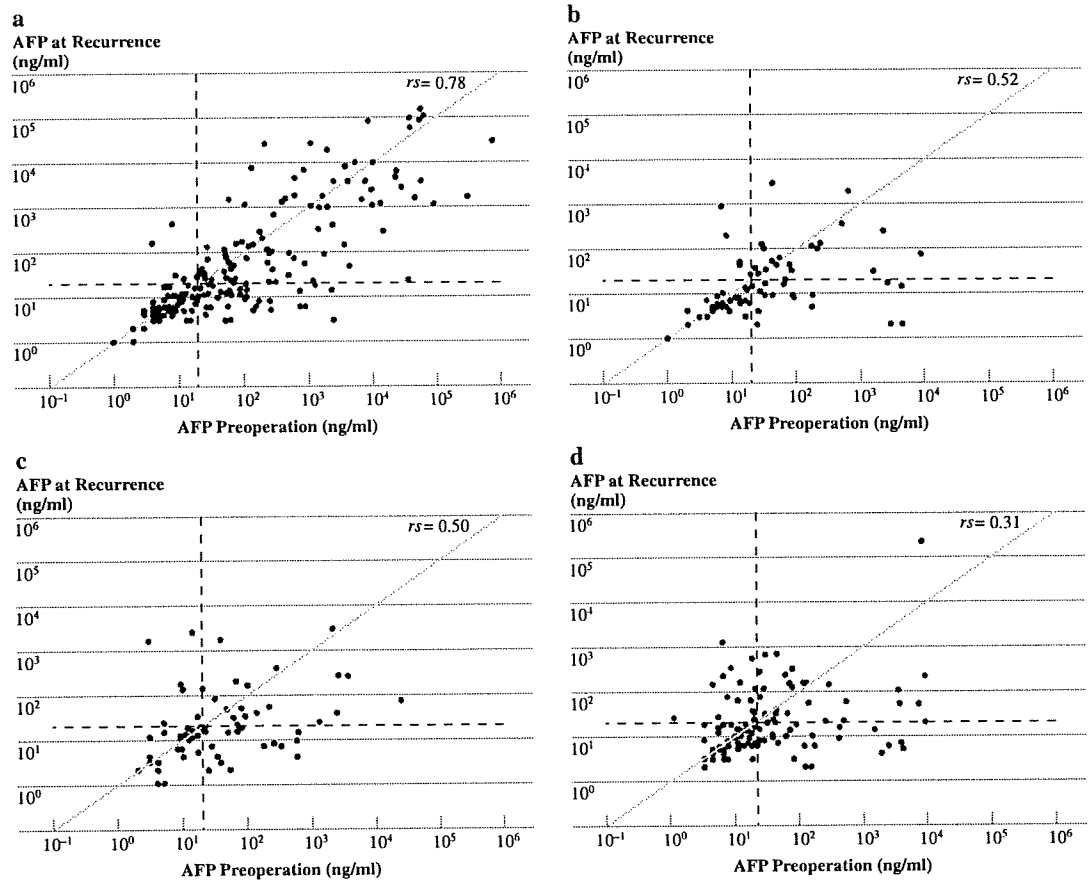

FIG. 3 Correlations between preoperative AFP values and AFP values at recurrence stratified according to period of recurrence: (**a**) recurrence ≤6 months (n = 190), (**b**) recurrence from 7 to 12 months (n = 70), (**c**) recurrence from 13 to 24 months (n = 70), and (**d**) recurrence >2 years (n = 114). The dotted lines represent 20 ng/ml

In addition, DCP is a superior marker for monitoring response to therapy, that is, it was confirmed that positive DCP status converted to negative status in 99.6% (245/246) of patients at 6 months post surgery in the absence of tumor recurrence; in contrast, conversion from AFP-positive to AFP-negative status was achieved in only 80.3% of the patients (184/229). This high false-positive rate of AFP is thought to reflect the observed elevation in the levels of this marker also in conditions such as acute and/or chronic hepatitis and cirrhosis, which is an inherent drawback of AFP as a HCC-specific tumor marker.[3] Whereas high DCP values have been reported in patients with vitamin K deficiency, such as in cases of obstructive jaundice or cases receiving vitamin K antagonists, e.g., warfarin, these uncommon clinical situations can be easily discriminated in HCC patients.[12,33] Rather, it must be noted that patients with chronic alcoholism, another high-risk cohort for HCC, often show nonspecific DCP elevation, reportedly in 5–8% of patients.[34,35] The higher DCP cutoff value adopted by Marrero et al. in their study (125 mAU/mL) may be partially ascribed to the fact that their cohort included a considerable proportion of alcoholic patients (5%).[20]

In the present study, no correlation was found between the levels of AFP and DCP. This observation is consistent with previous reports.[11–17,21] These results strongly suggest that these markers are complementary to each other and that, although DCP might be superior to AFP as a single marker, the two should be evaluated in combination in clinical practice.

Although the association of tumor markers with various clinicopathological variables has been evaluated in many studies, the majority of these works assessed the associations solely with variables of interest and/or exclusively for AFP or DCP. Bearing this in mind, we investigated these associations in a comprehensive manner. While serum DCP values increased with increasing tumor size, no similar association was found for AFP (Table 2). This result is consistent with the results of previous

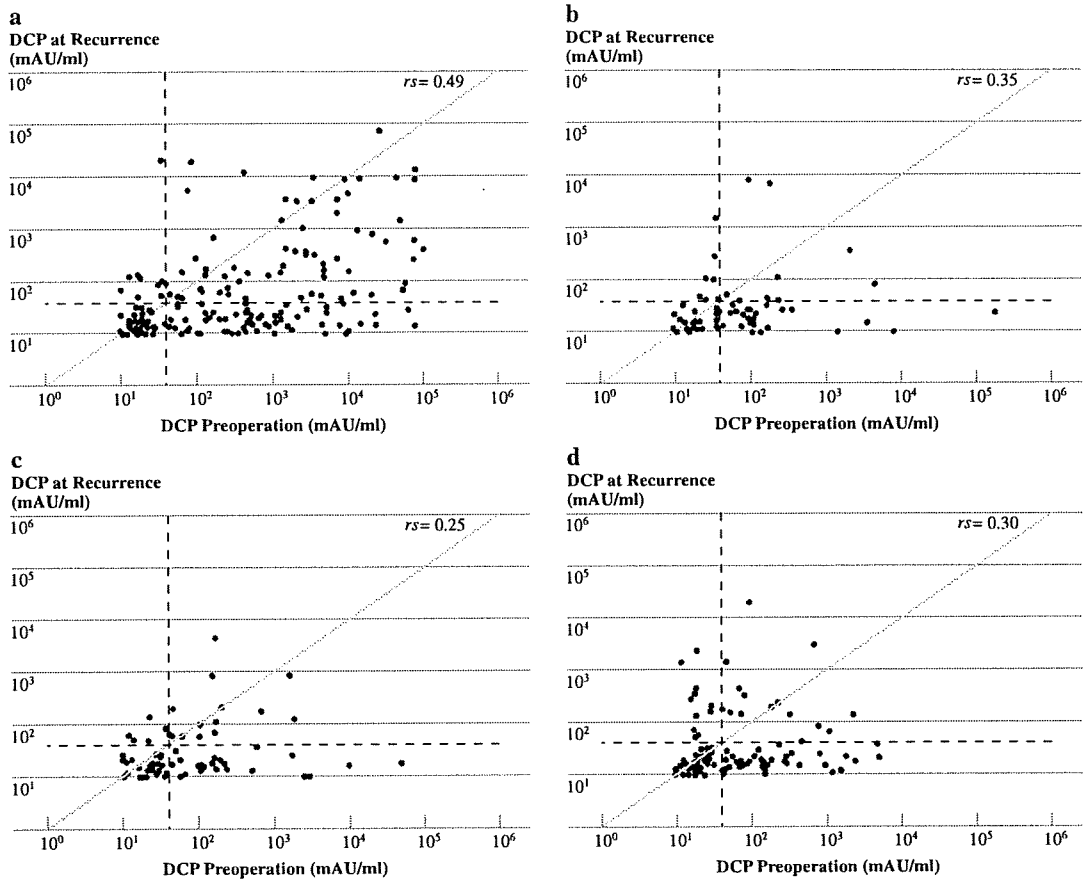

FIG. 4 Correlations between preoperative DCP values and DCP values at recurrence stratified according to period of recurrence: (**a**) recurrence ≤6 months (n = 190), (**b**) recurrence from 7 to 12 months (n = 70), (**c**) recurrence from 13 to 24 months (n = 70), and (**d**) recurrence >2 years (n = 114). The dotted lines represent 40 mAU/ml

studies.[8,13,14,16,17,26,36] These findings suggest that the interindividual variations in the capacity of the tumor cells to synthesize AFP far exceed the elevation in the marker values with increasing tumor cell number.

While serum AFP levels tended to increase with increasing tumor number, this association was not observed for plasma DCP (Table 2). This finding is consistent with those of Kasahara et al. and Carr et al., who found a significant relationship between AFP and tumor number.[13,22] Considering that tumor number is thought to be a variable representing the degree of carcinogenicity in the background liver, the finding of the association for AFP but not for DCP is most probably explained by the elevation of AFP with advancing severity of background liver disease.[3,36,37]

In the present cohort (n = 714), both increased AFP and DCP values were related to presence of indices of tumor invasiveness, such as vascular invasion, and intrahepatic metastases. To date, several studies with 72–161 patients have investigated the association of AFP and/or DCP with these indices, three of which assessed these pathological variables on surgically resected specimens.[14,21,24] A closer and/or specific relationship between these indices and DCP has been reported. Thus, the results of the present and former studies were partially contradictory. In our study, the AFP and DCP values were associated to a similar extent with the tumor cell differentiation grade (Table 2). Again, this observation is partially contradictory to the results of previous studies with 56–354 patients that claimed a specific close association with AFP or DCP.[24,26,27] The results of the present large cohort strongly suggests that both increased levels of AFP and DCP indicate the overall presence of pathological indices representing tumor invasiveness and/or increased malignant potential; however, they do not necessarily signify the presence of any specific entity.

Elevated preoperative AFP and/or DCP levels were correlated with early postoperative recurrence (≤6 months), and recurrence in the early phase was characterized by high serum levels of tumor markers. These results can most reasonably be interpreted as follows: high tumor marker levels signify an increased malignant

potential of the tumor, and the majority of recurrences in the early phase represent recurrence by metastasis, while the later phase of recurrences most often represent secondary de novo tumors whose malignant potential has not yet increased during the process of multistep carcinogenesis. This contention is further supported by the observed association of elevated tumor marker levels with a higher frequency of extrahepatic recurrence.

Two different underlying mechanisms are thought to contribute to postoperative HCC recurrence. In theory, recurrence by metastasis takes place in the early period after surgery, whereas recurrence in the late phase largely represents a new primary lesion.[37,38] Likewise, it can be hypothesized that (1) metastatic recurrence exhibits similar tumor characteristics to the primary lesion, while de novo lesions are independent of the primary tumors in terms of the marker expression profile, and (2) tumor marker levels in recurrent tumors in the early phase show a close relationship with those before hepatectomy, while this relationship becomes obscure in recurrent tumors in the late phase. Chronological alterations in the correlation coefficients (Figs. 3 and 4) support this hypothesis. Moreover, this correlation was stronger for AFP than for DCP across all the study groups. This observation suggests that the increased AFP values both before hepatectomy and at the time of recurrence are at least partially accounted for by the background liver diseases.

A limitation of this investigation is that all of the study patients underwent curative liver resections. They would therefore be supposed to exhibit relatively well-preserved liver function, despite the presence of cirrhosis, from the viewpoint of screening. Likewise, they would be expected to have relatively early stage of HCC as compared with patients undergoing transcatheter arterial embolization, from the standpoint of prediction of response to therapies.

In conclusion, although DCP might be more accurate than AFP for the differentiation of HCC from nonmalignant chronic liver disease, the two markers are complementary to each other. The levels of both markers increased with tumor growth, but no specific association of either with any specific pathological entities was noted. The observed relationship between the preoperative marker values and the values measured at the time of recurrence may serve as a basis for predicting the pattern of recurrence of HCC, i.e., recurrence by metastasis or de novo secondary lesions.

ACKNOWLEDGMENT This work was supported by Grant-in-Aid for Scientific Research (B) (20390352).

