**Table 7.** Comparison of Adverse Reaction Incidence and Pharmacokinetic Parameters of Gemcitabine Between Two Patient Groups With and Without Haplotype **3*

| | | Incidence of Neuropenia (nadir)* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | ≥ Grade 3 | | | ≥ Grade 4 | | | |
| Chemotherapy | Genotype | No. of Cases | Total No. of Patients | Probability | No. of Cases | Total No. of Patients | Probability | AUC† (hr·μg/mL) |
| Monotherapy | *non *3/non *3* | 66 | 167 | 0.40 | 8 | 67 | 0.05 | 9.91 |
| | *non *3/*3* | 6 | 10 | 0.60 | 1 | 10 | 0.10 | 13.13 |
| | *P* | | | 0.205 | | | 0.514 | **0.0017** |
| With fluorouracil | *non *3/non *3* | 3 | 12 | 0.25 | 2 | 12 | 0.17 | 8.11 |
| | *non *3/*3* | 2 | 2 | 1.00 | 1 | 2 | 0.50 | 11.98 |
| | *P* | | | **0.029** | | | 0.327 | 0.055 |
| With carboplatin | *non *3/non *3* | 9 | 13 | 0.69 | 1 | 13 | 0.08 | 9.87 |
| | *non *3/*3* | 3 | 3 | 1.00 | 2 | 3 | 0.67 | 12.48 |
| | *P* | | | 0.163 | | | **0.033** | **0.031** |
| With cisplatin | *non *3/non *3* | 8 | 28 | 0.29 | 2 | 28 | 0.07 | 9.53 |
| | *non *3/*3* | 1 | 1 | 1.00 | 0 | 1 | 0.00 | 11.71 |
| | **3/*3* | 1 | 1 | 1.00 | 1 | 1 | 1.00 | 52.86 |
| | *P*‡ | | | **0.030** | | | 0.128 | 0.061 |

Note. No analyses were performed in patients who received gemcitabine with vinorelbine, because only one patient bore the haplotype **3*. Boldfacing indicates a statistically significant difference ($P < .05$).
*$\chi^2$-test.
†Kruskal-Wallis test.
‡A *P* value for comparison between *non*3/non*3* and (*non*3/*3* + **3/*3*).

## REFERENCES


1. Noble S, Goa KL: Gemcitabine: A review of its pharmacology and clinical potential in non-small cell lung cancer and pancreatic cancer. Drugs 54:447-472, 1997
2. Burris HA III, 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. Rauchwerger DR, Firby PS, Hedley DW, et al: Equilibrative-sensitive nucleoside transporter and its role in gemcitabine sensitivity. Cancer Res 60:6075-6079, 2000
4. Mackey JR, Mani RS, Selner M, et al: Functional nucleoside transporters are required for gemcitabine influx and manifestation of toxicity in cancer cell lines. Cancer Res 58:4349-4357, 1998
5. Mackey JR, Yao SY, Smith KM, et al: Gemcitabine transport in xenopus oocytes expressing recombinant plasma membrane mammalian nucleoside transporters. J Natl Cancer Inst 91:1876-1881, 1999
6. Baldwin SA, Yao SY, Hyde RJ, et al: Functional characterization of novel human and mouse equilibrative nucleoside transporters (hENT3 and mENT3) located in intracellular membranes. J Biol Chem 280:15880-15887, 2005
7. Mangravite LM, Badagnani I, Giacomini KM: Nucleoside transporters in the disposition and targeting of nucleoside analogs in the kidney. Eur J Pharmacol 479:269-281, 2003
8. Ritzel MW, Ng AM, Yao SY, et al: Molecular identification and characterization of novel human and mouse concentrative Na+-nucleoside cotransporter proteins (hCNT3 and mCNT3) broadly selective for purine and pyrimidine nucleosides (system cib). J Biol Chem 276:2914-2927, 2001
9. Plunkett W, Huang P, Gandhi V: Preclinical characteristics of gemcitabine. Anticancer Drugs 6:S7-S13, 1995 (suppl 6)
10. Plunkett W, Huang P, Searcy CE, et al: Gemcitabine: Preclinical pharmacology and mechanisms of action. Semin Oncol 23:S3-S15, 1996 (suppl 10)
11. Heinemann V, Xu YZ, Chubb S, et al: Inhibition of ribonucleotide reduction in CCRF-CEM cells by 2',2'-difluorodeoxycytidine. Mol Pharmacol 38:567-572, 1990
12. Kiani A, Kohne CH, Franz T, et al: Pharmacokinetics of gemcitabine in a patient with end-stage renal disease: Effective clearance of its main metabolite by standard hemodialysis treatment. Cancer Chemother Pharmacol 51:266-270, 2003
13. Watanabe S, Uchida T: Expression of cytidine deaminase in human solid tumors and its regulation by 1 alpha, 2, 5-dihydroxyvitamin D3. Biochim Biophys Acta 1312:99-104, 1996
14. Ho DH: Distribution of kinase and deaminase of 1-beta-D-arabinofuranosylcytosine in tissues of man and mouse. Cancer Res 33:2816-2820, 1973
15. Kirch HC, Schroder J, Hoppe H, et al: Recombinant gene products of two natural variants of the human cytidine deaminase gene confer different deamination rates of cytarabine in vitro. Exp Hematol 26:421-425, 1998
16. Schroder JK, Kirch C, Seeber S, et al: Structural and functional analysis of the cytidine deaminase gene in patients with acute myeloid leukaemia. Br J Haematol 103:1096-1103, 1998
17. Gilbert JA, Salavaggione OE, Ji Y, et al: Gemcitabine pharmacogenomics: Cytidine deaminase and deoxycytidylate deaminase gene resequencing and functional genomics. Clin Cancer Res 12:1794-1803, 2006
18. Yue L, Saikawa Y, Ota K, et al: A functional single-nucleotide polymorphism in the human cytidine deaminase gene contributing to ara-C sensitivity. Pharmacogenetics 13:29-38, 2003
19. Aapro MS, Martin C, Hatty S: Gemcitabine: A safety review. Anticancer Drugs 9:191-201, 1998
20. Gallelli L, Nardi M, Prantera T, et al: Retrospective analysis of adverse drug reactions induced by gemcitabine treatment in patients with non-small cell lung cancer. Pharmacol Res 49:259-263, 2004
21. Bokemeyer C, Gerl A, Schoffski P, et al: Gemcitabine in patients with relapsed or cisplatin-refractory testicular cancer. J Clin Oncol 17:512-516, 1999
22. Locker GJ, Wenzel C, Schmidinger M, et al: Unexpected severe myelotoxicity of gemcitabine in pretreated breast cancer patients. Anticancer Drugs 12:209-212, 2001
23. Yonemori K, Ueno H, Okusaka T, et al: Severe drug toxicity associated with a single-nucleotide polymorphism of the cytidine deaminase gene in a Japanese cancer patient treated with gemcitabine plus cisplatin. Clin Cancer Res 11:2620-2624, 2005
24. Colly LP, Peters WG, Richel D, et al: Deoxycytidine kinase and deoxycytidine deaminase values correspond closely to clinical response to cytosine arabinoside remission induction therapy in patients with acute myelogenous leukemia. Semin Oncol 14:S257-S261, 1987 (suppl 1)
25. Steuart CD, Burke PJ: Cytidine deaminase and the development of resistance to arabinosyl cytosine. Nat New Biol 233:109-110, 1971
26. Tattersall MH, Ganeshaguru K, Hoffbrand AV: Mechanisms of resistance of human acute leukaemia cells to cytosine arabinoside. Br J Haematol 27:39-46, 1974
27. Chiba P, Tihan T, Szekeres T, et al: Concordant changes of pyrimidine metabolism in blasts of two cases of acute myeloid leukemia after repeated treatment with ara-C in vivo. Leukemia 4:761-765, 1990
28. Nakamura T, Saito Y, Murayama N, et al: Apparent low frequency of sequence variability within the proximal promoter region of the cytochrome P450(CYP)3A5 gene in established cell lines from Japanese individuals. Biol Pharm Bull 24:954-957, 2001
29. Kitamura Y, Moriguchi M, Kaneko H, et al: Determination of probability distribution of diplotype configuration (diplotype distribution) for each subject from genotypic data using the EM algorithm. Ann Hum Genet 66:183-193, 2002

**30.** Richards DA, Sherwood RA, Ndebele D, et al: Determination of plasma cytidine deaminase activity by HPLC. Biomed Chromatogr 2:148-151, 1987

**31.** Benjamini Y, Hochberg Y: Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Statist Soc Ser B 57:289-300, 1995

**32.** Bhargava P, Marshall JL, Fried K, et al: Phase I and pharmacokinetic study of two sequences of gemcitabine and docetaxel administered weekly to patients with advanced cancer. Cancer Chemother Pharmacol 48:95-103, 2001

**33.** Johansson E, Mejlhede N, Neuhard J, et al: Crystal structure of the tetrameric cytidine deaminase from Bacillus subtilis at 2.0: A resolution. Biochemistry 41:2563-2570, 2002

**34.** Costanzi S, Vincenzetti S, Vita A, et al: Human cytidine deaminase: Understanding the catalytic mechanism. Nucleosides Nucleotides Nucleic Acids 22:1539-1543, 2003

**35.** Kreis W, Lesser M, Budman DR, et al: Phenotypic analysis of 1-B-D-arabinofuranosylcytosine deamination in patients treated with high doses and correlation with response. Cancer Chemother Pharmacol 30:126-130, 1992

