lymphoma. Blood **84** : 2457-2466, 1994
3) McLaughlin P, Liles, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, Heman MR, Bence-Bruckler I, White CA, Cabanillas F, Jain V, Ho AD, Lister J, Wey K, Shen D, Dallaire BK : Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma : half of patients responded to a four-dose treatment program. J Clin Oncol **16** : 2825-2833, 1998
4) Vose JM, Link BK, Grossbard ML, Czuczman M, Grillo-Lopez A, Gliman P, Lowe A, Kunkel LA, Fisher RI : Phase II study of rituximab in combination with CHOP chemotherapy in patients with previously untreated, aggressive non-Hodgkin's lymphoma. J Clin Oncol **19** : 389-397, 2001
5) Tobinai K, Kobayashi Y, Narabayashi M, Ogura M, Kagami Y, Morishima Y, Ohtsu T, Igarashi T, Sasaki Y, Kinoshita T, Murate T : Feasibility and pharmacokinetic study of a chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in relapsed B-cell lymphoma. Ann Oncol **9** : 527-534, 1998
6) Tobinai K : Clinical trial of a mouse-human chimeric anti-CD20 monoclonal antibody (rituximab) for B-cell lymphoma in Japan. Cancer Chemother Phamacol **98** (Suppl 1) : S85-S90, 2001
7) Park JW, Stagg R, Lewis GD, Carter P, Maneval D, Slamon DJ, Jaffe H, Shepard HM : Anti-p185HER2 monoclonal antibodies : biological properties and potential for immunotherapy. Cancer Treat Res **61** : 193-211, 1992
8) Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, Colombat P, Watier H : Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor Fcgamma RIIIa gene. Blood **99** : 754-758, 2002
9) Smith GP : Filamentous fusion phage : novel expression vectors that display cloned antigens on the virion surface. Science **228** : 1315-1317, 1985
10) Marks JD, Hoogenboom HR, Bonnert TP, McCafferty J, Griffiths AD, Winter G : By-passing immunization human antibodies from V-gene libraries displayed on phage. J Mol Biol **222** : 581-597, 1991
11) Winter G, Griffiths AD, Hawkins RE, Hoogenboom HR : Ann Rev Immunol **12** : 433-455, 1994
12) Glover RD : 12th Annual International Conference on Antibody Engineering 2001, (San Diego)
13) Jerne NK : Clonal selection ina lymphocyte network. Soc Gen Physiol Ser **29** : 39-48, 1974
14) http://www.anthrax.osd.mil/vaccine/schedule.asp
15) Manard JA, Maassen CB, Leppla SH, Brasky K, Patterson JL, Iverson BL, Georgiou G : Protection against anthrax toxin by recombinant antibody fragments correlates with antigen affinity. Nat.Biotechnol **20** : 597-601, 2002
16) Kasuya K, Boyer JL, Tan Y, Alipui DO, Hackett NR, Crystal RG : Passive immunotherapy for anthrax toxin mediated by an adenovirus expressing an anti-protective antigen single chain antibody. Mol Ther **11** : 237-244, 2005
17) Chadd HE, Chamow SM : Therapeutic antibody expression therapy. Curr Opin Biotechnol **12** : 188-194, 2001
18) Weiner LM, Clark JI, Ring DB, Alpaugh RK : Clinical development of 2B1 a bispecific murine monoclonal antibody targeting c-erbB-2 and FcγRIII. J Hematother **4** : 453-456, 1995
19) McCall AM, Adams GP, Amoroso AR, Nielsen UB, Zhang L, Horak E, Simmons H, Schier R, Marks JD, Weiner LM. Isolation and characterization of an anti-CD16 single chain-Fv fragment and construction of an anti-HER2/neu/anti-CD16 bispecific scFv that triggers CD16-dependent tumor cytolysis. Mol Immunol **36** : 433-446, 1999
20) Vitetta ES : The development of immunotoxins for the therapy of cancer, AIDS, and immune dysfunction. Princess Takamatsu Symp **19** : 333-340, 1988
21) Vitetta ES : Immunotoxins : new therapeutic reagents for autoimmunity, cancer, and AIDS. J Clin Immunol **10** (6 Suppl) : 15S-18S, 1990
22) Torchilin VP, Klibanov AL, Huang L, O'Donnell S, Nossiff ND, Khaw BA : Targeted accumulation of polyethylene glycol-coated immunoliposomes in infracted rabbit myocardium. FASEB J **6** : 2716-2719, 1992
23) Hosokawa S, Tagawa T, Niki H, HIrakawa Y, Nohga K, Nagaike K : Efficacy of immunoliposome on cancer models in a cell-surface-antigen-density-dependent manner. Br J Cancer **89** : 1543-1551, 2003
24) Uziely B, Jeffers S, Isacson R, Kutsch K, Wei-Tsao D, Yehoshua Z, Libson E, Muggia FM, Gabizon A : Liposomal doxorubicin : antitumor activity and unique toxicities during two complementary phase I studies. J Clin Oncol **13** : 1777-1785, 1995

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ORIGINAL ARTICLE

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Detection and evaluation of epithelial cells in the blood of colon cancer patients using RT-PCR

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Abstract
Background. As a mode of colorectal cancer recurrence, liver metastasis plays an important role. One of the factors reported to predict liver metastasis is the detection of trace amounts of tumor cells in the blood. For this purpose, cancer cell-induced cytokeratins (CKs) are generally identified, using the reverse transcriptase-polymerase chain reaction (RT-PCR). In the present study, we aimed to detect trace amounts of tumor cells, based on CK20, in the circulating venous blood, and we examined pathological factors, liver metastasis, and prognosis.
Methods. The subjects were 57 colorectal cancer patients who had undergone operation. We examined the cancer-induced marker (CK20) in circulating venous blood by RT-PCR and investigated the relationships between this marker, pathological factors, and prognosis.
Results. Detection ratio of CK20 mRNA was 42.1%, and CK20 was significantly correlated with the pathological factor of lymph node metastasis($P = 0.037$). The 5-year survival rate for CK20-positive patients was 62.5% and that for the CK20-negative patients was 87.5%; there was a significant difference($P = 0.048$) between the two groups. Recurrence was recognized in six patients; two were positive for CK20 and four were negative for CK20.
Conclusions. These findings indicate that CK20 is strongly related to lymph node metastasis and prognosis, suggesting its usefulness for the diagnosis of colorectal cancer recurrence. However, CK20 did not predict liver metastasis.

Key words Colorectal cancer · Cancer cells in circulating venous blood · Cytokeratin 20 · RT-PCR

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Introduction

The primary strategy in treating colorectal cancer is complete resection of the lesion; this sometimes includes wide resection of surrounding organs, extended lymph node dissection, and procedures that consider the patient's quality of life (QOL). However, after radical resection, some patients have recurrences, which are presumed to be due to residual micrometastatic foci. However, with classical morphological diagnostic methods, it is difficult to detect micrometastases; therefore, genetic diagnosis is being investigated. Real-time polymerase chain reaction (PCR) is generally used for this purpose, with genes for cytokeratins (CKs) and carcinoembryonic antigen (CEA) – regarded as cancer cell-induced genes – generally being identified using reverse transcriptase-PCR (RT-PCR). However, the detection rates vary, and there are also problems with false-positive and false-negative results depending on the target genes and the detection technology. In the present study, we aimed to detect trace amounts of tumor cells, based on the detection of CK19, CK20, and CEA mRNA in circulating venous blood.

Patients and methods

The subjects were 57 patients with colorectal cancer who had undergone surgical resection of the carcinoma. There were 29 men and 28 women with a mean age of 66.1 ± 10.9 years. The degree of cancer progression, by Dukes classification, was Dukes A (7 patients), Dukes B (26 patients), Dukes C (14 patients), and Dukes D (10 patients). Findings for cancer differentiation were: well-differentiated adenocarcinoma, 38 patients; moderately differentiated adenocarcinoma, 17 patients; and mucinous adenocarcinoma, 2 patients (Table 1) .The observation period ranged from 70 months to 84 months. The study was approved by the institution's ethics committee. The significance of the study and its safety were explained to the patients, and we obtained their informed consent.

Specimens for measurement were prepared as follows. Samples of circulating venous blood were collected during surgical resection of the carcinoma; the initial 5 ml of the sample, and the syringe, were discarded and the remaining 15 ml was employed.[1]

CK19 and CK20 were measured, using RT-PCR, as follows. Monocytes were separated from 5 ml of the blood specimen, and total RNA was extracted using acid quanidinium thiocynate-phenol-chlorotorm Nippon Gene Tokyo Japan (AGPC) and Isogen (Nippon Gene, Tokyo, Japan). From the total RNA, cDNA was synthesized, using both reverse transcriptase and random primer (25 mM Tris-Cl, pH 7.5; 75 mM KCl; 3 mM $MgCl_2$; 10 mM dithiothreitol [DTT]; 0.5 mM dNTP; 1 nM random primer; 1 U/μl RNase inhibitor; and 1 U/μl Super Script II RTase (GIBCO BRL Life Technologies, CA, USA).

The preparation was amplified using a CK19 amplification primer set (5′-AGC TAA CCA TGC AGA ACC TCAa-3′ and 5′-CTT CAG GCC TTC GAT CTG CAT-3′). If CK19 existed in the test product, the band was observed at 383 bp.

The preparation was also amplified using a CK20 amplification primer set (5′-CAG ACA CAC GGT GAA CTA TGG-3′ and 5′-GAT CAG CTT CCACTG TTA GACG-3′) in order to induce a PCR reaction (95°C/30 s, 60°C/30 s, and 72°C/30 s: 40 cycles of amplification). The PCR product was separated using electrophoresis with 25% agarose, and photographed under UV irradiation. If CK20 existed in the test product, the band was observed at 371 bp.[2]

CEA was measured using RT-nested PCR, as used to prevent a non-specific amplification. Total RNA was extracted using Isogen, and cDNA was synthesized, using 1.0 μg of the total RNA, at 40°C for 60 min. The synthesized cDNA was added to 50 μl of first-PCR solution (10 × Ampli Taq Gold Gold Enzyme ABI Calfornia USA) PCR solution, 0.2 mM dNTP, 30 pmol outer forward primer [CEA: 5′-GGA CCT ATG CCT GTT TTG TCT C-3′], 30 pmol reverse primer [CEA: 5′-GTT GCA AAT GCT TTA AGG AAG AAGC-3′], and 1.0 U Ampli taq gold PCR solution] and 35 cycles of PCR were carried out (one cycle: 95°C/30 s, 60°C/30 s, and 72°C/30 s). After completion of the PCR reaction, 5.0 μl of the first-PCR amplification product was added to 50 μl of the second-PCR solution (10 × Ampli taq gold PCR solution, 0.2 mM dNTP, 30 pmol inner forward primer [CEA: 5′ TTC TCC TGG TCT CTC AGC TGG-3′], 30 pmol reverse primer, and 1.0 U Amplitaq gold PCR solution], and 30 cycles of the PCR reaction were carried out (one cycle: 95°C/30 s, 60°C/30 s, and 72°C/30 s). After the reaction was completed, the PCR amplification product was electrophoresed with 3% agarose gel and ethidium bromide staining was performed; then the amplified product was confirmed using a UV transilluminator. If CEA existed in the test product, the band was confirmed at 145 bp. As an internal standard, glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA was detected.

