AUTHOR CONTRIBUTIONS

Conception and design: Hideki Ueno, Tatsuya Ioka, Shinichi Ohkawa, Narikazu Boku, Kenji Yamao, Kazuhiro Mizumoto, Junji Furuse, Akihiro Funakoshi, Takashi Hatori, Taketo Yamaguchi, Shinichi Egawa, Atsushi Sato, Yasuo Ohashi, Takuji Okusaka, Masao Tanaka
Provision of study materials or patients: Masayuki Kitano, Masao Tanaka
Collection and assembly of data: Hideki Ueno, Tatsuya Ioka, Masafumi Ikeda, Shinichi Ohkawa, Hiroaki Yanagimoto, Narikazu Boku, Akira Fukutomi, Kazuya Sugimori, Hideo Baba, Kenji Yamao, Tomotaka Shimamura, Masayuki Sho, Masayuki Kitano, Ann-Lii Cheng, Kazuhiro Mizumoto, Jen-Shi Chen, Junji Furuse, Akihiro Funakoshi, Takashi Hatori, Taketo Yamaguchi, Shinichi Egawa, Takuji Okusaka, Masao Tanaka
Data analysis and interpretation: Hideki Ueno, Tatsuya Ioka, Shinichi Ohkawa, Narikazu Boku, Kenji Yamao, Kazuhiro Mizumoto, Junji Furuse, Akihiro Funakoshi, Takashi Hatori, Taketo Yamaguchi, Shinichi Egawa, Atsushi Sato, Yasuo Ohashi, Takuji Okusaka, Masao Tanaka
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Jemal A, Bray F, Center MM, et al: Global cancer statistics. CA Cancer J Clin 61:69-90, 2011

2. Burris HA 3rd, Moore MJ, Andersen J, 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 15:2403-2413, 1997

3. Moore MJ, Goldstein D, Hamm J, et al: Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: A phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 25:1960-1966, 2007

4. Conroy T, Desseigne F, Ychou M, et al: FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 364:1817-1825, 2011

5. Saif MW, Chabot J: Chemotherapy: Metastatic pancreatic cancer—Is FOLFIRINOX the new standard? Nat Rev Clin Oncol 8:452-453, 2011

6. Saif MW, Syrigos KN, Katirtzoglou NA: S-1: A promising new oral fluoropyrimidine derivative. Expert Opin Investig Drugs 18:335-348, 2009

7. Shirasaka T: Development history and concept of an oral anticancer agent S-1 (TS-1): Its clinical usefulness and future vistas. Jpn J Clin Oncol 39:2-15, 2009

8. Ueno H, Okusaka T, Ikeda M, et al: An early phase II study of S-1 in patients with metastatic pancreatic cancer. Oncology 68:171-178, 2005

9. Okusaka T, Funakoshi A, Furuse J, et al: A late phase II study of S-1 for metastatic pancreatic cancer. Cancer Chemother Pharmacol 61:615-621, 2008

10. Nakamura K, Yamaguchi T, Ishihara T, et al: Phase II trial of oral S-1 combined with gemcitabine in metastatic pancreatic cancer. Br J Cancer 94:1575-1579, 2006

11. Ueno H, Okusaka T, Furuse J, et al: Multicenter phase II study of gemcitabine and S-1 combination therapy (GS Therapy) in patients with metastatic pancreatic cancer. Jpn J Clin Oncol 41:953-958, 2011

12. Therasse P, Arbuck SG, Eisenhauer EA, et al: New guidelines to evaluate the response to treatment in solid tumors: European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216, 2000

13. EuroQol: A new facility for the measurement of health-related quality of life—The EuroQol Group. Health Policy 16:199-208, 1990

14. Spiegelhalter DJ, Freedman LS, Parmar MKB: Bayesian approaches to randomized trials. J R Stat Soc 157:357-387, 1994

15. Brookmeyer R, Crowley J: A confidence interval for the median survival time. Biometrics 38:29-41, 1982

16. Kalbfleisch JD, Prentice RL: The Statistical Analysis of Failure Time Data. New York, NY, Wiley, 1980, p. 14

17. Philip PA, Mooney M, Jaffe D, et al: Consensus report of the national cancer institute clinical trials planning meeting on pancreas cancer treatment. J Clin Oncol 27:5660-5669, 2009

18. Heinemann V, Quietzsch D, Gieseler F, et al: Randomized phase III trial of gemcitabine plus cisplatin compared with gemcitabine alone in advanced pancreatic cancer. J Clin Oncol 24:3946-3952, 2006

19. Herrmann R, Bodoky G, Ruhstaller T, et al: Gemcitabine plus capecitabine compared with gemcitabine alone in advanced pancreatic cancer: A randomized, multicenter, phase III trial of the Swiss Group for Clinical Cancer Res and the Central European Cooperative Oncology Group. J Clin Oncol 25:2212-2217, 2007

20. Cunningham D, Chau I, Stocken DD, et al: Phase III randomized comparison of gemcitabine versus gemcitabine plus capecitabine in patients with advanced pancreatic cancer. J Clin Oncol 27: 5513-5518, 2009

21. Poplin E, Feng Y, Berlin J, et al: Phase III, randomized study of gemcitabine and oxaliplatin versus gemcitabine (fixed-dose rate infusion) compared with gemcitabine (30-minute infusion) in patients with pancreatic carcinoma E6201: A trial of the Eastern Cooperative Oncology Group. J Clin Oncol 27:3778-3785, 2009

22. Philip PA, Benedetti J, Corless CL, et al: Phase III study comparing gemcitabine plus cetuximab versus gemcitabine in patients with advanced pancreatic adenocarcinoma: Southwest Oncology Group-directed intergroup trial S0205. J Clin Oncol 28:3605-3610, 2010

23. Kindler HL, Niedzwiecki D, Hollis D, et al: Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: Phase III trial of the Cancer and Leukemia Group B (CALGB 80303). J Clin Oncol 28:3617-3622, 2010

24. Kindler HL, Ioka T, Richel DJ, et al: Axitinib plus gemcitabine versus placebo plus gemcitabine in patients with advanced pancreatic adenocarcinoma: A double-blind randomised phase 3 study. Lancet Oncol 12:256-262, 2011

25. Morizane C, Okusaka T, Furuse J, et al: A phase II study of S-1 in gemcitabine-refractory metastatic pancreatic cancer. Cancer Chemother Pharmacol 63:313-319, 2009

26. Haller DG, Cassidy J, Clarke SJ, et al: Potential regional differences for the tolerability profiles of fluoropyrimidines. J Clin Oncol 26:2118-2123, 2008

27. Chuah B, Goh BC, Lee SC, et al: Comparison of the pharmacokinetics and pharmacodynamics of S-1 between Caucasian and East Asian patients. Cancer Sci 102:478-483, 2011

Affiliations

Hideki Ueno and Takuji Okusaka, National Cancer Center Hospital; Junji Furuse, Kyorin University; Takashi Hatori, Tokyo Women's Medical University; Atsushi Sato, Showa University Hospital; Yasuo Ohashi, The University of Tokyo, Tokyo; Tatsuya Ioka, Osaka Medical

Center for Cancer and Cardiovascular Diseases, Osaka; Masafumi Ikeda, National Cancer Center Hospital East, Kashiwa; Shinichi Ohkawa, Kanagawa Cancer Center, Yokohama; Hiroaki Yanagimoto, Kansai Medical University, Hirakata; Narikazu Boku and Akira Fukutomi, Shizuoka Cancer Center, Sunto-gun; Kazuya Sugimori, Yokohama City University Medical Center, Yokohama; Hideo Baba, Kumamoto University, Kumamoto; Kenji Yamao, Aichi Cancer Center Hospital, Nagoya; Tomotaka Shimamura, Saitama Cancer Center, Saitama; Masayuki Sho, Nara Medical University, Kashihara; Masayuki Kitano, Kinki University, Osakasayama; Kazuhiro Mizumoto and Masao Tanaka, Kyushu University; Akihiro Funakoshi, Fukuoka Sanno Hospital, Fukuoka; Taketo Yamaguchi, Chiba Cancer Center, Chiba; Shinichi Egawa, Tohoku University, Sendai, Japan; Ann-Lii Cheng, National Taiwan University Hospital, Taipei; and Jen-Shi Chen, Linkou Chang Gung Memorial Hospital and Chang Gung University, Tao-Yuan, Taiwan.

■ ■ ■

Acknowledgment

Appendix

Members of the Gemcitabine and S-1 Trial Group

Steering committee. T. Okusaka, S. Egawa, J. Furuse, T. Yamaguchi, H. Ueno, T. Hatori, S. Ohkawa, N. Boku, K. Yamao, T. Ioka, A. Funakoshi, K. Mizumoto, M. Tanaka.

Independent data and safety monitoring committee. A. Nakao, I. Hyodo, S. Morita; Medical Advisor: A. Sato; Statistical Analyst: Y. Ohashi.

Principal investigators. National Cancer Center Hospital: T. Okusaka; Osaka Medical Center for Cancer and Cardiovascular Diseases: T. Ioka; National Cancer Center Hospital East: M. Ikeda, K. Nakachi; Kanagawa Cancer Center: S. Ohkawa; Kansai Medical University: H. Yanagimoto; Yokohama City University Medical Center: K. Sugimori; Shizuoka Cancer Center: A. Fukutomi; Kumamoto University: H. Baba; Saitama Cancer Center: T. Shimamura, H. Hara; Aichi Cancer Center Hospital: K. Yamao; Nara Medical University: M. Sho; Kinki University, Department of Gastroenterology and Hepatology: M. Kitano; Sapporo-Kosei General Hospital: H. Miyagawa; Kyushu University, First Department of Surgery: K. Mizumoto; Jichi Medical University Hospital: H. Fujii; National Hospital Organization Osaka National Hospital: S. Nakamori; Kinki University, Department of Medical Oncology: T. Satoh, S. Ueda; Tochigi Cancer Center: Y. Hamamoto, E. Warita; Kyushu University, Department of Hepatology and Pancreatology: T. Ito; Teine-Keijinkai Hospital: H. Maguchi; Kyorin University: J. Furuse; Kyoto University Hospital: S. Matsumoto; Saitama Medical University International Medical Center: Y. Sasaki; Hokkaido University Hospital: Y. Komatsu; Tokyo Women's Medical University: M. Yamamoto; Saku Central Hospital: T. Hisa; Osaka City Juso Hospital: T. Yamazaki, O. Kurai; Kochi Health Sciences Center: A. Tsuji; National Kyushu Cancer Center: A. Funakoshi, M. Furukawa; Niigata Cancer Center Hospital: Y. Tsuchiya; Chiba Cancer Center: T. Yamaguchi; Osaka Red Cross Hospital: Y. Okabe; Tohoku University, Division of Gastroenterology: K. Sato; Tohoku University, Division of Gastroenterological Surgery: F. Motoi; Matsusaka Chuo General Hospital: H. Naota; Kyoto Second Red Cross Hospital: K. Yasuda; Hyogo College of Medicine: J. Fujimoto; Toyama University Hospital: A. Hosokawa; Fukuoka University Chikushi Hospital: T. Ueki; Hokkaido Social Insurance Hospital: K. Furuya; Kameda Medical Center: Y. Oyama; Nagoya Medical Center: H. Iwase; Shinshu University Hospital: N. Arakura; Yodogawa Christian Hospital: A. Watanabe; Osaka Medical College Hospital: H. Takiuchi; Kitano Hospital: S. Yazumi; Sakai Municipal Hospital: H. Ohzato; Kawasaki Medical School Hospital: K. Yoshida; Onomichi General Hospital: K. Hanada; Kagawa University Hospital: F. Goda; Shikoku Cancer Center: H. Iguchi; Keio University Hospital: T. Hibi; Osaka City General Hospital: H. Nebiki; Chiba University Hospital: T. Ishihara; Nippon Medical School Hospital: E. Uchida; Tokai University Hospital: T. Imaizumi; Nagoya City University Hospital: H. Ohara; Aichi Cancer Center Aichi Hospital: H. Kojima; Osaka City University Hospital: N. Yamada; Wakayama Medical University Hospital: H. Yamaue; Tokyo Medical University Hospital: F. Moriyasu; Showa University Northern Yokohama Hospital: K. Shimada; Shizuoka General Hospital: K. Matsumura; Hyogo Cancer Center: H. Nishisaki; Kanazawa University Hospital: S. Yano; Hiroshima Prefectural Hospital: K. Shinozaki; University of Miyazaki Hospital: H. Inatsu; Linkou Chang Gung Memorial Hospital and Chang Gung University: Jen-Shi Chen; National Taiwan University Hospital: Chiun Hsu; Taipei Veterans General Hospital: Jin-Hwang Liu; Chang Gung Medical Foundation, Kaohsiung: Kun-Ming Rau; Chung-Ho Memorial Hospital, Kaohsiung Medical University: Sheng-Fung Lin; China Medical University Hospital: Chang-Fang Chiu; Mackay Memorial Hospital, Taipei: Ruey-Kuen Hsieh; Changhua Christian Hospital: Cheng-Shyong Chang; Chi Mei Medical Center, Yong Kang: Wei-Shou Huang; Chi Mei Medical Center, Liou Ying: Wen-Tsun Huang; National Cheng Kung University Hospital: Wu-Chou Su.