REFERENCES

1. Bruix J, Sherman M. Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. *Hepatology.* 2005;42:1208–36.
2. Abelev GI, Perova SD, Khramkova NI, et al. Production of embryonal alpha-globulin by transplantable mouse hepatomas. *Transplantation.* 1963;1:174–80.
3. Collier J, Sherman M. Screening for hepatocellular carcinoma. *Hepatology.* 1998;27:273–8.
4. Oka H, Tamori A, Kuroki T, et al. Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. *Hepatology.* 1994;19:61–6.
5. Bruix J, Sherman M, Llovet JM, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol.* 2001;35:421–30.
6. Liebman HA, Furie BC, Tong MJ, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. *N Eng J Med.* 1984;310:1427–31.
7. Tanabe Y, Ohnishi K, Nomura F, et al. Plasma abnormal prothrombin levels in patients with small hepatocellular carcinoma. *Am J Gastroenterol.* 1988;83:1386–9.
8. Fujiyama S, Izuno K, Gohshi K, et al. Clinical usefulness of des-gamma-carboxy prothrombin assay in early diagnosis of hepatocellular carcinoma. *Dig Dis Sci.* 1991;36:1787–92.
9. Grazi GL, Mazziotti A, Legnani C, et al. The role of tumor markers in the diagnosis of hepatocellular carcinoma, with special reference to the des-gamma-carboxy prothrombin. *Liver Transpl Surg.* 1995;1:249–55.
10. Nomura F, Ishijima M, Kuwa K, et al. Serum des-gamma-carboxy prothrombin levels determined by a new generation of sensitive immunoassays in patients with small-sized hepatocellular carcinoma. *Am J Gastroenterol.* 1999;94:650–4.
11. Tsai SL, Huang GT, Yang PM, et al. Plasma des-gamma-carboxyprothrombin in the early stage of hepatocellular carcinoma. *Hepatology.* 1990;11:481–8.
12. Deyashiki Y, Nishioka Y, Takahashi K, et al. Evaluation of des-gamma-carboxy prothrombin as a marker protein of hepatocellular carcinoma. *Cancer.* 1989;64:2546–51.
13. Kasahara A, Hayashi N, Fusamoto H, et al. Clinical evaluation of plasma des-gamma-carboxy prothrombin as a marker protein of hepatocellular carcinoma in patients with tumors of various sizes. *Dig Dis Sci.* 1993;38:2170–6.
14. Inoue S, Nakao A, Harada A, et al. Clinical significance of abnormal prothrombin (DCP) in relation to postoperative survival and prognosis in patients with hepatocellular carcinoma. *Am J Gastroenterol.* 1994;89:2222–6.
15. Lamerz R, Runge M, Stieber P, et al. Use of serum PIVKA-II (DCP) determination for differentiation between benign and malignant liver diseases. *Anticancer Res.* 1999;19:2489–93.
16. Cui R, He J, Zhang F, et al. Diagnostic value of protein induced by vitamin K absence (PIVKA-II) and hepatoma-specific band of serum gamma-glutamyl transferase (GGTII) as hepatocellular carcinoma markers complementary to alpha-fetoprotein. *Br J Cancer.* 2003;88:1878–82.
17. Wang CS, Lin CL, Lee HC, et al. Usefulness of serum des-gamma-carboxy prothrombin in detection of hepatocellular carcinoma. *World J Gastroenterol.* 2005;11:6115–9.
18. Mita Y, Aoyagi Y, Yanagi M, et al. The usefulness of determining des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. *Cancer.* 1998;82:1643–8.
19. Nakamura S, Nouso K, Sakaguchi K, et al. Sensitivity and specificity of des-gamma-carboxy prothrombin for diagnosis of patients with hepatocellular carcinoma varies according to tumor size. *Am J Gastroenterol.* 2006;101:2038–43.
20. Marrero JA, Su GL, Wei W, et al. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in American patients. *Hepatology.* 2003;37:1114–21.

21. Suehiro T, Sugimachi K, Matsumata T, et al. Protein induced by vitamin K absence or antagonist II as a prognostic marker in hepatocellular carcinoma. Comparison with alpha-fetoprotein. *Cancer.* 1994;73:2464–71.
22. Carr BI, Kanke F, Wise M, et al. Clinical evaluation of lens culinaris agglutinin-reactive alpha-fetoprotein and des-gamma-carboxy prothrombin in histologically proven hepatocellular carcinoma in the United States. *Dig Dis Sci.* 2007;52:776–82.
23. Johnson PJ. The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. *Clin Liver Dis.* 2001;5:145–59.
24. Imamura H, Matsuyama Y, Miyagawa Y, et al. Prognostic significance of anatomical resection and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma. *Br J Surg.* 1999;86:1032–8.
25. Hagiwara S, Kudo M, Kawasaki T, et al. Prognostic factors for portal venous invasion in patients with hepatocellular carcinoma. *J Gastroenterol.* 2006;41:1214–9.
26. Okuda H, Nakanishi T, Takatsu K, et al. Serum levels of des-gamma-carboxy prothrombin measured using the revised enzyme immunoassay kit with increased sensitivity in relation to clinicopathologic features of solitary hepatocellular carcinoma. *Cancer.* 2000;88:544–9.
27. Oishi K, Itamoto T, Amano H, et al. Clinicopathologic features of poorly differentiated hepatocellular carcinoma. *J Surg Oncol.* 2007;95:311–6.
28. Takayama T, Sekine T, Makuuchi M, et al. Adoptive immunotherapy to lower postsurgical recurrence rate of hepatocellular carcinoma: a randomized trial. *Lancet.* 2000;356:802–7.
29. Tateishi R, Yoshida H, Matsuyama Y, et al. Diagnostic accuracy of tumor markers for hepatocellular carcinoma: a systematic review. *Hepatol Int.* 2008;2:17–30.
30. Liver Cancer Study Group in Japan. The general rules for the clinical and pathological study of primary liver cancer, 4th ed. Tokyo: Kanehara;2000.
31. Metz CE. Basic principles of ROC analysis. *Semin Nucl Med.* 1998;8:283–98.
32. Pepe MS, Etzioni R, Feng Z, et al. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst.* 2001;93:1054–61.
33. Nakao A, Suzuki Y, Issiki K, et al. Clinical evaluation of plasma abnormal prothrombin (des-gamma-carboxy prothrombin) in hepatobiliary malignancies and other diseases. *Am J Gastroenterol.* 1991;86:62–6.
34. Lefrere JJ, Gozin D, Lerable J, et al. Des-gamma-carboxyprothrombin assay in alcoholic cirrhotic patients with alpha-fetoprotein level slightly increased. *Hepatology.* 1986;6:1066–7.
35. Ohhira M, Ohtake T, Saito H, et al. Increase of serum des-gamma-carboxy prothrombin in alcoholic liver disease without hepatocellular carcinoma. *Alcohol Clin Exp Res.* 1999;23:67S–70S.
36. Nakagawa T, Seki T, Shiro T, et al. Clinicopathologic significance of protein induced vitamin K absence or antagonist II and alpha-fetoprotein in hepatocellular carcinoma. *Int J Oncol.* 1999;14:281–6.
37. Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, et al. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol.* 2003;38:200–7.
38. Poon RT, Fan ST, Ng IO, et al. Different risk factors and prognosis for early and late intrahepatic recurrence after resection of hepatocellular carcinoma. *Cancer.* 2000;89:500–7.

British Journal of Cancer (2009) 101, 908–915

www.bjcancer.com

A randomised phase III trial comparing gemcitabine with surgery-only in patients with resected pancreatic cancer: Japanese Study Group of Adjuvant Therapy for Pancreatic Cancer

H Ueno*[,1], T Kosuge[2], Y Matsuyama[3], J Yamamoto[4], A Nakao[5], S Egawa[6], R Doi[7], M Monden[8], T Hatori[9], M Tanaka[10], M Shimada[11] and K Kanemitsu[12]

[1]*Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, Tokyo, Japan;* [2]*Hepatobiliary and Pancreatic Surgery Division, National Cancer Center Hospital, Tokyo, Japan;* [3]*Department of Biostatistics, Tokyo University, Tokyo, Japan;* [4]*Department of Gastrointestinal Surgery, Cancer Institute Hospital, Tokyo, Japan;* [5]*Department of Surgery II, Nagoya University, Aichi, Japan;* [6]*Division of Gastroenterology, Tohoku University, Miyagi, Japan;* [7]*Department of Surgery, Kyoto University, Kyoto, Japan;* [8]*Department of Surgery and Clinical Oncology, Osaka University, Osaka, Japan;* [9]*Department of Surgery, Tokyo Women's Medical University, Tokyo, Japan;* [10]*Department of Surgery and Oncology, Kyushu University, Fukuoka, Japan;* [11]*Department of Surgery, Tokushima University, Tokushima, Japan;* [12]*Department of Gastroenterological Surgery, Kumamoto University, Kumamoto, Japan*

BACKGROUND: This multicentre randomised phase III trial was designed to determine whether adjuvant chemotherapy with gemcitabine improves the outcomes of patients with resected pancreatic cancer.

METHODS: Eligibility criteria included macroscopically curative resection of invasive ductal carcinoma of the pancreas and no earlier radiation or chemotherapy. Patients were randomly assigned at a 1 : 1 ratio to either the gemcitabine group or the surgery-only group. Patients assigned to the gemcitabine group received gemcitabine at a dose of 1000 mg m^{-2} over 30 min on days 1, 8 and 15, every 4 weeks for 3 cycles.

RESULTS: Between April 2002 and March 2005, 119 patients were enrolled in this study. Among them, 118 were eligible and analysable (58 in the gemcitabine group and 60 in the surgery-only group). Both groups were well balanced in terms of baseline characteristics. Although heamatological toxicity was frequently observed in the gemcitabine group, most toxicities were transient, and grade 3 or 4 non-heamatological toxicity was rare. Patients in the gemcitabine group showed significantly longer disease-free survival (DFS) than those in the surgery-only group (median DFS, 11.4*versus* 5.0 months; hazard ratio = 0.60 (95% confidence interval (CI): 0.40–0.89); $P = 0.01$), although overall survival did not differ significantly between the gemcitabine and surgery-only groups (median overall survival, 22.3 *versus* 18.4 months; hazard ratio = 0.77 (95% CI: 0.51–1.14); $P = 0.19$).

CONCLUSION: The current results suggest that adjuvant gemcitabine contributes to prolonged DFS in patients undergoing macroscopically curative resection of pancreatic cancer.

British Journal of Cancer (2009) **101,** 908–915. doi:10.1038/sj.bjc.6605256 www.bjcancer.com
Published online 18 August 2009

Keywords: pancreatic cancer; phase III; adjuvant chemotherapy; gemcitabine

Pancreatic cancer remains to be one of the most challenging malignancies to treat. Surgical resection offers the only opportunity for cure. However, as no valid method for early detection of this disease has been established, 80% or more of patients present with unresectable disease at the time of diagnosis. Furthermore, even when resection is performed, the recurrence rate is extremely high, resulting in the 5-year survival rate of patients with resected pancreatic cancer being no more than 20% (Evans *et al*, 1997). As surgical resection alone has limitations, development of non-surgical treatments, including adjuvant therapy, is needed to improve the prognosis of patients with pancreatic cancer.

Several previous studies have suggested the efficacy of adjuvant chemoradiotherapy and/or chemotherapy for the treatment of resected pancreatic cancer (Kalser and Ellenberg, 1985; Neoptolemos *et al*, 2004; Stocken *et al*, 2005; Hazard *et al*, 2007). In the United States, adjuvant chemoradiation with fluorouracil has become the standard of care after the Gastrointestinal Tumour Study Group study showed a statistically significant improvement in survival as compared with surgery-only (median overall survival, 20 *versus* 11 months; 2-year survival rate, 42 *versus* 15%) (Kalser and Ellenberg, 1985). Recently, an evaluation of Medicare patients derived from the SEER database showed a survival advantage for patients who received adjuvant chemoradiotherapy as compared with patients who did not (3-year survival rate, 45 *versus* 30%) (Hazard *et al*, 2007). On the other

*Correspondence: Dr H Ueno, Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan; E-mail: hiueno@ncc.go.jp
A part of the content of this report was presented at the 14th meeting of ECCO.
Revised 08 July 2009; accepted 21 July 2009; published online 18 August 2009

hand, no survival benefit of adjuvant chemoradiation was shown by the European Study Group for Pancreatic Cancer (ESPAC)-1 trial, a large-scale phase III study conducted in Europe (Neoptolemos *et al*, 2004). In the ESPAC-1, chemotherapy using fluorouracil plus leucovorin, but not chemoradiation, showed efficacy in the adjuvant setting: for patients who received postoperative chemotherapy compared with those who did not, the 2-year survival rates (40 *versus* 30%) and the 5-year survival rates (21 *versus* 8%) were significantly greater. Therefore, although the benefit of adjuvant therapy has become more apparent in recent years, the optimal treatment modality remains controversial (Ueno and Kosuge, 2008; Zuckerman and Ryan, 2008).