Statistical analysis was done using the *t*-test, the χ^2 test, multivariate analysis by the proportional-hazard model, Spearman's test, and the Kaplan-Meier 5-year survival test. Significance was defined as $P < 0.05$.

Table 1. Patients' characteristics

Sex (M/F)	M29/F28
Age (years)	66.1 ± 10.9
Stage	Dukes A, 7; Dukes B, 26; Dukes C, 14; Dukes D, 10
Histology	Well 38
	Moderate 17
	Mucinous adenocarcinoma 2

Results

CEA, CK19, and CK20 were measured by each examination in healthy volunteers. CEA showed false-positive in 4 patients (57.1%) of 7 of the volunteers, but there were no false-positive results for CK19 and CK20 in 14 volunteers.

Preliminary analysis was carried out in 40 patients. CK19 was not a remarkable prognostic factor because its positive rate was very low and it was not relevant to tumor progression. The positive rates for CK20 and CEA were very similar (Table 2) and the identical ratio between CEA and CK 20 was 62.8% We also recognized some false-positive results for CEA by RT-PCR and decided to measure CK20 and to investigate this factor further of the patients.

CK20 in circulating blood was detected in 24 (42.1%) of the 57 patients. No correlation was observed between CK20 expression and the pathological factors of tumor progression, lymphatic invasion, vessel invasion, or liver metastasis. A correlation was seen between CK20 expression and the presence or absence of lymph node metastasis ($P = 0.037$; Table 3). There were two patients with recurrence in the CK20-positive group (11.8%). One patient had hepatic metastasis 28 months after operation, and the other had pulmonary metastasis at 26 months after operation. They died at 43 months and 34 months, respectively, after operation. There were four patients with recurrence in the CK-

Table 2. Relationship between cancer cells in blood and staging

	CK19		CK20		CEA	
	Positive	Negative	Positive	Negative	Positive	Negative
Dukes A	1	3	2	2	1	3
Dukes B	1	18	7	12	9	10
Dukes C	3	7	6	4	4	6
Dukes D	0	7	3	4	3	4
Positive rate	12.5%		45.0%		42.5%	

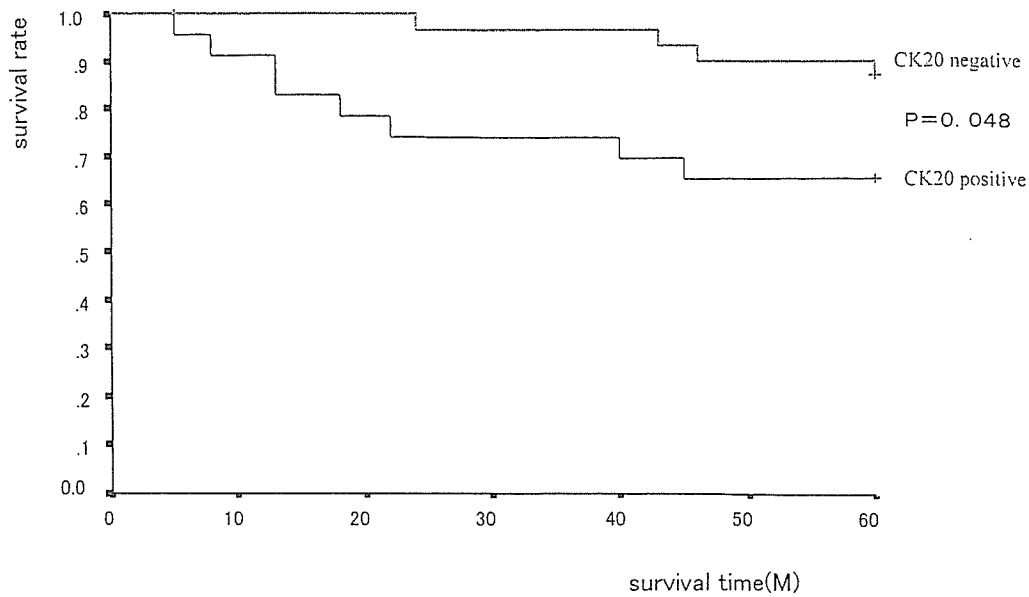

Fig. 1. Comparison of 5-year survival curves between CK20-negative and CK20-positive patients. *m*, moths. The 5-year survival rate for CK20 positive patients was 62.5% and CK negative patients was 87.5%. There was a significant difference ($P = 0.047$)

Table 3. Relationship between CK20 mRNA and pathological factors

		CK20-positive (n = 24, 42.1%)	CK20-negative (n = 33, 57.9%)	
Dukes	A	2	5	
	B	8	18	$P = 0.065$
	C	8	6	
	D	6	4	
Lymphatic invasion	Negative	20	20	
	Positive	4	13	
Vessel invasion	Negative	2	6	
	Positive	22	27	
Lymph node metastasis	Negative	10	24	$P = 0.037$
	Positive	14	9	
Liver metastasis	H0	18	29	
	H1	0	2	$P = 0.067$
	H3	6	2	

20-negative group (14.3%). Two patients had pulmonary metastasis, which occurred at 25 and 26 months after operation. One of the other two patients had a hepatic metastasis (10 months after operation) and the other had a brain metastasis at 25 months. The four patients died at 34, 53, 43, and 46 months, respectively, after operation. There were no differences between the two groups in recurrence rates.

The 5-year survival rate in the CK20-positive group was 65.2% and that in the CK20-negative group was 87.5%. There was a significant difference ($P = 0.048$) between the two groups (Fig. 1).

In the multivariate analysis, tumor progression and lymph node metastasis correlated with the prognosis ($P = 0.044$). Lymphatic invasion ($P = 0.940$),vessel invasion ($P = 0.313$), CK20 ($P = 0.632$), and CEA in serum ($P = 0.237$) showed no correlation with prognosis (Table 4).

Table 4. Analysis of maximum likelihood estimates for recurrence of colorectal cancer

Variable[a]	χ^2	$Pr^b > \chi^2$	Hazard ratio
Stage	4.063	0.044*	8.300
Wall-infiltrating	0.248	0.619	0.525
Lymphatic invasion	0.006	0.940	1.079
Vessel invasion	1.097	0.313	0.472
CK20	0.230	0.632	1.636
CEA > 2.5/CEA ≦ 2.5	1.400	0.237	0.245

Multivariate analysis by proportional-hazard model
[a] Lymph node metastasis was excluded from this model because it had a linear correlation with stage
[b] Pr, provability

Discussion

It has been reported that, even when no lymph node metastasis is observed pathologically, immunostaining examination may show that there is micrometastasis in the lymph nodes, which is related to cancer recurrence and prognosis.[3] It is difficult to prove that there is micrometastasis in the liver. In patients whose tumor cells are detected in circulating blood, metastasis in distant organs, including the liver, occurs at a high incidence, indicating that tumor cell detection in circulating blood can serve as an index of cancer recurrence. Few molecular biology studies have shown can-

cer cells in circulating blood, or the relationship between the micrometastasis in the liver and macroscopic recurrence or prognosis, although some studies[4–6] have shown the prognostic usefulness of detecting tumor cells in circulating blood. Nevertheless, some patients with obvious metastasis show no evidence of tumor cell presence in circulating blood, based on molecular biology.[7] To identify tumor cells in circulating blood, RT-PCR diagnosis, using monoclonal antibodies, is now being tried, i.e., using antibodies specific to epithelial and tumor cells. Tumor markers for detecting tumor cells in circulating blood are classified into markers of genetic alteration, markers of tissue-specific forms, markers of cancer-specific forms, and markers of viral transformation. Tumor markers for colorectal cancer reportedly include K-ras and p53 point mutation in the genetic alteration group; CKs-18, 19, and 20 for the tissue-specific forms; and CEA for the cancer-specific form. However, when RT-PCR is extremely sensitive, these tumor markers may yield false-positive results. Therefore, it is reportedly necessary to employ several procedures, e.g., to decrease the degree of amplification as well as using different gene index procedures.[8] There are other methods for detecting tumor cells in circulating blood, using either mutant-allele-specific amplification (MASA) or PCR-restriction fragment length polymorphism (RFLP) analysis; these procedures have lower sensitivity but higher specificity for genetic alterations.[9] These methods are reportedly useful for detecting K-*ras* gene alterations in pancreatic cancer.[10] At present, real-time PCR is usually carried out for these tumor markers to measure. But, unfortunately, when we measured tumor markers, it could not be done, because it had not been developed yet.

Of the tissue-specific forms of representative tumor markers, such as CK18, CK19, and CK20, CK20 is reportedly the most representative of gastrointestinal epithelium. However, PCR-single-strand conformation polymorphism (SSCP) showed force positive results. Therefore in the present study, we useless improved PCR-SSCP reported by Gunn Jeremy[11] and accuracy became to be higher.[2] Our results showed that CK19 had a low positive rate and that the positive rates for CK20 and CEA were very similar. Because there were no false-positive cases in the CK20 group, we judged that the specificity of CK20 for colon cancer was high. Previously, tumor markers were measured only in Dukes C patients in order to examine the relation with metastasis. However the detection of these markers in Dukes A and B patients has not been clarified because there are small amount of patients in Dukes A and B. Therefore, measurements at all stages are needed. We found a correlation between CK20 and lymph node metastasis and prognosis, indicating that this marker expression was specific to colorectal cancer. Many reports have shown no relationship between tumor markers and cancer recurrence or prognosis,[5,6] although one report[12] indicated that there were differences in malignant grade between patients with and without tumor marker expression. Thus, no conclusion has been established on this subject to date.

CEA, in contrast to the CKs, is an epithelial tumor marker which appears nonspecifically in epithelial tumors, although this marker has been confirmed in normal gastrointestinal membrane, indicating that CEA may appear in most patients with digestive-tract cancers.[13] Our present study showed that CK20 and CEA positive rates were very similar and there was no relationship between CEA in serum and CEA mRNA in the blood by Spearman's test ($P = 0.297$). But there were some false-positive cases in our healthy volunteers and there were no relationships between CEA and the pathological factors we examined.

In our study, we examined the existence of tumor cells using RT-PCR, but it became apparent that metastasis depends on the numbers of tumor cells and their features, and on factors at the metastatic organ site. Reportedly it is possible to determine tumor cell count using a real-time PCR assay.[14] Tumor cells reportedly spread in the blood from the primary focus due to the dysfunction of either cadherin or catenin. It has also been reported that tumor cell spread in the blood is related to patient prognosis.[15] Another report has shown that tumor cells spreading in the blood appear to be destroyed within 24 h by mechanical stress and by immunocytes, whereas, in metastasis from gastric and colorectal cancers to the liver, cadherin is still expressed, suggesting that the tumor cells form tumor masses in order to ensure their survival.[16] It has been reported that tumor cell spread in the blood results in the following processes, in association with various factors: tumor cell agglutination induced by cadherin and SLX; adhesion to endothelium induced by endotherial leucocyte adhesion molecule-1 (ELAM-1), sialyl Lewis (SLX), and intercellular adhesion molecule-1 (ICAM-1); and target organ infiltration induced by cadherin.[17,18] In general, the procedures for quantifying tumor cells in blood are being improved using real-time PCR, but no reports have yet clarified the features of the tumor cells themselves. Some reports show a relationships between tumor cells detected in blood, cancer recurrence, and patient prognosis. We also examined these relationships, and although there were no conclusive findings associated with recurrence, there were significant differences in pathological factors and prognosis between patients who were CK20-positive and those who were CK2-negative.