Details of Adequate Organ Functions in Enrollment Criteria and Main Exclusion Criteria

Adequate organ functions were defined as follows: leukocyte count $\geq$ 3,500/μL, neutrophil count $\geq$ 2,000/μL, platelet count $\geq$ 100,000/μL, hemoglobin level $\geq$ 9.0 g/dL, serum creatinine level $\leq$ 1.2 mg/dL, creatinine clearance $\geq$ 50 mL/min, serum AST and ALT levels $\leq$ 150 U/L, and serum total bilirubin level $\leq$ 2.0 mg/dL or $\leq$ 3.0 mg/dL if biliary drainage was performed.

Main exclusion criteria were as follows: pulmonary fibrosis or interstitial pneumonia; watery diarrhea; active infection; marked pleural effusion or ascites; and serious complications such as heart failure, peptic ulcer bleeding, or poorly controlled diabetes. Pancreatic cancers other than adenocarcinoma or adenosquamous carcinoma (eg, anaplastic carcinoma) were excluded from the study.

Dosage Adjustment Guideline for Toxicities

All treatment cycles were repeated until disease progression, unacceptable toxicity, or patient refusal. If patients had a leukocyte count of less than 2,000/μL, a neutrophil count of less than 1,000/μL, a platelet count of less than 70 $\times$ 10^3/μL, or grade 3 or worse rash, the administration of anticancer agents was postponed. S-1 was temporarily halted both in S-1 and in GS groups if patients had a creatinine level of 1.5 mg/dL or higher or grade 2 or worse diarrhea or stomatitis. Treatment was discontinued if these events did not resolve within 4 weeks after treatment suspension. In patients who experienced febrile neutropenia, grade 4 leukopenia, neutropenia, or thrombocytopenia or grade 3 or worse rash, the dose of gemcitabine was reduced by 200 mg/m^2. In patients with febrile neutropenia; grade 4

leukopenia, neutropenia, or thrombocytopenia; a creatinine level of 1.5 mg/dL or higher; or grade 3 or worse diarrhea, stomatitis, or rash, the dose of S-1 was reduced by 20 mg/d.

Sample Size Determination: Statistical Methods

In the initial plan, the total target number of patients was set at 600, given a statistical power of 80%, an enrollment period of 3 years, and a follow-up period of 2 years. However, because patient enrollment was faster than expected, the target number of patients was revised to 750 to provide the study with a statistical power of 90%. Consequently, the final analysis was performed after the occurrence of 680 events had been confirmed. An interim analysis was not performed. Although the actual median OS in the gemcitabine group was better than initially expected, because an adequate number of patients had been enrolled, a power of ≥ 90% was maintained on recalculation of the power on the basis of the actual results.

Quality of Life

To assess the quality of life, the health status of patients on the EQ-5D questionnaire was converted into a single simple utility index ranging from 0 for death to 1 for complete health. Quality-adjusted life-years (QALYs) for individual patients were estimated as the product of the utility index during follow-up and survival time and were compared between the groups, using the generalized Wilcoxon test.

As a result, median QALYs were 0.401 in the gemcitabine group, 0.420 in the S-1 group, and 0.525 in the GS group. The QALY value in the S-1 group was similar to that in the gemcitabine group, and there was no statistically significant difference between the two groups ($P = .56$). The QALY value in the GS group was significantly better than that in the gemcitabine group ($P < .001$). The details of quality-of-life assessments will be reported elsewhere.
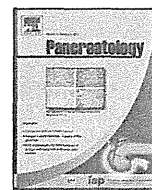
Original article

Treatment outcome for systemic chemotherapy for recurrent pancreatic cancer after postoperative adjuvant chemotherapy

Tomoko Katsui Taniyama [a], Chigusa Morizane [a,*], Kohei Nakachi [b], Satoshi Nara [c], Hideki Ueno [a], Shunsuke Kondo [a], Tomoo Kosuge [c], Kazuaki Shimada [c], Minoru Esaki [c], Masafumi Ikeda [b], Shuichi Mitsunaga [b], Taira Kinoshita [d], Masaru Konishi [d], Shinichiro Takahashi [d], Takuji Okusaka [a]

[a] Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
[b] Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital East, Chiba, Japan
[c] Hepatobiliary and Pancreatic Surgery Division, National Cancer Center Hospital, Tokyo, Japan
[d] Hepatobiliary and Pancreatic Surgery Division, National Cancer Center Hospital East, Chiba, Japan

ABSTRACT

Objectives: A global consensus on how to treat recurrent pancreatic cancer after adjuvant chemotherapy with gemcitabine (ADJ-GEM) does not exist.
Methods: We retrospectively reviewed the clinical data of 41 patients with recurrences who were subsequently treated with chemotherapy.
Results: The patients were divided into two groups according to the time until recurrence after the completion of ADJ-GEM (ADJ-Rec): patients with an ADJ-Rec < 6 months ($n = 25$) and those with an ADJ-Rec $\geq$ 6 months ($n = 16$). The disease control rate, the progression-free survival after treatment for recurrence and the overall survival after recurrence for these two groups were 68 and 94% ($P = 0.066$), 5.5 and 8.2 months ($P = 0.186$), and 13.7 and 19.8 months ($P = 0.009$), respectively. Furthermore, we divided the patients with an ADJ-Rec < 6 months into two groups: patients treated with gemcitabine ($n = 6$) and those treated with alternative regimens including fluoropyrimidine-containing regimens ($n = 19$) for recurrent disease. Patients treated with the alternative regimens had a better outcome than those treated with gemcitabine.
Conclusions: Fluoropyrimidine-containing regimens may be a reasonable strategy for recurrent disease after ADJ-GEM and an ADJ-Rec < 6 months.

1. Introduction

Pancreatic cancer patients have an extremely poor prognosis. Although surgical resection is the only curative treatment, only 15%–20% of patients are candidates for resection. Even if a curative resection is performed, the 5-year-survival rate is only 10%–25%, and the median survival period is 11–20 months [1,2].

Various adjuvant chemotherapy or chemoradiotherapy regimens after surgical resection have been evaluated [2–6]. Recently, The Charite' Onkologie (CONKO)-001 trial was designed to determine the benefits of gemcitabine for patients with resected

pancreatic cancer. Adjuvant chemotherapy with gemcitabine (ADJ-GEM) significantly improved the disease-free survival period, compared with surgery alone, in patients with resected pancreatic cancer. Although no significant difference in overall survival was seen at the time of publication, analysis after a longer follow-up period demonstrated a survival advantage for gemcitabine over observation-only (median progression-free survival, 22.8 months for ADJ-GEM vs. 20.2 months for observation-only; $P = 0.005$). At approximately the same time as the CONKO-001 trial, the Japanese Study Group of Adjuvant Therapy for Pancreatic Cancer (JSAP) conducted a randomized clinical trial evaluating adjuvant gemcitabine. Although no significant difference in overall survival was seen, the patients in the gemcitabine arm demonstrated a significantly longer disease-free survival period than the patients in the observation-only arm. These results were similar to those of the CONKO-001 trial and supported the concept that adjuvant chemotherapy using gemcitabine was effective in an Asian

* Corresponding author. Tel.: +81 3 3542 2511; fax: +81 3 3542 3815.
E-mail address: cmorizan@ncc.go.jp (C. Morizane).

population [2,5]. Therefore, adjuvant therapy using gemcitabine for resected pancreatic cancer is now firmly established as a therapy that offers a modest but real improvement in overall survival [5,7].

In approximately 50% of patients, recurrent disease was reportedly seen within a year, even after receiving ADJ-GEM [5], and no global consensus exists regarding treatment strategies for recurrent disease after ADJ-GEM. If the length of time from the completion of adjuvant therapy until the detection of recurrence is less than 6 months, the NCCN guidelines recommend alternative chemotherapy using a fluoropyrimidine-based chemotherapy regimen. When this period is 6 months or greater, they recommend an alternative regimen or the same regimen as the previous therapy [8]. However, these recommendations have not been substantiated by actual clinical data.

In Japan, the oral fluoropyrimidine derivative S-1 is often used as an alternative regimen for gemcitabine-refractory cases. S-1 showed a non-inferiority to gemcitabine in terms of overall survival in a phase III trial and is considered an alternative to gemcitabine for chemonaïve patients with advanced pancreatic cancer [9]. Additionally, in gemcitabine-refractory metastatic cases, a recent phase II study of S-1 yielded results that demonstrated preferable activity, including a response rate of 9.5%–15% and a median overall survival time of 4.5–6.3 months [10,11]. Therefore, S-1 is widely used for the treatment of advanced pancreatic cancer in first-line and second-line settings in Japan.

We studied the current status of treatments for recurrent pancreatic cancer after curative resection followed by ADJ-GEM. The objective of this study was to examine the adequacy of the

Table 1
Patient characteristics at resection (n = 41).