**36.** Abbruzzese JL, Grunewald R, Weeks EA, et al: A phase I clinical, plasma, and cellular pharmacology study of gemcitabine. J Clin Oncol 9:491-498, 1991

**37.** Nishida M: Pharmacological and clinical properties of Xeloda (capecitabine), a new oral active derivative of fluoropyrimidine [Japanese]. Nippon Yakurigaku Zasshi 122:549-553, 2003

**38.** Fukunaga AK, Marsh S, Murry DJ, et al: Identification and analysis of single nucleotide polymorphisms in the gemcitabine pharmacologic pathway. Pharmacogenomics J 4:307-314, 2004

**39.** Jahns-Streubel G, Reuter C, Auf der Landwehr U, et al: Activity of thymidine kinase and of polymerase alpha as well as activity and gene expression of deoxycytidine deaminase in leukemic blasts are correlated with clinical response in the setting of granulocyte-macrophage colony-stimulating factor-based priming before and during TAD-9 induction therapy in acute myeloid leukemia. Blood 90: 1968-1976, 1997

**40.** Sherwood RA: The measurement of nucleoside deaminases by high performance liquid chromatography and their use in clinical chemistry. Biomed Chromatogr 5:235-239, 1991

***Acknowledgment***

We thank Emiko Jimbo, Miho Akimoto, Atsuko Watanabe, Tomoko Chujo, Makiyo Iwamoto, and Mamiko Shimada for assistance in sample collection and management, and Chie Sudo for secretarial assistance.

Vol 25, No 30 October 20, 2007

*Reprinted from*

JOURNAL OF CLINICAL ONCOLOGY

# Genotype-Based Methods for Anticipating Gemcitabine-Related Severe Toxicities May Lead to False-Negative Results

## IN REPLY:

*Nahoko Kaniwa, Emiko Sugiyama, Su-Ryang Kim, Yoshiro Saito, Jun-ichi Sawada, Junji Furuse, Hiroshi Ishii, Teruhiko Yoshida, Hideki Ueno, Takuji Okusaka, and Nagahiro Saijo*

www.jco.org

Official Journal of the American Society of Clinical Oncology

ASCO®

IN REPLY: We appreciate the comments raised by Mercier et al and the opportunity to respond to them. We agree that the reduced intracellular CDA level is one of the major factors increasing gemcitabine-mediated toxicities. We also recognize that the genotyping based on *CDA* 208G>A (Ala70Thr) itself gives false-negative results with respect to the prediction of hematological toxicities (Table 7 in our article[1]), as is often the case with genotyping. Thus, phenotype-based methods are useful for identification of patients at a higher risk toward gemcitabine-mediated toxicities. However, as far as Japanese patients are concerned, the genetic method is fairly useful for predicting severe toxicities of gemcitabine because *CDA* 208G>A, a tagging SNP of haplotype *CDA*3*, is one of the factors that reduce CDA activity as clearly demonstrated by us.[1]

According to the letter by Mercier et al, four patients displayed severe hematologic toxicities (> grade 3) without any associations with *CDA* genotypes in their study. Their observations are quite reasonable from the following points: *CDA* 208G>A has not been detected in white people, and its allele frequency is relatively low in other populations (probably variable within African populations[2,3]; only nine Africans and one Asian were included in their study); all other genetic polymorphisms that we detected, including *CDA* 79A>C (*2, Lys27Gln),[4,5] failed to show any significant associations with altered pharmacokinetics and toxicities of gemcitabine and plasma CDA activity.[1] Therefore, we consider that, in white people, no validated genotype is currently available for predicting gemcitabine toxicities.

Mercier et al pointed out that little correlation was evident among the various diplotype groups, the pharmacokinetic parameters of gemcitabine, and the occurrence of severe toxicities, other than the **3/*3* diplotype recorded in the single patient. However, as presented in our article,[1] significant differences were observed between **3/*1* and **1/*1* for pharmacokinetic parameters (our Fig 2), and the incidences of grade ≥ 3 or grade 4 neutropenia in the combined chemotherapies with fluorouracil or platinum-containing drugs were mostly higher in the non-**3/*3* patients than in the non-**3*/non-**3* patients (Table 7). Our Figures 3A (gemcitabine as a substrate) and 3B (cytidine as a substrate) show that when plasma CDA activities of the **3/*1* and **3/*2* patients were compared with those of the **1/*1* patients by Dunn's multiple comparison test, statistically significant differences were obtained ($P < .001$ and $<0.05$ for **3/*1* and **3/*2* groups, respectively, in Fig 3A; $P < .001$ for **3/*1* group in Fig 3B; $P$ values were not provided in our report).[1]

In order to reply to the comments by Mercier et al, we re-evaluated the association between grade 4 neutropenia and gemcitabine area under the curve (AUC) or CDA activity (one patient with an extremely high level was excluded) either for the monotherapy or the combined therapy (fluorouracil, carboplatin, or cisplatin) group by the Mann-Whitney test. The median values of AUC were higher in the grade 4 group than in the grade ≤ 3 group (Δ, +9% for the monotherapy; Δ, +30% for the combined therapy), and the median values of plasma CDA levels were lower in the grade 4 group than in the grade ≤ 3 group (Δ, −29% for the monotherapy; Δ, −40% for the combined therapy). Both the increase in AUC and decrease in plasma CDA activity observed in the grade 4 group who received the combined therapies were mainly attributable to the **3*-bearing patients. Appropriate cutoff values could not be set for both AUC and plasma CDA activity to effectively screen grade 4 neutropenia since the median values of the two patient groups were not sufficiently different in our hands. Notably, these biomarkers successfully identified the patient who encountered life-threatening toxicities, because he had **3/*3* and showed extremely high AUC and low plasma CDA activity. As for the relationship between plasma CDA activities and AUC values (gemcitabine exposure levels), a moderate but statistically significant correlation was obtained ($r = -0.30$; $P = .0009$). It was reported that CDA released from damaged neutrophils diffuses into blood, and thus CDA activity in the blood is considered to be one of the markers of inflammatory diseases.[5] It must be noted that pretreatment neutrophil counts also showed a moderate correlation with CDA activity ($r = 0.37$; $P < .0001$; gemcitabine used as a substrate). Moreover, aging and sex influence on the pharmacokinetic parameters of gemcitabine.[1] Therefore, it is not surprising that very strong correlations were not obtained between plasma CDA activity and the pharmacokinetic parameters of gemcitabine.

Taken together, both predictive genotype (*3) and phenotype markers, gemcitabine AUC and plasma CDA activity, could predict grade 4 neutropenia, but with some false-negative cases and with increased false-positive cases for AUC and plasma CDA. At least, *CDA* 208G>A is a useful marker to predict gemcitabine toxicities in Japanese and probably East Asians.

*Nahoko Kaniwa, Emiko Sugiyama, Su-Ryang Kim, Yoshiro Saito, and Jun-ichi Sawada*
Project Team for Pharmacogenetics, National Institute of Health Sciences; Division of Medicinal Safety Sciences, National Institute of Health Sciences; Division of Biochemistry and Immunochemistry, National Institute of Health Sciences, Setagaya, Tokyo, Japan

*Junji Furuse and Hiroshi Ishii*
Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital East, Kashiwa, Chiba, Japan

*Teruhiko Yoshida*
Genetics Division, Research Institute, National Cancer Center, Tsukiji, Tokyo, Japan

*Hideki Ueno and Takuji Okusaka*
Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, Tsukiji, Tokyo, Japan

*Nagahiro Saijo*
National Cancer Center Hospital East, Kashiwa, Chiba, Japan

**ACKNOWLEDGMENT**
Supported in part by the Program for the Promotion of Fundamental Studies in Health Sciences, and the Health and Labour Sciences Research grant (Research on Human Genome, Tissue Engineering) from the Ministry of Health, Labour and Welfare.

**AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**
The author(s) indicated no potential conflicts of interest.

**REFERENCES**

1. Sugiyama E, Kaniwa N, Kim S-R, et al: Pharmacokinetics of gemcitabine in Japanese cancer patients: The impact of a cytidine deaminase polymorphism. J Clin Oncol 25:32-42, 2007
2. Gilbert JA, Salavaggione OE, Ji Y, et al: Gemcitabine pharmacogenomics: Cytidine deaminase and deoxycytidylate deaminase gene resequencing and functional genomics. Clin Cancer Res 12:1794-1803, 2006
3. Fukunaga AK, Marsh S, Murry DJ, et al: Identification and analysis of single-nucleotide polymorphisms in the gemcitabine pharmacologic pathway. Pharmacogenomics J 4:307-314, 2004
4. Kirch HC, Schroder J, Hoppe H, et al: Recombinant gene products of two natural variants of the human cytidine deaminase gene confer different deamination rates of cytarabine in vitro. Exp Hematol 26:421-425, 1998
5. Thompson PW, Jones DD, Currey HLF: Cytidine deaminase activity as a measure of acute inflammation in rheumatoid arthritis. Annals Rheum Dis 45:9-14, 1986

DOI: 10.1200/JCO.2007.13.4577

Cancer Chemother Pharmacol (2008) 61:615–621
DOI 10.1007/s00280-007-0514-8

ORIGINAL ARTICLE

# A late phase II study of S-1 for metastatic pancreatic cancer

**Takuji Okusaka · Akihiro Funakoshi · Junji Furuse · Narikazu Boku · Kenji Yamao · Shinichi Ohkawa · Hiroshi Saito**

Received: 11 February 2007 / Accepted: 30 April 2007 / Published online: 23 May 2007
© Springer-Verlag 2007

**Abstract** This study evaluated the antitumor effect and safety of S-1, an oral fluoropyrimidine derivative, in patients with metastatic pancreatic cancer. Chemo-naive patients with pancreatic adenocarcinoma, and measurable metastatic lesions were enrolled. S-1 was administered orally twice daily after meals at a dose of 80, 100, or 120 mg/day for body surface areas (BSAs) of less than 1.25 $m^2$, between 1.25 $m^2$ and less than 1.5, or 1.5 $m^2$ or greater, respectively, for 28 consecutive days, followed by a 14-day rest. Fifteen (37.5%) of 40 patients responded to treatment, including 1 complete response and 14 partial responses. The median time to progression and the overall survival time were 3.7 months (95% confidence interval, 2.2–5.6 months) and 9.2 months (95% confidence interval, 7.5–10.8 months), respectively. The major adverse events were anorexia, fatigue, hemoglobin reduction, nausea and pigmentation change, although most were tolerable and reversible. Although disseminated intravascular coagulation occurred in two patients, the condition resolved with anticoagulant therapy. S-1 is an effective and well-tolerated drug. The effectiveness of this drug should be confirmed in a phase III study.