Conclusion

Our study indicated that CK20 in the blood of colorectal cancer patients was a specific marker for this disease and was useful for determining prognosis. But it was not an independent prognostic factor. If a correlation between CK20 in the blood and cancer recurrence were established by other studies, our findings could be better evaluated.

References

1. Wharton RQ, Patel H, Jonas SK, et al. (2000) Venesection needle coring increases positive results with RT-PCR for detection of

circulating cells expressing CEA mRNA. Clin Exp Metastasis 18:291–294
2. Hisatomi Y, Sugawara E, Nakano H, et al. (1999) Usefulness of various mRNAs detected as hematogeneously metastatic markers. SRL HOKAN (SRL Treasury) 23:109–112
3. Nordgard O, Aloysius TA, Todnem K, et al. (2003) Detection of lymph node micro metastases in colorectal cancer. Scand J Gastroenterol 2:125–132
4. Mellado B, Colomer D, Castel T, et al. (1996) Detection of circulating neoplastic cells by reverse-transcriptase polymerase chain reaction in malignant melanoma: association with clinical stage and prognosis. J Clin Oncol 14:2091–2097
5. Peilin H, Jingmei W, Ying G, et al. (2003) Molecular detection of disseminated tumor cells in the peripheral blood in patients with gastrointestinal cancer. J Cancer Res Clin Oncol 129:192–198
6. Maria GP, Filippo N, Daniela B, Piva MG, Navaglia F, Basso D (2000) CEA mRNA identification in peripheral blood is feasible for colorectal, but not for gastric or pancreatic cancer staging. Oncology 59:323–328
7. Masson D, Denis MG, Lustenberger P (2000) Limitation of CD44v6 amplification for the detection of tumor cells in the blood of colorectal cancer patients. Br J Cancer 82:1283–1289
8. Stathopoulou A, Mavroudis D, Perraki M, et al. (2003) Molecular detection of cancer cells in the peripheral blood of patients with breast cancer: comparison of CK-19, CEA and Maspin as detection markers. Anticancer Res 23:1883–1890
9. Takeda S, Ichii S, Nakamura Y (1993) Detection of K-ras mutation in sputum by mutant-allele-specific amplification (MASA). Hum Mutat 2:112–117
10. Nomoto S, Nakao A, Kasai Y, et al. (1996) Detection of ras gene mutation in preoperative peripheral blood with pancreatic adenocarcinoma. Jpn J Cancer Res 87:793–797
11. Gunn J, McCall JL, Yunk K, et al. (1996) Detection of micro metastases in colorectal cancer patients by CK19 and CK20 reverse transcription polymerase chain reaction. Lab Invest 75:611–616
12. Marc GD, Cecile L, Joel L, Paul-Antoine L, Denis MG, Lipart C, Leborgue J (1997) Detection of disseminated tumor cells in peripheral blood of colorectal cancer patients. Int J Cancer 74:540–544
13. Maria GP, Filippo N, Daniela B, et al. (2000) CEA mRNA identification in peripheral blood is feasible for colorectal, but not for gastric or pancreatic cancer staging. Oncology 59:323–328
14. Miura M, Ichikawa Y, Tanaka K, et al. (2003) Real-time PCR (TaqMan PCR) quantification of carcinoembryonic antigen (CEA) mRNA in the peripheral blood of colorectal cancer patients. Anticancer Res 23:1271–1276
15. Tabuchi Y, Nakae S, Imanishi K, et al. (1984) A clinicopathological study on the hematogenous recurrence of colorectal cancer – with special reference to the prediction and prevention of the recurrence. J Jpn Surg Soc 85:1359–1369
16. Maruyama K, Ochiai A, Nakamura S, et al. (1998) Dysfunction of E-cadherin system in invasion and metastasis of colorectal cancer. J Jpn Surg Soc 99:402–408
17. Schipper JH, Frixen UH, Behrens J, et al. (1991) E-cadherin expression in squamous cell carcinomas of head and neck: inverse correlation with tumor differentiation and lymph node metastasis. Cancer Res 52:6328–6337
18. Katsumata K, Yamamoto K, Murano A, et al. (1999) Correlation between adhesion molecules (laminin, sialyl Le^a, intercellular adhesion molecule-1), agglutination activity of platelet (11-dehydro-thromboxane B^2), and metastasis. J Jpn Soc Colo-Proctol 52:200–2002

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ORIGINAL ARTICLE

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Phase II study of single-agent gemcitabine in patients with advanced biliary tract cancer

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Abstract *Purpose*: This phase II study was conducted to evaluate the efficacy and toxicity of single-agent gemcitabine in patients with advanced or metastatic biliary tract cancer. *Patients and methods*: Gemcitabine 1,000 mg/m^2 was administered as an intravenous 30-min infusion on days 1, 8, and 15 for every 28 days. *Results*: Forty chemonaive patients with a median age of 61 (range 33–73) were enrolled, and all 40 patients were involved in efficacy and safety analyses. Seven (17.5%) achieved partial response; 15 (37.5%) had stable disease; 17 (42.5%) had progressive disease; and 1 (2.5%) was not evaluated. The median survival time was 7.6 months, and the 1-year survival rate was 25.0%. Grade 3/4 neutropenia occurred in 12 patients (30.0%), leukopenia in five patients (12.5%), and anemia in four patients (10.0%). The most common grade 3/4 nonhematologic toxicities were elevated ALT (15.0%) and elevated γ-GTP (12.5%). One patient had grade 4 hemolytic uremic syndrome and recovered after discontinuation of gemcitabine. *Conclusions*: In single-agent therapy, gemcitabine demonstrated moderate efficacy with manageable toxicity in patients with advanced or metastatic biliary tract cancer. Further evaluations are warranted, including the exact impact of gemcitabine on the management of advanced or metastatic biliary tract cancer.

Keywords Biliary tract cancer · Chemotherapy · Clinical trial · Gallbladder cancer · Gemcitabine

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Introduction

The incidence of biliary tract cancer has increased markedly in Japan over the past several decades. In 2002, biliary tract cancer was the sixth leading cause of cancer death in Japan with approximately 16,000 deaths and a mortality rate of 12.5 per 100,000. A continued sharp increase in age-adjusted mortality is predicted over the next 10 years [22, 25, 30].

Of all the treatment modalities for biliary tract cancer, only resection offers the opportunity for cure. However, because of metastases or invasion of the tumor directly into the adjacent liver or the hepatic artery, only a small minority of biliary tract cancer patients are candidates for resection with curative intent. The prognosis for these patients is dismal, and the impact of existing chemotherapy is virtually negligible. Therefore, there is a clear need for new, effective, chemotherapeutic regimens in the management of biliary tract cancer.

Gemcitabine is a novel nucleoside analogue, which requires to be phosphorylated to its active metabolite, gemcitabine triphosphate. Gemcitabine triphosphate competes with deoxycytidine triphosphate for incorporation into DNA, inhibiting DNA synthesis [16]. Gemcitabine has shown broad activity in a variety of tumors and is currently approved for use in non-small-cell lung

cancer and pancreatic cancer in Japan. Based on the results obtained in early phase studies in other locales and the established safety profile of the agent [3, 7, 8, 12, 24, 34, 35, 40], our group has conducted a multicenter, phase II trial of single-agent gemcitabine to investigate the response rate, toxicity, and time-to-event variables (progression-free survival, duration of tumor response, and survival time) in patients with advanced or metastatic biliary tract cancer.

Patients and methods

Eligibility criteria

Enrolled patients had histologically or cytologically confirmed adenocarcinoma of biliary tract, extrahepatic bile duct, gallbladder, or ampulla of Vater. Each patient was required to meet the following eligibility criteria: unresectable biliary tract cancer with at least one bidimensionally measurable tumor; no history of prior chemotherapy; no history of prior antitumor treatment for biliary tract cancer except resection and intraoperative or postoperative adjuvant radiotherapy; an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; 20–74 years of age; estimated life expectancy ≥2 months; adequate renal function (creatinine ≤ upper limit of normal [ULN]); adequate liver function (bilirubin ≤ 2 times ULN and aspartate/alanine transaminases [AST/ALT] ≤ 2.5 times ULN); adequate bone marrow reserve (white blood cells ≤ 4,000/mm^3, neutrophils ≥2,000/mm^3, platelets ≥100,000/mm^3, and hemoglobin ≥10 g/dl); and written informed consent. Patients with pre-existing obstructive jaundice were also eligible after their bilirubin levels met the criteria by biliary stent insertion or percutaneous biliary drainage.

Patients were excluded from the study if they had pulmonary fibrosis, interstitial pneumonia, New York Heart Association class III or IV congestive heart failure, myocardial infarction within the preceding 6 months, diabetes mellitus with severe complications, marked pleural or pericardial effusion, marked peripheral edema, or active infection. Additional exclusion criteria included pregnant or lactating females, patients of reproductive potential who did not use effective contraception, severe drug hypersensitivity, central nervous system metastases, active concomitant malignancy, other serious medical conditions, or patients receiving any investigational drug within 30 days before enrollment.

The study was conducted in accordance with the ethical principles stated in the most recent version of the Declaration of Helsinki or the applicable guidelines on good clinical practice, whichever represented the greater protection of the individual. In addition, the study design was approved by the appropriate ethical review boards.

Study treatment

Gemcitabine (supplied by Eli Lilly, Japan) 1,000 mg/m^2 was administered as an intravenous 30-min infusion on days 1, 8, and 15 for every 28 days. The treatment was continued until evidence of disease progression or unacceptable toxicity.

For white blood cells < 2,000/mm^3, neutrophils < 1,000/mm^3, platelets < 70,000/mm^3, bilirubin > 3 times ULN, or AST/ALT > 5 times ULN, gemcitabine was omitted on that day and postponed to the next scheduled treatment day.

In subsequent cycles, gemcitabine was reduced to 800 mg/m^2 if neutrophils < 500/mm^3 for 4 days, white blood cells < 1,000/mm^3 for 4 days, platelets < 25,000/mm^3, bilirubin > 3 times ULN, or AST/ALT > 5 times ULN. Gemcitabine was also reduced to 800 mg/m^2 if a platelet transfusion was performed due to thrombocytopenia or if gemcitabine was omitted twice in succession due to toxicity. No dose adjustment was allowed during the same cycle. The treatment was discontinued if a second dose reduction was needed, if bilirubin > 5.0 times ULN, AST/ALT > 20 times ULN, or tumor progression was observed. The use of granulocyte colony-stimulating factor (G-CSF) was permitted for any grade 4 leukopenia or neutropenia or grade 3 neutropenia with high fever (38.0°C). Prophylactic administration of antiemetics was allowed.

Baseline and treatment assessments

Pretreatment evaluation included complete history and physical examination. In addition, complete blood count, biochemistry tests, urinalysis, and chest X-ray were performed. Performance status and laboratory tests, except for urinalysis, were assessed weekly. Urinalysis was performed during days 15–28 in each cycle. Tumor size was measured by CT scan or MRI during days 22–28 in each cycle. Carcinoembryonic antigen (CEA) and carbohydrate antigen 19–9 (CA19–9) were quantified every 4 weeks. All 40 patients who received at least one dose of gemcitabine were involved in the efficacy analyses.Objective tumor response was assessed every 4 weeks using WHO criteria [41]. The duration of response was calculated from the first day of treatment until documentation of disease progression. Survival was measured from the first day of treatment.