	Variables	n (%) All patients n = 41	ADJ-Rec < 6 months n = 25	ADJ-Rec ≥ 6 months n = 16	P value
Age (years)	Median (range)	65 (38–78)	64 (38–78)	65 (50–77)	0.96
Gender	Male	27 (66)	16 (64)	11 (69)	1.00
	Female	14 (34)	9 (36)	5 (31)	
PS[a] at recurrence	0	30 (73)	20 (80)	10 (63)	0.34
	1	5 (12)	3 (12)	2 (12)	
	Unknown	6 (15)	2 (8)	4 (25)	
Primary site	Head	26 (63)	17 (68)	9 (56)	0.51
	Body or -tail	15 (37)	8 (32)	7 (44)	
Type of Resection	PD[b]	26 (64)	17 (68)	9 (56)	0.66
	DP[c]	12 (29)	6 (24)	6 (38)	
	TP[d]	3 (7)	2 (8)	1 (6)	
Resection status	R0	36 (88)	22 (88)	14 (88)	1.00
	R1	5 (12)	3 (12)	2 (12)	
Histology	Adenocarcinoma	39 (95)	23 (92)	16 (100)	0.51
	Adenosquamous carcinoma	2 (5)	2 (8)	0 (0)	
Stage[e] at resection	IIA	5 (12)	0 (0)	5 (31)	0.006
	IIB	36 (88)	25 (100)	11 (69)	
CEA[f] (ng/mL)	Median (range)	2.7 (0.7–51.8)	2.7 (0.7–21.0)	2.4 (1.2–51.8)	0.98
CA19-9[g] (U/mL)	Median (range)	202 (0.5–6450)	212 (0.5–6450)	138 (17–3203)	0.56
Histological grade	Well	5 (12)	3 (12)	2 (12.5)	0.83
	Moderately	28 (71)	17 (68)	12 (75)	
	Poorly	7 (17)	5 (20)	2 (12.5)	
Lymph node ratio[h]	0	5 (12)	0 (0)	5 (31)	0.008
	0.1–0.199	23 (56)	14 (56)	9 (57)	
	0.2–0.299	8 (20)	7 (28)	1 (6)	
	0.3–	4 (10)	4 (16)	0 (0)	
	Unknown	1 (2)	0 (0)	1 (6)	
Recurrent pattern[i]	Locoregional	21 (51)	10 (40)	11 (69)	0.15
	Liver	18 (44)	14 (56)	4 (25)	
	Peritoneum	4 (10)	4 (16)	0 (0)	
	Lungs	11 (27)	7 (28)	4 (25)	
	Bones	1 (2)	1 (4)	0 (0)	
Cycles of ADJ-GEM	Median (range)	6 (3–9)	6 (3–6)	6 (3–9)	0.88
ADJ-Rec[j] (months)	Median (range)	3.7 (0.1–36.1)	1.3 (0.1–4.9)	11.5 (6.3–36.1)	
Chemotherapy[k]	GEM	21 (51)	6 (24)	15 (94)	0.00
	Alternatives[l]	20 (49)	19 (76)	1 (6)	
	(S1)	17 (41)	17 (68)	1 (6)	
	(GEM + S1)	1 (2)	0 (0)	0 (0)	
	(S1 + Radiation)	1 (2)	1 (4)	0 (0)	
	(S1 + oxaliplatin)	1 (2)	1 (4)	0 (0)	

[a] PS, performance status.
[b] PD, pancreaticoduodenectomy.
[c] DP, distal pancreatectomy.
[d] TP, total pancreatectomy.
[e] Stage, UICC 7th.
[f] CEA, carcinoembryonic antigen at resection.
[g] CA-19-9, carbohydrate antigen 19-9 at resection.
[h] Lymph node ratio, number of metastatic lymph nodes divided by number of examined nodes.
[i] Recurrent pattern, numbers of locoregional, extra-pancreatic, and combined recurrences were 11, 20, and 10 patients.
[j] ADJ-Rec, period between the last date of ADJ-GEM and recurrence.
[k] Chemotherapy, chemotherapy for recurrent disease after adjuvant chemotherapy.
[l] Alternatives, all alternative regimens consisted of fluoropyrimidine-containing regimens.

NCCN guidelines for recurrent pancreatic cancer after adjuvant chemotherapy, which recommend that the treatment options should be determined by the period between the last date of ADJ-GEM and recurrence (ADJ-Rec), with a threshold of 6 months.

2. Patients and methods

2.1. Patients

A retrospective review was conducted for 113 pancreatic cancer patients who underwent curative resection followed by ADJ-GEM at the National Cancer Center Hospital (NCCH) and NCCH East in Japan between April 2002 and October 2010. Forty-two patients with no recurrence after ADJ-GEM, 10 patients with withdrawal from ADJ-GEM within 2 cycles, 6 patients with recurrence during ADJ-GEM, and 14 patients who changed hospitals after recurrence were excluded. We finally retrieved the clinical data of 41 patients with recurrences who were subsequently treated with chemotherapy at our hospitals.

2.2. Treatment

After resection, we started ADJ-GEM within 10 weeks. An initial gemcitabine dose of 1000 mg/m^2 was administrated intravenously for 30 min on days 1, 8 and 15 every 4 weeks for 3 to 6 cycles, in principle. A computed tomography examination was performed every 3–6 months. Once evidence of recurrence was revealed, treatment for recurrent disease was initiated.

2.3. Data collection and evaluation of tumor response

The following data were collected from the medical records: patient characteristics at resection, the resection status, the ADJ-Rec, the treatment regimen, and the outcome of treatment after the recurrence. We also compared the treatment outcomes according to the length of the ADJ-Rec and the treatment regimens. Tumor responses were evaluated according to the RECIST criteria, Ver.1.1. We evaluated the best overall response and the disease control rate (DCR). The DCR was defined as the rate of complete response + partial response + stable disease. When the disease status was stably maintained for more than 8 weeks, the patient was considered to have stable disease.

2.4. Statistical analysis

The Fisher exact test was used to assess the hypothesis of independence between categorical variables. For quantitative data such as age and the carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) levels, we used the Mann–Whiney test. ADJ-Rec was defined as the period between the last date of the administration of ADJ-GEM and the date on which local or distant recurrence was noted. The date of recurrence was defined as the date of documentation of recurrent disease using diagnostic imaging techniques. Progression-free survival (PFS) was defined as the period between the start of treatment for recurrent disease and the date of progression, the last follow-up visit, or death from any cause. Overall survival after recurrence (r-OS) was defined as the period between the start of treatment for recurrent disease and death from any cause or the last follow-up. Patients who were lost to follow-up were treated as censored cases. Survival curves were estimated using the Kaplan–Meier method, and the significances were evaluated using a log-rank test. All the analyses were performed using Stata/SE, Version 11.1 (StataCorp, USA).

3. Results

3.1. Patient characteristics

The characteristics at resection of the 41 eligible patients are listed in Table 1. R0 resection (complete resection with no microscopic residual tumor) was performed in 36 patients (88%). Concerning the pathological stage, 5 (12%) of the patients had stage IIA disease and 36 (88%) had stage IIB. The sites of recurrence were locoregional (21 patients), the liver (18 patients), and the lung (11 patients). Patients with an ADJ-Rec ≥ 6 months (16 patients) had a significantly better status than patients with an ADJ-Rec < 6 months (25 patients) with regard to disease stage ($P = 0.006$) and the lymph node ratio (the number of metastatic lymph nodes divided by the number of examined nodes) ($P = 0.0075$). As for the treatments for recurrent disease, 21 patients were treated with gemcitabine monotherapy and 20 patients were treated with alternative regimens. All the alternative regimens were fluoropyrimidine-containing regimens (17 patients received S-1 and 1 patient each received GEM + S-1, S-1 + radiation, and S-1 + oxaliplatin). The treatment strategy after recurrence depended on each oncologist's plan, without a unified policy. Among the 25 patients with an ADJ-Rec < 6 months, 6 were treated with gemcitabine monotherapy and 19 were treated with alternative regimens. Among the 16 patients with an ADJ-Rec ≥ 6 months, 15 were treated with gemcitabine monotherapy and 1 was treated with an alternative regimen.

3.2. Treatment efficacy and survival analysis of treatments for recurrence

Overall, 2 of the 41 patients responded to the treatments for recurrent disease (4.9%; 2 partial responses; 95% confidence interval (95% CI), 0.60%–16.53%). The DCR was 78% (32 of the 41 patients; 95% CI, 62.39%–89.44%). The median PFS and median r-OS were 5.5 months (95% CI, 3.7–8.1 months) and 18.3 months (95% CI, 13–19.8 months), respectively (Fig. 1).

We divided the patients into two groups according to the length of the ADJ-Rec: patients with an ADJ-Rec < 6 months ($n = 25$), and patients with an ADJ-Rec ≥ 6 months ($n = 16$). The DCRs were 68% and 94% ($P = 0.066$), and the median PFS periods were 5.5 and 8.2 months ($P = 0.186$; Fig. 2A), respectively. The median r-OS of the patients with an ADJ-Rec < 6 months was significantly shorter than
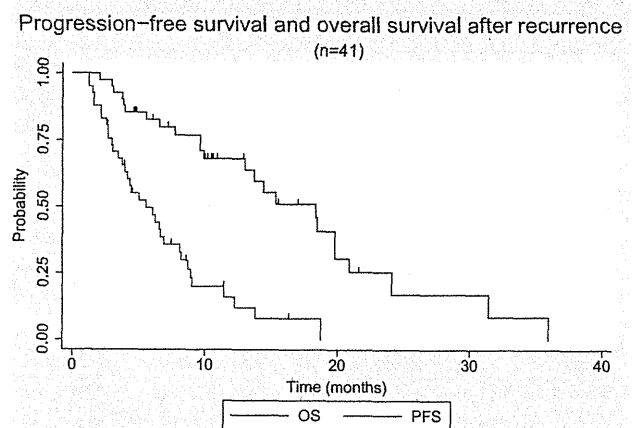

Fig. 1. Progression-free survival (PFS) and overall survival after recurrence (r-OS) in all patients ($n = 41$). The median PFS and r-OS were 5.5 and 18.3 months, respectively.
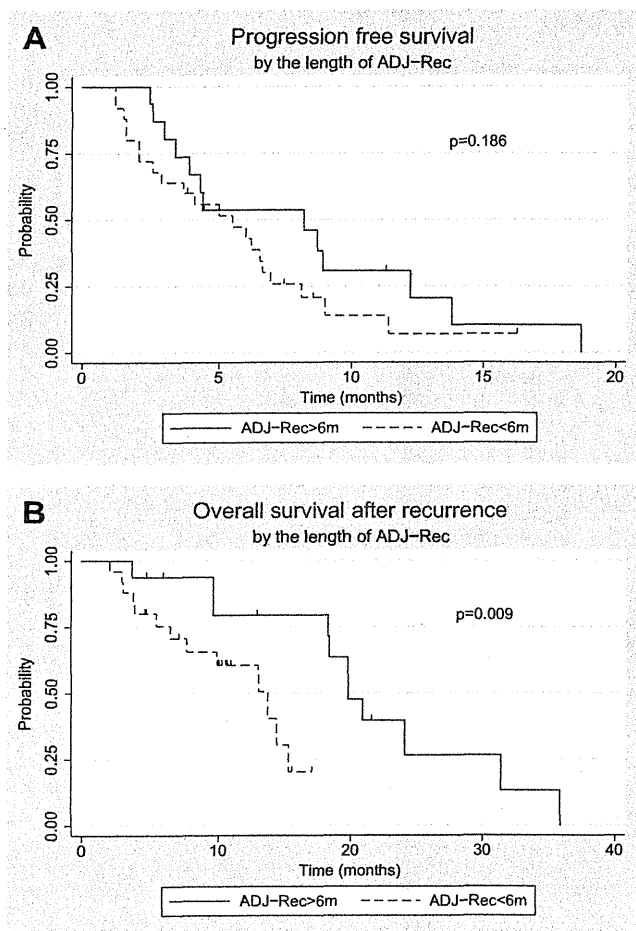
A

Progression free survival
by the length of ADJ–Rec

p=0.186

ADJ-Rec>6m ----- ADJ-Rec<6m

B

Overall survival after recurrence
by the length of ADJ–Rec

p=0.009

ADJ-Rec>6m ----- ADJ-Rec<6m

Fig. 2. Progression-free survival (PFS) and overall survival after recurrence (r-OS) according to the length of the ADJ-Rec: patients with an ADJ-Rec < 6 months (n = 25); and patients with an ADJ-Rec ≥ 6 months (n = 16). (A) The median PFS for each group was 5.5 and 8.2 months (P = 0.186), respectively. (B) The median r-OS was 13.7 and 19.8 months (P = 0.009), respectively.

that of the patients with an ADJ-Rec ≥ 6 months (13.7 and 19.8 months, P = 0.009; Fig. 2B).