**Keywords** Pancreatic cancer · Phase II study · Chemotherapy · S-1

This work was presented in part at the 41st Annual Meeting of the American Society of Clinical Oncology, Orlando, Florida USA, May 13–17, 2005.

T. Okusaka (✉)
Hepatobiliary and Pancreatic Oncology Division,
National Cancer Center Hospital, 5-1-1 Tsukiji,
Chuo-ku, Tokyo 104-0045, Japan
e-mail: tokusaka@ncc.go.jp

A. Funakoshi
National Kyushu Cancer Center, Fukuoka, Japan

J. Furuse
National Cancer Center Hospital East, Kashiwa, Japan

N. Boku
Shizuoka Cancer Center, Suntou-gun, Japan

K. Yamao
Aichi Cancer Center Hospital, Nagoya, Japan

S. Ohkawa
Kanagawa Cancer Center, Yokohama, Japan

H. Saito
Yamagata Prefectural Central Hospital, Yamagata, Japan

## Introduction

Pancreatic cancer is a major leading cause of cancer-related mortality worldwide: it ranks as the fifth leading cause of death in Japan, with an annual incidence of approximately 20,000 cases and a similar mortality rate [1]. Of all the treatments available for pancreatic cancer, only resection offers a chance for a cure. However, owing to the high frequency of local extension and/or metastatic disease at the time of diagnosis, only a small minority of patients are candidates for curative resection. Moreover, surgery alone is limited, with an unsatisfactory prognosis and a high incidence of postoperative recurrence. To improve the survival of patients with pancreatic cancer, effective non-surgical treatments are urgently needed.

A randomized controlled study demonstrated that treatment with gemcitabine exhibited a better clinical benefit response (CBR) (23.8 vs. 4.8%) and median survival period (5.65 vs. 4.41 months) than bolus 5-fluorouracil (5-FU) [2].

However, chemotherapy for pancreatic cancer must be substantially improved because gemcitabine monotherapy offers only a limited survival benefit. Gemcitabine administration via a fixed-dose-rate infusion [3] and gemcitabine-based combined regimens have been investigated, but a meaningful impact on survival, compared with that of gemcitabine monotherapy, was not obtained. Randomized phase III studies of gemcitabine plus erlotinib [4] and gemcitabine plus capecitabine [5] have demonstrated significant survival benefits, but a worldwide consensus regarding these results has not been established.

S-1 is an oral anticancer drug consisting of tegafur (FT), a prodrug of 5-FU, and two biochemical modulators, 5-chloro-2,4-dihydroxypyridine (CDHP) and potassium oxonate (Oxo) [6]. CDHP is a competitive inhibitor of dihydropyrimidine dehydrogenase, which is involved in the degradation of 5-FU, and allows efficacious concentrations of 5-FU to be maintained in the plasma and tumor tissues. Oxo, a competitive inhibitor of orotate phosphoribosyltransferase, inhibits the phosphorylation of 5-FU in the gastrointestinal tract and reduces the gastrointestinal toxicity of 5-FU. S-1 has been clinically shown to have a potent antitumor activity against various solid tumors [7–15].

S-1 was also effective against human pancreatic cancer xenografts implanted into nude rats [16]. Furthermore, an early phase II study of S-1 showed promising results, with a 21% response rate and a manageable toxicity profile in 19 patients with metastatic pancreatic cancer [17]. Therefore, we conducted a multi-institutional late phase II study of S-1 to confirm these previous results.

## Patients and methods

### Patients

Patients with inoperable pancreatic cancer or who were unable to receive radiotherapy were considered for enrollment. The eligibility criteria were as follows: capable of oral intake, histologically or cytologically confirmed pancreatic adenocarcinoma, between 20 and 74 years old, no history of prior treatment other than pancreatic resection, measurable metastatic lesions, a Karnofsky performance status (KPS) of 80–100%, an adequate hematological profile (hemoglobin $\geq$10.0 g/dl; leukocyte count, 4,000–12,000/mm$^3$; neutrophil count $\geq$2,000/mm$^3$; platelet count $\geq$100,000/mm$^3$), adequate hepatic function (total bilirubin level $\leq$3 times the upper limit of normal, transaminases levels $\leq$ 2.5 times the upper limit of normal), adequate renal function (normal serum creatinine level), and a life expectancy $\geq$2 months. The exclusion criteria were as follows: participation in another clinical study; treatment with phenytoin, potassium warfarin or flucytosine; active infection; serious complications; clinically significant ascites or pleural effusion; brain metastasis; abnormal bowel movements, like watery diarrhea or chronic constipation; active secondary malignancies; pregnancy or lactation; and men who were trying to father a child. The study was conducted in accordance with the Helsinki Declaration and Good Clinical Practice and was approved by the institutional review boards at each hospital. Written informed consent was obtained from all patients before their participation.

### Treatment plan

S-1 (Taiho Pharmaceutical Co. Ltd., Tokyo, Japan) was administered orally at a dose of 40 mg/m$^2$ twice daily, after breakfast and dinner, for 28 consecutive days followed by a 14-day rest one course. The three initial doses were determined according to the body surface area (BSA) as follows: BSA $<$1.25 m$^2$, 40 mg/dose; 1.25 m$^2$ $\leq$ BSA $<$ 1.5 m$^2$, 50 mg/dose; 1.5 m$^2$ $\leq$ BSA, 60 mg/dose. Treatment cycles were repeated until the appearance of disease progression, unacceptable toxicities, or the patient's refusal to continue treatment. If a grade 3 or higher hematological toxicity or a grade 2 or higher non-hematological toxicity was observed, dose reduction by 10 mg/dose (minimum, 40 mg/dose) or temporary interruption of S-1 administration was recommended. To enhance treatment efficacy, the rest period was shortened to 7 days or the dose was escalated one step during the next course (maximum, 75 mg/dose), unless adverse events were observed. If a rest period of more than 28 days was required, the study treatment was stopped. Prophylactic granulocyte colony-stimulating factor was not used.

### Response and safety

Patients who received at least one dose of S-1 were evaluated for response and toxicity. Tumor response was assessed using computed tomography or magnetic resonance imaging after each course according to the Japan Society for Cancer Therapy (JSCT) Criteria [18], which are similar to the World Health Organization Criteria. Primary pancreatic lesions were considered assessable, but not measurable. The response was secondarily assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) [19]. Carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) levels were quantified in each course.

The CBR was evaluated using the KPS and pain score, as described below [2]. The KPS was recorded weekly by the attending physician. Pain was evaluated by measuring the change from the baseline pain intensity and the daily dose of morphine or morphine-equivalent (doses of analgesic agents were converted to morphine-equivalent doses,

i.e., 5.0 mg fentanyl patch = 60 mg morphine). The pain intensity was graded from 0 (no pain) to 100 (worst pain) using a visual analog scale and was recorded on a pain assessment card everyday. Patients who fulfilled at least one of the following criteria were defined as eligible for the CBR analysis: (1) baseline pain intensity ≥20, or (2) baseline morphine consumption ≥10 mg/day. Moreover, all the patients underwent a 'pain stabilization period' for 2 days to ensure that the baseline values were stable before treatment: when the variation in the morphine consumption between 2 days was within 5 mg and the variation of the pain intensity was within 10, the patient was considered eligible for inclusion in the CBR analysis. Any adverse events were evaluated for grading, duration and S-1 causality according to the National Cancer Institute Common Toxicity Criteria, version 2.0. Physical findings were assessed weekly, blood biochemistry and urinalysis were assessed biweekly, and vital signs were assessed as necessary. An independent review committee confirmed the responses and the adverse events.

### Statistics

The primary measure of efficacy was the overall response rate, as defined by the tumor measurement. Other measures included the response duration, median survival time (MST) and time to progression (TTP), according to the JSCT Criteria. Response duration was calculated from the first documentation of a response until progressive disease (PD). The MST and median TTP were estimated using the Kaplan–Meier method [20]. The threshold rate was defined as 5%, and the expected rate was set at 20% because the response rate in the previous study had been 21.1% [17]. If the response rate to S-1 was 20%, a sample size of 40 patients would ensure a power of at least 80% at a one-sided significance level of 2.5% to reject the null hypothesis that the response rate was ≤5%. If the lower limit of the 95% confidence interval (95% CI) of the response rate exceeded the 5% threshold, a response rate of 6 out of 40 patients would be required.