Toxicity was assessed using the National Cancer Institute Common Toxicity Criteria version 2.0 [27]. A monitoring committee independently evaluated the efficacy and safety of the study.

Statistical analysis

Considering the results of previous trials using gemcitabine for advanced or metastatic biliary tract cancer, we expected an overall response rate of 15–20% in this

study. With this population, response rates typically have not exceeded 10% in patients treated with 5-fluorouracil (5-FU); therefore, a response rate of at least 15% in our study would suggest a potential benefit.

Our goal was to enroll 40 eligible patients. If no response occurred in the first 18 patients, accrual was terminated because the chance of a 15% response rate was only 5.3%. If the response rate was 15%, the statistical power (the probability of a 5% response rate) would be 73% with type I error of 5% (one-sided). For a response rate of 17.5%, the statistical power would be 85%, and the statistical power would be 92% for a response rate of 20%.

All time-to-event measures were calculated using the Kaplan–Meier method.

Results

Patient characteristics and disposition

From October 2001 to September 2003, 21 males and 19 females, with a median age of 61 years (range 33–73 years), were enrolled. Table 1 shows the baseline patient characteristics. Twenty-three patients (57.5%) had no prior therapy, and 17 (42.5%) relapsed after resection for primary lesion. The major metastatic lesions were the abdominal lymph nodes (67.5%) and liver (55.0%). Prior to the initiation of study treatment, obstructive jaundice was palliated with percutaneous transhepatic catheter placement (11 patients) or endobiliary stent placement (3 patients).

The reasons for the treatment discontinuation included progressive disease (34 patients), elevated blood pressure associated with worsening of renal function (one patient), hemolytic uremic syndrome (one patient), blood bilirubin increased with progressive disease (one patient), relapse of pre-existing schizophrenia (one patient), patient's refusal due to nausea/vomiting (one patient), and general fatigue (one patient).

Table 1 Baseline patient characteristics ($n=40$)

Characteristic	
Gender, *n* (%)	
Male	21 (52.5)
Female	19 (47.5)
Age, years	
Median (range)	61 (33–73)
ECOG performance status	
0	24 (60.0)
1	16 (40.0)
Primary lesion	
Extrahepatic bile duct	12 (30.0)
Gallbladder	22 (55.0)
Ampulla of Vater	6 (15.0)
CA19–9, *n* (U/ml)	
Median (range)	448.6 (1–77,820)
CEA, *n* (ng/ml)	
Median (range)	10.9 (0.5–1,790)
Metastatic sites, *n* (%)	
Abdominal lymph nodes	27 (67.5)
Liver	22 (55.0)
Peritoneum	4 (10.0)
Lung	2 (5.0)
Bone	1 (2.5)

ECOG Eastern Cooperative Oncology Group; *CA19–9* carbohydrate antigen 19–9; *CEA* carcinoembryonic antigen

Efficacy

All 40 patients were evaluated for efficacy and according to WHO criteria, seven patients achieved a partial response for an overall response rate of 17.5% (95% CI, 7.3–32.8%). The median duration of the response was 9.4 months (range, 2.6–9.4 months). Fifteen patients (37.5%) had stable disease, and 17 patients (42.5%) had progressive disease. Tumor response was not determined in one patient because she was transferred to another hospital before response evaluation. The serum CA 19–9 level was reduced by less than half in 11 (33%) of 33 patients who had a pretreatment level of above upper normal limit, and the CEA level was reduced by less than half in 6 (24%) of 25 patients. Of the 11 patients whose CA 19–9 level was reduced, 4 (36%) showed a partial response. Five (83%) of the six patients with the CEA response achieved a partial response.

At the time of analysis, 35 of 40 patients had died of cancer and two of five patients lived longer than 24 months after the initial administration of gemcitabine. The median progression-free interval was 2.6 months (95% CI, 1.7–3.8 months), and the median survival time was 7.6 months (95% CI, 5.4–9.3 months) (Fig. 1). The 1-year survival rate was 25.0%.

Toxicity

All 40 patients were evaluable for toxicity (Table 2). No toxic deaths occurred. Hematologic toxicity was reversible and manageable. Patients reported grade 3/4 neutropenia (30.0%), leukopenia (12.5%), and anemia (10.0%). Three patients had red blood cell transfusions due to hemolytic uremic syndrome, hemorrhagic shock, and anemia. No grade 3/4 thrombocytopenia was reported. Although two patients were treated with G-CSFs, there was no febrile neutropenia.

The most common nonhematologic toxicities, grades 1–4 were nausea (52.5%) and anorexia (52.5%), but only four patients (10%) required intravenous infusion due to these toxicities. The most common grade 3/4 nonhematologic toxicities were elevated ALT (15.0%) and elevated γ-glutamyltransferase (γ-GTP) (12.5%). Grade 4 elevated γ-GTP was observed in one patient, which was considered to be gemcitabine-related because the level returned to normal after treatment discontinuation. The patient, who had grade 3 uremia, grade 2 serum creatinine elevation, and grade 2 thrombocytopenia, was diagnosed with grade 4 hemolytic uremic syndrome and also recovered from these toxicities by

transfusion without dialysis after discontinuing gemcitabine. In another patient on day 25 of cycle 1, hemorrhagic shock occurred following unexpected hematemesis, which was unlikely to be gemcitabine related. Endoscopic examination showed acute gastric mucosal lesions, and prescribed nonsteroidal anti-inflammatory drugs to control abdominal pain were suspected to be the cause of hemorrhagic shock.

Dose intensity

A median of three cycles was administered (range, 1–14). Eleven patients (27.5%) completed one cycle; eight patients (20.0%) completed two cycles; and five patients (12.5%) completed three cycles. The planned mean dose intensity of gemcitabine was 750 mg/m^2; however, the actual mean dose intensity of gemcitabine was 688.7 mg/m^2. Thus, the dose intensity was 91.8% for gemcitabine. Of the 476 planned infusions, 37 dose omissions (7.8%) occurred, mainly due to neutropenia. There were no dose reductions.

Discussion

The vast majority of patients with biliary tract cancer are candidates for chemotherapy; however, chemotherapy for biliary tract cancer currently has only limited value in clinical practice. 5-FU is the mainstay of palliative chemotherapy, although response rates range from 0 to 13% in phase II trials [6, 11, 39]. It is generally accepted that combinations with 5-FU have little superiority over single-agent 5-FU, and the considerable toxicity often outweighs the benefit for the patients [11, 39]. Except for gemcitabine, no individual agent has shown a reproducible response rate over 15% [1, 12, 19, 29, 31, 33, 37]. Therefore, new agents need to be developed for truly effective chemotherapeutic regimens against this disease.

In a prospective randomized trial [4], gemcitabine is the only agent showing significant efficacy in respect to survival prolongation and symptom relief for patients with advanced pancreatic cancer; these results prompted trials for biliary tract cancer, which, to some extent, shares embryological and clinical features with pancreatic cancer. Several early-phase studies of single-agent gemcitabine at doses of 1,000–2,200 mg/m^2 have reported response rates of 8–60%, and median survival durations ranging from 6.5 to 11.5 months. [3, 7, 8, 14, 21, 24, 34, 35].

In our trial, gemcitabine 1,000 mg/m^2 was administered for 3 weeks with 1 week of rest; this schedule is currently approved in Japan for non-small-cell lung cancer and pancreatic cancer and is considered to be a standard regimen worldwide. Our overall response rate of 17.5% appeared to be comparable to previous trials with gemcitabine or other combination regimens and appeared near the highest results in single-agent therapy. In recent phase II trials of various single agents, responses were 8% in a study with cisplatin [29], 0% in paclitaxel [19], 0–25% in docetaxel [2, 31, 33], 11% in irinotecan [12], and 19% in capecitabine [23]. Our median overall survival of 7.6 months was also comparable to other trials of single-agent therapy, which ranged from 4.5 to 8.0 months [2, 12, 19, 23, 29, 31, 33, 37], and for combination therapies, which ranged from 5.0 to 14.0 months [5, 9, 10, 15, 18, 20, 26, 28, 32, 35, 36, 38]. However, it seemed to be longer when compared with other phase II trials for Japanese patients with advanced or metastatic biliary tract cancer, which was 5.3 months in uracil/tegafur, 5.9 months in cisplatin/

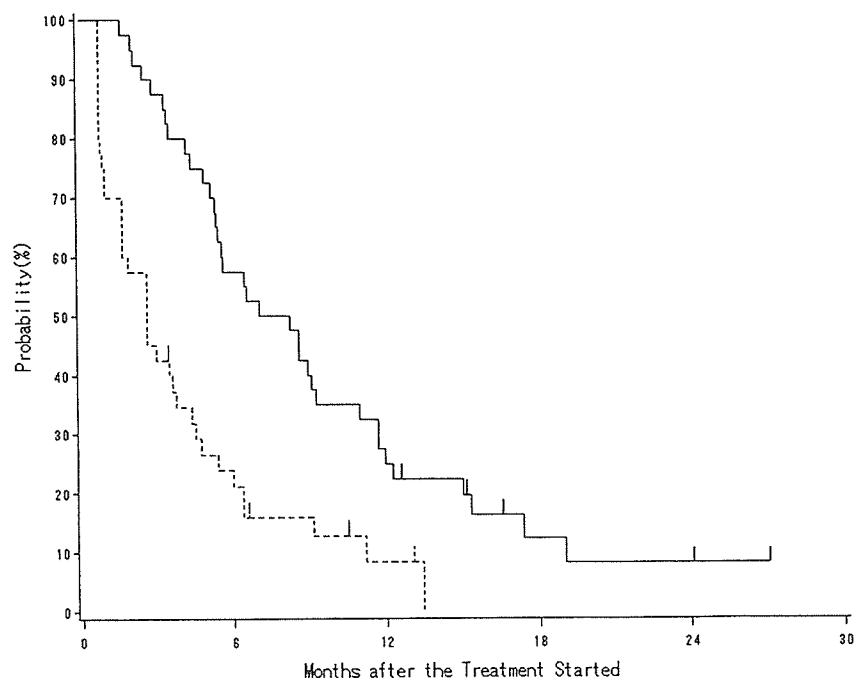

Fig. 1 Progression-free survival (*dashed line*) and overall survival (*solid line*) curves of patients with advanced biliary tract cancer receiving systemic chemotherapy with gemcitabine

Table 2 Adverse drug reaction

Adverse drug reaction	Grade 3		Grade 4	
	n	(%)	*n*	(%)
Hematologic toxicities				
Neutropenia	10	25.0	2	5.0
Leukopenia	5	12.5	0	0.0
Anemia	3	7.5	1	2.5
Thrombocytopenia	0	0.0	0	0.0
Nonhematologic toxicities				
Elevated ALT	6	15.0	0	0.0
Elevated γ-GTP	4	10.0	1	2.5
Elevated AST	2	5.0	0	0.0
Decreased serum sodium	2	5.0	0	0.0
Increased serum ALP	2	5.0	0	0.0
Urinary occult blood positive	1	2.5	0	0.0
Increased serum bilirubin increased	0	0.0	0	0.0
Increased serum creatinine	0	0.0	0	0.0
Proteinuria	0	0.0	0	0.0
Hematuria	0	0.0	0	0.0
Hemolytic uremic syndrome	0	0.0	1	2.5
Constipation	3	7.5	0	0.0
Vomiting	3	7.5	0	0.0
Nausea	2	5.0	0	0.0
Hematemesis	0	0.0	1	2.5
Diarrhoea	0	0.0	0	0.0
Stomatitis	0	0.0	0	0.0
Fatigue	0	0.0	0	0.0
Edema	0	0.0	0	0.0
Pyrexia	0	0.0	0	0.0
Biliary tract infection	1	2.5	0	0.0
Anorexia/Appetite impared	3	7.5	1	2.5
Rash	1	2.5	0	0.0
Alopecia	0	0.0	0	0.0
Hypertension	1	2.5	0	0.0
Hemorrhagic shock	0	0.0	1	2.5

ALT Alanine aminotransferase, *γ-GTP* γ-glutamyltransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase

epirubicin/5-FU, and 5.5 months in a study with cisplatin [18, 26, 29].