Additionally, we divided the patients with an ADJ-Rec < 6 months into two groups according to the treatment regimens for recurrent disease: patients treated with gemcitabine (n = 6) and patients treated with alternative regimens (n = 19). The outcomes are shown in Table 2 and Fig. 3. For the patients treated with gemcitabine and those treated with alternative regimens, the DCR, median PFS and median r-OS were 67% and 68% (P = 0.651), 2.9 and

6.5 months (P = 0.065; Fig. 3A), and 7.7 and 13.0 months (P = 0.242; Fig. 3B), respectively.

4. Discussion

In this study, at first we examined the current status of the treatment strategy for pancreatic cancer patients with recurrence after adjuvant chemotherapy. Most patients with ADJ-Rec ≥ 6 months were placed on gemcitabine. Even for patients with an ADJ-Rec < 6 months, gemcitabine was resumed in 24% of these patients. Generally, patients who relapse within a short period after receiving adjuvant chemotherapy should be considered as being resistant to those drugs. The NCCN guidelines also recommend that the options for recurrent disease after adjuvant therapy should be assessed according to the ADJ-Rec. However, these guidelines are only the recommendation of the panel, and these strategies have not yet been substantiated by actual clinical data. In the case of ovarian cancer, a consensus based on actual clinical data exists with regard to the treatment strategy for relapsed disease. Patients who have relapsed within an interval of less than 6 months since the previous paclitaxel-plus-platinum chemotherapy should be considered as platinum resistant [12,13]. However, the chemosensitivity and the key drugs are quite different between pancreatic cancer and ovarian cancer. Therefore, actual clinical data for pancreatic cancer is needed.

The outcome of patients with a short ADJ-Rec was worse than that of the patients with a long ADJ-Rec. This finding suggests that patients with a long ADJ-Rec may owe their period of prolonged sensitivity to the adjuvant gemcitabine treatment, slow tumor growth, and a smaller quantity of residual tumor. Concerning advanced pancreatic cancer, similar findings have been reported in a previous study, which indicated that the progression-free survival period after first-line chemotherapy was an independent prognostic factor [14]. Additionally, patients with pathological stage IIA or a lymph node ratio of 0 had a long ADJ-Rec in the present study, possibly influencing the outcome. However, our results should be interpreted with caution because biases introduced by the different selection of treatment regimens between the two groups may exist.

Among the patients with an ADJ-Rec ≥ 6 months, we were unable to compare the treatment outcome according to regimens, since most of them (15 out of 16) received gemcitabine mono-therapy and seldom received alternative options such a fluoropyr-imidine-based regimens. In the present study, the patients treated with gemcitabine had a better DCR, PFS and r-OS than the metastatic or recurrent pancreatic cancer patients treated with gemcitabine in past studies [15,16]. Even after considering the possibility that an ADJ-Rec ≥ 6 months may be a good prognostic factor, these preferable outcomes suggest the appropriateness of a re-challenge with gemcitabine.

Among the patients with an ADJ-Rec < 6 months, patients receiving alternative regimens tended to have a better DCR, PFS,

Table 2
Outcomes of patients according to ADJ-Rec and treatment regimens.

ADJ-Rec	<6 months				≥6 months			
	All	GEM	Alternative	P value	All	GEM	Alternative	P value
n	25	6	19		16	15	1	
DCR (%)	68	67	68	1.00	94	93	(100)	1.00
95% CI	62.4–89.4	22.3–95.7	43.5–87.4		69.8–99.8	68.1–99.8	2.5–100	
Median PFS (m)	5.5	2.9	6.5	0.06	8.2	8.2	(12.2)	0.69
95% CI	2.6–6.6	1.5–	2.1–8.1		3.4–12.2	3.0–13.8		
Median r-OS(m)	13.7	7.7	13.0	0.24	19.8	20.9	(19.8)	0.67
95% CI	6.5–15.3	2.9–	6.5–		9.6–31.4	9.6–31.4		

ADJ-Rec, period between the last date of ADJ-GEM and recurrence; DCR, disease control rate; PFS, progression-free survival time; r-OS, survival time from recurrence; Alternative*, including S-1, GEM + S-1, S-1 + radiation, and S-1 + oxaliplatin.

and r-OS than those receiving gemcitabine monotherapy. Although the optimal ADJ-Rec threshold was not clarified, the present results support the recommendations of the NCCN guidelines, which recommend alternative regimens for patients with an ADJ-Rec < 6 months after previous treatment with gemcitabine. These findings suggest that a certain proportion of patients with a short ADJ-Rec may already have a gemcitabine-refractory status at the time of ADJ-GEM.

This study had some limitations. This study was a retrospective analysis with an insufficient sample size, and the treatment strategy after recurrence depended on each oncologist's plan, with no unified policy. Another limitation concerns the alternative treatment options after recurrence. The NCCN guidelines recommend alternative regimens as second-line therapies for metastatic disease. The recommended regimens consist of fluoropyrimidine-based therapies, such as 5-FU/leucovorin (LV)/oxaliplatin (Oxal) [17] or capecitabine/Oxal [18]. The CONKO-003 study revealed the survival advantage of 5-FU + LV + Oxal for gemcitabine-refractory pancreatic cancer. In Japan, these drugs have not yet been approved under the Japanese medical insurance system for the treatment of pancreatic cancer. S-1 monotherapy was mainly used as the alternative option in our study. Although S-1 demonstrated a non-inferiority to gemcitabine as a first-line treatment [8,9] and had a marginal activity as a second-line regimen for gemcitabine-refractory pancreatic cancer

[10,11], it has not been accepted as a global standard therapy for gemcitabine-refractory pancreatic cancer.

In conclusion, patients with an ADJ-Rec ≥ 6 months had a relatively favorable outcome when treated with a gemcitabine rechallenge. Among the patients with an ADJ-Rec < 6 months, those patients receiving alternative regimens tended to have a better DCR, PFS, and r-OS, compared with those receiving gemcitabine. As a result, our results did not deny the appropriateness of strategies outline in the NCCN guidelines. A well-designed prospective study with a sufficient sample size is needed to identify the optimal regimen for the treatment of recurrent pancreatic cancer after postoperative adjuvant chemotherapy.

Grant support

None declared.

Conflict of interest

Takuji Okusaka had research findings and honoraria to disclose from Taiho pharmaceutical co. and Eli Lilly Japan.

Hideki Ueno had honoraria to disclose from Taiho pharmaceutical co. and Eli Lilly Japan, and had a consultation or advisory relationship to disclose from Taiho phatmaceutical co.

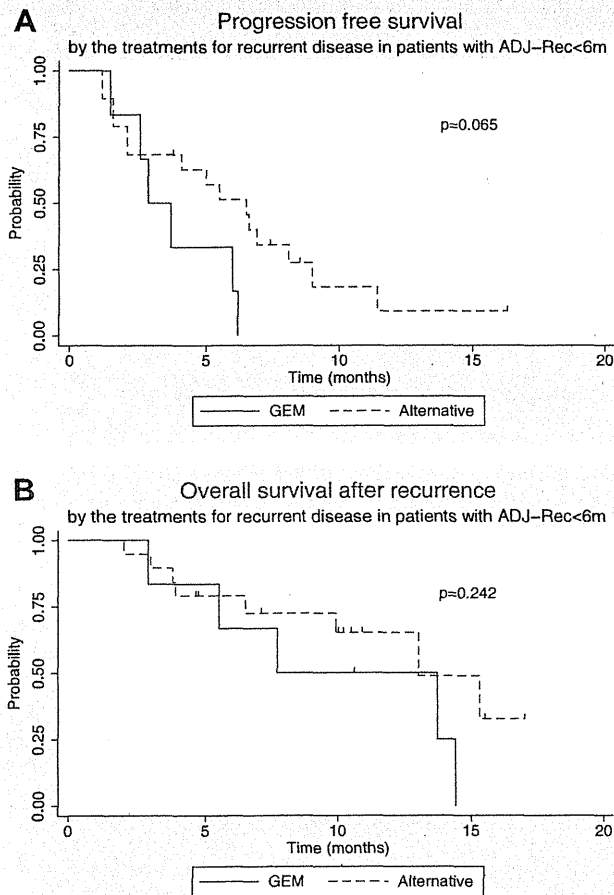Tomoo Kosuge had honoraria to disclose from Taiho pharmaceutical co. and Eli Lilly Japan.

Fig. 3. Progression-free survival (PFS) and overall survival after recurrence (r-OS) according to treatments for recurrent disease in patients with an ADJ-Rec < 6 months: patients treated with gemcitabine (n = 6), and patients treated with alternative regimens (n = 19). (A) The median PFS for each group was 2.9 and 6.5 months (P = 0.065), respectively. (B) The median r-OS was 7.7 and 13.0 months (P = 0.242), respectively.

References

[1] Yeo Charles J, Cameron JL, Lillemoe KD. Pancreaticoduodenectomy for cancer of the head of the pancreas. Ann Surg 1995;221:721–33.
[2] Oettle H, Post S, Neuhaus P, Gellert K, Langrehr J, Ridwelski K, et al. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. JAMA 2007;297:267–77.
[3] Kalser MH, Ellenberg SS. Pancreatic cancer. Adjuvant combined radiation and chemotherapy following curative resection. Arch Surg 1985;120:899–903.
[4] Neoptolemos JP, Stocken DD, Friess H, Bassi C, Dunn JA, Hickey H, et al. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. N Engl J Med 2004;350:1200–10.
[5] Ueno H, Kosuge T, Matsuyama Y, Yamamoto J, Nakao A, Egawa S, et al. 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. Br J Cancer 2009;101:908–15.
[6] Regine WF, Winter KA, Abrams RA, Safran H, Hoffman JP, Konski A, et al. Fluorouracil vs gemcitabine chemotherapy before and after fluorouracil-based chemoradiation following resection of pancreatic adenocarcinoma: a randomized controlled trial. JAMA 2008;299:1019–26.
[7] Riess H, Neuhaus P, Post S, Gellert K, Ridwelski K, Schramm H, et al. 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). Ann Oncol 2008;19:45–6.
[8] NCCN clinical practice guidelines in oncology (NCCN guidelines®). Available at: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp; December 7, 2011 [Accessed February 2012].
[9] Ioka T, Ikeda M, Ohkawa S, Yanagimoto H, Fukutomi A, Sugimori K, et al. Randomized phase III study of gemcitabine plus S-1 (GS) versus S-1 versus gemcitabine (GEM) in unresectable advanced pancreatic cancer (PC) in Japan and Taiwan: GEST study. J Clin Oncol 2011;29(suppl.). abstr 4007.
[10] Morizane C, Okusaka T, Furuse J, Ishii H, Ueno H, Ikeda M, et al. A phase II study of S-1 in gemcitabine-refractory metastatic pancreatic cancer. Cancer Chemother Pharmacol 2009;63:313–9.
[11] Sudo K, Yamaguchi T, Nakamura K, Denda T, Hara T, Ishihara T, et al. Phase II study of S-1 in patients with gemcitabine-resistant advanced pancreatic cancer. Cancer Chemother Pharmacol 2011;67:249–54.
[12] Harries M, Gore M. Part II: chemotherapy for epithelial ovarian cancer-treatment of recurrent disease. Lancet Oncol 2002;3:537–45.
[13] Parmar MK, Ledermann JA, Colombo N, du Bois A, Delaloye JF, Kristensen GB, et al. Paclitaxel plus platinum-based chemotherapy versus conventional platinum-based chemotherapy in women with relapsed ovarian cancer: the ICON4/AGO-OVAR-2.2 trial. Lancet 2003;361:2099–106.
[14] Reni M, Berardi R, Mambrini A, Pasetto L, Cereda S, Ferrari VD, et al. A multi-centre retrospective review of second-line therapy in advanced pancreatic adenocarcinoma. Cancer Chemother Pharmacol 2008;62:673–8.
[15] Van Cutsem E, van de Velde H, Karasek P, Oettle H, Vervenne WL, Szawlowski A, et al. Phase III trial of gemcitabine plus tipifarnib compared

with gemcitabine plus placebo in advanced pancreatic cancer. J Clin Oncol 2004;22:1430–8.