## Results

### Patient characteristics

Between January 2003 and April 2004, 41 patients from 7 institutions were enrolled in the present study. S-1 was not administered in 1 patient because of rapid disease progression: thus, toxicity and response were evaluated in 40 patients. The patient characteristics are listed in Table 1. Most patients had a good Karnofsky performance status of 90–100%. Among the five patients who had undergone resections, three patients received pancreaticoduodenectomies and two patients received distal pancreatectomies. The major sites of metastases were the liver and distal lymph nodes. Ten of the 40 patients fulfilled the eligibility criteria for the CBR evaluation.

### Treatment

A total of 144 courses were administered to 40 patients, with a median of 3.0 courses per patient (range 1–16 courses). The S-1 dose was reduced in eight patients for the following reasons: grade 3 hepatotoxicity (one patient); grade 3 gastrointestinal toxicity, including anorexia, nausea and vomiting (one patient each); grade 2 gastrointestinal toxicity (1 patient); grade 2 abdominal pain (one patient); grade 1 pancytopenia (one patient); and a body weight loss of less than 5% (one patient: the body weight of the patient was originally close to the boundary between the 50 and 60 mg dose categories). The dose was increased in eight patients because no adverse events that might have posed an impediment to dose escalation were observed; thereafter, three of the eight patients required a dose reduction to their original dose. Thirty-five (90%) of the 39 patients who completed this study were subsequently treated with gemcitabine, although the treatment periods and responses were not monitored.

### Responses and survival

The responses of the 40 patients are shown in Table 2. The overall response rate, as evaluated using the JSCT criteria, was 37.5% (95% CI 22.7–54.2%), including 1 complete response (CR) and 14 partial responses (PRs). The response in the patient who showed a CR according to the JSCT criteria was judged as a PR according to the RECIST criteria because the serum CEA level did not decrease to normal. The serum CA 19-9 level decreased by more than half in 15 (48%) of the 31 patients who had pretreatment levels over 100 U/ml, and the serum CEA level decreased by more than half in 4 (29%) of the 14 patients who had pretreatment levels over 15 U/ml. The median duration of response was 6.9 months (range 4.0–18.6 months). The median TTP, MST, and 1-year survival rate were 3.7 months (95% CI 2.2–5.6 months), 9.2 months (95% CI 7.5–10.8 months), and 32.5% (13/40), respectively (Fig. 1). S-1 treatment was ongoing in 1 of the 40 patients who showed no evidence of disease progression at the time of analysis (617 days).

### Clinical benefits

The CBR scores of four (40%) of the ten evaluated patients improved after S-1 therapy. The pain intensity of all four patients decreased, although their daily analgesic consump-

**Table 1** Patient characteristics

| Characteristics | Median (Range) | No. of patients | (%) |
|---|---|---|---|
| No. of patients enrolled | | 41 | |
| Assessable for response and toxicity | | 40 | |
| Sex | | | |
| Male | | 21 | 52.5 |
| Female | | 19 | 47.5 |
| Age, years | 59.5 (41–74) | | |
| Karnofsky performance status, % | | | |
| 100 | | 18 | 45.0 |
| 90 | | 21 | 52.5 |
| 80 | | 1 | 2.5 |
| First dose, mg | | | |
| 40 | | 3 | 7.5 |
| 50 | | 18 | 45.0 |
| 60 | | 19 | 47.5 |
| Pancreatectomy | | | |
| (+) | | 5 | 12.5 |
| (−) | | 35 | 87.5 |
| Metastatic sites | | | |
| Liver | | 36 | 90.0 |
| Distant lymph nodes | | 10 | 25.0 |
| Lung | | 4 | 10.0 |
| Peritoneum | | 1 | 2.5 |
| CA 19-9, U/ml | 1,020 (1.0–250,000) | | |
| No. of cases with more than 100 U/ml | | 31 | 77.5 |
| CEA, U/ml | 6.95 (1.0–498) | | |
| No. of cases with more than 15 U/ml | | 14 | 35.0 |

tion and KPS scores did not change. In the remaining six patients, the CBR remained unchanged in one patient and increased in five patients. The responses according to the JSCT criteria of the four patients with improved CBR scores were two PR and two no change (NC).

## Safety

Treatment-related adverse events are listed in Table 3. The major adverse events were anorexia, fatigue, hemoglobin reduction, nausea, and pigmentation change; however, most

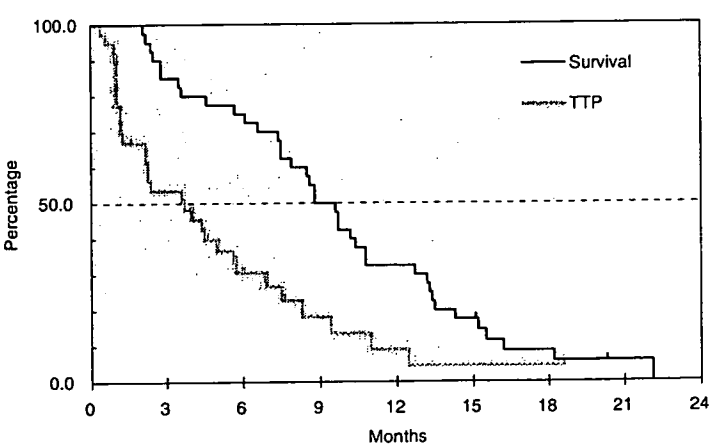


**Fig. 1** Kaplan–Meier curves for overall survival (*solid line*) and time to progression (*dotted line*)

**Table 2** Tumor response ($n = 40$)

| Tumor response | JSCT (%) | RECIST (%) |
|---|---|---|
| Complete response | 1 (2.5) | 0 (0.0) |
| Partial response | 14 (35.0) | 15 (37.5) |
| No change/stable disease | 11 (27.5) | 11 (27.5) |
| Progressive disease | 13 (32.5) | 13 (32.5) |
| Not evaluable[a] | 1 (2.5) | 1 (2.5) |
| Overall response | 15 (37.5) | 15 (37.5) |

[a] Radiographic assessment was not determined

**Table 3** Treatment-related adverse events ($n = 40$): worst grade reported during the treatment period

| Toxicity | Grade | | | | Grades 1–4 | Grades 3–4 |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | (%) | (%) |
| Hematological | | | | | | |
| Leukopenia | 10 | 7 | 0 | 0 | 42.5 | 0 |
| Neutropenia | 4 | 4 | 5 | 0 | 32.5 | 12.5 |
| Hemoglobin reduction | 8 | 13 | 1 | 1 | 57.5 | 5.0 |
| Thrombocytopenia | 13 | 1 | 1 | 0 | 37.5 | 2.5 |
| Non-Hematological | | | | | | |
| Anorexia | 10 | 10 | 4 | 1 | 62.5 | 12.5 |
| Nausea | 11 | 6 | 3 | 0 | 50.0 | 7.5 |
| Vomiting | 8 | 6 | 2 | 0 | 40.0 | 5.0 |
| Diarrhea | 12 | 4 | 3 | 0 | 47.5 | 7.5 |
| Fatigue | 16 | 9 | 0 | 0 | 62.5 | 0 |
| Stomatitis | 9 | 1 | 0 | 0 | 25.0 | 0 |
| Skin rash | 6 | 4 | 0 | 0 | 25.0 | 0 |
| Pigmentation change | 20 | 0 | 0 | 0 | 50.0 | 0 |
| DIC[a] | 0 | 0 | 2 | 0 | 5.0 | 5.0 |
| Colitis | 0 | 0 | 1 | 0 | 2.5 | 2.5 |
| Hypotension | 0 | 0 | 1 | 0 | 2.5 | 2.5 |
| Prothrombin time | 0 | 0 | 1 | 0 | 2.5 | 2.5 |
| T-bilirubin elevation | 5 | 8 | 2 | 1 | 40.0 | 7.5 |
| AST elevation | 3 | 4 | 1 | 0 | 20.0 | 2.5 |
| ALT elevation | 5 | 4 | 1 | 0 | 25.0 | 2.5 |
| γ-GTP elevation | 0 | 0 | 1 | 0 | 2.5 | 2.5 |
| Albumin reduction | 5 | 3 | 0 | 0 | 20.0 | 0 |
| T-protein reduction | 6 | 2 | 0 | 0 | 20.0 | 0 |
| Weight loss | 6 | 1 | 0 | 0 | 17.5 | 0 |
| LDH elevation | 4 | 1 | 0 | 0 | 12.5 | 0 |

Events with a frequency of more than 10.0% or high-grade events (grades 3, 4) are listed

[a] Disseminated intravascular coagulation

of these events were tolerable and reversible. Treatment was discontinued in six patients because of treatment-related adverse events: grade 4 elevation in total bilirubin, grade 4 anorexia, grade 3 disseminated intravascular coagulation (DIC), and grade 3 colitis during the first course, grade 4 anemia (hemoglobin reduction) during the third course, and grade 2 nausea during the fourth course. Most of the events resolved with the cessation of S-1 administration, although an elevated total bilirubin level persisted in 1 patient until his death 41 days after the discontinuation of S-1 and anorexia persisted in 1 patient until the initiation of radiotherapy as a second-line treatment 13 days after the discontinuation of S-1.