As for unresectable advanced pancreatic cancer, gemcitabine has been widely employed, since Burris *et al* (1997) reported results of a phase III study. The results of this study suggested that patients receiving gemcitabine experienced improved survival as compared with those receiving fluorouracil (median overall survival, 5.65*versus* 4.42 months; $P=0.0025$). The efficacy and tolerability of gemcitabine for advanced pancreatic cancer have been confirmed by several subsequent studies (Berlin *et al*, 2002; Moore *et al*, 2003; Rocha Lima *et al*, 2004), and gemcitabine has become the standard therapy for unresectable pancreatic cancer. These facts led investigators to evaluate gemcitabine in the adjuvant setting for patients with resected pancreatic cancer.

In 2005, a large phase III study, CONKO-001 (Charité Onkologie), was presented at the American Society of Clinical Oncology (ASCO) Annual Meeting by a German group (Oettle *et al*, 2007). CONKO-001 compared a gemcitabine therapy group with a surgery-only group after macroscopically curative resection of pancreatic cancer. In CONKO-001, disease-free survival (DFS) was significantly longer in the gemcitabine than in the observation group (median DFS, 13.4 *versus* 6.9 months; $P<0.001$). However, overall survival did not differ significantly between the gemcitabine and surgery-only groups, although the survival period tended to be longer in the gemcitabine than in the observation group (median, 22.1 *versus* 20.2 months; $P=0.06$).

Coincidentally, at approximately the same time as the CONKO-001, our multicentre randomised phase III trial, JSAP-02 (Japanese Study Group of Adjuvant Therapy for Pancreatic Cancer), was being conducted to test whether the addition of adjuvant gemcitabine to surgery would improve the outcomes of patients with resected pancreatic cancer. The JSAP-02 study design basically resembled that of CONKO-001, except for the planned number of gemcitabine cycles: six cycles of gemcitabine were used in CONKO-001 and three cycles in our study. To our knowledge, this is the first randomised phase III trial of adjuvant gemcitabine in an Asian population.

PATIENTS AND METHODS

Trial design

JSAP-02 was conducted at 10 centres in Japan. The trial was supported by funding from the Health and Labour Sciences Research Grant for Clinical Cancer Research from the Ministry of Health, Labour and Welfare, Japan.

The primary end point was overall survival. Secondary end points were DFS and gemcitabine safety. The ethics boards of all institutions approved the protocol and all patients provided a written, informed consent. The trial was conducted in accordance with the World Medical Association Declaration of Helsinki and Japanese Good Clinical Practice guidelines. The trial was monitored for excessive toxicity by the Data Monitoring Committee, which functions independently of the JSAP. Data were collected using the web-based clinical trial management system at the data centre (EPS Co., Ltd., Osaka, Japan), and additional changes were locked out of the database on 31 March 2009.

Patient eligibility

Patients who underwent macroscopically curative resection of pancreatic cancer were enrolled in the study 3 to 10 weeks after surgery. The other eligibility criteria were histologically proven invasive ductal carcinoma of the pancreas; no history of earlier chemotherapy or radiotherapy for pancreatic cancer except intraoperative radiotherapy; age 20–74 years; Karnofsky performance status of 50 or more; and adequate organ function (WBC count $\geqslant 4000$ and $\leqslant 12\,000\,mm^{-3}$; neutrophil count $\geqslant 2000\,mm^{-3}$; platelet count $\geqslant 100\,000\,mm^{-3}$; haemoglobin level $\geqslant 9.0\,g$ per 100 ml; serum total bilirubin level $\leqslant 3.0\,mg$ per 100 ml; serum aspartate aminotransferase and serum alanine aminotransferase level $\leqslant 5$ times the upper limit of the normal range; and serum creatinine level lesser than or equal to the upper limit of the normal range). The exclusion criteria were pulmonary fibrosis or interstitial pneumonia; clinically significant pleural effusions; presence of distant metastasis (except distant lymph node metastasis confirmed by resected specimen); other concomitant malignant disease; active infection; history of serious complications related to surgery; active gastrointestinal ulcers; history of myocardial infarction within 3 months; severe mental disorder; pregnant or lactating women; and other serious concomitant systemic disorders incompatible with the trial in the investigator's judgment.

Treatment plan

Patients were enrolled, within 10 weeks after surgery, through fax by the staff at the data centre. Patients were randomly assigned at a 1 : 1 ratio to either the gemcitabine group or the surgery-only group using the minimisation method stratified by resection status (R0 *versus* R1), pathological stage (I–II *versus* III–IV) and enrollment centre. Stage classification and the evaluation of resected specimens were performed in accordance with the fifth edition of the tumour–node–metastasis classification system of the International Union Against Cancer. Patients assigned to the gemcitabine group received gemcitabine at a dose of $1000\,mg\,m^{-2}$ over 30 min on days 1, 8 and 15 every 4 weeks. This 4-week cycle was repeated for 3 cycles. If patients developed leukocyte counts of $<2000\,mm^{-3}$ or $>12\,000\,mm^{-3}$, or platelet counts of $<75\,000\,mm^{-3}$ during chemotherapy, gemcitabine administration was stopped until recovery. When patients had grade 4 leukopenia or neutropenia, febrile neutropenia or infection with grade 3 leukopenia or neutropenia, a platelet count of $<25\,000\,mm^{-3}$, or non-heamatological toxic effects of grade 3 or greater, a dose reduction of gemcitabine from $1000\,mg\,m^{-2}$ to $800\,mg\,m^{-2}$ was allowed. The surgery-only group received no anticancer treatment after surgery, unless there was a confirmed relapse.

Assessments

Baseline assessments included medical history, physical examination, vital signs, chest radiography, ECG, routine laboratory tests, and the tumour markers CEA and CA19-9. Patients in the gemcitabine group underwent laboratory tests and assessment of clinical symptoms every week during the treatment period and every 3 months after completing adjuvant chemotherapy. Patients in the surgery-only group underwent similar examinations every 3 months. Adverse events were assessed according to the Common Toxicity Criteria of the National Cancer Institute (version 2.0). Patients in both groups underwent computed tomography and/or ultrasonography at 3-month intervals after surgery, unless there was a confirmed relapse. Tumour markers, CEA and CA19-9, were also measured every 3 months until relapse.

Statistical analysis

A total of 116 patients were required to detect a hazard ratio of 0.55 with 80% power at a two-sided 0.05 significance level, which

corresponds to a 20% increase in the 2-year overall survival rate in the surgery-only group *versus* the gemcitabine group (15 *versus* 35%, respectively).

All randomised and eligible patients were included in the intent-to-treat (ITT) population for efficacy analyses. Efficacy analyses were also performed in subpopulations stratified by resection status (R0 *versus* R1) and pathological stage (I–II *versus* III–IV). For safety analyses of gemcitabine, only patients who received adjuvant gemcitabine were included. Overall survival was defined as the period between randomisation and death. All deaths, including those from other diseases, were considered to be events. Disease-free survival was defined as the period between randomisation and the occurrence of an event—relapse or death—whichever came first. Data for patients who had not had an event were censored, as of the date of the final observation. The Kaplan–Meier method was used to estimate the overall survival or DFS and the log-rank test was used for comparisons between the two groups. The Wilcoxon test, Fisher's exact test and the Mantel trend test were used to compare differences among pretreatment characteristics between the two groups. *P*-values of less than 0.05 were considered to indicate statistical significance. All statistical analyses were performed using SAS version 9.1 statistical software (SAS Institute Inc, Cary, NC, USA).

RESULTS

Characteristics of patients

Between April 2002 and March 2005, 119 patients in total were enrolled at 10 centres. After randomisation, one patient in the gemcitabine group was found to be ineligible because of a low WBC count at baseline. Therefore, 118 eligible patients (58 in the gemcitabine group and 60 in the surgery-only group) were included in the ITT population for efficacy analyses (Figure 1). No patients assigned to the surgery-only group received post-operative anticancer treatment until a confirmed relapse. The two groups were well balanced with regard to baseline characteristics (Table 1). In total, 16% of the patients had a microscopically positive margin (R1) and 69% had nodal metastases (N1). The median follow-up period for surviving patients was 60.4 months (range, 40.6–77.1 months) on the analysis cut-off date of 31 March 2009.

Treatment administration

Among the 58 patients in the gemcitabine group, one withdrew from the study before treatment because of a postoperative complication. Six patients (10%) discontinued treatment within 1 cycle, 7 (12%) after 2 cycles and 44 (76%) completed the scheduled 3 cycles of treatment. The reasons for withdrawal from treatment included adverse events or complications (10 patients), the detection of recurrent disease (2 patients) and patient preference (2 patients). The dose of gemcitabine was decreased in one patient because of neutropenia. The median number of cycles and the median number of gemcitabine doses administered were 3 and 8, respectively. The median dose intensity of gemcitabine was 667 mg m^{-2} per week, and the median relative dose intensity was 89%.

Safety

Of the 58 eligible patients assigned to the gemcitabine group, adverse events were analysed in 57 patients who received at least one dose of gemcitabine. Major grade 3 or 4 adverse events observed during the treatment are listed in Table 2. Adjuvant gemcitabine was generally well tolerated. Although high frequencies of grade 3 or 4 leukopenia and neutropenia were experienced (25 and 70%, respectively), most myelosuppression resolved promptly without complications.

Three fatal events occurred during the study period: two in the gemcitabine group and one in the surgery-only group. Of the two, an association with gemcitabine could not be ruled out in one patient who developed an abdominal abscess without neutropenia after two treatment cycles and died from gastrointestinal bleed 183 days after the final gemcitabine administration.

DFS and overall survival

At the time of analysis, 44 patients in the gemcitabine group and 53 in the surgery-only group had recurrent disease. The common sites of first recurrence were the liver, peritoneum and local recurrence (Table 3). The recurrence pattern was similar in the two groups. DFS was significantly longer in the gemcitabine group than in the surgery-only group, with an estimated hazard ratio of 0.60 (95% confidence interval (CI), 0.40–0.89; $P=0.01$;

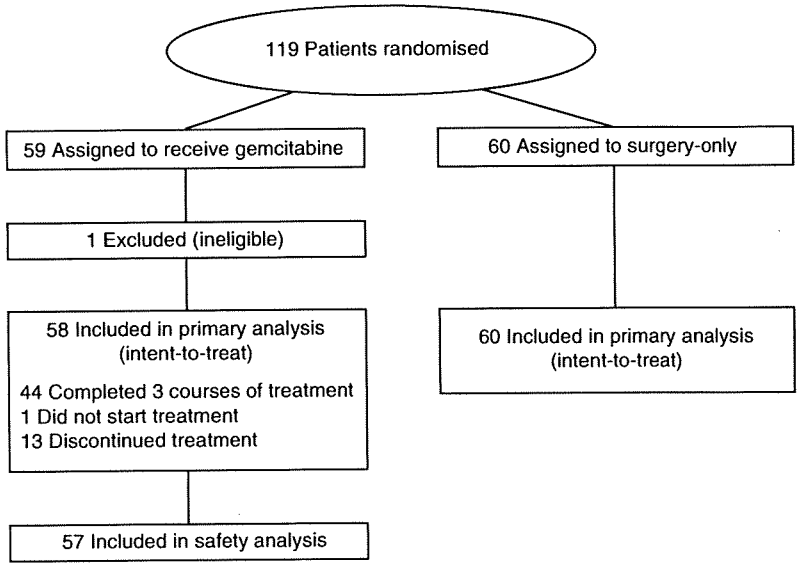

Figure 1 Flow chart of study subjects.