[16] Hashimoto K, Ueno H, Ikeda M, Kojima Y, Hagihara A, Kondo S, et al. Do recurrent and metastatic pancreatic cancer patients have the same outcomes with gemcitabine treatment? Oncology 2009;77: 217–23.

[17] Pelzer U, Stieler J, Schwaner I, Heil G, Seraphin J, Gorner M, et al. Results of the conko 003 trial. A randomized second line trial in patients with gemcitabine refractory advanced pancreatic cancer. Onkologie 2008;31:98.

[18] Xiong HQ, Varadhachary GR, Blais JC, Hess KR, Abbruzzese JL, Wolff RA. Phase 2 trial of oxaliplatin plus capecitabine (XELOX) as second-line therapy for patients with advanced pancreatic cancer. Cancer 2008;113:2046–52.

Phase I/II study of gemcitabine as a fixed dose rate infusion and S-1 combination therapy (FGS) in gemcitabine-refractory pancreatic cancer patients

Chigusa Morizane · Takuji Okusaka · Hideki Ueno · Shunsuke Kondo · Masafumi Ikeda ·
Junji Furuse · Ohkawa Shinichi · Kohei Nakachi · Shuichi Mitsunaga · Yasushi Kojima ·
Eiichiro Suzuki · Makoto Ueno · Tomohiro Yamaguchi

Abstract

Purpose There is no standard regimen for gemcitabine
(Gem)-refractory pancreatic cancer (PC) patients. In a
previous phase II trial, S-1 was found to exhibit marginal
efficacy. Gem administration by fixed dose rate infusion of
10 mg/m^2/min (FDR-Gem) should maximize the rate of
intracellular accumulation of gemcitabine triphosphate and
might improve clinical efficacy. We conducted the phase
I/II of FDR-Gem and S-1 (FGS) in patients with Gem-
refractory PC.
Methods The patients received FDR-Gem on day 1 and
S-1 orally twice daily on days 1–7. Cycles were repeated
every 14 days. Patients were scheduled to receive Gem
(mg/m^2/week) and S-1 (mg/m^2/day) at four dose levels in
the phase I: 800/80 (level 1), 1,000/80 (level 2), 1,200/80
(level 3) and 1,200/100 (level 4). Forty patients were
enrolled in the phase II study at recommended dose.
Results The recommended dose was the level 3. In the
phase II, a partial response has been confirmed in seven
patients (18%). The median overall survival time and
median progression-free survival time are 7.0 and
2.8 months, respectively. The common adverse reactions
were anorexia, leukocytopenia and neutropenia.
Conclusion This combination regimen of FGS is active
and well tolerated in patients with Gem-refractory PC.

Keywords Chemotherapy · Pancreatic carcinoma ·
Second-line · Gemcitabine · S-1 · Salvage · Fixed dose rate
infusion

C. Morizane (✉) · T. Okusaka · H. Ueno · S. Kondo ·
T. Yamaguchi
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

M. Ikeda · K. Nakachi · S. Mitsunaga · Y. Kojima
Division of Hepatobiliary and Pancreatic Oncology,
National Cancer Center Hospital, East, Kashiwa, Japan

J. Furuse · E. Suzuki
Division of Medical Oncology,
Kyorin University School of Medicine, Tokyo, Japan

O. Shinichi · M. Ueno
Division of Hepatobiliary and Pancreatic Oncology,
Kanagawa Cancer Center, Yokohama, Japan

Introduction

Gemcitabine monotherapy or gemcitabine-containing
combination chemotherapy is the standard first-line therapy
for advanced pancreatic cancer. In the recent phase III
study, the first-line FOLFIRINOX regimen (5-fluorouracil,
leucovorin, irinotecan and oxaliplatin) led to a median
survival of 11.1 months compared with 6.8 months in the
gemcitabine group [4]. However, the FOLFIRINOX regi-
men was quite toxic (e.g., 5.4% of patients had grade 3 or 4
febrile neutropenia), and a survival benefit was shown only
among a highly select population with a good performance
status, an age of 75 years or younger, and normal or nearly
normal bilirubin levels [13]. Therefore, this combination
therapy was considered to be one of the treatment options
for patients in good general condition, and gemcitabine
remains the mainstay of care for patients with advanced
pancreatic cancer. However, after disease progression
during first-line gemcitabine-containing chemotherapy, the

options for further anticancer treatment are limited. S-1 is an orally administered anticancer drug that consists of a combination of tegafur, 5-chloro-2,4-dihydroxypyridine and oteracil potassium in a 1 : 0.4 : 1 molar ratio [27]. The antitumor effect of S-1 has already been demonstrated in a variety of solid tumors including pancreatic cancer [7, 11, 12, 14, 20, 21, 25, 26, 32, 33]. In patients with chemo-naïve pancreatic cancer, an overall response rate of 21.1% was achieved, and the median time-to-progression and median overall survival period were 3.7 and 8.3 months, respectively [32]. In gemcitabine-refractory metastatic pancreatic cancer, our recent phase II study of S-1 yielded results that demonstrated marginal activity including a response rate of 15%, a median progression-free survival time of 2.0 months and a median overall survival time of 4.5 months, with a favorable toxicity profile [17]. In addition, other reports also demonstrated marginal antitumor activity [1, 28]. Gemcitabine administration via infusion at a fixed dose rate of 10 $mg/m^2/min$ (FDR-Gem) has been found to increase the intracellular drug concentrations, compared with gemcitabine at a standard dose rate infusion over a period of 30 min. A recent phase II study of combination therapy consisting of FDR-Gem and oxaliplatin (GEMOX) yielded results that demonstrated activity in gemcitabine-refractory advanced pancreatic cancer [5], although oxaliplatin is inactive against pancreatic cancer when used as a single agent [6]. The increased intracellular concentrations of gemcitabine as a result of FDR infusion and/or the synergistic effect of gemcitabine and oxaliplatin may play an important role in the antitumor effect of GEMOX. This finding is of interest when considering the effect of combination therapy consisting of FDR-Gem and some other agent that exhibits a synergistic effect with gemcitabine in patients with metastatic pancreatic cancer who failed standard dose rate gemcitabine.

The inhibition of ribonucleotide reductase by gemcitabine is considered to enhance the effect of the 5-FU metabolite 5-FdUMP by reducing the concentration of its physiological competitor [10]. Preclinical studies have demonstrated a synergy between gemcitabine and 5-FU in tumor cell lines, including pancreatic cancer cells [3, 23]. S-1 is a fluoropyrimidine, and several phase II studies of S-1 and gemcitabine combination therapy have yielded results that demonstrated a promising activity in chemo-naïve advanced pancreatic cancer patients, including a response rate of 32–48% and a median survival times of 7.89–12.5 months [16, 18, 19, 31].

Therefore, we conducted the present phase I/II study to determine the recommended doses of FDR-Gem and S-1 (FGS) to use for combination therapy and to evaluate the toxicity and efficacy at the recommended doses in patients with gemcitabine-refractory pancreatic cancer.

Materials and methods

Eligibility criteria

The eligibility criteria were histologically proven pancreatic adenocarcinoma with measurable metastatic lesions, disease progression during gemcitabine-based first-line chemotherapy, age 20 years or over, ECOG performance status of 0–2 points, more than 2-week interval between the final dose of the prior chemotherapy regimen and study entry, adequate bone marrow function (leukocyte count $\geq$ 3,500/mm^3, neutrophil count $\geq$ 1,500/mm^3, platelet count $\geq$ 100,000/mm^3, hemoglobin concentration $\geq$ 9.0 g/dL), adequate renal function (serum creatinine level $\leq$ 1.1 mg/dL) and adequate liver function (serum total bilirubin level $\leq$ 2.0 mg/dL, transaminase levels $\leq$ 100 U/L). Patients with obstructive jaundice or liver metastasis were considered eligible if their total bilirubin level $\leq$ 3.0 mg/dL and transaminase levels could be reduced to 150 U/L by biliary drainage. The exclusion criteria were regular use of phenytoin, warfarin or flucytosine, history of fluorinated pyrimidine use, severe mental disorder, active infection, ileus, watery diarrhea, interstitial pneumonitis or pulmonary fibrosis, refractory diabetes mellitus, heart failure, renal failure, active gastric or duodenal ulcer, massive pleural or abdominal effusion, brain metastasis, and 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 of the National Cancer Center of Japan.

Treatment

Considering the patients' quality of life, we adopted biweekly schedule. Gemcitabine (Eli Lilly Japan K.K., Kobe, Japan) was administered by FDR intravenous infusion of 10 $mg/m^2/min$ on day 1. S-1 (Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) was administered orally twice daily on day 1 to day 7, followed by a 1-week rest. Treatment cycles were repeated every 2 weeks until disease progression or unacceptable toxicity occurred. If blood examination revealed leukocytopenia < 2,000/mm^3, thrombocytopenia < 75,000/mm^3, total bilirubin > 3.0 mg/dL, aspartate aminotransferase or alanine aminotransferase level > 150 U/L, or creatinine > 1.5 mg/dL, both gemcitabine and S-1 were withheld until recovery. If a patient experienced dose-limiting toxicity (DLT), the dose of gemcitabine and S-1 was reduced by one level in the subsequent cycle. If a rest period of more than 15 days was required because of toxicity, the patient was withdrawn from the study. Patients were scheduled to receive gemcitabine and S-1 at four dosage levels (Table 1). Two dosage levels of S-1 were established according to the body

Table 1 Dosage levels of gemcitabine and S-1

Dosage level	Gemcitabine	S-1
Level 0	600 mg/m^2/60 min	Dosage A
Level 1[a]	800 mg/m^2/80 min	Dosage A
Level 2	1,000 mg/m^2/100 min	Dosage A
Level 3	1,200 mg/m^2/120 min	Dosage A
Level 4	1,200 mg/m^2/120 min	Dosage B

[a] Starting dosage

surface area as dosage A, about 80 mg/m^2/day, and dosage B, about 100 mg/m^2/day (Table 2). At the first dose level (level 1), gemcitabine was administered at a dosage of 800 mg/m^2 administered as a 80-min infusion, and S-1 was administered at dosage A. At the next dose level (level 2), the gemcitabine dosage was increased to 1,000 mg/m^2 administered as a 100-min infusion, and S-1 was administered at the same dosage. At the next dose level (level 3), the gemcitabine dosage was increased to 1,200 mg/m^2 administered as a 120-min infusion, and S-1 was administered at the same dosage. At the final dosage level (level 4), gemcitabine administered at the same dosage, and S-1 was administered at dosage B.