Although DIC also occurred in one patient during the first course, it resolved soon after the start of anticoagulant therapy; nonetheless, the S-1 therapy had to be discontinued because of disease progression after the patient recovered from the DIC. Febrile neutropenia or treatment-related deaths did not occur. Ileus, which occurred in three patients during the early phase II study, did not occur in this study. Most of the patients were treated as outpatients.

## Discussion

A variety of chemotherapy regimens for the treatment of advanced pancreatic cancer have been evaluated since the introduction of gemcitabine, which aroused renewed interest in clinical research. However, little evidence of significant activity against this disease has been demonstrated, and few agents have reproducibly provided high response rates or a meaningful impact on patient survival or quality of life.

In phase II and III studies for advanced pancreatic cancer, gemcitabine monotherapy produced response rates ranging from 4 to 17% and an MST ranging from 5.4 to 7.3 months [21, 22]. In phase II trials of oral fluoropyrimidines, UFT yielded no objective response (0/21), with an MST of 4.2 months [23], and capecitabine yielded a response rate of 9.5% (4/42), with an MST of 182 days (6.0 months) [24]. For gemcitabine combined therapy, response rates of up to 29% were reported in phase III studies, with MST values ranging from 3.74 to 9.0 months [21, 22].

An early phase II study of S-1 produced a response rate of 21% and an MST of 5.6 months [17]. The present phase II study concluded that S-1 was a promising agent for advanced pancreatic cancer, with a response rate of 37.5%, an MST of 9.2 months, and an acceptable toxicity profile. The efficacy of S-1 in the present study was more favorable than that in the previous study. The reasons for this discrepancy could not be definitively identified because of the small numbers of patients involved, although differences in the patients' backgrounds probably affected the results. A logistic regression analysis suggested that a larger proportion of female patients, fewer measurable lesions, and a lower morphine consumption, compared with the early phase II study, might have contributed to the superior response rate in the present study, although the differences were not statistically significant (data not shown). Moreover,

the larger proportion of patients receiving second-line chemotherapy may have contributed to the longer MST in the present study: the proportion of patients receiving second-line chemotherapy was 26% (5/19, 3 patients receiving 5-FU plus cisplatin, 2 patients receiving gemcitabine) in the previous study and 90% (35/39, 35 patients receiving gemcitabine) in the present study. Gemcitabine was approved for the treatment of pancreatic cancer in Japan in April 2001, after enrollment in the previous study had been completed. Although some divergences in the response rates and survival periods were noted, the results of both studies seemed to favor S-1 over other agents for the treatment of advanced pancreatic cancer.

The toxicity profiles in the previous and present studies on S-1 were similar. However, gastrointestinal toxicities like anorexia and vomiting tended to occur more frequently in the studies for pancreatic cancer than in those for other cancers. We speculated that the higher frequency of toxicity may be related to the clinical features of pancreatic cancer itself, since gastrointestinal symptoms like anorexia are observed in many patients at the time of the initial diagnosis. No treatment-related deaths were observed, but three patients developed ileus during the previous phase II study and two patients developed DIC during the present study. DIC was a noteworthy complication, although this complication can occur even in patients with pancreatic cancer who are receiving only supportive care without chemotherapy. Although the cause of the DIC could not be determined, the possibility that it was caused by the S-1 treatment cannot be excluded. Periodic monitoring of the patients' physical conditions and laboratory parameters is recommended for the early diagnosis of serious complications in patients treated outside of clinical trials, even though most patients were treated as outpatients without any serious complaints.

S-1, an oral anticancer agent, may offer clinical advantages while maintaining quality of life [25]. Since a promising anticancer effect and a relatively long MST were observed in this study, S-1 may be a potentially useful alternative to gemcitabine as a first-line drug for the treatment of advanced pancreatic cancer. Furthermore, S-1 may be useful when administered in combination with gemcitabine, since its toxicity is generally mild and its toxicological profile is distinct from that of gemcitabine. We previously conducted a phase I study to determine the recommended dose of S-1 and gemcitabine in a combination regimen for the treatment of advanced pancreatic cancer [26]. Currently, we are conducting a multi-institutional phase II study. Nakamura et al. [27] reported a 48% (16/33) response rate and an MST of 12.5 months for metastatic pancreatic cancer in a single-institute phase II study of S-1 and gemcitabine. Randomized trials are essential for determining whether chemotherapy with S-1 is equivalent or superior in efficacy to gemcitabine as an initial treatment for advanced pancreatic cancer.

In conclusion, S-1 administered as a single agent showed a promising anticancer effect with acceptable toxicity in patients with metastatic pancreatic cancer. A randomized phase III trial to evaluate the effectiveness of S-1 for advanced pancreatic cancer is warranted.

**Acknowledgments** We thank Drs. M. Kurihara, S. Matsuno, O. Ishikawa and T. Taguchi for their kind advice and Drs. H. Saisho, N. Moriyama and W. Koizumi for the extramural review. We also thank Mr. M. Noguchi and Dr. R. Azuma for their assistance in the data collection and analysis.

*Conflict of interest* The study was supported by Taiho Pharmaceutical Co., Ltd. (Tokyo). For all authors, there is no potential conflict of interest, relevant to this article.

## References

1. Lowenfels AB, Maisonneuve P (2004) Epidemiology and prevention of pancreatic cancer. Jpn J Clin Oncol 34:238–244
2. Burris HA 3rd, 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
3. Tempero M, Plunkett W, Ruiz Van Haperen V, Hainsworth J, Hochster H, Lenzi R et al (2003) Randomized phase II comparison of dose-intense gemcitabine: thirty-minute infusion and fixed dose rate infusion in patients with pancreatic adenocarcinoma. J Clin Oncol 21:3402–3408
4. Moore MJ (2005) Brief communication: a new combination in the treatment of advanced pancreatic cancer. Semin Oncol 32(6 Suppl 8):S5–S6
5. Cunningham D, Chau I, Stocken D, Davies C, Dunn J, Valle J et al (2005) Phase III randomised comparison of gemcitabine (GEM) versus gemcitabine plus capecitabine (GEM-CAP) in patients with advanced pancreatic cancer. Eur J Cancer Suppl 3:12
6. Shirasaka T, Shimamato Y, Ohshimo H, Yamaguchi M, Kato T, Yonekura K et al (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
7. Shirao K, Ohtsu A, Takada H, Mitachi Y, Hirakawa K, Horikoshi N et al (2004) Phase II study of oral S-1 for treatment of metastatic colorectal carcinoma. Cancer 100:2355–2361
8. Ohtsu A, Baba H, Sakata Y, Mitachi Y, Horikoshi N, Sugimachi K et al (2000) Phase II study of S-1, a novel oral fluoropyrimidine derivative, in patients with metastatic colorectal carcinoma. Br J Cancer 83:141–145
9. Kawahara M, Furuse K, Segawa Y, Yoshimori K, Matsui K, Kudoh S et al (2001) Phase II study of S-1, a novel oral fluorouracil, in advanced non-small-cell lung cancer. Br J Cancer 85:939–943
10. Furuse K, Kawahara M, Hasegawa K, Kudoh S, Takada M, Sugiura T et al (2001) Early phase II study of S-1, a new oral fluoropyrimidine, for advanced non-small-cell lung cancer. Int J Clin Oncol 6:236–241
11. 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. Oncology 58:191–197

12. Sugimachi K, Maehara Y, Horikoshi N, Shimada Y, Sakata Y, Mitachi Y et al (1999) An early phase II study of oral S-1, a newly developed 5-fluorouracil derivative for advanced and recurrent gastrointestinal cancers. Oncology 57:202–210
13. 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
14. 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
15. Saeki T, Takashima S, Sano M, Horikoshi N, Miura S, Shimizu S et al (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
16. Fukushima M, Satake H, Uchida J, Shimamoto Y, Kato T, Takechi T et al (1998) Preclinical antitumor efficacy of S-1: a new oral formulation of 5-fluorouracil on human tumor xenografts. Int J Oncol 13:693–698
17. 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
18. Japan Society for Cancer Therapy (1993) Criteria for the evaluation of the clinical effects of solid cancer chemotherapy. J Jpn Soc Cancer Ther 28:101–130
19. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L et al (2000) New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 92:205–216
20. Kaplan EL, Meier P (1958) Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–481
21. Kindler HL (2005) Front-line therapy of advanced pancreatic cancer. Semin Oncol 32(6 Suppl 9):S33–S36
22. Hochster HS, Haller DG, de Gramont A, Berlin JD, Philip PA, Moore MJ et al (2006) Consensus report of the International Society of Gastrointestinal Oncology on therapeutic progress in advanced pancreatic cancer. Cancer 107:676–685
23. Ueno H, Okada S, Okusaka T, Ikeda M, Kuriyama H (2002) Phase II study of uracil-tegafur in patients with metastatic pancreatic cancer. Oncology 62:223–227
24. Cartwright TH, Cohn A, Varkey JA, Chen YM, Szatrowski TP, Cox JV et al (2002) Phase II study of oral capecitabine in patients with advanced or metastatic pancreatic cancer. J Clin Oncol 20:160–164
25. O'Neill VJ, Twelves CJ (2002) Oral cancer treatment: developments in chemotherapy and beyond. Br J Cancer 87:933–937
26. Ueno H, Okusaka T, Ikeda M, Ishiguro Y, Morizane C, Matsubara J et al (2005) A phase I study of combination chemotherapy with gemcitabine and oral S-1 for advanced pancreatic cancer. Oncology 69:421–427
27. 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

International Journal of Surgical Pathology
http://ijs.sagepub.com

**Nuclear Expression of STAT5 in Intraductal Papillary Mucinous Neoplasms of the Pancreas**

Tatsuki R. Kataoka, Tatsuya Ioka, Yoshitane Tsukamoto, Makiko Matsumura, Shingo Ishiguro and Yasuko Nishizawa

*Int J Surg Pathol* 2007; 15; 277
DOI: 10.1177/1066896907302233

The online version of this article can be found at:
http://ijs.sagepub.com/cgi/content/abstract/15/3/277

Published by:
SAGE Publications
http://www.sagepublications.com

**Additional services and information for *International Journal of Surgical Pathology* can be found at:**

**Email Alerts:** http://ijs.sagepub.com/cgi/alerts

**Subscriptions:** http://ijs.sagepub.com/subscriptions

**Reprints:** http://www.sagepub.com/journalsReprints.nav

**Permissions:** http://www.sagepub.com/journalsPermissions.nav

**Citations** (this article cites 16 articles hosted on the SAGE Journals Online and HighWire Press platforms):
http://ijs.sagepub.com/cgi/content/refs/15/3/277

Downloaded from http://ijs.sagepub.com by guest on February 24, 2008
© 2007 SAGE Publications. All rights reserved. Not for commercial use or unauthorized distribution.