Table 1 Baseline characteristics

Characteristic	Gemcitabine ($n=58$) No.	%	Surgery-only ($n=60$) No.	%	*P*-value
Age (years)					
Median	65		64		0.62
Range	41–74		36–74		
Sex					
Women	18	31	26	43	0.19
Men	40	69	34	57	
Days from surgery to randomisation (days)					
Median	44		47		0.45
Range	22–71		22–70		
Karnofsky performance status					
Median	90		90		0.83
Range	70–100		70–100		
Intra-operative radiotherapy					
Yes	27	47	34	57	0.36
No	31	53	26	43	
Primary site					
Head	42	72	42	70	0.84
Body-tail	16	28	18	30	
Maximal tumour size (cm)					
Median	3.5		3.5		0.27
Range	1.0–10.0		1.2–7.0		
Resection status					
R0	47	81	52	87	0.46
R1	11	19	8	13	
Primary tumour size					
T1	6	10	6	10	0.90
T2	1	2	4	6	
T3	31	53	28	47	
T4	20	35	22	37	
Nodal status					
N0	19	33	18	30	0.84
N1	39	67	42	70	
Pathological stage[a]					
I	3	5	4	7	0.82
II	10	17	10	17	
III	21	36	22	37	
IV	24	41	24	40	
Grading					
1	18	31	16	27	0.80
2	33	57	36	60	
3	5	9	4	7	
Unknown	2	3	4	7	
Histology					
Adenocarcinoma	56	97	56	93	0.68
Other	2	3	4	7	
CEA (ng ml^{-1})					
Median	3.7		4.6		0.34
Range	0.9–252		0.5–74		
CA19–9 (U ml^{-1})					
Median	33.2		37.5		0.40
Range	0–10435		0–46100		

Abbreviations: CEA = carcinoembryonic antigen; CA 19–9 = carbohydrate antigen 19–9. [a]UICC fifth edition.

Table 2 Grade 3 and 4 adverse events in the gemcitabine group ($n=57$)

Adverse event	Gemcitabine Grade 3[a] No.	%	Grade 4[a] No.	%
Haematological				
Leukopenia	13	23	1	2
Neutropenia	32	56	8	14
Anaemia	2	4	0	0
Thrombocytopenia	1	2	0	0
Non-haematological				
Diarrhoea	1	2	0	0
Fever	1	2	0	0
Nausea	0	0	1	2
Anorexia	1	2	1	2
Fatigue	1	2	0	0
AST	3	5	0	0
ALT	4	7	0	0
Abscess	0	0	1	2

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase. [a]NCI Common Toxicity Criteria, version 2.0.

Table 3 Patterns of initial recurrence

	Gemcitabine No.	%	Surgery-only No.	%
Local	10	23	17	32
Liver	13	30	16	30
Peritoneum	8	18	7	13
Other	12	27	12	23
Unknown	1	2	1	2

Figure 2). Median DFS was 11.4 months (95% CI, 8.0–14.5) in the gemcitabine group *versus* 5.0 months (95% CI, 3.7–8.9) in the surgery-only group. The estimated DFS rates at 6, 12 and 24 months were 70.7, 49.0 and 27.2% in the gemcitabine group, and 43.3, 26.7 and 16.7% in the surgery-only group, respectively. Subgroup analyses showed that the beneficial effect of adjuvant gemcitabine on DFS was evident for R0, N0 and stage I–II patients (Table 4, Figure 3).

At the time of analysis, 98 patients (83%) had died (45 patients in the gemcitabine group and 53 in the surgery-only group). The causes of death in the gemcitabine group and surgery-only groups were as follows: relapse (41 and 52 patients, respectively), adverse events (2 and 1 patients, respectively) and unknown causes (2 and 0 patients, respectively). Log-rank analysis revealed no statistically significant difference in survival estimates between the treatment groups (hazard ratio, 0.77 (95% CI, 0.51–1.14); $P=0.19$; Figure 4). Median overall survival was 22.3 months in the gemcitabine group (95% CI, 16.1–30.7) *versus* 18.4 months in the surgery-only group (95% CI, 15.1–25.3). The estimated overall survival rates at 6, 12, 18, 24 and 60 months were 94.8, 77.6, 58.6, 48.3 and 23.9% in the gemcitabine group, and 85.0, 75.0, 53.3, 40.0 and 10.6% in the surgery-only group, respectively. Subgroup analyses failed to show the beneficial effect of adjuvant gemcitabine on overall survival, although the survival period tended to be longer in the gemcitabine than in the observation group for R0, N0 and stage I–II patients (Table 4).

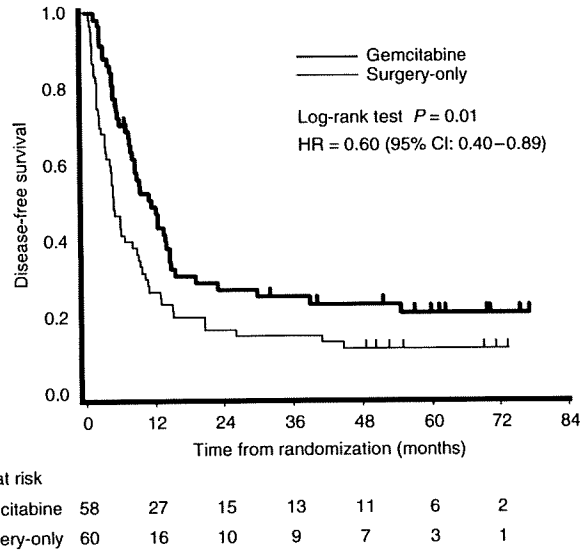

Figure 2 Kaplan–Meier estimates of disease-free survival. Intent-to-treat analysis.

DISCUSSION

We found that DFS in patients with resected pancreatic cancer was significantly improved with three cycles of adjuvant gemcitabine as compared with surgery-only, with an estimated hazard ratio of 0.60 ($P = 0.01$). However, a statistically significant improvement in overall survival was not shown in this study, although median overall survival, and 2-year and 5-year survival rates were favourable in the gemcitabine group as compared with the surgery-only group. These results were similar to those of the previously reported phase III trial of adjuvant gemcitabine, CONKO-001 (Oettle *et al*, 2007).

CONKO-001 compared six cycles of gemcitabine with surgery-only after macroscopically curative resection of pancreatic cancer. Table 5 shows a comparison of our study (JSAP-02) and CONKO-001. The study design of JSAP-02 basically resembled that of CONKO-001 except for the planned sample size, number of gemcitabine cycles, weeks from surgery to randomisation and eligibility criteria determined by postoperative tumour markers. Baseline patient characteristics, including resection status and nodal status, were similar between the two studies. As for efficacies, although both studies failed to show a statistically significant improvement in overall survival, a significantly better DFS was shown with the adjuvant gemcitabine. The data on DFS and overall survival reported in JSAP-02 were comparable with those in CONKO-001.

Table 4 Disease-free and overall survivals in the total entire population and subgroups

	No.		Disease-free survival				Overall survival			
			Median (months)				Median (months)			
	GEM	Surgery-only	GEM	Surgery-only	HR (95% CI)	*P*-value	GEM	Surgery-only	HR (95% CI)	*P*-value
All patients	58	60	11.4	5.0	0.60 (0.40–0.89)	0.01	22.3	18.4	0.77 (0.51–1.14)	0.19
R0	47	52	11.4	5.1	0.58 (0.37–0.91)	0.02	26.8	19.1	0.70 (0.45–1.09)	0.11
R1	11	8	9.5	3.4	0.39 (0.15–1.06)	0.05	18.3	17.6	1.05 (0.41–2.72)	0.92
N0	19	18	—	9.0	0.38 (0.16–0.86)	0.02	32.0	28.4	0.63 (0.29–1.37)	0.24
N1	39	42	8.6	4.5	0.73 (0.46–1.16)	0.19	17.1	17.3	0.84 (0.53–1.34)	0.84
Stage I–II	13	14	—	10.0	0.27 (0.08–0.85)	0.02	67.8	—	0.42 (0.15–1.22)	0.10
Stage III–IV	45	46	8.9	4.6	0.68 (0.44–1.05)	0.08	18.3	16.3	0.82 (0.53–1.26)	0.36

Abbreviations: CI = confidence interval; GEM = gemcitabine; HR = hazard ratio.

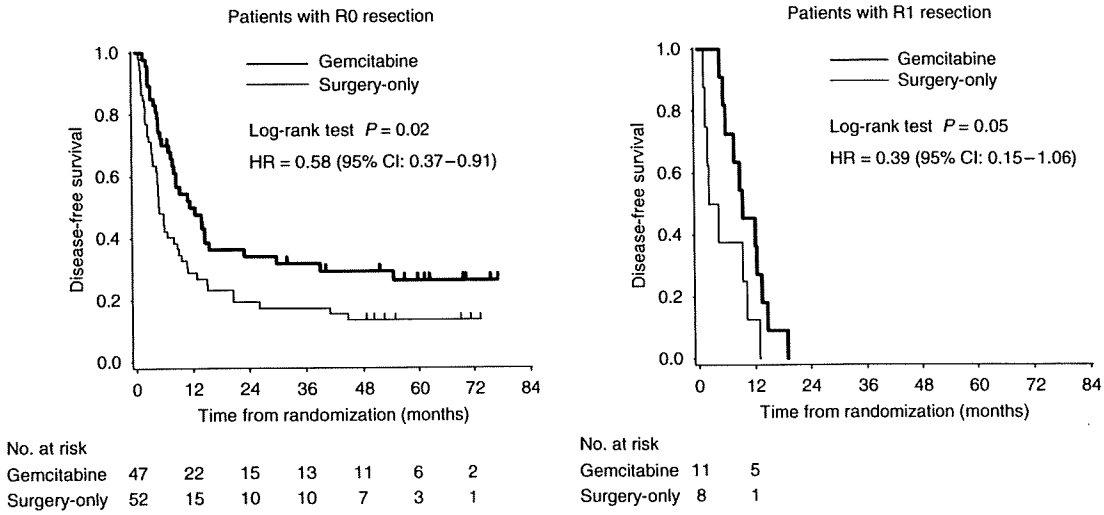

Figure 3 Kaplan–Meier estimates of disease-free survival in patients with R0 or R1 resection. Intent-to-treat analysis.

The CONKO-001 data were re-analysed in March 2008, and presented at the ASCO Annual Meeting of that year as the final results (Neuhaus *et al*, 2008). Although the improvement in overall survival did not reach statistical significance ($P=0.06$) in the previous report (Oettle *et al*, 2007), the new CONKO-001 report showed a significant difference in overall survival between the gemcitabine and surgery-only groups after long-term observation (median overall survival, 22.3 months *versus* 20.2 months; 5-year survival rate, 21.0% *versus* 9.0%; $P=0.005$). In contrast to these final results of CONKO-001, our study, though the data were analysed after an adequate observation period, failed to show a survival benefit of adjuvant gemcitabine. As the JSAP-02 survival curve itself resembled that of CONKO-001, the main reason for this discrepancy may be the underpowered nature of our study. The planned sample size for the JSAP-02, which was less than one-third that of CONKO-001, might have been too small to detect a significant difference in overall survival. Other factors that differed between the two studies, including race, number of gemcitabine cycles and patient selection based on postoperative tumour markers, may also have influenced the outcome of our study. Further study is needed to clarify the impacts of these factors on adjuvant gemcitabine. Although intra-operative radiotherapy was allowed only in our study and 52% of patients actually received this treatment, its influence may be very small because a recent phase III trial failed to show any benefits of intra-operative radiotherapy in patients with resected pancreatic cancer (Kinoshita *et al*, 2009).

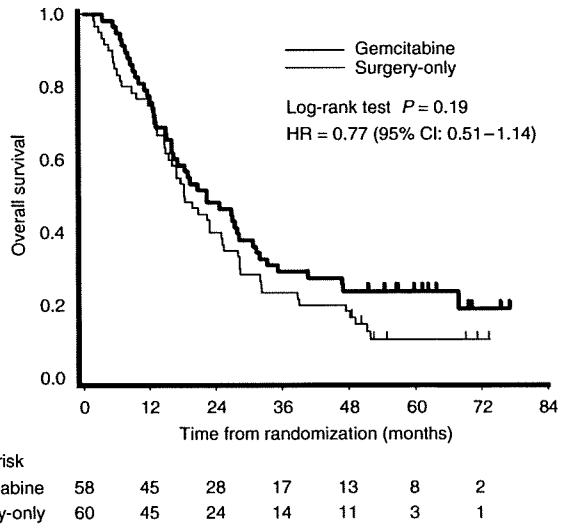

Figure 4 Kaplan–Meier estimates of overall survival. Intent-to-treat analysis.