Study design

This study was an open-label, four-center, single-arm phase I/II study performed in two steps. The objective of step 1 (phase I) was to evaluate the frequency of DLT during first 2 cycles (4 weeks) and then use the frequency of DLT to determine which of the four dosages tested to recommend (Table 1). At least 3 patients were enrolled at each dosage level. If DLT was observed in the initial three patients, up to three additional patients were entered at the same dosage level. The highest dosage level that did not cause DLT in 3 of the 3 or ≥3 of the 6 patients treated at that level during the first two cycles of treatment was considered the maximum-tolerated dosage (MTD). DLT was defined as (1) grade 4 leucopenia or grade 4 neutropenia or febrile neutropenia, (2) grade 4 thrombocytopenia or thrombocytopenia requiring transfusion, (3) grade 3 or 4 non-hematological toxicity excluding hyperglycemia and electrolyte disturbances, (4) serum transaminases levels, γ-glutamyl

Table 2 Dosage of S-1 (tegafur equivalent)

Body surface area (m^2)	Dosage A (≒80 mg/m^2/day)	Dosage B (≒100 mg/m^2/day)
<1.25	40 mg × 2/day	50 mg × 2/day
1.25–<1.5	50 mg × 2/day	60 mg × 2/day
≥1.5	60 mg × 2/day	75 mg × 2/day

transpeptidase level and alkaline phosphatase level ≥10 times UNL, (5) serum creatinine level ≥ 2.0 mg/dL and (6) any toxicity that necessitated a treatment delay of more than 15 days. Toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. In step 2, the recommended dosages (RD) of FGS were then administered, and the effect of this combination therapy on objective tumor response was evaluated in patients who were given the RD (phase II). The number of patients to be enrolled in phase II was determined by using a SWOG's standard design (attained design) [8, 9]. The phase II included the patients who received the RD in the step 1. The null hypothesis was that the overall response rate would be ≤5%, and the alternative hypothesis was that the overall response rate would be ≥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 in previous reports [5, 15, 24, 30, 34]. 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, an additional 20 patients were to be included in a second stage of accrual to more precisely estimate the actual response rate. If the number of objective responses after completing the trial was 5 or more among the 40 patients, then we would reject the null hypothesis and conclude that FGS was effective, and we would proceed to the next large-scale study. The severity of adverse events and progression-free survival and overall survival were investigated as secondary objectives in phase II.

Results

Patient characteristics

Between June 2006 and March 2009, 49 patients were enrolled in this study. Fifteen patients (level 1: 3 patients, level 2: 3 patients, level 3: 6 patients, level 4: 3 patients) were enrolled into the phase I (STEP 1), and an additional 34 patients were enrolled into the phase II (STEP2) at dose level 3. Table 3 shows the baseline characteristics of the patients in step 1 and step 2. A total of the 40 patients who were given the recommended dose, 6 patients and 34 patients who entered into the study at phase I and phase II, respectively, were evaluated for efficacy and detailed safety profile.

Phase I (STEP 1)

No DLT occurred during the first 2 cycles (4 weeks) at level 1 or level 2. At dose level 3, three patients were

Table 3 Patient characteristics

Characteristic	Step 1				Step 2	Total at the recommended dose (level 3)
	Level 1	Level 2	Level 3	Level 4	Level 3	
No. of patients	3	3	6	3	34	40
Age, years						
Median	66	58	64	62	63.5	64
Range	55–69	51–58	48–71	52–70	40–80	40–80
Sex, n (%)						
Male	1 (33)	3 (100)	4 (67)	1 (33)	19 (56)	23 (58)
Female	2 (67)	0	2 (33)	2 (67)	15 (44)	17 (48)
ECOG performance status, n (%)						
0	2 (67)	2 (67)	5 (83)	2 (67)	22 (65)	27 (68)
1	1 (33)	1 (33)	1 (17)	1 (33)	12 (35)	13 (33)
Primary tumor, n (%)						
Head	1 (33)	2 (67)	2 (33)	2 (67)	17 (50)	19 (48)
Body/tail	2 (67)	1 (33)	4 (67)	1 (33)	17 (50)	21 (53)
Metastatic site, n (%)						
Liver	3 (100)	3 (100)	6 (100)	1 (33)	25 (74)	31 (78)
Lung	1 (33)	0	0	2 (67)	7 (21)	7 (18)
Peritoneum	1 (33)	1 (33)	0	1 (33)	11 (32)	11 (28)
Lymph node	0	2 (67)	0	0	11 (32)	11 (28)
Tumor stage at the start of prior treatment, n (%)						
Locally advanced	0	0	0	1 (33)	7 (21)	7 (18)
Metastatic	3 (100)	3 (100)	6 (100)	2 (67)	27 (79)	33 (83)
Prior treatment, n (%)						
Gemcitabine alone	3 (100)	3 (100)	5 (83)	3 (100)	26 (76)	31 (78)
Gem + Axitinib	0	0	0	0	2 (6)	2 (5)
Gem + Erlotinib	0	0	1 (17)	0	6 (18)	7 (18)

evaluated first, and none developed DLT. Since all 3 patients experienced DLT at dose level 4 (grade 4 neutropenia in two patients, grade 3 stomatitis in one patient), 3 additional patients were evaluated at dose level 3. A DLT (grade 4 neutropenia) was experienced by 2 of the 3 patients in this additional cohort in dose level 3, and dose level 3 was determined to be the MTD. Based on these results, the RD was determined to be level 3.

Phase II (efficacy and safety profile in the 40 patients treated at dose level 3)

In step 2, the RD of FDR-Gem and S-1 was administered to an additional 34 patients, and a total 40 patients were treated at dose level 3 to evaluate the objective tumor response to this combination therapy. As of the date of the analysis, the protocol treatment had been concluded in 39 of the 40 patients, and a total of 286 courses (median: 5 courses; range 1–31 courses) had been administered at level 3. The actual mean weekly dose administered were gemcitabine 545 mg/m^2/week (90.8% of planned dosage)

and 90.1% of planned dosage of S-1. Dose reduction was required in 10 patients because of grade 4 neutropenia (five patients), grade 3 fatigue (1 patient), grade 2 fatigue with grade 2 appetite loss (one patient), grade 2 nausea (two patients) and grade 3 rash (1). The reasons for treatment discontinuation in phase II were radiological disease progression (33 patients), clinical disease progression (two patients), recurrent grade 4 neutropenia despite dose reduction due to grade 4 neutropenia (two patients), grade 4 myocardial infarction (one patients) and patient request to return to his distant hometown (one patient). All patients who discontinued treatment because of adverse events recovered from the toxicities after discontinuation. Twelve patients received third-line chemotherapy after discontinuation of FGS: S-1 monotherapy in four patients, gemcitabine + S-1 combination therapy on another treatment schedule in three patients, chemoradiotherapy with S-1 in one patient and new molecularly targeted agents in four patients who participated in a different clinical trial. Twenty-two patients received best supportive care, the other five patients transferred to another hospital, and no

information is available about their treatment after discontinuation of FGS.

Toxicity

All patients in steps 1 and 2 were evaluated for toxicity. In step 1, grade 3/4 non-hematological toxicity was observed in two patients (grade 3 fatigue during the third course in one patient, grade 3 stomatitis during the second course in one patient). No grade 4 leukocytopenia was observed at any dose level, but grade 4 neutropenia was observed in one out of three patients at dose level 1, none of the three patients at dose level 2, two of the six patients at dose level 3 and all three of the patients at dose level 4. Grade 3 thrombocytopenia was observed in one patient at dose level 2.

Table 4 summarizes the toxicities in the 40 patients who received the RD (level 3). All 40 eligible patients were assessable for toxicities, and FGS combination therapy at the RD was generally well tolerated. The most common

toxicities were leukocytopenia (60%) and neutropenia (60%), but most of these toxicities were tolerable and reversible. Grade 4 neutropenia was noted as hematological toxicity in five patients (13%). Grade 3 non-hematological toxicities consisted of fatigue (one patient), vomiting (one patient), rash (one patient) and liver abscess (one patient). The patient who developed the grade 3 liver abscesses recovered after appropriate treatment with intravenous antibiotic alone. One female patient, who had hypercholesterolemia and history of smoking of 30 cigarettes/day, experienced a grade 4 acute myocardial infarction on day 1 of the third course of treatment, after gemcitabine had been administered but before the start of oral S-1. Emergency coronary angiography showed total occlusion of the left anterior descending coronary artery. The patient recovered from the cardiogenic shock due to myocardial infarction after coronary stent implantation and appropriate supportive treatment. S-1 monotherapy for the pancreatic cancer was started about 1 month after the infarction. No other severe or unexpected toxicities were noted in any of the patients.

Table 4 Treatment-related adverse events among the 40 patients who received the recommended dosages: highest grade reported during the treatment period

	Grade				Grade 1–4	Grade 3–4
	n					
	1	2	3	4	n (%)	n (%)
Hematological toxicities						
Leukocytes	11	4	9	0	24 (60)	9 (23)
Neutrophils	10	1	8	5	24 (60)	13 (33)
Hemoglobin	5	11	1	0	17 (43)	1 (3)
Platelets	11	2	1	0	14 (35)	1 (3)
Non-hematological toxicities					(0)	
Aspartate aminotransferase	8	1	0	0	9 (23)	0 (0)
Alanine aminotransferase	8	3	0	0	11 (28)	0 (0)
Alkaline phosphatase	5	2	0	0	7 (18)	0 (0)
Total bilirubin	3	0	0	0	3 (8)	0 (0)
Fatigue	15	2	1	0	18 (45)	1 (3)
Nausea	13	4	0	0	17 (43)	0 (0)
Vomiting	8	1	1	0	10 (25)	1 (3)
Anorexia	19	6	0	0	27 (68)	0 (0)
Stomatitis	4	0	0	0	4 (10)	0 (0)
Alopecia	8	0	–	–	8 (20)	–
Diarrhea	7	2	0	0	9 (23)	0 (0)
Rash	3	4	1	0	8 (20)	1 (3)
Hyperpigmentation	9	1	–	–	10 (25)	–
Hand-foot skin reaction	1	2	0	0	3 (8)	0 (0)
Watery eye	2	0	0	–	2 (5)	0 (0)
Hoarseness	1	0	0	0	1 (3)	0 (0)
Infection liver abscess	0	0	1	0	1 (3)	1 (3)
Myocardial infarction	0	0	0	1	1 (3)	1 (3)

Three patients died within 30 days after the final dose of the study drug. All 3 of the deaths were attributed to disease progression, and there were no treatment-related deaths.