International Journal of Surgical Pathology
Volume 15 Number 3
July 2007 277-281
© 2007 Sage Publications
10.1177/1066896907302233
http://ijsp.sagepub.com
hosted at
http://online.sagepub.com

# Nuclear Expression of STAT5 in Intraductal Papillary Mucinous Neoplasms of the Pancreas

Tatsuki R. Kataoka, MD, Tatsuya Ioka, MD, Yoshitane Tsukamoto, MD, Makiko Matsumura, MD, Shingo Ishiguro, MD, and Yasuko Nishizawa, MD

Intraductal papillary mucinous neoplasms (IPMNs) are noninvasive lesions of the pancreas and classified as intraductal papillary mucinous adenomas (IPMAs), borderline IPMNs, and intraductal papillary mucinous carcinomas (IPMCs). Expression patterns of the specific genes alter during IPMN progression. Based on the evidence that signal transducers and activators of transcription (STAT) 5 play important roles in tumor development, we tested STAT5 expression in IPMAs, borderline IPMNs, and IPMCs by immunohistochemical method. STAT5 frequently expressed in the nuclei of tumor cells of borderline IPMNs or IPMCs but was not observed in those of IPMAs. Nuclear expression of STAT5 protein correlated to the Ki-67 labeling index of the examined IPMNs. STAT5 protein could contribute to the progression and proliferation of IPMNs.

**Keywords:** IPMN; STAT5; Ki-67; tumorigenesis; immunohistochemistry

## Introduction

Intraductal papillary mucinous neoplasms (IPMNs) are noninvasive lesions of the pancreas.[1] IPMNs are characterized by mucin (MUC) production, cystic dilation of the pancreatic ducts, and intraductal papillary growth of epithelium.[1] IPMNs can be classified into 4 subtypes on the basis of immunohistochemical reactivities for some types of MUCs and morphological features.[2] IPMNs are also divided into 3 categories: intraductal papillary mucinous adenomas (IPMAs), borderline IPMNs, and intraductal papillary mucinous carcinomas (IPMCs) according to atypism of tumor cells.[1] Some alterations of expression of the specific genes were observed during the progression of IPMNs.[1,3] Almost all the changes were contributed to enhanced proliferation of IPMNs.[1,3]

Signal transducers and activators of transcription (STAT) proteins are also involved in tumor progression and cell proliferation.[4,5] Seven members of STAT proteins have been identified in mammalian cells; STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6.[4,5] Extracellular stimulation induces phosphorylation following by dimerization of STAT proteins. STAT proteins translocate from cytoplasm to nucleus, and transactivate specific target genes. Aberrant activation of STATs, especially STAT3 and STAT5, has been reported in many kinds of neoplasms.[4,5] STAT3 also plays some role in pancreatic cancer progression,[6,7] but, to our knowledge, there is no report on STAT5 involvement in pancreatic cancer biology.

In the present study, we examined the status of STAT5 proteins in noninvasive pancreatic tumors, IPMNs, by immunohistochemical method. We were not able to detect STAT5A expression in either normal pancreatic duct or all examined IPMNs. STAT5B expression was confirmed in some borderline IPMNs

From the Department of Pathology (TRK, YT, MM, SI, YN); the Department of Hepatobiliary and Pancreatic Oncology (TI, MM), Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; and the Department of Pathology, Medical School/Graduate School of Frontier Bioscience (TRK); the Laboratory of Clinical and Experimental Pathophysiology, Graduate School of Phamaceutical Science (YN), Osaka University, Osaka, Japan.

Address correspondence to: Tatsuki R. Kataoka, MD, Department of Pathology, Room C2, Osaka University Medical School, Yamada-oka 2-2, Suita, Osaka, 565-0871, Japan; e-mail: trkata@patho.med.osaka-u.ac.jp.



Downloaded from http://ijs.sagepub.com by guest on February 24, 2008
© 2007 SAGE Publications. All rights reserved. Not for commercial use or unauthorized distribution.

or IPMCs but not in normal pancreatic duct or IPMAs. Almost all cases expressing STAT5B showed positive staining in the nuclei of tumor cells with borderline malignant or malignant atypia. The presence of nuclear expression of STAT5B was related to Ki-67 labeling index in the lesions. Taken together, STAT5B proteins could be activated and involved in tumor cell proliferation during IPMN progression.

## Materials and Methods

### Patients

We selected 44 patients who underwent curative resection at the Osaka Medical Center for Cancer and Cardiovascular Diseases from January 1990 to July 2003. Informed consent from patients and approval from our institutional review board were obtained. Surgically resected specimens were fixed in 10% formalin and embedded in paraffin. Sections (cut to 3-μm thickness) were stained with hematoxylin and eosin (H&E). Histological examination was performed by TRK and SI. The classification of the IPMNs was based on their morphological appearance and their immunohistochemical reactivities for MUC1, MUC2, and MUC5.2.

### Immunohistochemical Analysis

After deparaffinization with xylene, tissue sections were rehydrated and pretreated with 0.3% hydrogen peroxide for 5 minutes Sections were set in AutoStainer plus (DakoCytomation, Glostrup, Denmark) after steam heating for 30 minutes The antibodies used were anti-MUC1 (Clone; Ma552, Novocastra Laboratories, Newcastle, UK, a dilution of 1:100), anti-MUC2 (Clone; Ccp58, Novocastra, 1:200), anti-MUC5 (Clone; CLH2, Novocastra, 1:500), anti-MUC6 (Clone; CLH5, Novocastra, 1:500), anti-STAT5A (Clone; L-20, Santa Cruz Biotechnology, Santa Cruz, CA, 1:100), anti-STAT5B (Clone; G-2, Santa Cruz Biotechnology, 1:100) and anti-Ki-67 (Clone; MIB-1, DakoCytomation, 1:1000). Staining was performed using the avidin–biotin complex peroxidase (DakoCytomation).

### Assessment of Immunostaining

We selected a representative section of each case and evaluated immunostaining. We used immunohistochemical reactivities to islet cells as inner control for anti-STAT5A or anti-STAT5B. Reactivities weaker than acinar cell staining were considered as non-specific staining. In each lesion, the presence of ductal cells with a visible nuclear reaction to anti-STAT5A

Table 1. Clinical features of IPMNs.

(A) IPMAs, borderline IPMNs, and IPMCs.

| | Number of cases | M:F | Age (years old) |
|---|---|---|---|
| IPMAs | 11 | 7:4 | 63.9 ± 2.17 |
| Borderline IPMNs | 12 | 10:2 | 65.9 ± 1.90 |
| IPMCs | 21 | 16:5 | 67.6 ± 1.57 |

(B) Gastric type, intestinal type, pancreatobiliary type, and oncocytic type.

| | Number of cases | M:F | Age (years old) |
|---|---|---|---|
| Gastric | 9 | 6:3 | 62.4 ± 2.25 |
| Intestinal | 27 | 24:3 | 67.3 ± 2.17 |
| Pancreatobiliary | 5 | 1:4 | 70.0 ± 4.38 |
| Oncocytic | 3 | 2:1 | 62.7 ± 3.84 |

Note: IPMN, intraductal papillary mucinous neoplasm; IPMA; intraductal papillary mucinous adenoma; IPMC, intraductal papillary mucinous carcinoma.

Table 2. Nuclear expression of STAT5 in IPMNs.

(A) IPMAs, borderline IPMNs, and IPMCs.

| | STAT5A (%) | STAT5B (%) |
|---|---|---|
| IPMAs | 0 case / 11 cases (0 %) | 0 case / 11 cases ( 0 %) |
| Borderline IPMNs | 0 case / 12 cases (0 %) | 7 cases / 12 cases (58 %) |
| IPMCs | 0 case / 21 cases (0 %) | 14 cases / 21 cases (67 %) |

(B) Gastric type, intestinal type, pancreatobiliary type, and oncocytic type.

| | STAT5A (%) | STAT5B (%) |
|---|---|---|
| Gastric | 0 case / 9 cases (0 %) | 0 case / 9 cases (0 %) |
| Intestinal | 0 case / 27 cases (0 %) | 15 case / 27 cases (56 %) |
| Pancreatobiliary | 0 case / 5 cases (0 %) | 4 case / 5 cases (80 %) |
| Oncocytic | 0 case / 3 cases (0 %) | 2 case / 3 cases (67 %) |

Note: STAT, signal transducers and activators of transcription; IPMA; intraductal papillary mucinous adenoma; IPMN, intraductal papillary mucinous neoplasm; IPMC, intraductal papillary mucinous carcinoma.