The toxicity profile in our study was generally acceptable. The major toxicities were myelosuppression; the incidences of grade 3/4 toxicities were 30.0% in neutropenia, 12.5% in leukopenia, and 10.0% in anemia. However, grade 4 toxicities were infrequent, and neither febrile neutropenia nor treatment-related deaths were observed. The toxicity profile in our study was consistent with past studies using gemcitabine in other tumors. For patients treated with cisplatin, epirubicin, and 5-FU [26], high incidences of grade 3/4 neutropenia (76.0%), leukopenia (59.0%), and death due to treatment-related sepsis 5.0% occurred despite a response rate (19%) similar to that in our study. There was only one episode of cholangitis in this study, although patients with biliary tract cancer are at high-risk for cholangitis, and sometimes severe sepsis occurs, which is derived from cholangitis during chemotherapy [26]. Transient elevations of hepatic enzymes have been reported in gemcitabine therapy for both pancreatic and biliary tract cancer; liver function may be easily affected by cholestasis due to existence of primary and/or metastatic tumors.

One patient developed hemolytic uremic syndrome, which was considered to be a manifestation of thrombotic microangiopathy, although gemcitabine-associated thrombotic microangiopathy is believed to be very rare, with estimated incidences of 0.008–0.31% [13, 17]. The event in this patient seemed to be a treatment-related adverse reaction; however, the patient recovered from hemolytic uremic syndrome without hemodialysis after discontinuation of gemcitabine. Grade 4 anemia was observed in one patient, who suffered grade 4 hematemesis and hemorrhagic shock. This was unlikely to be related to gemcitabine because no thrombocytopenia was observed in this patient. Also, upper gastrointestinal endoscopy revealed acute gastric mucosal lesions as the origin of the bleeding, which seemed to be related to prescribed non-steroidal anti-inflammatory drugs.

Our study was conducted among the largest group of patients with biliary tract cancer to date. In our study, gemcitabine was administered to patients who had biliary stent insertion or percutaneous biliary drainage, and no particular drug-related toxicity was observed in these patients. The result of our study is promising for patients with biliary tract cancer.

In conclusion, chemotherapy with single-agent gemcitabine was feasible and appeared to show efficacy in advanced or metastatic biliary tract cancer. Gemcitabine may provide a more favorable prognosis in patients with this disease compared to other chemotherapeutic regimes or best supportive care.

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Appendix

Case Judgment Committee

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References

1. Aabo K, Pedersen H, Rorth M (1985) Cisplatin in the treatment of advanced gastric carcinoma: a phase II study. Cancer Treat Rep 69:449–450
2. Agelaki S, Papakostas P, Stathopoulos G, Aravantinos G, Kalbakis K, Sarra E et al (1999) Phase II study of docetaxel with G-CSF support as first-line treatment for unresectable or advanced biliary tract carcinoma: a multicenter phase II trial. Proc Am Soc Clin Oncol 18:276a (Abstract No: 1058)
3. Arroyo G, Gallardo J, Rubio B, Orlandi L, Yañez M, Gamargo C et al (2001) Gemcitabine (GEM) in advanced biliary tract cancer (ABTC). Experience from Chile and Argentina in phase II trials. Proc Am Soc Clin Oncol 20:157a (Abstract 626)
4. Burris HA III, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR et al (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
5. Chen JS, Lin YC, Jan YY, Liau CT (2001) Mitomysin C with weekly 24-h infusion of high-dose 5-fluorouracil and leucovorin in patients with biliary tract and periampullar carcinomas. Anticancer Durgs 12:339–343
6. Davis HL Jr, Ramirez G, Ansfield FJ (1974) Adenocarcinomas of stomach, pancreas, liver, and biliary tracts. Survival of 328 patients treated with fluoropyrimidine therapy. Cancer 33:193–197
7. Dobrila-Dintinjana R, Kovac D, Depolo A, Uravic M, Dintinjana M (2000) Gemcitabine in patients with nonresectable cancer of the biliary system or advanced gallbladder cancer. Am J Gastroenterol 95:2476
8. Eng C, Ramathan RK, Wong MK, Remick SC, Dai L, Wade-Oliver KT et al (2004) A phase II trial of fixed dose rate gemcitabine in patients with advanced biliary tree carcinoma. Am J Clin Oncol 276:565–569
9. Ducreux M, Rougier P, Fandi A, Clavero-Fabri MC, Villing AL, Fassone F et al (1998) Effective treatment of advanced biliary tract carcinoma using 5-fluorouracil continuous infusion with cisplatin. Ann Oncol 9:653–656
10. Ellis PA, Norman A, Hill A, O'Brien ME, Nicolson M, Hickish T et al (1995) Epirubicin, cisplatin and infusional 5-fluorouracil (5-FU) (ECF) in hepatobiliary tumors. Eur J Cancer 31A:1594–1598
11. Falkson G, MacIntyre JM, Moertel CG (1984) Eastern Cooperative Oncology Group experience with chemotherapy for inoperable gallbladder and bile duct cancer. Cancer 54:965–969
12. Fishkin P, Alberts S, Mahoney M, Sargent D, Goldberg R, Burgart L et al (2001) Irinotecan in patients with advanced gallbladder carcinoma: a North Central Cancer Treatment Group (NCCTG) phase II study. Proc Am Soc Clin Oncol 20:155a (Abstract No: 618)
13. Fung MC, Storniolo AM, Nguyen B, Arning M, Brookfield W, Vigil J (1999) A review of hemolytic uremic syndrome in patients treated with gemcitabine therapy. Cancer 85:2023–2032
14. Gebbia V, Giuliani F, Verderame F, Boerellino N, Mauceri G, Tirrito M, et al (2001) Treatment of inoperable and/or metastatic biliary tree carcinomas with single-agent gemcitabine or in combination with levofolinic acid and infusional fluorouracil: results of a multicenter phase II study. J Clin Oncol 19:4089–4091
15. Harvey JH, Smith FP, Schein PS (1984) 5-Fluorouracil, mitomycin, and doxorubicin (FAM in carcinoma of the biliary tract. J Clin Oncol 2:1245–1248
16. Huang P, Chubb S, Hertel LW, Grindey GB, Plunkett W (1991) Action of 2×,2×-difluorodeoxycytidine on DNA synthesis. Cancer Res 51:6110–6117
17. Humphreys BD, Sharman JP, Henderson JM, Clark JW, Marks PW, Rennke HG et al (2004) Gemcitabine-associated thrombotic microangiopathy. Cancer 100:2664–2670
18. Ikeda M, Okusaka T, Ueno H, Furuse J, Ishii H, Morizane C, et al (2004) Phase II study of UFT for advanced extra- and intrahepatic cholangiocarcinoma (in Japanese). J Jpn Biliary Assoc 18:421
19. Jones DV Jr, Lozano R, Hoque A, Markowitz A, Patt YZ (1996) Phase II study of paclitaxel therapy for unresectable biliary tree carcinomas. J Clin Oncol 14:2306–2310
20. Kajanti M, Pyrhonen S (1994) Epribicin-sequential methotrexate-5-fluorouracil-leucovorin treatment in advanced cancer of the extrahepatic biliary system. A phase II study. Am J Clin Oncol 17:223–226
21. Kubicka S, Tietze MK, Rudolph L, Manns MP (1999) Phase II study of gemcitabine in patients with nonresectable cancer of the biliary system. Eur J Cancer 35(suppl 4):S151 (Abstract 559)
22. Kuroishi T, Hirose K, Tajima K, Tominaga S (1999) Prediction of cancer death in Japan. In: Tominaga S, Oshima A, Kuroishi T, Aoki K, (eds) Cancer statistics-1999 (in Japanese). Shinohara Shuppan, Tokyo , pp 171–86
23. Lozano R, Patt Y, Hassan M, Frome A, Vauthey J, Ellis L et al (2000) Oral capecitabine (Xeloda) for the treatment of hepatobiliary cancers (hepatocellular carcinoma, cholangiocarcinoma, and gallbladder cancer). Proc Am Soc Clin Oncol 19:264a (Abstract No: 1025)
24. Mezger J, Sauerbruch T, Ko Y, Wolter H, Funk C, Glasmacher A (1998) A phase II trial of gemcitabine in gallbladder and biliary tract carcinomas. Onkologie 21:232–234