Adjuvant gemcitabine was well tolerated in our study. A total of 44 patients (76%) completed the three scheduled treatment cycles, and the median relative dose intensity of gemcitabine was as good as 89%. Although 70% of patients experienced grade 3 or 4 neutropenia during adjuvant gemcitabine therapy, most of these toxicities were transient, and serious adverse events were rare. The frequencies of grade 3 or 4 neutropenia induced by gemcitabine monotherapy are reportedly 20–30% (Aapro *et al*, 1998). The reasons for marked heamatological toxicities occurring in our study are unclear, although surgical stress might have exacerbated myelosuppression. Onoue *et al* (2004) reported that administering gemcitabine to patients after surgical resection resulted in more severe leukopenia, as compared with patients not undergoing resection (grade 3 or 4 leukopenia, 57 *versus* 25%; $P=0.048$). Although our study, similar to CONKO-001, showed the safety of adjuvant gemcitabine, cautious selection of patients and careful observation of treatment will be necessary when giving this agent to patients with resected pancreatic cancer.

Other than gemcitabine, fluorouracil-based chemotherapy is now considered to be an option for adjuvant therapy for resected pancreatic cancer based on the results of ESPAC-1. The ESPAC-1

Table 5 Comparison between the current Japanese study and CONKO-001

	Current study (JSAP-02)		CONKO-001[a]	
	Gemcitabine	**Surgery-only**	**Gemcitabine**	**Surgery-only**
Study design				
Planned sample size	116		368	
Planned number of gemcitabine cycles	3	—	6	—
Planned weeks from surgery to randomisation	⩽10		⩽6	
Selection based on postoperative tumour markers	No requirement		⩽2.5 times the upper limit of normal	
Baseline patient characteristics				
No. of patients	58	60	179	175
Median age (years)	65	64	62	61
Sex: men	69%	57%	59%	56%
Median Karnofsky PS	90	90	80	80
Resection status: R0	81%	87%	81%	85%
Nodal status: N0	33%	30%	29%	27%
Median days from surgery to randomisation	44	47	22	24
Results				
Median DFS (months)	11.4	5.0	13.4	6.9
1-year DFS rate	49%	27%	58%	31%
2-year DFS rate	27%	17%	31%	15%
P-value	0.01		<0.01	
Median OS (months)	22.3	18.4	22.1	20.2
1-year OS rate	78%	75%	73%	73%
2-year OS rate	48%	40%	48%	42%
5-year OS rate	24%	11%	23%	12%
P-value	0.19		0.06	

Abbreviations: DRF = disease-free survival; OS = overall survival; PS = performance status. [a]Previously reported in Oettle *et al* (2007).

study showed a survival benefit of adjuvant fluorouracil plus leucovorin in 289 patients with resected pancreatic cancer (Neoptolemos *et al*, 2004). The ESPAC group also performed a pooled analysis using data from 458 patients who were enrolled in ESPAC-1, ESPAC-1 plus or early ESPAC-3(v1) (Neoptolemos *et al*, 2009a). The overall survival was superior in patients randomised to fluorouracil plus leucovorin, as compared with those randomised to observation (pooled hazard ratio, 0.70; $P=0.003$, median overall survival, 23.2 *versus* 16.8 months), indicating the validity of using fluorouracil plus leucovorin as adjuvant therapy.

With regard to the comparison between gemcitabine and fluorouracil-based chemotherapy in the adjuvant setting, two large phase III trials, RTOG 97-04 and ESPAC-3(v2), were recently reported (Regine *et al*, 2008; Neoptolemos *et al*, 2009b). RTOG 97-04 examined whether survival could be extended by substituting gemcitabine for fluorouracil before and after fluorouracil-based radiation. When the data from the entire population were analysed, no significant difference in the survival period was noted between the fluorouracil and gemcitabine groups, but the gemcitabine group had better outcomes when the analysis was confined to patients with pancreatic head cancer (median overall survival, 20.5 *versus* 16.9 months, $P=0.033$). ESPAC-3(v2) was designed to compare fluorouracil plus leucovorin and gemcitabine in patients with resected pancreatic cancer. In total, 1088 patients were randomised in ESPAC-3(v2), and Neoptolemos *et al* reported no significant difference in survival between adjuvant fluorouracil plus leucovorin and adjuvant gemcitabine at the 2009 ASCO Annual Meeting (hazard ratio, 0.94; $P=0.39$, median overall survival 23.0 *versus* 23.6 months). Although no significant difference in survival was shown, gemcitabine may be suitable for clinical use as adjuvant therapy because the rate of serious adverse events in patients treated with gemcitabine was significantly lower than that in patients treated with fluorouracil plus leucovorin (7.5 *versus* 14%, $P<0.001$).

In recent years, new approaches, including novel cytotoxic or molecular-targeting agents, have been actively applied in the adjuvant setting for pancreatic cancer. In Japan, S-1, an oral fluoropyrimidine derivative, has attracted the attention of investigators on the basis the promising results of clinical trials for advanced pancreatic cancer (Ueno *et al*, 2007; Okusaka *et al*, 2008). We are now conducting a phase I/II trial of gemcitabine plus S-1 for resected pancreatic cancer (JSAP-03 trial). As well as developing new effective treatments, individualised approaches based on individual differences in drug metabolism are also important in selecting patients who are more likely to benefit from adjuvant gemcitabine. Several recent studies have suggested that tumour-specific expression of human equilibrative nucleoside transporter 1 may be a promising predictive biomarker of outcome in pancreatic cancer patients receiving gemcitabine chemotherapy (Spratlin *et al*, 2004; Giovannetti *et al*, 2006; Farrell *et al*, 2009; Maréchal *et al*, 2009). Further investigation of and progress in these new strategies are expected in the future.

In conclusion, adjuvant chemotherapy with gemcitabine significantly improved DFS, as compared with surgery-only in patients with resected pancreatic cancer. Our study supports the conclusions of the CONKO-001 as well as the validity of using gemcitabine as adjuvant therapy for resected pancreatic cancer.

ACKNOWLEDGEMENTS

We are grateful to Drs M Sasako, Y Matsumura and T Sato, who served as Independent Data Monitoring Committee. We thank Drs A Saiura, H Ishii and S Takeda for their support. We also thank Ms K Kondo, Ms R Mukouyama and Ms T Okada for their assistance in data management. This work was supported by funding from the Health and Labour Sciences Research Grant for Clinical Cancer Research from the Ministry of Health, Labour and Welfare, Japan.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Aapro MS, Martin C, Hatty S (1998) Gemcitabine—a safety review. *Anticancer Drugs* **9:** 191–201

Berlin JD, Catalano P, Thomas JP, Kugler JW, Haller DG, Benson 3rd AB (2002) Phase III study of gemcitabine in combination with fluorouracil *versus* gemcitabine alone in patients with advanced pancreatic carcinoma: Eastern Cooperative Oncology Group Trial E2297. *J Clin Oncol* **20:** 3270–3275

Burris 3rd HA, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD (1997) Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* **15:** 2403–2413

Evans DB, Abbruzzese JL, Willett CG (1997) Cancer of the pancreas. In *Cancer: Principles and Practice of Oncology (ed 6)*, De Vita VT Jr, Hellman S, Rosenberg SA (eds), Pp 1126–1162. Lippincott Williams and Wilkins: Philadelphia

Farrell JJ, Elsaleh H, Garcia M, Lai R, Ammar A, Regine WF, Abrams R, Benson AB, Macdonald J, Cass CE, Dicker AF, Mackey JR (2009) Human Equilibrative Nucleoside Transporter 1 Levels Predict Response to Gemcitabine in Patients With Pancreatic Cancer. *Gastroenterology* **136:** 187–195

Giovannetti E, Del Tacca M, Mey V, Funel N, Nannizzi S, Ricci S, Orlandini C, Boggi U, Campani D, Del Chiaro M, Iannopollo M, Bevilacqua G, Mosca F, Danesi R (2006) Transcription analysis of human equilibrative nucleoside transporter-1 predicts survival in pancreas cancer patients treated with gemcitabine. *Cancer Res* **66:** 3928–3935

Hazard L, Tward JD, Szabo A, Shrieve DC (2007) Radiation therapy is associated with improved survival in patients with pancreatic adenocarcinoma: results of a study from the Surveillance, Epidemiology, and End Results (SEER) registry data. *Cancer* **110:** 2191–2201

Kalser MH, Ellenberg SS (1985) Pancreatic cancer adjuvant combined radiation and chemotherapy following curative resection. *Arch Surg* **120:** 899–903

Kinoshita T, Uesaka K, Shimizu Y, Sakamoto H, Kimura W, Sunada S, Imaizumi T, Ozawa I, Okamoto A, Oda T (2009) Effects of adjuvant intraoperative radiation therapy after curative resection in pancreatic cancer patients: results of a randomized study by 11 institutions in Japan. *J Clin Oncol* **27:** 15s (suppl; abstr 4622)

Maréchal R, Mackey JR, Lai R, Demetter P, Peeters M, Polus M, Cass CE, Young J, Salmon I, Devière J, Van Laethem JL (2009) Human equilibrative nucleoside transporter 1 and human concentrative nucleoside transporter 3 predict survival after adjuvant gemcitabine therapy in resected pancreatic adenocarcinoma. *Clin Cancer Res* **15:** 2913–2919

Moore MJ, Hamm J, Dancey J, Eisenberg PD, Dagenais M, Fields A, Hagan K, Greenberg B, Colwell B, Zee B, Tu D, Ottaway J, Humphrey R, Seymour L (2003) Comparison of gemcitabine *versus* the matrix metalloproteinase inhibitor BAY 12-9566 in patients with advanced or metastatic adenocarcinoma of the pancreas: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* **21:** 3296–3302

Neoptolemos JP, Stocken DD, Friess H, Bassi C, Dunn JA, Hickey H, Beger H, Fernandez-Cruz L, Dervenis C, Lacaine F, Falconi M, Pederzoli P, Pap A, Spooner D, Kerr DJ, Buchler MW (2004) A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. *N Engl J Med* **350:** 1200–1210

Neoptolemos JP, Stocken DD, Tudur Smith C, Bassi C, Ghaneh P, Owen E, Moore M, Padbury R, Doi R, Smith D, Büchler MW (2009a) Adjuvant 5-fluorouracil and folinic acid vs observation for pancreatic cancer:

composite data from the ESPAC-1 and -3(v1) trials. *Br J Cancer* **100:** 246–250

Neoptolemos JP, Büchler M, Stocken DD, Ghaneh P, Smith D, Bassi C, Moore M, Cunningham D, Dervenis C, Goldstein D (2009b) ESPAC-3(v2): A multicenter, international, open-label, randomized, controlled phase III trial of adjuvant 5-fluorouracil/folinic acid (5-FU/FA) *versus* gemcitabine (GEM) in patients with resected pancreatic ductal adenocarcinoma. *J Clin Oncol* **27:** 18s (suppl; abstr LBA4505)

Neuhaus P, Riess H, Post S, Gellert K, Ridwelski K, Schramm H, Zuelke C, Fahlke J, Langrehr J, Oettle H (2008) CONKO-001: final results of the randomized, prospective, multicenter phase III trial of adjuvant chemotherapy with gemcitabine *versus* observation in patients with resected pancreatic cancer (PC). *J Clin Oncol* **26:** 15s (suppl; abstr LBA4504)

Oettle H, Post S, Neuhaus P, Gellert K, Langrehr J, Ridwelski K, Schramm H, Fahlke J, Zuelke C, Burkart C, Gutberlet K, Kettner E, Schmalenberg H, Weigang-Koehler K, Bechstein WO, Niedergethmann M, Schmidt-Wolf I, Roll L, Doerken B, Riess H (2007) Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. *JAMA* **297:** 267–277

Okusaka T, Funakoshi A, Furuse J, Boku N, Yamao K, Ohkawa S, Saito H (2008) A late phase II study of S-1 for metastatic pancreatic cancer. *Cancer Chemother Pharmacol* **61:** 615–621

Onoue M, Terada T, Okuda M, Fujimoto K, Doi R, Imamura M, Inui K (2004) Surgical resection deteriorates gemcitabine-induced leukopenia in pancreatic cancer. *Int J Clin Oncol* **9:** 174–178

Regine WF, Winter KA, Abrams RA, Safran H, Hoffman JP, Konski A, Benson AB, Macdonald JS, Kudrimoti MR, Fromm ML, Haddock MG, Schaefer P, Willett CG, Rich TA (2008) Fluorouracil vs gemcitabine chemotherapy before and after fluorouracil-based chemoradiation following resection of pancreatic adenocarcinoma: a randomized controlled trial. *JAMA* **299:** 1019–1026