Downloaded from http://ijs.sagepub.com by guest on February 24, 2008
© 2007 SAGE Publications. All rights reserved. Not for commercial use or unauthorized distribution.

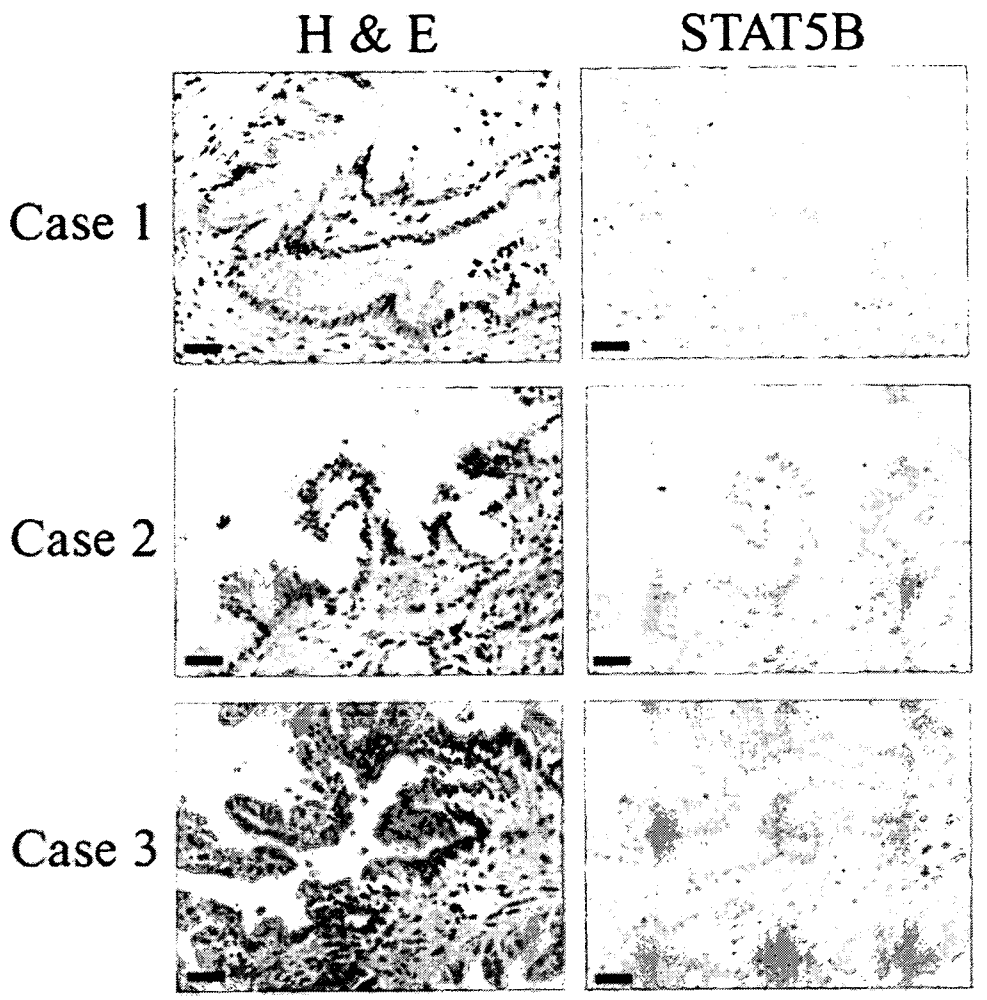


Figure 1. Histological appearance of IPMNs and immunostaining for STAT5B. We diagnosed Case 1 as IPMA (gastric type), Case 2 as borderline IPMN (intestinal type), and Case 3 as IPMC (pancreatobiliary type). Cases 2 and 3 were nuclear positive for STAT5B. Scale bars: 50 μm.

or anti-STAT5B antibodies were regarded as positively labeled. No stained ductal cells or cells with cytoplasmic stain by anti-STAT5A or anti-STAT5B antibodies were considered as negative. Staining with anti-Ki-67 antibody was estimated in the same field using the evaluation of anti-STAT5A or anti-STAT5B staining.

## Results

Forty-four IPMNs (11 IPMAs, 12 borderline IPMNs, and 21 IPMCs / 9 gastric type IPMNs, 27 intestinal type IPMNs, 5 pancreatobiliary type IPMNs, 3 oncocytic type IPMNs) were examined. Features of patients with IPMNs were summarized in Tables 1A and 1B. None of the patients received any treatment when the samples were resected.

Table 3. The Association Between Ki-67 Index and Nuclear Expression of STAT5B in IPMNs

| | Ki-67 Index (%) |
|---|---|
| Nuclear expression (−) | 7.13 ± 2.07 |
| Nuclear expression (+) | 16.4 ± 3.57 |

Note: Data are expressed as the means ± SE; $P$ value = .00519.

First, we examined STAT5A expression in these samples by immunohistochemical method. None of these specimens were stained by anti-STAT5A antibody

Downloaded from http://ps.sagepub.com by guest on February 24, 2008
© 2007 SAGE Publications. All rights reserved. Not for commercial use or unauthorized distribution.

(Tables 2A and 2B), though 5-week-old C57BL/6 mouse mammary gland specimen was stained with this antibody (data not shown).[8]

Next, we performed immunohistochemical staining of IPMN samples by anti-STAT5B antibody. This antibody showed the immunohistochemical reactivity to the nuclei of some islet cells in the specimen (data not shown). Normal ductal cells in all examined specimen were not stained by STAT5B staining. None of the ductal cells of 11 IPMAs or 9 gastric type IPMNs were stained by anti-STAT5B antibody (Tables 2A and 2B, Fig. 1). Some borderline IPMNs and IPMCs were STAT5B-positive (borderline IPMNs 7 cases/12 cases [58%] and IPMCs 14/21 [67%]) (Table 2A, Fig. 1). Some IPMN cases classified into intestinal type, pancreatobiliary type, or oncocytic type showed positive reactivities to anti-STAT5B antibody (intestinal type 15 cases/27 cases [56%], pancreatobiliary type 4 cases/5 cases [80%], and 2 cases/3 cases [67%]) (Table 2B, Fig. 1) In all positive-stained cases, more than half the nuclei of tumor cells were stained. One case of IPMCs was negative, but cytoplasms of some cancer cells of this IPMC were stained by anti-STAT5B antibody (data not shown). There was no reactivity to the ductal cells without atypia in borderline IPMN or IPMC samples.

STAT5 was reported to be involved in the proliferation of tumor cells.[4,5] Therefore, we tested the association between STAT5B nuclear localization and the proliferative property of IPMNs. We used Ki-67 labeling index as the proliferative marker. IPMNs with nuclear STAT5B staining showed significant higher Ki-67 index than those without STAT5B staining (Table 3). In our specimen, STAT5B and Ki-67 were not always coexpressed in IPMN cells.

## Discussion

Some alterations of cell cycle regulators in the progression of IPMNs have been described.[1,3] In the present data, we showed the change of STAT5B expression pattern during IPMN development. STAT5 is also involved in cell proliferation,[4,5,9,10] therefore this observation seems to be reasonable. Cell proliferation activity increases during IPMN development.[11] STAT5 could contribute to this process. STAT5 controls cell proliferation by regulating the expression of some cell cycle regulators,[4,5] including $p21^{Cip1/WAF1}$.[9,10] p21 expression increases during the development of intraductal lesions of pancreas.[12,13] We also examined whether STAT5 nuclear expression and p21 expression were related. There was no significant relationship between them (data not shown). p21 do not seem to be involved in STAT5-mediated IPMN proliferation.

STAT5 plays some role in breast cancer progression.[14-16] Intraductal lesions of breast cancer express STAT5 like IPMNs of the pancreas.[14] In breast cancer, STAT5 inhibits invasive property.[15] STAT5B expression was also examined in 10 cases of invasive tubular adenocarcinoma of the pancreas after chemoradiation therapy, and we detected nuclear expression of STAT5B in 2 of 10 cases (data not shown). STAT5B did not seem to be associated with pancreatic cancer invasion because STAT5B expression in invasive cancers decreased when compared with that in borderline IPMNs or IPMCs.

STAT5 activation in breast cancer closely relates to favorable prognosis.[16] We also examined the relationship between STAT5B expression and IPMN prognosis. None of the patients examined had nodal or distant metastasis. Five patients had experienced local relapse during our follow-up (Case 1 [IPMA], 60 months after operation; Case 2 [IPMC], 44 months after operation; Case 3 [IPMC], 116 months after operation; Case 4 [IPMC], 89 months after operation; and Case 5 [IPMC], 125 months after operation). Cases 1, 2, and 3 were STAT5B-negative, and Cases 4 and 5 expressed STAT5B in the nuclei of IPMNs. There was no significant association between the status of STAT5B and the occurrence of relapse of this disease.