Efficacy

It was possible to assess all 40 eligible patients who received the RD for response. Thirty-four patients had died by the completion of the follow-up period. There were no complete responses, but a partial response was achieved in seven patients (18, 95% confidence interval, 7.3–32.8%). Stable disease was noted in 19 patients (48%) and progressive disease in 14 patients (35%). Tumor responses to second-line FGS therapy are classified according to the tumor responses to first-line gemcitabine in Table 5. Three of 10 patients whose best response was progression disease in first-line chemotherapy achieved partial response in FGS therapy. The median progression-free survival time was 2.8 months. The median overall survival time after the start of second-line therapy was 7.0 months (range 1.3–18.9+),

Table 5 Objective tumor response

Response (2nd line)	n (%)	Response (1st line)		
		PR	SD	PD
PR	7 (18)	1	3	3
SD	19 (48)	3	12	4
PD	14 (35)	2	9	3
Total	40 (100)	6	24	10

Response rate: 18% (95% CI: 7.3–32.8)
RECIST criteria

—— Median OS (after second-line chemotherapy): 7.0 months
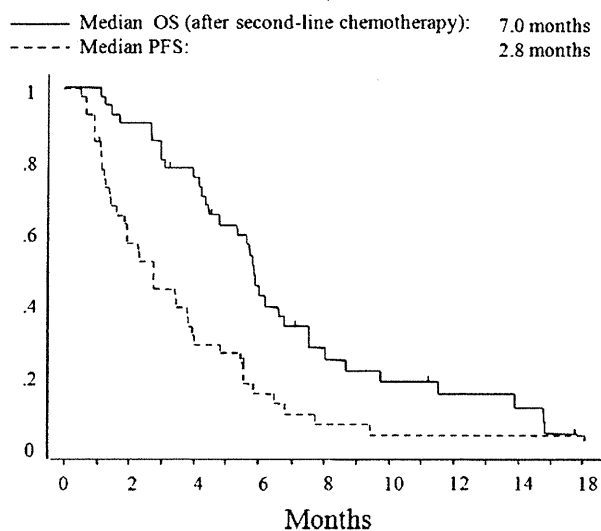----- Median PFS: 2.8 months

Fig. 1 Survival curves. Survival (n = 40). Progression-free survival (*dashed line*) and overall survival time (*solid line*) curves of patients with gemcitabine-refractory pancreatic cancer receiving systemic chemotherapy with FGS

and the 1-year survival rate was 18% (Fig. 1). The median overall survival time after the start of first-line therapy was 13.9 months (range 5.2–31.4).

Discussion

In the last decade, several clinical trials (mainly phase II) have been conducted in patients with advanced pancreatic cancer after failure of first-line gemcitabine or a gemcitabine-based combination regimen. The results of a randomized trial (n = 168) comparing fluorouracil and folinic acid versus oxaliplatin, fluorouracil and folinic acid (OFF) indicated that OFF improved progression-free survival and overall survival as a second-line chemotherapy. The median progression-free survival time and median survival time of OFF were 3 and 6 months, respectively [22]. In the present study, FGS yielded a median progression-free survival time of 2.8 months and a median overall survival time of 7.0 months, similar to the data mentioned above. Furthermore, the response rate of 18% in the present study was above the pre-established boundary (objective response in five or more of the 40 patients) required for the regimen to be considered effective. However, the gap between the median overall survival time and the median progression-free survival time in the present study was relatively large. Although the reason for this gap is unknown, a bias arising from the selection of patients with a good general condition or with a small tumor burden may explain these findings.

Whether gemcitabine as an FDR infusion is active even after progression during treatment with the standard 30-min administration of gemcitabine was the critical clinical question examined in this study. Differentiating between the relative roles of gemcitabine and S-1 in overcoming tumor resistance is difficult. The efficacy and survival data obtained in the present study seem to be better than those of previous studies for oral fluoropyrimidine monotherapy as a salvage chemotherapy for advanced pancreatic carcinoma (Table 6) [1, 2, 17, 28, 29]. However, since all the data were obtained in single-arm studies, a randomized study is needed to make these suggestions reliable. Furthermore, whether the combined regimen in the present study is superior to other regimens, such as the OFF regimen, remains an essential clinical question.

Safety and convenience as well as antitumor efficacy are critically important issues with regard to second-line chemotherapy. One patient experienced an acute myocardial infarction. Although she had other risk factors, such as a smoking habit and hyperlipidemia, a relation between gemcitabine and the acute myocardial infarction cannot be ruled out because gemcitabine had been administered on the day of the infarction. The toxicity profile of FGS

Table 6 Comparison between the current study and previous studies of oral fluoropyrimidine monotherapy as salvage chemotherapy for advanced pancreatic carcinoma

Study	References	Phase	Regimen	n	PR + CR (%)	Median PFS (months)	Median OS (months)
Morizane et al.	[12]	II	S-1	40	15	2.0	4.5
Abbruzzese et al.	[29]	II	S-1	45	0	1.4	3.1
Sudo et al.	[31]	II	S-1	21	9.5	4.1	6.3
Todaka et al.	[32]	Retrospective	S-1	52	4	2.1	5.8
Boeck et al.	[30]	II	Capecitabine	39	0	2.3	7.6
Morizane et al.	Current study	II	FGS	40	18	2.8	7.0

therapy in the other patients was acceptable, and the most common grade 1–4 adverse reactions were anorexia (68%), leukocytopenia (60%) and neutropenia (60%), although most episodes were tolerable and reversible. The safety profile in this study suggests that FGS can be safely administered to pancreatic cancer patients even in a second-line setting, at least in select populations. The biweekly schedule allows enough time to recover from myelosuppression and non-hematological toxicities before the following cycle, enabling patients to receive treatment as scheduled. Actually, the relative dose intensities of gemcitabine and S-1 in our study were high (90.8 and 90.1%, respectively). Furthermore, because of the biweekly schedule, patients do not need to come to the hospital for treatment as often compared with the first-line standard schedule of gemcitabine therapy. Our new treatment schedule may therefore improve the patients' quality of life during anticancer treatment.

We concluded that combination therapy consisting of gemcitabine as a fixed dose rate infusion and S-1 (FGS) provided a promising antitumor activity and tolerable toxicity in patients with gemcitabine-refractory metastatic pancreatic cancer. A larger randomized controlled trial is needed to confirm the clinical benefits of FGS following gemcitabine failure.

References

1. Abbruzzese JL, Lenz H, Hanna W, Kindler HL, Scullin D, Nemunaitis J, Kudva G, Zhang J, Zergebel C, Urrea P (2009) Open-label phase II study of S-1 as second-line therapy for patients with metastatic pancreatic cancer. 2009 Gastrointestinal Cancers Symposium Abstract No: 243
2. Boeck S, Wilkowski R, Bruns CJ, Issels RD, Schulz C, Moosmann N, Laessig D, Haas M, Golf A, Heinemann V (2007) Oral capecitabine in gemcitabine-pretreated patients with advanced pancreatic cancer. Oncology 73:221–227
3. Bruckner HW, Zhou G, Haenel P, Szraijer L, Greenspan E, Kurbacher CM (1998) Ex vivo ATP tumor testing of gemcitabine for combination chemotherapy and biochemical modulation. Proc Am Assoc Cancer Res 39
4. Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R, Becouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardiere C, Bennouna J, Bachet JB, Khemissa-Akouz F, Pere-Verge D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M (2011) FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 364:1817–1825
5. Demols A, Peeters M, Polus M, Marechal R, Gay F, Monsaert E, Hendlisz A, Van Laethem JL (2006) Gemcitabine and oxaliplatin (GEMOX) in gemcitabine refractory advanced pancreatic adenocarcinoma: a phase II study. Br J Cancer 94:481–485
6. Ducreux M, Mitry E, Ould-Kaci M, Boige V, Seitz JF, Bugat R, Breau JL, Bouche O, Etienne PL, Tigaud JM, Morvan F, Cvitkovic E, Rougier P (2004) Randomized phase II study evaluating oxaliplatin alone, oxaliplatin combined with infusional 5-FU, and infusional 5-FU alone in advanced pancreatic carcinoma patients. Ann Oncol 15:467–473
7. Furuse J, Okusaka T, Boku N, Ohkawa S, Sawaki A, Masumoto T, Funakoshi A (2008) S-1 monotherapy as first-line treatment in patients with advanced biliary tract cancer: a multicenter phase II study. Cancer Chemother Pharmacol 62:849–855
8. Green SJ, Benedetti J, Crowley J (1997) Clinical trials in oncology, 2nd edn. Chapman and Hall/CRC, London, pp 53–58
9. Green SJ, Dahlberg S (1992) Planned versus attained design in phase II clinical trials. Stat Med 11:853–862
10. Heinemann V, Xu YZ, Chubb S, Sen A, Hertel LW, Grindey GB, Plunkett W (1990) Inhibition of ribonucleotide reduction in CCRF-CEM cells by 2', 2'-difluorodeoxycytidine. Mol Pharmacol 38:567–572
11. Inuyama Y, Kida A, Tsukuda M, Kohno N, Satake B (2001) Late phase II study of S-1 in patients with advanced head and neck cancer. Gan To Kagaku Ryoho 28:1381–1390
12. Kawahara M, Furuse K, Segawa Y, Yoshimori K, Matsui K, Kudoh S, Hasegawa K, Niitani H (2001) Phase II study of S-1, a novel oral fluorouracil, in advanced non-small-cell lung cancer. Br J Cancer 85:939–943
13. Kim R (2011) FOLFIRINOX: a new standard treatment for advanced pancreatic cancer? Lancet Oncol 12:8–9
14. Koizumi W, Kurihara M, Nakano S, Hasegawa K (2000) Phase II study of S-1, a novel oral derivative of 5-fluorouracil, in advanced gastric cancer. For the S-1 cooperative gastric cancer study group. Oncology 58:191–197
15. Kozuch P, Grossbard ML, Barzdins A, Araneo M, Robin A, Frager D, Homel P, Marino J, DeGregorio P, Bruckner HW (2001) Irinotecan combined with gemcitabine, 5-fluorouracil, leucovorin, and cisplatin (G-FLIP) is an effective and noncross-resistant treatment for chemotherapy refractory metastatic pancreatic cancer. Oncologist 6:488–495
16. Lee GW, Kim HJ, Ju JH, Kim SH, Kim HG, Kim TH, Jeong CY, Kang JH (2009) Phase II trial of S-1 in combination with gemcitabine for chemo-naive patients with locally advanced or

metastatic pancreatic cancer. Cancer Chemother Pharmacol 64:707–713

17. Morizane C, Okusaka T, Furuse J, Ishii H, Ueno H, Ikeda M, Nakachi K, Najima M, Ogura T, Suzuki E (2009) A phase II study of S-1 in gemcitabine-refractory metastatic pancreatic cancer. Cancer Chemother Pharmacol 63:313–319

18. Nakamura K, Yamaguchi T, Ishihara T, Sudo K, Kato H, Saisho H (2006) Phase II trial of oral S-1 combined with gemcitabine in metastatic pancreatic cancer. Br J Cancer 94:1575–1579

19. Oh DY, Cha Y, Choi IS, Yoon SY, Choi IK, Kim JH, Oh SC, Kim CD, Kim JS, Bang YJ, Kim YH (2010) A multicenter phase II study of gemcitabine and S-1 combination chemotherapy in patients with unresectable pancreatic cancer. Cancer Chemother Pharmacol 65:527–536

20. Ohtsu A, Baba H, Sakata Y, Mitachi Y, Horikoshi N, Sugimachi K, Taguchi T (2000) Phase II study of S-1, a novel oral fluorophyrimidine derivative, in patients with metastatic colorectal carcinoma. S-1 cooperative colorectal carcinoma study group. Br J Cancer 83:141–145