25. Ministry of Health, Labour and Welfare. Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour and Welfare. Vital Statistics of Japan 2002 (in Japanese)
26. Morizane C, Okada S, Okusaka T, Ueno H, Saisho T (2003) Phase II study of cisplatin, epirubicin, and continuous-infusion 5-fluorouracil for advanced biliary tract cancer. Oncology 64:475–476
27. National Cancer Institute (1999) Common Toxicity Criteria (version 2). Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda
28. Nehls O, Klump B, Arkenau HT, Hass HG, Greschniok A, Gregor M, et al (2002) Oxaliplatin, fluorouracil and leucovorin for advanced biliary system adenocarcinomas: a prospective phase II trial. Br J cancer 87:702–704
29. Okada S, Ishii H, Nose H, Yoshimori M, Okusaka T, Aoki K et al (1994) A phase II study of cisplatin in patients with biliary tract carcinoma. Oncology 51:515–517
30. Okusaka T (2002) Chemotherapy for biliary tract cancer in Japan. Semin Oncol 29:51–53
31. Papakostas P, Kouroussis C, Androulakis N, Samelis G, Aravantinos G, Kalbakis K et al (2001) First-line chemotherapy with docetaxel for unresectable or metastatic carcinoma of the biliary tract. A multicentre phase II study. Eur J Cancer 37:1833–1838
32. Patt YZ, Hassan MM, Lozano RD, Waugh KA, Hoque AM, Frome AI, et al (2001) Phase II trail of cisplatin, interferon α-2b, doxorubicin, and 5-fluorouracil for biliary tract cancer. Clin Cancer Res 7:3375–3380
33. Pazdur R, Royce ME, Rodriguez GI, Rinaldi DA, Patt YZ, Hoff PM et al (1999) Phase II trial of docetaxel for cholangiocarcinoma. Am J Clin Oncol 22:78–81
34. Penz M, Kornek GV, Raderer M, Ulroch-Pur H, Fiebiger W, Lenauer A et al (2001) Phase II trial of two-weekly gemcitabine in patients with advanced biliary tract cancer. Ann Oncol 12:183–186
35. Raderer M, Hejna MH, Valencak JB, Kornek GV, Weinländer GS, Bareck E, et al (1999) Two consecutive phase II studies of 5-fluorouracil/leucovorin/mitomycin C and of gemcitabine in patients with advanced biliary tract cancer. Oncology 56:177–180
36. Sanz-Altamira PM, Ferrante K, Jenkins R, Lewis WD, Huberman MS, Stuart KE (1998) A phase II trial fo 5-fluorouracil, leucovorin, and carboplatin in patients with unresectable biliary tree carcinoma. Cancer 82:2321–2325
37. Taal BG, Audisio RA, Bleiberg H, Blijham GH, Neijt JP, Veenhof CH et al (1993) Phase II trial of mitomycin C (MMC) in advanced gallbladder and biliary tree carcinoma. An EORTC Gastrointestinal Tract Cancer Cooperative Group Study. Ann Oncol 4:607–609
38. Taieb J, Mitry E, Boige V, Artru P, Ezenfis J, Lecomte T et al (2002) Optimization of 5-fluorouracil (5-FU)/cisplatin combination chemotherapy with a new schedule of leucovorin, 5-FU and cisplatin (LV5FU2-P regimen) in patients with biliary tract carcinoma. Ann Oncol 13:1192–1196
39. Takada T, Kato H, Matsushiro T, Nimura Y, Nagakawa T, Nakayama T (1994) Comparison of 5-fluorouracil, doxorubicin and mitomycin C with 5-fluorouracil alone in the treatment of pancreatic-biliary carcinomas. Oncology 51:396–400
40. Valencak J, Kornek GV, Raderer M, Ulrichi-Pur H, Krauss G, Greul R et al (1999) Gemcitabine for the treatment of advanced biliary tract carcinomas: Evaluation of two different dose regimens. Onkologie 22:498–501
41. World Health Organization (1979) WHO handbook for reporting results of cancer treatment. Offset Publication 48. Geneva: World Health Organization

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A Phase I/II Study of Combination Chemotherapy with Gemcitabine and 5-Fluorouracil for Advanced Pancreatic Cancer

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A Phase I/II Study of Combination Chemotherapy with Gemcitabine and 5-Fluorouracil for Advanced Pancreatic Cancer

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Background: In an effort to improve efficacy of single-agent gemcitabine in pancreatic cancer, several studies have examined the effects of 5-FU combined with gemcitabine. However, no studies to date have been performed in Japanese patients. We thus conducted a phase I/II study of gemcitabine and infusional 5-FU in Japanese patients to determine a recommended dosage for this combination and clarify efficacy and toxicity.
Methods: Phase I evaluated the frequency of dose limiting toxicity of two 5-FU dosages (400 and 500 mg/m^2/day) infused continuously over 5 days combined with gemcitabine 1000 mg/m^2 × 3 every 4 weeks. Results from phase I determined the recommended dosage to be examined in phase II for effect on survival period, clinical benefit response (CBR), tumor response and safety.
Results: A total of 34 chemo-naive patients were entered into the study. All had a Karnofsky performance of ⩾50 points and distant metastases. Dose limiting toxicities in phase I determined the recommended 5-FU dosage at 400 mg/m^2/day. Grade 3–4 hematological toxicities (neutropenia, leukopenia and thrombocytopenia) were the most common severe toxicities. For the 28 patients administered the recommended dosage, 1-year survival rate was 14.3%, median survival time 7.1 months and progression free survival 3.2 months. Seven patients achieved a 25% overall response rate and three showed 27.3% improvement in CBR.
Conclusion: Although a meaningful survival benefit over single-agent gemcitabine was not demonstrated, 5-FU 400 mg/m^2/day infused continuously over 5 days in combination with gemcitabine 1000 mg/m^2 × 3 every 4 weeks appeared to be a moderately effective palliative treatment with low toxicity in Japanese patients with metastatic pancreatic cancer.

Key words: pancreatic cancer – phase I/II study – chemotherapy – gemcitabine – 5-FU

INTRODUCTION

Pancreatic cancer is a virulent disease with an extremely poor prognosis. Of all the treatment modalities for pancreatic cancer, only surgical resection offers the opportunity for a cure. However, because of local extension and/or metastatic disease, only a small minority of pancreatic cancer patients are candidates for curative resection. Moreover, even for these selected patients, prognosis remains unsatisfactory because of the postoperative recurrence, indicating that surgery alone has only limited value in the treatment of pancreatic cancer. Accordingly, to improve the overall survival of patients with pancreatic cancer, there is an urgent need to develop an effective non-surgical treatment for this disease.

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Previously a randomized controlled study demonstrated that gemcitabine, a nucleoside analogue, was effective in palliating symptoms and prolonging survival in patients with advanced pancreatic cancer (1). In the present study, gemcitabine showed a statistically significant advantage in both clinical benefit response (CBR) (23.8% versus 4.8%, $P = 0.0022$) and median survival (5.65 versus 4.41 months, $P = 0.0025$) compared with weekly bolus 5-fluorouracil (5-FU). Although single-agent gemcitabine has been accepted worldwide as the first-line therapy for advanced pancreatic cancer, there is substantial room for improvement in chemotherapy for pancreatic cancer because single-agent gemcitabine provides only limited benefit with a median survival of 4–6 months (1–3).

One approach has been to look for possible agents to use in combination with gemcitabine. A promising candidate has been the fluoropyrimidine, 5-FU, a key chemotherapeutic agent for pancreatic cancer before introduction of gemcitabine. Initially two *in vitro* studies in HT-29 colon cancer cells

using fluoropyrimidines in combination with gemcitabine suggested at least additive activity (4,5). Several phase II trials of gemcitabine combined with bolus 5-FU were then conducted, all of which showed promising results (6–9). Based on these findings the Eastern Cooperative Oncology Group (ECOG) conducted a phase III trial to compare gemcitabine plus bolus 5-FU with gemcitabine alone in patients with advanced pancreatic cancer (10). Although the overall survival in the combination arm tended to be superior to that in the gemcitabine alone arm, it was not possible to show a statistically significant difference. Since bolus 5-FU was adopted in this trial, we considered that administering infusional 5-FU might increase the efficacy of the regimen because (i) infusional 5-FU had previously demonstrated a superior antitumor effect to bolus 5-FU in colon cancer (11) and (ii) the effectiveness of infusional 5-FU in the combination with gemcitabine had not been elucidated in pancreatic cancer. Furthermore, since little information is available on the combination of gemcitabine and infusional 5-FU in Japanese patients, we decided to conduct a phase I/II study to determine the recommended dosage of this combination and to clarify its efficacy and toxicity in patients with metastatic pancreatic cancer.

PATIENTS AND METHODS

Eligibility Criteria

The present study included patients with a histological or cytological diagnosis of distant metastatic pancreatic adenocarcinoma not amenable to curative surgical resection or radiation therapy. Patients were required to have no history of chemotherapy, radiation therapy, curative resection or any other therapy for cancer; be between 20 and 74 years of age with a Karnofsky Performance Status (KPS) of 50 or higher; and have an estimated life expectancy of at least 3 months. Indicators of major organ functions were also required to be at normal levels: hemoglobin $\geq$ 9.5 g/dL, WBC $\geq$ 4000/mm^3, neutrophils $\geq$ 2000/mm^3, platelets $\geq$ 100 000/mm^3, alanine transaminase and aspartate transaminase levels [ALT (GPT), AST (GOT)] $\leq$ 2.5 times upper normal limit (UNL) (or $\leq$5 times UNL in patients with obstructive jaundice or liver metastasis), total bilirubin $\leq$ 2 times UNL, serum creatinine $\leq$ UNL and $PaO_2 \geq 70$ torr. Written informed consent was obtained from all patients in the study.

Patients were excluded from the study if they had pulmonary fibrosis, interstitial pneumonia, heart failure or difficult to control arrhythmia, refractory diabetes mellitus, hypercalcemia (serum Ca $\geq$ 11.5 mg/dL) or active infection. Other exclusion criteria included pregnant or lactating females, or females of childbearing age not using effective contraception, severe drug hypersensitivity, brain metastases, obvious neuropathy or mental disorders, active concomitant malignancy, other serious medical conditions or patients who received any investigational drug within 30 days before enrollment.

Study Design

This was a phase I/II study performed in two steps. The objective of Step 1 (phase I) was to evaluate the frequency of dose limiting toxicity (DLT) and then use this to determine which of the three possible 5-FU dosages (400, 500, 600 mg/m^2/day) would be recommended for continuous 24 h infusion over 5 days in combination with gemcitabine at its approved dosage (1000 mg/m^2/day). In Step 2 (phase II), this recommended 5-FU dosage was then administered in combination with gemcitabine at its approved dosage to evaluate the effect of this combination therapy on survival period. Effects on CBR, objective tumor response and the frequency and severity of adverse events were investigated as secondary objectives in Step 2. CBR, objective tumor response and survival period were also examined in those patients from Step 1 who were administered the recommended dosage.

Study Treatment

Step 1 (Phase I)

Gemcitabine (Eli Lilly and Company; Indianapolis, IN; USA) at a dose of 1000 mg/m^2 was administered as an intravenous 30-min infusion on days 1, 8 and 15 every 28 days. Continuous 24-h infusion of 5-FU (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) began immediately after completion of gemcitabine administration on Day 1 and continued for 5 days (Days 1–5). This 28 day period constituted one administration course.

Three possible dosage levels of 5-FU (Level 0: 400 mg/m^2/day, Level 1: 500 mg/m^2/day, Level 2: 600 mg/m^2/day) were assigned for Step 1. The first patient to enter the study began at Level 1. At least three patients were treated at this level and observed for DLT (see below for definition). If three or more patients experienced DLT at Level 1, the 5-FU dosage was reduced to 400 mg/m^2/day (Level 0) in the next three to six patients. Otherwise, patients were assigned to either Level 1 or 2 until at least three, but not more than six, patients had been assigned to two sequential levels. The dosage of 5-FU was considered tolerable according to the general method used for phase I trials of anticancer agents, i.e. DLT frequency not higher than 50%.

Treatment was discontinued if there was clear evidence of disease progression or unacceptable toxicity. Another administration course could be initiated if laboratory values met specifically defined criteria (WBC $\geq$ 4000/mm^3, neutrophils $\geq$ 2000/mm^3, platelets $\geq$ 100 000/mm^3, total bilirubin $\leq$2 times UNL, serum creatinine $\leq$ UNL, diarrhea $\leq$ Grade 1, mucosal disorders $\leq$ Grade 1). The next administration course could be delayed up to 8 weeks. Patients who experienced possible DLT received 800 mg/m^2 of gemcitabine in subsequent courses, although no dose adjustment was allowed during the same course. When patients experienced adverse effects such as Grade 3 diarrhea, Grade 3 mucosal disorders, Grade 3 hand-foot syndrome, serum transaminase of 10 times UNL, Grade 3 hepatic toxicity, or a total bilirubin level of 5.0 times UNL in patients with

obstructive jaundice or liver metastasis, the 5-FU dosage could be reduced to a lower dosage level in subsequent courses or 5-FU could be omitted in subsequent courses when the lowest dosage (400 mg/m^2/day) of 5-FU was given. When patients had leukocytopenia (<2000/mm^2) or thrombocytopenia (70 000/mm^2) on day 7–8 or day 14–15, gemcitabine administration was omitted on that day and postponed to the next scheduled treatment day (12).