Rocha Lima CM, Green MR, Rotche R, Miller Jr WH, Jeffrey GM, Cisar LA, Morganti A, Orlando N, Gruia G, Miller LL (2004) Irinotecan plus gemcitabine results in no survival advantage compared with gemcitabine monotherapy in patients with locally advanced or metastatic pancreatic cancer despite increased tumor response rate. *J Clin Oncol* **22:** 3776–3783

Spratlin J, Sangha R, Glubrecht D, Dabbagh L, Young JD, Dumontet C, Cass C, Lai R, Mackey JR (2004) The absence of human equilibrative nucleoside transporter 1 is associated with reduced survival in patients with gemcitabine-treated pancreas adenocarcinoma. *Clin Cancer Res* **10:** 6956–6961

Stocken DD, Buchler MW, Dervenis C, Bassi C, Jeekel H, Klinkenbijl JH, Bakkevold KE, Takada T, Amano H, Neoptolemos JP (2005) Meta-analysis of randomised adjuvant therapy trials for pancreatic cancer. *Br J Cancer* **92:** 1372–1381

Ueno H, Okusaka T, Furuse J, Yamao K, Funakoshi A, Boku N, Ohkawa S, Makimoto A, Sato T (2007) A multicenter phase II study of gemcitabine and S-1 combination therapy (GS therapy) in patients with metastatic pancreatic cancer. *J Clin Oncol* **25:** 18s (suppl; abstr 4550)

Ueno H, Kosuge T (2008) Adjuvant treatments for resectable pancreatic cancer. *J Hepatobiliary Pancreat Surg* **15:** 468–472

Zuckerman DS, Ryan DP (2008) Adjuvant therapy for pancreatic cancer: a review. *Cancer* **112:** 243–249

British Journal of Cancer (2009) 100, 870–873

www.bjcancer.com

Full Paper

Homozygous *CDA**3 is a major cause of life-threatening toxicities in gemcitabine-treated Japanese cancer patients

H Ueno*[,1], N Kaniwa[2,3], T Okusaka[1], M Ikeda[1], C Morizane[1], S Kondo[1], E Sugiyama[2,3], SR Kim[2], R Hasegawa[3], Y Saito[2,4], T Yoshida[5], N Saijo[6] and J Sawada[2,4]

[1]*Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan;* [2]*Project Team for Pharmacogenetics, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan;* [3]*Division of Medicinal Safety Sciences, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan;* [4]*Division of Functional Biochemistry and Genomics, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan;* [5]*Genetics Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan;* [6]*National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa-shi, Chiba 277-0882, Japan*

Among 242 Japanese pancreatic cancer patients, three patients (1.2%) encountered life-threatening toxicities, including myelosuppression, after gemcitabine-based chemotherapies. Two of them carried homozygous *CDA**3 (*CDA*208G>A [Ala70Thr]), and showed extremely low plasma cytidine deaminase activity and gemcitabine clearance. Our results suggest that homozygous *3 is a major factor causing gemcitabine-mediated severe adverse reactions among the Japanese population.
British Journal of Cancer (2009) **100,** 870–873. doi:10.1038/sj.bjc.6604971 www.bjcancer.com

Keywords: gemcitabine; toxicity; *CDA*208G>A; pancreatic cancer; pharmacogenomics; polymorphism

Gemcitabine (2′,2′-difluorodeoxycytidine) is a nucleoside anticancer drug for various solid tumours (Noble and Goa, 1997). Gemcitabine exerts its cytotoxic effect through phosphorylation by nucleotide kinases, including the deoxycytidine kinase (DCK), whereas most of the administered gemcitabine is rapidly degraded by cytidine deaminase (CDA) into its inactive metabolite, 2′,2′-difluorodeoxyuridine (Plunkett *et al*, 1995). Various genetic variations have recently been reported in human *DCK* and *CDA* genes (Ueno *et al*, 2007).

Our earlier prospective pharmacogenetic study using 256 Japanese cancer patients treated with gemcitabine-based chemotherapies revealed that one of the *CDA* single-nucleotide polymorphisms (SNPs), *CDA**3 (*CDA*208G>A [Ala70Thr], rs60369023), showed significant associations with reduced CDA activity, reduced gemcitabine clearance, increased gemcitabine area under the concentration–time curve (AUC), and an increased incidence of severe neutropaenia (Sugiyama *et al*, 2007). Most notably, one patient who had developed life-threatening toxicities, including severe myelosuppression, was found to be homozygous for *CDA**3 (*CDA**3/*3), and excessive exposure to gemcitabine was considered responsible for the severe toxicities (Yonemori *et al*, 2005; Sugiyama *et al*, 2007).

Owing to a low allele frequency of *CDA**3 (3.7% in the Japanese population), only one homozygous patient was found in the earlier study, necessitating further examination. For this purpose, we have carefully monitored toxicities in gemcitabine-treated patients in the National Cancer Center Hospital for 4.5 years. Three patients with life-threatening adverse reactions, including serious myelosuppression, were identified, and their *CDA* genotypes, plasma CDA activities, and pharmacokinetic parameters (when available) were determined and compared with those of the earlier cases.

*Correspondence: Dr H Ueno; E-mail: hiueno@ncc.go.jp
Received 28 November 2008; revised 11 February 2009; accepted 13 February 2009

PATIENTS AND METHODS

The ethics committees of the National Cancer Center and the National Institute of Health Sciences approved this study. Written informed consent was obtained from each participant. Of 176 and 66 pancreatic cancer patients who received gemcitabine monotherapy and gemcitabine-based combination chemotherapies, respectively, at the National Cancer Center Hospital between 1 September 2003 and 31 March 2008, three showed severe and prolonged myelosuppression with other complications. Characteristics of the three patients designated A, B, and C are summarised in Table 1. All these patients received a 30 min intravenous gemcitabine infusion at an initiation dose of 1000 mg m^{-2}. Patient A initially received gemcitabine and S-1 combination therapy, whereas patients B and C were given gemcitabine alone.

Measurement of plasma CDA activity towards gemcitabine and genotyping of *CDA* and *DCK* were carried out in the three patients as reported earlier (Sugiyama *et al*, 2007; Kim *et al*, 2008). After recovery from severe adverse reactions, chemotherapy was resumed in two patients: patient A received gemcitabine monotherapy instead of gemcitabine plus S-1, whereas the gemcitabine dose was reduced in patient C. Gemcitabine was not resumed in patient B because of disease progression. Pharmacokinetics were carried out when 1000 and 450 mg m^{-2} of gemcitabine were administered to patients A and C, respectively. Blood sampling schedule and the measurement method of gemcitabine in plasma were reported earlier (Sugiyama *et al*, 2007). Pharmacokinetic parameters were estimated using WinNonlin ver 4.01 (Pharsight Corporation, Mountain View, CA, USA).

RESULTS

Although 30 patients (about 12.4%) among the 242 developed grade 4 neutropaenia, the toxicity was transient and required no supportive treatment in most patients except in patients A, B, and C. Pretreatment organ functions, including bone marrow, renal, and hepatic functions, were preserved in the three patients (Table 1). Observed toxicities in the patients are summarised in Table 2. The serious haematotoxicities requiring intensive supportive treatments during hospitalisation were recognised in these patients: patient A was treated with antibiotics because of febrile neutropaenia, patient B received a platelet transfusion, and patient C received a red blood cell transfusion, a platelet transfusion, and a granulocyte colony-stimulating factor. Both the neutrophil and platelet nadir appeared at approximately day 15 of the first course of treatment in patients A and B, whereas in patient C, the nadir occurred on day 15 of the second course of treatment that was resumed after reducing the dose of gemcitabine. The symptomatic non-haematologic toxicities shown in Table 2 appeared before severe myelosuppression.

Patients B and C were found to be *CDA*3/*3*, whereas patient A did not have *CDA*3* (Table 2). No SNPs of *DCK*, including *DCK*364C>T (Pro122Ser), which were reported to have reduced enzymatic activity (Lamba *et al*, 2007), were identified in our three patients. Plasma CDA activities of patients A, B, and C were compared with those of 121 patients in our earlier study (Sugiyama *et al*, 2007) (Figure 1A). Patient A without *CDA*3* showed relatively high plasma CDA activity, whereas plasma CDA activities in the *CDA*3/*3* patients (patients B and C) were comparably low to those in the earlier reported *CDA*3/*3* patient.

Pharmacokinetic parameters of patients A and C were also shown in Table 2, and their gemcitabine clearances were compared with those of the earlier reported 250 patients (Sugiyama *et al*, 2007) (Figure 1B). Although the gemcitabine dose for patient C was low (450 mg m^{-2}), her gemcitabine AUC was higher than the average value in the *CDA*3*-negative patients who were administered 1000 mg m^{-2} gemcitabine. When it was assumed that patient

Table 1 Patient characteristics at baseline

	Patient A	Patient B	Patient C
Sex	Female	Male	Female
Age (years)	57	70	70
Performance status	1	2	1
Stage[a]	IV	IV	IV
Previous treatment	Surgery	None	None
Body surface area (m^2)	1.3	1.9	1.1
Laboratory data			
Leukocyte (mm^{-3})	6300	9700	4300
Neutrophil (mm^{-3})	4200	6400	2700
Haemoglobin (g dl^{-1})	12.4	16.0	10.8
Platelet (mm^{-3})	116 000	163 000	185 000
Total bilirubin (mg dl^{-1})	0.6	1.4	0.8
ALT (IU l^{-1})	26	35	24
Creatinine (mg dl^{-1})	0.6	1.2	0.4
Initial regimen	Gemcitabine+S-1[b]	Gemcitabine alone	Gemcitabine alone

ALT = alanine aminotransferase. [a]UICC sixth edition. [b]An oral product containing tegafur, gimeracil, and oteracil potassium.

Table 2 Toxicities, treatment, genotype, and PK analysis

	Patient A		Patient B		Patient C	
	Grade	Value	Grade	Value	Grade	Value
Haematologic toxicities[a]						
Leukocyte (mm^{-3})	4	800	3	1100	3	1000
Neutrophil (mm^{-3})	4	200	4	300	4	100
Haemoglobin (g dl^{-1})	2	8.1	1	13.2	4	6.3
Platelet (mm^{-3})	3	26 000	4	10 000	3	28 000
Non-haematologic toxicity[a]						
Fatigue	2		3		2	
Anorexia	3		3		2	
Diarrhoea	3		0		1	
Stomatitis	3		0		0	
Rash	2		0		2	
Febrile neutropaenia	3		0		0	
Treatment						
Resumption of chemotherapy	Yes		No		Yes	
Total number of gemcitabine doses	10		2		10	
Final dose (mg m^{-2})	600		1000		270	
Genotype						
CDA haplotype[b]	*1a/*1j		*3a/*3a		*3a/*3a	
DCK haplotype[c]	*1a/*1a		*1a/*1a		*1a/*1a	
PK analysis						
Regimen at PK study	Gemcitabine alone				Gemcitabine alone	
Dose of gemcitabine (mg m^{-2})	1000				450	
C_{max} (mg l^{-1})	29.0		Not available		22.5 (49.7[d])	
AUC (mg h l^{-1})	16.2				26.8 (59.0[d])	
Clearance (l h^{-1} m^{-2})	61.8				16.6	

AUC = area under the concentration–time curve; C_{max} = maximum plasma concentration; CDA = cytidine deaminase; DCK = deoxycytidine kinase; PK = pharmacokinetics. [a]Toxicities were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. [b]*CDA* haplotype was reported earlier in Sugiyama *et al* (2007). [c]*DCA* haplotype was reported earlier in Kim *et al* (2008). [d]On the basis of the assumption that patient C received 1000 mg m^{-2} of gemcitabine.

