver. 1.0 [15]. All patients provided written informed consent before treatment. The treatment protocol was approved by the ethics committee of the institution.

Diagnosis of HCC

Intrahepatic lesions, vascular invasion, and extrahepatic metastasis of HCC were diagnosed with contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI), considering hyperattenuation in the arterial phase with washout in the late phase as the definitive sign of HCC [16, 17]. Ultrasound-guided tumor biopsy was also performed when radiological findings were atypical. Bone scintigraphy was added when bone metastasis was suspected because of symptoms but was not confirmed on CT or MRI.

Treatment

One cycle of this treatment consisted of 4 weeks (days 1–28). Peginterferon alfa-2a (90 μg) was administered subcutaneously on days 1, 8, 15, and 22, and 5-FU (500 mg/day) was systemically administered via continuous intravenous infusion, using a portable infusion pump, on days 1–5 and 8–12. Treatment was continued until disease progression, unacceptable toxicity, or patient refusal occurred. This protocol had no treatment interval, and the next cycle started on the day after day 28 of the previous cycle. The first one or two treatment cycles were provided during hospitalization and 5-FU was administered through a peripheral intravenous catheter. Patients who could be expected to survive for a relatively long period underwent implantation of an indwelling central intravenous catheter and were treated on an outpatient basis thereafter. Indwelling central intravenous catheters were inserted by ultrasound-guided subclavian vein puncture and the catheter tip was placed into the superior vena cava using a guidewire under fluoroscopic guidance. When adverse events caused by 5-FU became clinically important, the dose of 5-FU was reduced by 50 %. As prevention and treatment for stomatitis, sodium gualenate hydrate and sodium bicarbonate were used as a gargle. Dexamethasone ointment was also used for stomatitis. Antidiarrheal agents such as loperamide hydrochloride were used for diarrhea.

Response and toxicity assessment

To assess the response to treatment, contrast-enhanced CT or MRI was performed at the end of the first and second cycles and every two cycles thereafter. In principle, treatment responses were evaluated according to the RECIST guidelines ver.1.0 [15]. The best overall response was adopted in the analysis. Complete response (CR) was defined as the disappearance of both intrahepatic lesions and extrahepatic metastasis. CR was confirmed by repeat assessments performed 4 weeks or more after the criteria for response were first met. Patients who had not completed the first cycle were regarded as having progressive disease (PD) if radiological disease progression was confirmed at the time, and as "not assessable (NA)" if imaging was not performed at the time. Toxicity was evaluated using the National Cancer Institute Common Toxicity Criteria version 3.0. During hospitalization, patients were interviewed about their symptoms and underwent a daily physical examination. Blood tests were performed every week. When treated as outpatients, they were required to visit the outpatient department at least once every 2 weeks.

Statistical analysis

We included in the analysis those patients who could not complete the first cycle. The categorical variables were compared by χ^2tests, whereas continuous variables were compared with an unpaired Student's *t*-test (parametric) or Mann–Whitney *U*-test (nonparametric). A P value of <0.05 was considered statistically significant. Overall survival and time to progression (TTP) were calculated using the Kaplan–Meier method. Patients were censored at the time of the last visit, when lost to follow up, or at the end of the study period. Follow up ended on June 30, 2010. The clinical data at baseline were assessed as predictors of survival using univariate and multivariate Cox proportional hazard regression analysis. The following variables were included in this analysis: age, sex, ECOG performance status, hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCVAb), Child–Pugh classification, platelet count, Barcelona-Clinic Liver Cancer (BCLC) staging classification [18], presence of viable intrahepatic lesions, macroscopic vascular invasion, extrahepatic metastasis, and a history of previous treatment. Stepwise variable selection with the Akaike information criterion (AIC) was used to find the best model in multivariate analysis. All analytical procedures were performed with S-plus Ver. 7.0 (Insightful, Seattle, WA, USA).

Results

Patients

A total of 223 patients, 176 male and 47 female, with an average age of 64.3 years, received this treatment. Patient characteristics are listed in Table 1. Child–Pugh classification was A in 166 patients (74.4 %) and B in 57 (25.6 %). Macroscopic vascular invasion was present in 103 patients (46.2 %). Extrahepatic metastasis was present in 166 (74.4 %) patients. Those patients without extrahepatic metastasis who were treated with this regimen had contraindications to intraarterial chemotherapy because of stenosis of the common hepatic artery, mainly due to repeated TACE. Two hundred and ten (94.2 %) patients had previously received some other treatment. The median number of cycles of the combination treatment was two (range 1–13). Four patients did not complete the first cycle because of deterioration of performance status, unacceptable toxicity, or patient refusal.