21. 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

22. Pelzer U, Kubica K, Stieler J, Schwaner I, Heil G, Görner M, Mölle M, Hilbig A, Dörken B, Riess H, Oettle H (2008) A randomized trial in patients with gemcitabine refractory pancreatic cancer. Final results of the CONKO 003 study. J Clin Oncol 26(15S) (May 20 Supplement), ASCO Annual Meeting Proceedings (Post-Meeting Edition)

23. Ren Q, Kao V, Grem JL (1998) Cytotoxicity and DNA fragmentation associated with sequential gemcitabine and 5-fluoro-2'-deoxyuridine in HT-29 colon cancer cells. Clin Cancer Res 4:2811–2818

24. Reni M, Cordio S, Milandri C, Passoni P, Bonetto E, Oliani C, Luppi G, Nicoletti R, Galli L, Bordonaro R, Passardi A, Zerbi A, Balzano G, Aldrighetti L, Staudacher C, Villa E, Di Carlo V (2005) Gemcitabine versus cisplatin, epirubicin, fluorouracil, and gemcitabine in advanced pancreatic cancer: a randomised controlled multicentre phase III trial. Lancet Oncol 6:369–376

25. Saek T, Takashima S, Sano M, Horikoshi N, Miura S, Shimizu S, Morimoto K, Kimura M, Aoyama H, Ota J, Noguchi S, Taguchi T (2004) A phase II study of S-1 in patients with metastatic breast cancer—a Japanese trial by the S-1 cooperative study group, breast cancer working group. Breast Cancer 11:194–202

26. Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitachi Y, Taguchi T (1998) Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur-0.4 M gimestat-1 M otastat potassium) in advanced gastric cancer patients. Eur J Cancer 34:1715–1720

27. Shirasaka T, Shimamato Y, Ohshimo H, Yamaguchi M, Kato T, Yonekura K, Fukushima M (1996) Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. Anticancer Drugs 7:548–557

28. Sudo K, Yamaguchi T, Nakamura K, Denda T, Hara T, Ishihara T, Yokosuka O (2011) Phase II study of S-1 in patients with gemcitabine-resistant advanced pancreatic cancer. Cancer Chemother Pharmacol 67:249–254

29. Todaka A, Fukutomi A, Boku N, Onozawa Y, Hironaka S, Yasui H, Yamazaki K, Taku K, Machida N, Sakamoto T, Tomita H (2010) S-1 monotherapy as second-line treatment for advanced pancreatic cancer after gemcitabine failure. Jpn J Clin Oncol 40:567–572

30. Tsavaris N, Kosmas C, Skopelitis H, Gouveris P, Kopterides P, Loukeris D, Sigala F, Zorbala-Sypsa A, Felekouras E, Papalambros E (2005) Second-line treatment with oxaliplatin, leucovorin and 5-fluorouracil in gemcitabine-pretreated advanced pancreatic cancer: a phase II study. Invest New Drugs 23:369–375

31. Ueno H, Okusaka T, Furuse J, Yamao K, Funakoshi A, Boku N, Ohkawa S, Yokosuka O, Tanaka K, Moriyasu F, Nakamori S, Sato T (2011) Multicenter phase II study of gemcitabine and S-1 combination therapy (GS Therapy) in patients with metastatic pancreatic cancer. Jpn J Clin Oncol 41:953–958

32. Ueno H, Okusaka T, Ikeda M, Takezako Y, Morizane C (2004) Phase II study of S-1 in patients with advanced biliary tract cancer. Br J Cancer 91:1769–1774

33. Ueno H, Okusaka T, Ikeda M, Takezako Y, Morizane C (2005) An early phase II study of S-1 in patients with metastatic pancreatic cancer. Oncology 68:171–178

34. Ulrich-Pur H, Raderer M, Verena Kornek G, Schull B, Schmid K, Haider K, Kwasny W, Depisch D, Schneeweiss B, Lang F, Scheithauer W (2003) Irinotecan plus raltitrexed vs raltitrexed alone in patients with gemcitabine-pretreated advanced pancreatic adenocarcinoma. Br J Cancer 88:1180–1184

BMC
Cancer

Circulating endothelial cells and other angiogenesis factors in pancreatic carcinoma patients receiving gemcitabine chemotherapy

Shunsuke Kondo[1*], Hideki Ueno[1], Jun Hashimoto[1], Chigusa Morizane[1], Fumiaki Koizumi[2], Takuji Okusaka[1] and Kenji Tamura[3]

Abstract

Background: Pancreatic carcinoma is a significant cause of cancer-related death in developed countries. As the level of circulating endothelial cells (CECs) is known to increase in response to various cancers, we investigated the predictive potential of CEC levels and the association of these levels with the expression of proangiogenic factors in pancreatic carcinoma patients.

Methods: Pancreatic carcinoma patients receiving gemcitabine chemotherapy were prospectively assigned to this study. CEC levels were measured using the CellTracks system, and the plasma levels of several angiogenesis factors were measured using multiplex immunoassay. Associations between clinical outcomes and the levels of these factors were evaluated.

Results: Baseline CEC levels were markedly higher in pancreatic carcinoma patients (n = 37) than in healthy volunteers (n = 53). Moreover, these high CEC levels were associated with decreased overall survival (median, 297 days versus 143 days, $P < 0.001$) and progression-free survival (median, 150 days versus 64 days, $P = 0.008$), as well as with high vascular endothelial growth factor, interleukin (IL)-8, and IL-10 expression in the pancreatic carcinoma patients.

Conclusions: Several chemokines and proangiogenic factors correlate with the release of CECs, and the number of CECs detected may be a useful prognostic marker in pancreatic carcinoma patients undergoing gemcitabine chemotherapy.

Trial registration: UMIN000002323

Keywords: Pancreatic carcinoma, Circulating endothelial cells, Angiogenesis factors

Background

Pancreatic carcinoma is one of the most lethal tumors and is the fourth leading cause of cancer-related death in developed nations [1]. As pancreatic carcinoma has a high propensity for both local invasion and distant metastasis, surgery is precluded as a treatment for most patients who present with advanced-stage disease. These patients have a median survival of only 6 months and an overall 5-year survival of less than 5%. The prognosis for advanced pancreatic carcinoma patients is therefore extremely poor, and the impact of standard therapy is only modest, despite many advances that have improved the outcome of this disease.

Pancreatic carcinoma is not a grossly vascular tumor; however, it overexpresses multiple mitogenic growth factors that are also angiogenic, such as epidermal growth factor (EGF), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), platelet-derived growth factor B chain (PDGF-BB), and vascular endothelial growth factor (VEGF). Angiogenesis often occurs in response to an imbalance in which proangiogenic factors predominate over antiangiogenic factors. For instance, VEGF expression has been shown to promote tumor growth in pancreatic carcinomas [2]. High VEGF expression is also

* Correspondence: shkondo@ncc.go.jp
[1]Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, Tokyo, Japan
Full list of author information is available at the end of the article

associated with increased microvessel density [3] and is a predictor of poor outcomes and early tumor recurrence after curative resection [4]. Although agents that target the VEGF signaling pathway have been shown to inhibit tumor growth, metastasis, and angiogenesis [5], treating advanced pancreatic carcinoma patients with axitinib—a selective inhibitor of VEGF receptors 1, 2, and 3—in combination with gemcitabine was not found to improve overall survival in a phase 3 trial [6]. Despite this finding, proangiogenic factors remain an important therapeutic target for the treatment of pancreatic carcinoma.

Circulating endothelial cells (CECs) are mature cells that are not associated with vessel walls but are detached from the endothelium and circulate within peripheral blood. The number of CECs present in the blood has been found to increase in response to cardiovascular disease, vasculitis, infectious disease, and various cancers [7,8]. Indeed, the level of CECs has been recognized as a useful biomarker for vascular damage. It has also been reported that the number of CECs found in non-small cell lung cancer patients treated with carboplatin plus paclitaxel is a promising predictive marker of the clinical efficacy of these drugs [9]. We believe that CEC levels may also be a potential biomarker for pancreatic carcinoma; therefore, we investigated the levels of CECs found in patients with different severities of pancreatic carcinoma, as well as the effects of gemcitabine treatment on CEC levels. Furthermore, the associations between CEC levels and the expression levels of several factors involved in angiogenesis and neovascularization were also examined in this study.

Methods
Study approval
This prospective study was approved by the Institutional Review Board of the National Cancer Center, and written informed consent was obtained from all patients. This study is registered with the University Hospital Medical Information Network in Japan (UMIN; number UMIN000002323) and has been completed.

Patients and blood sample collection
A total of 37 chemotherapy-naïve patients with histologically or cytologically confirmed invasive ductal pancreatic carcinoma were prospectively enrolled in this study between April 2009 and March 2010 and received gemcitabine chemotherapy. Patients with coexisting infections and/or cardiovascular illness were excluded. The detailed history of all the patients was obtained and a physical examination was performed before beginning gemcitabine treatment. Pretreatment baseline laboratory parameters were also assessed for all patients. The baseline tumor status of each patient was evaluated using computed tomography (CT) scans of the chest, abdomen, and pelvis, while peripheral blood sampling was performed both prior to treatment initiation (baseline) and at day 28 ± 7 after starting chemotherapy. A dose of 1000 mg/m^2 gemcitabine was administered intravenously for 30 min on days 1, 8, and 15 of a 28-day cycle until disease progression, unacceptable toxicity, or patient refusal occurred. The data collected included those pertaining to standard demographics and disease characteristics, the date of initial treatment, the best response to treatment, date of progression, and the date of death or last follow-up. The tumors were evaluated every 6–8 weeks after starting each course of gemcitabine, and best responses were documented according to the Response Evaluation Criteria in Solid Tumors (RECIST).

CEC enumeration
Blood samples from advanced pancreatic carcinoma patients were drawn into 10 mL CellSave Preservative Tubes (Immunicon Corp. Huntingdon Valley, PA) for CEC enumeration. Samples were obtained both before starting chemotherapy (baseline) and at 28 ± 7 days after starting chemotherapy. Samples were kept at room temperature and processed within 42 h of collection. All of the evaluations were performed without knowledge of the clinical status of the patients. The CellTracks system (Veridex, LLC), which consists of the CellTracks AutoPrep system and the CellSpotter Analyzer system, was used for endothelial cell enumeration. In this system, CECs are defined as $CD146^+/DAPI^+/CD105\text{-}PE^+/CD45APC^-$ cells. Briefly, $CD146^+$ cells were captured immunomagnetically by using ferrofluids coated with CD146 antibodies. The enriched cells were then labeled with the nuclear dye 4 V, 6-diamidino-2-phenylindole (DAPI), CD105 antibodies were conjugated to phycoerythrin (CD105-PE), and the pan-leukocyte antibody CD45 was conjugated to allophycocyanin (CD45-APC). Cells with the $DAPI^+/CD105^+/CD45^-$ phenotype were enumerated. We evaluated morphological cell viability and excluded dead cells from the cell count. The number of CECs in each sample was determined twice, and the mean value was calculated.

Antibody suspension bead array system
Peripheral blood was drawn into prechilled tubes containing ethylenediaminetetraacetic acid; was immediately subjected to centrifugation at 1000 g and 4°C for 15 min, plasma was transferred to microtubes and subjected to further centrifugation at 10,000 g and 4°C for 10 min to remove contaminating platelets. Plasma samples were collected from patients before gemcitabine treatment was initiated and were stored at −80°C until they were used for testing. The plasma concentrations of 7 biological markers (interleukin [IL]-6, IL-8, IL-10,