STEP 2 (PHASE II)

Step 2 began once the recommended dosage was determined in Step 1. Administration proceeded with the recommended dosage using the same dosing schedule as in Step 1.

STUDY ASSESSMENTS

The objectives of Step 1 were to evaluate DLT frequency and to determine a recommended 5-FU dosage to be used with the standard dosage of gemcitabine in Step 2. The criteria of DLT included Grade 4 leukopenia or neutropenia, Grade 3 or higher neutropenia accompanied by fever (≥38°C) or infection (clinically or biologically confirmed), thrombocytopenia (<25 000/mm^3) or transfusion given to patient, Grade 3 non-hematological toxicity (except nausea/vomiting, anorexia, fatigue, hyperglycemia), AST and ALT > 10 times UNL, total bilirubin > 5 times UNL (patients with obstructive jaundice or liver metastasis) or gemcitabine administration omitted twice in succession. The primary endpoint of Step 2 was to evaluate the 1-year survival rate with the recommended dosage since statistically significant improvement was not recognized in objective tumor response (5% versus 0%), but was observed in survival period in a randomized phase III study comparing gemcitabine and 5-FU (1). The secondary endpoint was to evaluate CBR and objective tumor response, as well as the frequency and severity of adverse events.

CBR was evaluated by KPS and pain, as described elsewhere (13–15). KPS was recorded weekly by the physician. Pain was evaluated by measuring changes from baseline in pain intensity and morphine consumption (analgesic use other than morphine was converted to an equivalent morphine dosage). Each patient recorded pain intensity on a pain assessment card everyday. Patients who met at least one of the following criteria were defined as eligible for evaluation of CBR: (i) baseline KPS of 50–70 points, (ii) baseline pain intensity ≥ 20 (out of 100) as measured by the pain assessment card, (iii) baseline morphine consumption ≥ 10 mg/day.

Objective tumor response was assessed every 4 weeks. In the present study, the sizes of metastatic lesions were measured to evaluate tumor response, although pancreatic masses were not considered to be measurable because of the difficulty of accurately determining pancreatic tumor size with current imaging technology (16).

The Japan Society for Cancer Therapy criteria, which are fundamentally similar to the World Health Organization criteria and NCI Common Toxicity Criteria, were used to evaluate tumor responses and adverse events (17,18). The duration of tumor response was calculated from the first day of treatment. Duration of survival was also calculated from the first day of treatment using the Kaplan–Meier method.

STATISTICAL ANALYSIS

The sample size for the recommended dosage was determined as follows. The 1-year survival rate of existing treatments was assumed to be 5% in view of the 1-year survival rate observed in the Ueno et al. (19) study. To demonstrate that the true 1-year survival rate of the recommended dosage exceeded 5% at a one-sided significance level of 10% with a power of 80% when a normal approximation test was used the sample size for the recommended dosage needed to be at least 28 patients.

RESULTS

PATIENTS AND TREATMENTS

Of the 36 patients who registered for the present study 34 patients were administered the study drugs: 12 patients completed Step 1 (phase I) and an additional 22 patients completed Step 2 (phase II). Table 1 shows the baseline characteristics for patients in Step 1 (Level 1: 6 patients and Level 0: 6 patients), Step 2 and the total number of patients (20) who received the recommended 5-FU dosage in combination with standard gemcitabine (Level 0). There were 20 males and 8 females (median age: 59) who completed at least one administration course at Level 0. All patients showed a good KPS of ≥80 points. The major metastatic lesions for patients who received the recommended dosage were liver (21 patients: 75.0%), lymph node (6 patients: 21.4%) and lung (5 patients: 17.9%).

In Step 1 the dosing criteria, as defined by observed DLT events, assigned patients to the starting (Level 1: 6 patients) and lower (Level 0: 6 patients) dosage levels. No patients were administered the study drugs at Level 2. The recommended dosage (Level 0) was determined by the DLT frequency observed for each level: Level 1 (3/6 patients), Level 0 (2/6 patients).

At Level 1 (Step 1), a total of 22 administration courses were given with a median of three courses for each patient. A total of 89 administration courses were administered at Level 0 (Steps 1 and 2) with a median of two courses for each patient. At the recommended dosage level (Level 0), 23 (8.7%) of 265 scheduled gemcitabine administrations and 1 (0.2%) of 445 scheduled 5-FU administrations were omitted. The dosage was reduced for two (0.8%) gemcitabine administrations, but no dosage reductions of 5-FU were needed. The actual weekly mean dosages administered were 653.4 mg/m^2 (87.1% of planned dosage) for gemcitabine and 478.7 mg/m^2 (95.7% of planned dosage) for 5-FU.

Table 1. Profile of pancreatic cancer patient population

Characteristics	Step 1		Step 2	Total at recommended dose (Level 0)
	Level 0	Level 1	Level 0	
No. of patients	6	6	22	28
Gender, *n* (%)				
Male	5 (83.3)	3 (50.0)	15 (68.2)	20 (71.4)
Female	1 (16.7)	3 (50.0)	7 (31.8)	8 (28.6)
Age, years				
Median	61	58	58	59
Range	50–69	50–63	43–72	43–72
KPS, *n* (%)				
100	1 (16.7)	1 (16.7)	4 (18.2)	5 (17.9)
90	5 (83.3)	5 (83.3)	13 (59.1)	18 (64.3)
80	0 (0.0)	0 (0.0)	5 (22.7)	5 (17.9)
Metastatic sites, *n* (%)				
Liver	5 (83.3)	5 (83.3)	16 (72.7)	21 (75.0)
Lung	1 (16.7)	1 (16.7)	4 (18.2)	5 (17.9)
Depth lymph node	1 (16.7)	0 (0.0)	5 (22.7)	6 (21.4)
Bone	0 (0.0)	0 (0.0)	1 (4.5)	1 (3.6)

KPS, Karnofsky performance status.

The reasons for treatment discontinuation in Steps 1 and 2 were disease progression (27 patients), Grade 3 hepatic dysfunction (2 patients), Grade 3 appetite loss and Grade 3 infection (1 patient), patient refusal due to Grade 3 gastric ulcer (1 patient), Grade 4 stomatitis (1 patient), patient refusal to be admitted to hospital (1 patient) and patient refusal to follow the study protocol (1 patient). All patients who discontinued the treatment due to adverse events recovered from these toxicities after treatment discontinuation.

Toxicity

All patients in Steps 1 and 2 were evaluated for toxicity. DLT in Step 1 was observed in three out of six patients at Level 1 and in two out of six patients at Level 0. At Level 1, neutropenia (Grade 4) occurred in two patients, and a combination of stomatitis (Grade 4), esophagitis (Grade 4) and increased gamma-glutamyltransferase (Grade 3) in one patient. Less severe DLT events were observed at Level 0: one patient had a gastric ulcer hemorrhage (Grade 3) and one patient a combination of infection (Grade 3) and neutropenia (Grade 3).

Table 2 summarizes the toxicities of all patients (20) who received the recommended dosage (Level 0). This combination therapy at the recommended dosage was generally well tolerated and no treatment-related toxic deaths were reported. Hematological toxicities, notably neutropenia and leukopenia, were the most common severe toxicities. The main Grade 3–4 hematological toxicities were neutropenia (53.6%), leukopenia (25.0%) and thrombocytopenia (10.7%). Hepatic dysfunction (elevated alanine aminotransferase: 17.9%), anorexia (7.2%) and nausea (25.0%) were also commonly observed as Grade 3–4 toxicities. However, the above reactions were all predictable since they are known to be associated with gemcitabine and/or 5-FU, and were well managed during the study.

Table 2. Adverse drug reactions at recommended dose

	Grade 1–4		Grade 3		Grade 4	
	n	%	*n*	%	*n*	%
Hematologic toxicities						
Neutropenia	19	67.9	14	50.0	1	3.6
Leukopenia	22	78.6	7	25.0	0	0.0
Thrombocytopenia	18	64.3	3	10.7	0	0.0
Anemia	19	67.9	2	7.1	0	0.0
Non-hematologic toxicities						
Elevated ALT	13	46.4	5	17.9	0	0.0
Elevated γ-GTP	5	17.9	2	7.1	0	0.0
Increased serum ALP	4	14.3	2	7.1	0	0.0
Elevated AST	11	39.3	1	3.6	0	0.0
Increased serum bilirubin	5	17.9	1	3.6	0	0.0
Increased serum uric acid	1	3.6	0	0.0	1	3.6
Nausea	17	60.7	7	25.0	0	0.0
Vomiting	11	39.3	2	7.1	0	0.0
Gastric ulcer hemorrhage	1	3.6	1	3.6	0	0.0
Fatigue	14	50.0	1	3.6	0	0.0
Malaise	3	10.7	1	3.6	0	0.0
Infection	1	3.6	1	3.6	0	0.0
Anorexia/appetite impaired	19	67.9	7	25.0	2	7.2
Rash	12	42.9	1	3.6	0	0.0

ALT, alanine aminotransferase; γ-GTP, γ-glutamyltransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase.

Efficacy

Table 3 summarizes efficacy at the recommended dosage. Of the 28 patients who were administered the recommended dosage, 26 had died by completion of the study follow-up period. Four of these were classified as early deaths, which were defined as deaths within 91 days after beginning the first administration or within 29 days after the last administration, but all deaths were due to disease progression and not related to treatment. The 1-year survival rate was 14.3% [95% Confidence Interval (CI): 1.3–27.2%], median survival time 7.1 months (95% CI: 6.1–8.6 months) and progression free survival 3.2 months (95% CI: 1.7–4.6 months: Figure 1).

All of the 28 patients administered the recommended dosage were evaluable for tumor response; of these, 7 patients achieved a partial response for an overall response rate of 25.0% (95% CI, 10.7–44.9%). The median duration of the response was 4.8 months (range, 1.9–6.3 months), and

Table 3. Efficacy at recommended dose

Therapeutic outcome		
Median survival time	7.1 months	(95% CI, 6.1–8.6)
1 year survival rate	14.3%	(95% CI, 1.3–27.2)
Progression free survival	3.2 months	(95% CI, 1.7–4.6)
Tumor response		
Response rate	25.0%	(95% CI, 10.7–44.9)
Complete response (*n*)	0	
Partial response (*n*)	7	
Minor response (*n*)	0	
No change (*n*)	10	
Progressive disease (*n*)	10	
Not evaluable (*n*)	1[a]	
Clinical benefit response[b]	27.3%	(95% CI, 6.0–61.0)

CI, confidence interval.
[a]One patient discontinued due to early death and could not be evaluated for antitumor effects.
[b]Eleven patients were evaluable.