Response to treatment

Six patients had CR (2.7 %), 15 (6.7 %) had a partial response (PR), 52 (23.3 %) had stable disease (SD), and

Table 1 Demographic and baseline characteristics of patients ($n = 223$)

Variable, *n* (%)	
Age (years)[a]	64.3 ± 10.6
Male sex	176 (78.9)
ECOG performance status	
0	159 (71.3)
1	57 (25.6)
2	7 (3.1)
Viral infection	
HBsAg, positive	58 (26.0)
Anti HCVAb, positive	125 (56.1)
Both positive	4 (1.8)
Both negative	36 (16.1)
Child–Pugh classification	
Class A	166 (74.4)
Class B	57 (25.6)
Platelet count (10^3/μl)[b]	127 (34–840)
BCLC stage	
B	22 (9.9)
C	201 (90.1)
Viable intrahepatic lesion, present	213 (95.5)
Macroscopic vascular invasion, present[c]	103 (46.2)
Portal vein	73
Hepatic vein or vena cava	51
Maximum tumor size (cm)[b]	5.2 (1.0–20.0)
AFP >100 ng/mL	143 (64.1)
AFP-L3 >15.0 %[d]	147 (66.2)
DCP >100 mAU/mL[e]	152 (68.8)
Extrahepatic metastasis, present[c]	166 (74.4)
Lung	91
Lymph node	52
Bone	33
Adrenal gland	11
Dissemination	20
Others	5
Previous therapy[c]	
None	13 (5.8)
Surgical resection	78 (35.0)
Percutaneous ablation	95 (42.6)
Transarterial chemoembolization	150 (67.3)
Radiotherapy	32 (14.3)
Transarterial chemotherapy	65 (29.1)
Systemic chemotherapy	46 (20.6)
Cycles of systemic 5-FU + IFN therapy[b]	2 (1–13)

ECOG Eastern Cooperative Oncology Group, *HBsAg* hepatitis B surface antigen, *HCVAb* hepatitis C virus antibody , *BCLC* Barcelona-Clinic Liver Cancer, *AFP* alpha fetoprotein, *DCP* des-gamma-carboxy prothrombin, *5-FU* 5-fluorouracil, *IFN* interferon

[a] Mean ± SD

[b] Median (range)

[c] Including overlap

[d] Missing in one case

[e] Missing in two cases

Table 2 Summary of efficacy measures ($n = 223$)

Level of response, *n* (%)	
Complete response	6 (2.7)
Partial response	15 (6.7)
Stable disease	52 (23.3)
Progressive disease	132 (59.2)
Not assessable	18 (8.1)
Response rate (%)	9.4
Disease-control rate (%)	32.7
Time to progression (months)	
Median	2.0
95 % confidence interval (CI)	2.0–3.1
Overall survival (months)	
Median	6.5
95 % CI	5.13–9.13
1-year survival rate (%)	31.2
2-year survival rate (%)	12.7
3-year survival rate (%)	7.1

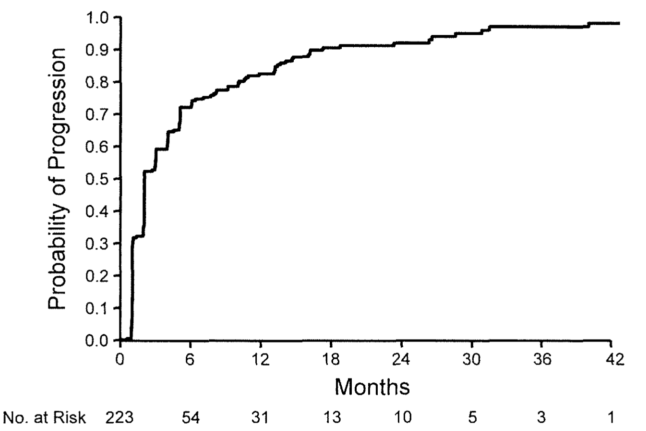

Fig. 1 Kaplan–Meier analysis of time to progression

132 (59.2 %) had PD. Treatment response was not assessable in the remaining 18 (8.1 %) patients due to symptomatic PD or their being lost to follow up before evaluation. The response rate was 9.4 % and the disease-control rate was 32.7 % (Table 2). The median TTP was 2.0 months (Fig. 1). There was no statistically significant difference in TTP between Child–Pugh class A and class B patients (median 3.0 vs. 2.0 months, $P = 0.19$).

Survival

The overall MST was 6.5 months (Fig. 2a). The survival rates at 1, 2, and 3 years were 31.2, 12.7, and 7.1 %, respectively (Table 2). MST was significantly longer in Child–Pugh class A as compared with class B patients (9.2 vs. 2.8 months, $P < 0.001$) (Fig. 2b). The MSTs of patients