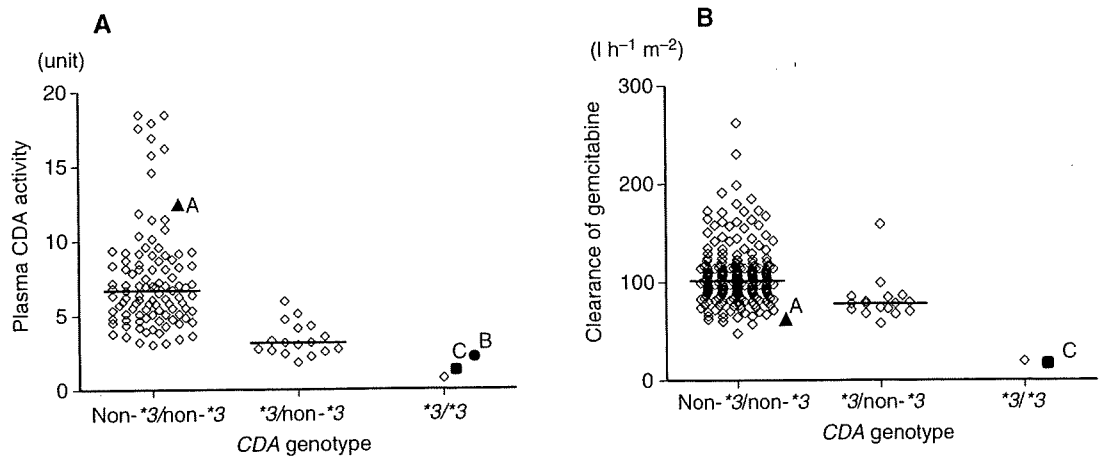

Figure 1 Effects of *CDA*3* on (**A**) plasma CDA activity towards gemcitabine and (**B**) gemcitabine clearance. The data obtained earlier (Sugiyama *et al*, 2007) are expressed as open diamonds, and those obtained in this study are as closed symbols. A, B, and C represent patients A, B, and C, respectively. Lines represent median values for non-*3/non-*3 patients and *3/non-*3 patients.

C received 1000 mg m^{-2} of gemcitabine, C_{max} and AUC values similar to those observed in the earlier reported *CDA*3/*3* patient were obtained. The value of gemcitabine clearance in patient C (*CDA*3/*3*) was less than one-fifth of the median value obtained earlier from *CDA*3*-negative patients. The clearance observed in patient A, who did not have *CDA*3*, was within the range obtained earlier from patients without *CDA*3* (Figures 1B).

DISCUSSION

We found two (patients B and C) of the three patients who experienced life-threatening adverse reactions to be homozygous for *CDA*3* and to have an extremely low CDA activity. Taken together with our earlier observations (Yonemori *et al*, 2005; Sugiyama *et al*, 2007), these life-threatening adverse reactions appear to have been caused by reduced deamination activity of CDA because of *CDA*3/*3* homozygosity. Sustained plasma gemcitabine elevations are most likely responsible for these severe adverse reactions. To date, we have had three *CDA*3/*3* patients, including one reported earlier (Yonemori *et al*, 2005; Sugiyama *et al*, 2007), and all experienced life-threatening severe adverse reactions. As genotyping of *CDA* was not carried out in the remaining 239 patients, we may have overlooked patients with *CDA*3/*3* who did not develop severe toxicities in this study. However, as the frequency of homozygous *CDA*3* in the Japanese population was estimated to be 0.14% in an earlier study (Sugiyama *et al*, 2007), the possibility of missing any such patients would be very low. Thus, *CDA*3/*3* is a potentially important biomarker for Japanese patients and for at least one African ethnic group (Fukunaga *et al*, 2004) for predicting severe gemcitabine-mediated adverse reactions including myelotoxicities.

We do not have sufficient pharmacokinetic data on *CDA*3/*3* to determine the optimal dose for this fraction of the patient population. However, the clearance and AUC values for the earlier reported patient (Sugiyama *et al*, 2007) and for patient C (dose adjusted), and the final dose for patient C (270 mg m^{-2}) suggest that a 75% reduction in the gemcitabine dose, at treatment initiation, may be appropriate for *CDA*3/*3* patients.

Patient A was given combined chemotherapy with oral S-1 (Table 1). As she could not tolerate gemcitabine monotherapy at the standard dose as shown in Table 2, gemcitabine itself appears to have been responsible for the life-threatening toxicities in this patient. Her gemcitabine clearance was within the range observed in patients without *CDA*3* (Sugiyama *et al*, 2007). Therefore, we concluded that there was no involvement of altered CDA activity in the severe neutropaenia experienced by patient A. Further investigation of gemcitabine pathway genotypes is needed to clarify factors contributing to this patient's adverse reactions.

In conclusion, in the Japanese population, *CDA*3/*3* is a major cause of gemcitabine-mediated life-threatening adverse reactions including myelosuppression. A substantial gemcitabine dose reduction is necessary for patients who are homozygous for *CDA*3*.

ACKNOWLEDGEMENTS

We thank Ms Emi Toshiro and Ms Tomoko Chujo for their assistance in sample collection and management. This study was supported in part by the Program for the Promotion of Fundamental Studies in Health Sciences from the National Institute of Biomedical Innovation (NiBio), and the Health and Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare.

Conflict of interest

Dr Saijo reported receiving honoraria from Eli Lilly. None of the other authors reported financial interest.

REFERENCES

Fukunaga AK, Marsh S, Murry DJ, Hurley TD, McLeod HL (2004) Identification and analysis of single-nucleotide polymorphisms in the gemcitabine pharmacologic pathway. *Pharmacogenomics J* 4: 307–314

Kim SR, Saito Y, Maekawa K, Sugiyama E, Kaniwa N, Ueno H, Okusaka T, Ikeda M, Morizane C, Yamamoto N, Yoshida T, Kamatani N, Furuse J, Ishii H, Saijo N, Ozawa S, Sawada J (2008) Twenty novel genetic variations and haplotype structures of the DCK gene encoding human deoxycytidine kinase (dCK). *Drug Metab Pharmacokinet* 23: 379–384

Lamba JK, Crews K, Pounds S, Schuetz EG, Gresham J, Gandhi V, Plunkett W, Rubnitz J, Ribeiro R (2007) Pharmacogenetics of deoxycytidine kinase: identification and characterization of novel genetic variants. *J Pharmacol Exp Ther* 323: 935–945

Noble S, Goa KL (1997) Gemcitabine. A review of its pharmacology and clinical potential in non-small cell lung cancer and pancreatic cancer. *Drugs* **54:** 447–472

Plunkett W, Huang P, Gandhi V (1995) Preclinical characteristics of gemcitabine. *Anticancer Drugs* 6(Suppl 6): 7–13

Sugiyama E, Kaniwa N, Kim SR, Kikura-Hanajiri R, Hasegawa R, Maekawa K, Saito Y, Ozawa S, Sawada J, Kamatani N, Furuse J, Ishii H, Yoshida T, Ueno H, Okusaka T, Saijo N (2007) Pharmacokinetics of gemcitabine in Japanese cancer patients: the impact of a cytidine deaminase polymorphism. *J Clin Oncol* **25:** 32–42

Ueno H, Kiyosawa K, Kaniwa N (2007) Pharmacogenomics of gemcitabine: can genetic studies lead to tailor-made therapy? *Br J Cancer* **97:** 145–151

Yonemori K, Ueno H, Okusaka T, Yamamoto N, Ikeda M, Saijo N, Yoshida T, Ishii H, Furuse J, Sugiyama E, Kim SR, Kikura-Hanajiri R, Hasegawa R, Saito Y, Ozawa S, Kaniwa N, Sawada J (2005) Severe drug toxicity associated with a single-nucleotide polymorphism of the cytidine deaminase gene in a Japanese cancer patient treated with gemcitabine plus cisplatin. *Clin Cancer Res* **11:** 2620–2624

British Journal of Cancer (2009) 100, 874–880

www.bjcancer.com

Expression of carbonic anhydrase IX suggests poor outcome in rectal cancer

E Korkeila*[,1], K Talvinen[2], PM Jaakkola[1,3,4], H Minn[1,5], K Syrjänen[1], J Sundström[2,6] and S Pyrhönen[1]

[1]Department of Oncology and Radiotherapy, Turku University Hospital, Savitehtaankatu 1, PB 52, Turku FIN-20521, Finland; [2]Department of Pathology, University of Turku, Kiinamyllynkatu 10, Turku FIN-20520, Finland; [3]Turku Center for Biotechnology, University of Turku, Tykistökatu 6, Turku FIN-20521, Finland; [4]Åbo Akademi University, Tuomiokirkontori 3, Turku FIN-20500, Finland; [5]Turku PET Centre, PO Box 52, Turku FIN-20521, Finland; [6]Department of Pathology, Turku University Hospital, Kiinamyllynkatu 10, Turku FIN-20520, Finland

The aim of the study is to assess the value of carbonic anhydrase isozyme IX (CA IX) expression as a predictor of disease-free survival (DFS) and disease-specific survival (DSS) in rectal cancer treated by preoperative radio- or chemoradiotherapy or surgery only. Archival tumour samples from 166 patients were analysed for CA IX expression by three different evaluations: positive/negative, proportion of positivity and staining intensity. The results of immunohistochemical analysis were confirmed by demonstrating CA IX protein in western blotting analysis. Forty-four percent of the operative samples were CA IX positive, of these 34% had weak and 66% moderate/strong staining intensity. In univariate survival analysis, intensity of CA IX expression was a predictor of DFS ($P = 0.003$) and DSS ($P = 0.034$), both being markedly longer in tumours with negative or weakly positive staining. In multivariate Cox model, number of metastatic lymph nodes and CA IX intensity were the only independent predictors of DFS. Carbonic anhydrase isozyme IX intensity was the only independent predictor of DSS, with HR = 9.2 for dying of disease with moderate-intense CA IX expression as compared with CA IX-negative/weak cases. Negative/weak CA IX staining intensity is an independent predictor of longer DFS and DSS in rectal cancer.

British Journal of Cancer (2009) **100,** 874–880. doi:10.1038/sj.bjc.6604949 www.bjcancer.com
Published online 24 February 2009

Keywords: rectal cancer; CA IX; prognosis; predictive factor; radiotherapy; chemotherapy

Colorectal cancer (CRC) is a common malignancy in Western countries and the incidence is rising: there were nearly 372 000 new cases of CRC in Europe in 2002 (Ferlay *et al*, 2004). The most important prognostic factors of rectal cancer are the type of surgery, depth of invasion and nodal status. Other prognostic factors have been widely tested (Bendardaf *et al*, 2006, 2007) but as yet have not achieved an established role in the management of CRC.

Angiogenesis and tumour hypoxia have been widely studied during the past decades to develop better treatment modalities and prognostic factors. Angiogenesis favours tumour growth and metastasis, whereas hypoxia renders a tumour resistant to radiation and often to chemotherapy as well (Brizel *et al*, 1996). Hypoxic regions are common in various solid cancers due to their rapid growth. Tumour cells adapt to hypoxic conditions by stabilising the hypoxia-inducible transcription factor (HIF-1α), which leads to upregulation of several genes involved in cell proliferation and angiogenesis (Harris, 2002; Semenza, 2003). One of the upregulated genes is *CA9* (Opavsky *et al*, 1996). *CA9* encodes the carbonic anhydrase isozyme IX (CA IX) (Wykoff *et al*, 2000; Niemelä *et al*, 2007). Carbonic anhydrase isozyme IX is shown to be strongly inducible by hypoxia in tumour cells (Wykoff *et al*, 2000).

In earlier studies, the pattern of membranous CA IX expression is seen in malignant cells and only rarely in normal or benign cells (Pastorek *et al*, 1994; Kivelä *et al*, 2001). Colorectal tumours show an abnormal CA IX expression, which is especially seen in areas of high proliferation (Saarnio *et al*, 1998). More diffuse staining is seen in carcinomas than in benign lesions (Saarnio *et al*, 1998). Carbonic anhydrase isozyme IX is involved in maintaining the extracellular pH (Ivanov *et al*, 2001) by catalysing the reversible chemical reaction in which carbon dioxide is hydrated to carbonic acid and further to bicarbonate (Wykoff *et al*, 2000; Brennan *et al*, 2006). Thus, it is an important enzyme for cancer cells in hypoxic and normoxic conditions (Wykoff *et al*, 2000; Robertson *et al*, 2004) in the regulation of acid–base balance (Hilvo *et al*, 2007). Interestingly, in CRC samples studied by cDNA microarray (Talvinen *et al*, 2006), *CA9* was found to be the most upregulated gene.

This study was designed to assess the prognostic and predictive value of CA IX in rectal cancer treated by either short- or long course of radiotherapy (RT) with or without chemotherapy. Operative samples obtained from non-irradiated patients were used as controls. Carbonic anhydrase isozyme IX expression was studied in relation to histopathological features and clinical data pertinent to disease-free survival (DFS) and disease-specific survival (DSS).