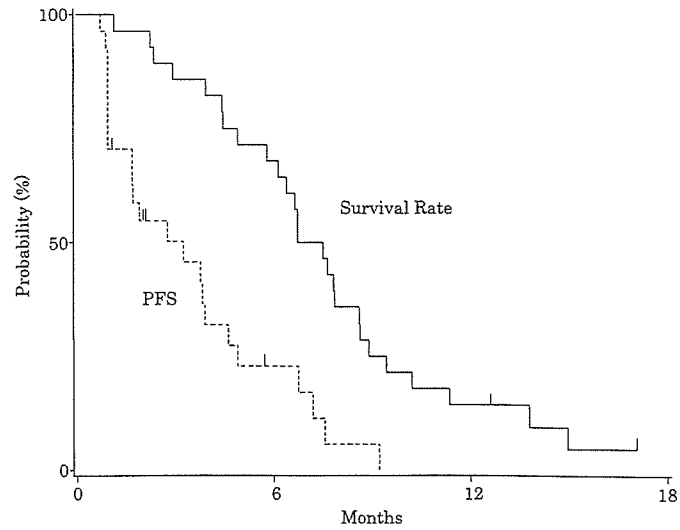

Figure 1. Survival rate and progression free survival (PFS) at recommended dose.

10 patients (35.7%) had stable disease and 10 patients (35.7%) had progressive disease. Tumor response was not determined in one patient due to a serious adverse event (hepatic dysfunction), which made it necessary for this patient to discontinue the study early.

Three of the 11 patients who met the CBR analysis criteria showed improvement in CBR for an overall improvement rate of 27.3% (95% CI: 6.0–61.0%). In all 3 patients, KPS was unchanged but pain intensity was reduced. Of the remaining eight patients, CBR was unchanged in three patients and aggravated in five patients.

DISCUSSION

Despite worldwide agreement about the role of gemcitabine as a first-line agent in advanced pancreatic cancer, therapies that can achieve more significant survival advantage are needed because the prognosis of patients with this disease remains very poor. Several phase II clinical trials combining gemcitabine with 5-FU have been performed using different sequences and schedules of administration (6–9,20–31). A review of the various combination regimens of gemcitabine and 5-FU used in these studies for the treatment of advanced pancreatic cancer found them to be well tolerated (32), although adding weekly intravenous bolus 5-FU to weekly gemcitabine did not confer a significant survival benefit in a randomized trial (10). This finding may be related to the power of the study or the mode of administration of 5-FU rather than to a lack of activity of 5-FU, and it may be possible that giving continuous infusional 5-FU would increase the efficacy of the regimen sufficiently to reach both clinical and statistical significance.

The primary objective of this trial was to find a recommended dosage of infusional 5-FU for use in combination with gemcitabine and to evaluate its efficacy and toxicity in Japanese patients with metastatic pancreatic cancer. Based on the results of our trial (Step 1), we found the recommended dosage to be 5-day continuous infusional 5-FU at 400 mg/m^2/day (Level 0). DLT findings seen in three of the six patients given 5-FU at 500 mg/m^2/day (Level 1) ruled this out as a recommended dosage. Neutropenia, which was observed as DLT in two patients at Level 1, was common in this combination. However, stomatitis and esophagitis in the remaining one patient, both of which were considered DLT and were also consistent with the toxicity profiles of 5-FU, might have been aggravated by Sjogren syndrome in this patient.

In 28 patients at the recommended dosage level, the most common toxicities were myelosuppression, liver dysfunction, appetite loss and nausea, all of which are well known as toxicities of these two agents. Four patients discontinued the treatment due to Grade 4 appetite loss, Grade 3 infection, and Grade 3 hepatic dysfunction, although most of these adverse reactions were transient and the overall toxicity profile in this regimen was acceptable. There appears to be no cumulative toxicity.

At the recommended dosage level, there was a 25% objective response rate with a 1-year survival rate of 14.3% and a median survival of 7.1 months. With respect to CBR, 3 of 11 evaluable patients (27.3%) showed a quality of life improvement. Compared with other reports of single-agent studies of gemcitabine or 5-FU, these results imply an additional benefit for the use of this scheme. Although the activity of this regimen seems to be consistent with results reported from previous studies that used infusional 5-FU in combination regimens (20–31), most of these have been associated with only a modest increase in response rate and/or survival. However, a definitive judgment of the superiority of this

combination is difficult because the majority of the data, including our results, represent only phase I or II trial outcomes.

Recently, Costanzo et al. (33) randomized patients with advanced pancreatic cancer to infusional 5-FU plus gemcitabine versus gemcitabine alone in a randomized phase II study. The results did not support better activity of the combination over gemcitabine alone. The overall response rate was 8% for gemcitabine alone and 11% for the combination, and the median survival time was 31 weeks and 30 weeks, respectively. Riess et al. (34) conducted a phase III study to compare the combination of gemcitabine and 5-FU administered as a continuous 24-h infusion, modulated by folinic acid, with gemcitabine monotherapy. This study also failed to demonstrate any benefit of the combination in terms of overall survival or time to tumor progression despite a manageable safety profile.

The concept of continuous 5-FU administration is evolving with the introduction of oral fluoropyrimidines. Herrmann et al. (35) compared the combination of gemcitabine plus capecitabine with gemcitabine alone in a randomized phase III study. However, no differences were observed with regard to response rate, progression free survival or overall survival. Recently, Cunningham reported a statistically significant survival benefit of capecitabine and gemcitabine combination over gemcitabine, although the role of fluoropyrimidines in the combination with gemcitabine remains controversial because the difference in the median survival time was only 1.4 months (36).

In conclusion, the regimen in the present study appears to be a moderately effective palliative treatment with a low toxicity profile for Japanese patients with metastatic pancreatic cancer. Since randomized trials failed to demonstrate a meaningful survival benefit for combinations of gemcitabine with fluoropyrimidine, including bolus 5-FU, infusional 5-FU and oral fluoropyrimidines such as capecitabine, caution should be taken before planning phase III studies until more promising regimens have been confirmed in phase II studies.

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References

1. Burris HA III, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997;15:2403–13.
2. Casper ES, Green MR, Kelsen DP, Heelan RT, Brown TD, Flombaum CD, et al. Phase II trial of gemcitabine (2,2′-difluorodeoxycytidine) in patients with adenocarcinoma of the pancreas. *Invest New Drugs* 1994;12:29–34.
3. Carmichael J, Fink U, Russell RC, Spittle MF, Harris AL, Spiessi G, et al. Phase II study of gemcitabine in patients with advanced pancreatic cancer. *Br J Cancer* 1996;73:101–5.
4. Schulz L, Schalhorn A, Wilmanns W, Heinemann V. Synergistic interaction of gemcitabine and 5-fluorouracil in colon cancer cells. *Proc Am Soc Clin Oncol* 1998;17: 251a.
5. Ren Q, Kao V, Grem JL. Cytotoxicity and DNA fragmentation associated with sequential gemcitabine and 5-fluoro-2′-deoxyuridine in HT-29 colon cancer cells. *Clin Cancer Res* 1998;4:2811–8.
6. Berlin JD, Adak S, Vaughn DJ, Flinker D, Blaszkowsky L, Harris JE, et al. A phase II study of gemcitabiene and 5-fluorouracil in metastatic pancreatic cancer: an eastern cooperative oncology group study (E3296). *Oncology* 2000;58:215–8.
7. Cascinu S, Silva RR, Barni S, Labianca R, Frontini L, Piazza E, et al. A combination of gemcitabine and 5-fluorouracil in advanced pancreatic cancer, a report from the Italian Group for the Study of Digestive Tract Cancer (GISCAD). *Br J Cancer* 1999;80:1595–8.
8. Jovtis S, Marantz A, Almira E, Balbiani L, Castilla L, Fein L, et al. Phase II trial of gemcitabine (GEM), 5-fluorouracil (5-FU) and leucovorin (LV) in advanced pancreatic cancer. *Eur J Cancer* 1999;35:S157.
9. Pastorelli D, Pedrazzoi S, Sperti C, Vicario G, Scelzi E, Santarossa S, et al. Phase II trial with gemcitabine (GEM) + 5-fluorouracil (5-FU) in advanced pancreatic cancer (APC). *Proc Am Soc Clin Oncol* 2000;19:284a.
10. Berlin JD, Catalano P, Thomas JP, Kugler JW, Haller DG, Benson III AB. Phase III study of gemcitabine in combination with fluorouracil versus gemcitabien alone in patients with advanced pancreatic carcinoma: Eastern cooperative oncology group trial E2297. *J Clin Oncol* 2002; 20:3270–5.
11. Meta-analysis Group In Cancer. Efficacy of intravenous continuous infusion of fluorouracil compared with bolus administration in advanced colorectal cancer. *J Clin Oncol* 1998;16:301–8.
12. Okada S, Ueno H, Okusaka T, Ikeda M, Furuse J, Maru Y. Phase I trial of gemcitabine in patients with advanced pancreatic cancer. *Jpn J Clin Oncol* 2001;31:7–12.
13. Rothenberg ML, Moore MJ, Cripps MC, Andersen JS, Portenoy RK, Burris HA III, et al. A phase II trial of gemcitabine in patients with 5-FU refractory pancreas cancer. *Ann Oncol* 1996;7:347–53.
14. Burris HA III, Moore MJ, Andersen JS, Green MR, Rothenberg ML, Modiano MR, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trail. *J Clin Oncol* 1997;15:2403–13.
15. Okusaka T, Okada S, Ishii H, Nose H, Nakasuka H, Nakayama H, et al. Clinical response to systemic combined chemotherapy with 5-fluorouracil and cisplatin (FP therapy) in patients with advanced pancreatic cancer. *Jpn J Clin Oncol* 1996;26:215–20.
16. Aoki K, Okada S, Moriyama N, Ishii H, Nose H, Yoshimori M, et al. Accuracy of computed tomography in determining pancreatic cancer tumor size. *Jpn J Clin Oncol* 1994;24:85–7.
17. Japan Society for Cancer Therapy. Criteria for the evaluation of the clinical effects of solid cancer chemotherapy. *Nippon Gan Chiryo Gakkai Shi* 1993;28:101–30.
18. Shibuya M. Adverse drug reaction criteria of the Japan Society for Cancer Therapy. *Gan To Kagaku Ryoho* 1997;24:2036–41.
19. Ueno H, Okada S, Okusaka T. Prognostic factors in patients with metastatic pancreatic adenocarcinoma receiving systemic chemotherapy. *Oncology* 2000;59:296–301.
20. Alabiso O, Buosi R, Clerico M, Pampallona S, Friess H, Ludwig CU, et al. Preliminary results of a phase II study with gemcitabine and continuous infusion 5FU in patients with advanced or metastatic pancreatic cancer. *Proc Am Soc Clin Oncol* 2001;20:A2331.
21. Hidalgo M, Castellano D, Paz-Ares L, Gravalos C, Diaz-Puente M, Hitt R, et al. Phase I-II study of gemcitabine and fluorouracil as a continuous infusion in patients with pancreatic cancer. *J Clin Oncol* 1999;17:585–92.
22. Rauch DP, Maurer CA, Aebi S, Pampallona S, Friess H, Ludwig CU, et al. Activity of gemcitabine and continuous infusion fluorouracil in advanced pancreatic cancer. *Oncology* 2001;60:43–8.
23. Anchisi S, Delaloye B, Petite J, Laurencet FL, Ambord Ch, Obrist R. Gemcitabine and continuous infusion 5-FU is active and well tolerated in advanced or metastatic pancreatic cancer. *Proc Am Soc Clin Oncol* 2000;9:A1280H.
24. Rodríguez-Lescure A, Carrato A, Massutí B, Garcia-Gomez J, Herrero J, Gallego J, et al. Phase I-II study of gemcitabine (GEM) and weekly