PATIENTS AND METHODS

Study population

This study consists of archival operative tumour samples of 166 consecutive patients with rectal cancer, treated according to the

*Correspondence: Dr E Korkeila; E-mail: eija.korkeila@tyks.fi
Received 21 October 2008; revised 23 January 2009; accepted 26 January 2009; published online 24 February 2009

Cancer Chemother Pharmacol (2009) 63:313–319
DOI 10.1007/s00280-008-0741-7

ORIGINAL ARTICLE

A phase II study of S-1 in gemcitabine-refractory metastatic pancreatic cancer

Chigusa Morizane · Takuji Okusaka · Junji Furuse · Hiroshi Ishii · Hideki Ueno · Masafumi Ikeda · Kohei Nakachi · Mina Najima · Takashi Ogura · Eiichiro Suzuki

Received: 27 December 2007 / Accepted: 11 March 2008 / Published online: 9 April 2008

Abstract
Purpose Gemcitabine monotherapy or gemcitabine-containing combination chemotherapy is the standard first-line therapy for advanced pancreatic cancer. After disease progression, there is no standard regimen available. In a previous phase II trial, S-1 has been reported to show considerable efficacy, achieving a response rate of 37.5% in chemo-naïve patients with pancreatic cancer. This study evaluated the efficacy and toxicity of S-1 in patients with gemcitabine-refractory metastatic pancreatic cancer.
Methods Eligibility criteria were histologically proven pancreatic adenocarcinoma with confirmation of progressive disease while receiving gemcitabine-based first-line chemotherapy, 20–74 years of age, Karnofsky performance status of 80–100 points, with measurable metastatic lesions, adequate hematological, renal and liver functions, and written informed consent. S-1 was administered orally at 40 mg/m^2 twice daily for 28 days with a rest period of 14 days as one course. Administration was repeated until the appearance of disease progression or unacceptable toxicity. The primary endpoint of this study was an objective response, and secondary endpoints included toxicity, progression-free survival (PFS) and overall survival, as well as clinical benefit response in symptomatic patients.
Results Forty patients from two institutions were enrolled between September 2004 and November 2005. The most common adverse reactions were fatigue and anorexia, although most of those adverse reactions were tolerable and reversible. One patient developed grade 3 pneumonitis without neutropenia and recovered with appropriate antibiotic treatment. Although no complete response was seen, partial response was obtained in six patients (15, 95% confidence interval, 3.9–26%). Stable disease was noted in 17 patients (43%), and progressive disease in 15 patients (38%). Out of 19 evaluable patients, a clinical benefit response was observed in four patients (21%). The median PFS was 2.0 months, and the median survival time was 4.5 months with a 1-year survival rate of 14.1%.
Conclusion S-1 as monotherapy had marginal anti-tumor activity with tolerable toxicity in patients with gemcitabine refractory metastatic pancreatic cancer.

Keywords Chemotherapy · Pancreatic carcinoma · Second-line · Salvage

C. Morizane (✉) · T. Okusaka · H. Ueno · M. Ikeda · M. Najima · T. Ogura
Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
e-mail: cmorizan@ncc.go.jp

J. Furuse · H. Ishii · K. Nakachi · E. Suzuki
Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital, East, Kashiwa, Japan

Background

The prognosis of patients with pancreatic carcinoma is extremely poor because of difficulty in the early detection of this disease, the high incidence of postoperative recurrence, and ineffectiveness of nonsurgical treatments. Gemcitabine has been established as providing clinical benefit and a modest survival advantage over treatment with bolus 5-FU [3]. However, the benefit provided was inadequate, with an objective response rate of less than 15% and a median survival of 5–7 months. To improve the prognosis of patients with pancreatic cancer, one of the strategies is to develop the effective first-line chemotherapy including

gemcitabine combinations. Among various combinations with gemcitabine plus other agents as a first-line chemotherapy, only a few regimens have shown any survival benefit over single-agent gemcitabine [6, 20, 25], although the worldwide consensus regarding the results of these studies has not been established. Another strategy is to develop an effective second-line chemotherapy regimen after disease progression during first-line chemotherapy. However, despite the fact that several studies have investigated second-line chemotherapy in pancreatic cancer, the therapeutic results have been disappointing with poor response rate and survival [1, 2, 4, 5, 7, 14, 16, 18, 19, 21, 26, 27, 33, 34, 36, 38] Effective treatment in patients failing gemcitabine-based chemotherapy is eagerly awaited.

S-1 is a novel orally administered drug that is a combination of tegafur (FT), 5-chloro-2,4-dihydroxypyridine (CDHP), and oteracil potassium (Oxo) in a 1:0.4:1 molar concentration ratio [31]. CDHP is a competitive inhibitor of dihydropyrimidine dehydrogenase, which is involved in the degradation of 5-FU, and acts to maintain efficacious concentrations of 5-FU in plasma and tumor tissues [35]. Oxo, a competitive inhibitor of orotate phosphoribosyltransferase, inhibits the phosphorylation of 5-FU in the gastrointestinal tract, reducing the serious gastrointestinal toxicity associated with 5-FU [32]. The antitumour effect of S-1 has already been demonstrated in a variety of solid tumors such as advanced gastric cancer [15, 30], colorectal cancer [23], non-small-cell lung cancer [13], head and neck cancer [11], and breast cancer [29].

Concerning pancreatic cancer, a recent late phase II study of S-1 for chemo-naïve advanced pancreatic cancer patients demonstrated promising results with a response rate of 37.5% and a favorable toxicity profile [24]. Furthermore, clinical studies have reported activity of gemcitabine in pancreatic cancer patients with refractoriness to 5-FU [28], suggesting the lack of crossresistance between the gemcitabine and fluorinated pyrimidine, including S-1. Therefore, we conducted the present phase II study to investigate the feasibility and efficacy of S-1 in patients with advanced pancreatic adenocarcinoma in a progressive state under gemcitabine-based first-line chemotherapy.

Patients and methods

Patients

All patients were required to show histologically proven pancreatic adenocarcinoma with measurable metastatic lesions. Additional criteria included the following: progressive disease under gemcitabine-based first-line chemotherapy, post operative recurrence or metastatic disease before the start of first-line chemotherapy, 20–74 years of age, Karnofsky performance status (KPS) of 80–100 points, more than 3 weeks intervals between the last administration of the prior chemotherapy regimen and study entry, adequate bone marrow function (white blood cell count $\geq$ 3,000/mm^3, neutrophil count $\geq$ 1500/mm^3, platelet count $\geq$ 100,000/mm^3, haemoglobin level $\geq$ 9.0 g/dl), adequate renal function (serum creatinine level $\leq$ 1.5 mg/dL), and adequate liver function (serum total bilirubin level $\leq$ 2.0 mg/dL, transaminases level $\leq$ 2.5 times the upper limits of normal). Patients who had obstructive jaundice or liver metastasis were considered eligible if their transaminases levels could be reduced to within 5 times the upper normal limit of normal after biliary drainage. The exclusion criteria were as follows: regular use of phenytoin, warfarin or frucitocin, history of fluorinated pyrimidine use, severe mental disorder, active infection, ileus, interstitial pneumonia or pulmonary fibrosis, refractory diabetes mellitus, heart failure, renal failure, active gastric or duodenal ulcer, massive pleural or abdominal effusion, brain metastasis, active concomitant malignancy. Pregnant or lactating women were also excluded. Written informed consent was obtained from all patients. This study was approved by the institutional review board at the National Cancer Center in Japan.

Treatments

S-1 (Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) was administered orally at a dose of 40 mg/m^2 twice daily after breakfast and dinner. Three initial doses were established according to the body surface area (BSA) as follows: BSA < 1.25 m^2, 80 mg/day; 1.25 m^2 $\leq$ BSA < 1.50 m^2, 100 mg/day; and 1.50 m^2 $\leq$ BSA, 120 mg/day. S-1 was administered at the respective dose for 28 days, followed by a 14-day rest period; this treatment course was repeated until the occurrence of disease progression, unacceptable toxicities, or the patient's refusal to continue. When a grade 3 or greater haematologic or grade 2 or greater nonhaeamatologic toxicity occurred, either the temporary interruption of the S-1 administrations until the toxicity decreased to grade 1 or less, or dose reduction by 20 mg/day (minimum dose, 80 mg/day) was recommended. If no toxicity occurred, the rest period was shortened to 7 days or the dose was gradually escalated in the next course (maximum dose, 150 mg/day), or both were permitted according to the judgment of the individual physicians. If a rest period of more than 28 days was required because of toxicity, the patient was withdrawn from the study. Patients were not allowed to receive concomitant radiation therapy, chemotherapy, or hormonal therapy during the study. Patients maintained a daily journal to record their intake of S-1 and any signs or symptoms that they experienced.

Response and toxicity evaluation

The response after each course was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST). Primary pancreatic lesions were not considered to be measurable lesions because the dimensions of such lesions are difficult to measure accurately. Physical examinations, complete blood cell counts, biochemistry tests, and urinalyses were performed at least weekly. Adverse events were evaluated according to the National Cancer Institute Common Toxicity Criteria, version 2.0.

Clinical benefit response

The clinical benefit response (CBR) was evaluated using the KPS and pain score, as described below [3]. The KPS was recorded weekly by the attending physician. Pain was evaluated by measuring the change from the baseline pain intensity and the daily dose of morphine or morphine-equivalent (doses of analgesic agents were converted to morphine-equivalent doses, i.e., 10 mg oxycodone = 15 mg morphine). The pain intensity was graded from 0 (no pain) to 100 (worst pain) using a visual analog scale and was recorded on a pain assessment card every day. Patients who fulfilled at least one of the following criteria were defined as eligible CBR analysis: (1) baseline pain intensity $\geq$20, or (2) baseline morphine consumption $\geq$10 mg/day. Moreover, all the patients underwent a 'pain stabilization period' for 2 days to ensure that the baseline values were stable before treatment: when the variation in the morphine consumption between 2 days was within 10 mg and the variation of the pain intensity was within 20, the patient was considered eligible for inclusion in the CBR analysis. For pain intensity, a positive response occurred when the score was improved by $\geq$50% from baseline, sustained for $\geq$4 weeks. For analgesic consumption, a positive response occurred when the weekly consumption was reduced by $\geq$50% from baseline, maintained for $\geq$4 weeks. A positive response for KPS was defined as an improvement of $\geq$20 points from baseline, sustained for at least 4 weeks. Any worsening from baseline, sustained for 4 weeks, was considered a negative response for each of the three domains. All the other results were considered stable. Pain intensity and analgesic consumption were compared to give a composite pain score. Each patient was classified positive, stable or negative for each of the primary measures (pain and KPS). In order to achieve a positive clinical benefit response, patients had to be positive for at least one parameter without being negative for any of the others for a minimum of 4 weeks. Patients who were stable in the two primary measures were classified as stable.

Statistical design

The primary endpoint of this study was objective response rate. The secondary endpoint of this study was clinical benefit response; toxicity; progression-free survival; and survival. The number of patients to be enrolled was planned using a SWOG's standard design (attained design) [8, 9]. The null hypothesis was that the overall response rate would be $\leq$5% and the alternative hypothesis was that the overall response rate would be $\geq$20%, the α error was 5% (one-tailed) and the β error was 10% (one-tailed). The alternative hypothesis was established based on the preferable data from previous reports [7, 16, 27, 36, 38]. Interim analysis was planned when 20 patients were enrolled. If none of the first 20 patients had a partial response or complete response, the study was to be ended. If a response was detected in any of the first 20 patients studied, an additional 20 patients were to be studied in a second stage of accrual to estimate more precisely the actual response rate. If the lower limit of the 90% confidence interval exceeded the 5% threshold (objective response in seven or more of the 40 patients), S-1 was judged to be effective and we would proceed to the next large-scale study.

The progression-free survival was calculated from the date of study entry to the date of documented disease progression or death due to any cause (whichever occurred first); and overall survival time was calculated from the date of study entry to the date of death or the last follow-up. The median probability of the survival period and progression-free survival were estimated using the Kaplan–Meier method. The relative dose intensity of S-1 was calculated according to the Hryniuk method [10].

Results

Patients

Forty consecutive patients with metastatic pancreatic cancer which was progressing under gemcitabine-based first-line chemotherapy were enrolled in this study between September 2004 and November 2005. The patient characteristics are shown in Table 1. Thirty-six of the forty patients showed a KPS of $\geq$90. Prior treatment was gemcitabine monotherapy in all patients. Thirty-six of the forty patients (90%) received gemcitabine as a standard 30 min infusion, and the remaining four patients (10%) received gemcitabine administered by fixed dose rate infusion. Of 40 patients, 4 patients (10%) showed a partial response, 21 patients (53%) showed stable disease, and 12 (30%) patients showed progressive disease in first-line gemcitabine therapy. Three patients had received first-line chemotherapy at another hospital and accurate data about