IPMNs are also divided into 4 subtypes according to their MUCs expression and their morphologies.[2] Among these subtypes, the gastric type IPMNs usually show mild atypia, and are negative for MUC2, which is a potent indicator for malignancy.[2,17] In this study, we examined 9 gastric type IPMNs which were composed of 8 IPMAs and 1 borderline IPMN, and revealed that all cases of gastric type were negative for STAT5B. Negative regulation of STAT5B seems to explain favorable phenotypes of the gastric type IPMNs, partially. We also observed that there was no significant difference among the proportion of STAT5B-positive cases of the intestinal type, that of the pancreatobiliary type, and that of the oncocytic type. STAT5B was not concerned with morphological features.

There are many reports on the relationship between STAT3 and tumorigenesis like STAT5[4,5]

Downloaded from http://ijs.sagepub.com by guest on February 24, 2008
© 2007 SAGE Publications. All rights reserved. Not for commercial use or unauthorized distribution.

and STAT3 activated pancreatic invasive adenocarcinoma progression.[6,7] Therefore, we also examined STAT3 expression in IPMNs. STAT3 expressed in the nuclei of 2 IPMN cases (one was borderline IPMN and the other was IPMC) and in the cytoplasms of all examined IPMN cases (data not shown). We were not able to clarify the relationship between STAT3 and IPMN progression.

In summary, STAT5B activation was correlated to progression and proliferation of IPMNs. STAT5B might be a useful marker for distinguishing IPMAs from borderline IPMNs or IPMCs for pathologists.

## Acknowledgments

The authors thank Ms M. Sugano and Ms T. Sawamura for technical assistance. This work is supported by grants from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

## References

1. Hruban RH, Takaori K, Klimstra DS, et al. An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. *Am J Surg Pathol.* 2004;28:977-987.
2. Furukawa T, Kloppel G, Adsay NV, et al. Classification of types of intraductal papillary-mucinous neoplasm of the pancreas: a consensus study. *Virchows Arch.* 2005;447: 794-799.
3. Bardeesy N, DePinho RA. Pancreatic cancer biology and genetics. *Nat Rev Cancer.* 2002;2:897-909.
4. Calo V, Migliavacca M, Bazan V, et al. STAT proteins: from normal control of cellular events to tumorigenesis. *J Cell Physiol.* 2003;197:157-168.
5. Benekli M, Baer MR, Baumann H, Wetzler M. Signal transducer and activator of transcription proteins in leukemias. *Blood.* 2003;101:2940-2954.
6. Greten FR, Weber CK, Greten TF, et al. Stat3 and NF-κB activation prevents apoptosis in pancreatic carcinogenesis. *Gastroenterology.* 2002;123:2052-2063.
7. Scholz A, Heinze S, Detjen KM, et al. Activated signal transducer and activator of transcription 3 (STAT3) supports the malignant phenotype of human pancreatic cancer. *Gastroenterology.* 2003;125:891-905.
8. Philp JAC, Burdon TG, Watson CJ. Differential activation of Stats 3 and 5 during mammary gland development. *FEBS Lett.* 1996;396:77-80.
9. Nosaka T, Kawashima T, Misawa K, Ikuta K, Mui AL, Kitamura T. STAT5 as a molecular regulator of proliferation, differentiation and apoptosis in hematopoietic cells. *EMBO J.* 1999;18:4754-4765.
10. Matsumura I, Ishikawa J, Nakajima K, et al. Thrombopoietin-induced differentiation of a human megakaryoblastic leukemia cell line, CMK, involves transcriptional activation of $p21^{WAF1/Cip1}$ by STAT5. *Mol Cell Biol.* 1997;17:2933-2943.
11. Terada T, Ohta T, Kitamura Y, Ashida K, Matsunaga Y. Cell proliferative activity in intraductal papillary-mucinous neoplasms and invasive ductal adenocarcinomas of the pancreas. An immunohistochemical study. *Arch Pathol Lab Med.* 1998;122:42-46.
12. Biankin AV, Kench JG, Morey AL, et al. Overexpression of $p21^{WAF1/Cip1}$ is an early event in the development of pancreatic intraepithelial neoplasia. *Cancer Res.* 2001; 61:8830-8837.
13. Biankin AV, Kench JG, Biankin SA, et al. Pancreatic intraepithelial neoplasia in association with intraductal papillary mucinous neoplasms of the pancreas. Implications for disease progression and recurrence. *Am J Surg Pathol.* 2004;28:1184-1192.
14. Shan L, Yu M, Clark BD, Snyderwine EG. Possible role of Stat5a in rat mammary gland carcinogenesis. *Breast Cancer Res Treat.* 2004;88:263-272.
15. Nevalainen MT, Xie J, Torhorst J, et al. Signal transducer and activator of transcription-5 activation and breast cancer prognosis. *J Clin Oncol.* 2004;22:2053-2060.
16. Saltan AS, Xie J, Le Baron MJ, Ealley EL, Nevalainen MT, Rui H. Stat5 promotes homotypic adhesion and inhibits invasive characteristics of human breast cancer cells. *Oncogene.* 2005;24:746-760.
17. Nakamura A, Horinouchi M, Goto M, et al. New classification of pancreatic intraductal papillary-mucinous tumour by mucin expression: its relationship with potential for malignancy. *J Pathol.* 2002;197:201-210.

Downloaded from http://ijs.sagepub.com by guest on February 24, 2008
© 2007 SAGE Publications. All rights reserved. Not for commercial use or unauthorized distribution.

# Concurrent Chemoradiotherapy for Advanced Pancreatic Cancer

## 1,000 mg/m² Gemcitabine can be Administered Using Limited-Field Radiotherapy

Hideya Yamazaki[1,2], Kinji Nishiyama[1], Masahiko Koizumi[1], Eiichi Tanaka[1], Tatsuya Ioka[3], Hiroyuki Uehara[3], Hiroyasu Iishi[3], Akihiko Nakaizumi[3], Hiroaki Ohigashi[4], Osamu Ishikawa[4]

**Purpose:** To examine the feasibility of concurrent use of full-dose gemcitabine (GEM) and radiotherapy for advanced pancreatic cancer.
**Patient and Methods:** 22 patients with advanced pancreatic cancer were subjected to concurrent chemoradiotherapy (GEM 1,000 mg/m² weekly, three times during 4 weeks). They received limited-field irradiation by three-dimensional radiotherapy planning.
**Results:** Of the 22 patients, 16 (72%) completed the treatment (50 Gy irradiation and at least three times concurrent administration of 1 g/m² GEM). One patient with unresectable tail cancer showed peritonitis carcinomatosa and both chemotherapy and radiotherapy had to be stopped. Dose reduction or omission of GEM was necessary in another four patients. In addition, radiotherapy was discontinued in one patient for fatigue. Grade 3 hematologic toxicity was detected in eight patients (36%), and grade 3 nonhematologic toxicity (anorexia) in one patient (5%). In total, the response rate amounted to 32% (seven partial responses), and the median survival time (MST) was 16 months. Among the twelve patients who received preoperative chemoradiotherapy, nine underwent surgery and showed a survival rate of 78% at 1 year. Another 13 patients without surgery showed 14 months of MST. No regional lymph node failure has appeared so far.
**Conclusion:** Limited-field radiotherapy enables the safe concurrent administration of 1,000 mg/m² GEM.

**Key Words:** Pancreatic cancer · Gemcitabine · Limited-field radiotherapy · 3D-CRT

Strahlenther Onkol 2007;183:301–6
DOI 10.1007/s00066-007-1641-1

**Simultane Radiochemotherapie bei fortgeschrittenem Pankreaskarzinom. 1000 mg/m² Gemcitabin können zusammen mit begrenzter Radiotherapie verabreicht werden**

**Ziel:** Untersuchung der Durchführbarkeit einer simultanen Verabreichung von Gemcitabin (GEM) in voller Dosis und Radiotherapie bei fortgeschrittenem Pankreaskarzinom.
**Patienten und Methodik:** 22 Patienten mit fortgeschrittenem Pankreaskarzinom wurden mit simultaner Radiochemotherapie (GEM 1000 mg/m² wöchentlich, dreimal während 4 Wochen) behandelt. Sie erhielten eine begrenzte Bestrahlung auf der Basis einer dreidimensionalen konformen Radiotherapie-Planung.
**Ergebnisse:** 16 der 22 Patienten (72%) schlossen die Behandlung ab (50 Gy Bestrahlung und simultane Verabreichung von mindestens dreimal 1 g/m² GEM). Ein Patient mit irresektablem Pankreasschwanzkarzinom wies eine Peritonitis carcinomatosa auf, und sowohl die Chemo- als auch die Radiotherapie mussten abgebrochen werden. Bei vier weiteren Patienten wurde eine Dosisverringerung oder Auslassung von GEM notwendig. Darüber hinaus wurde die Radiotherapie bei einem Patienten wegen Erschöpfung abgebrochen. Eine hämatologische Toxizität Grad 3 wurde bei acht (36%) und eine nichthämatologische Toxizität Grad 3 (Anorexie) bei einem Patienten (5%) beobachtet. Die Ansprechrate betrug insgesamt 32% (siebenmal partielles Ansprechen) und die mediane Überlebenszeit (MST) 16 Monate. Von den zwölf Patienten, die sich einer präoperativen Radiochemotherapie unterzogen, wurden neun operiert; diese Patienten wiesen nach 1 Jahr eine Überlebensrate von 78% auf. Bei den 13 Patienten ohne operativen Eingriff ergab sich eine MST von 14 Monaten. Bisher ist es zu keinem Ausfall der regionären Lymphknotenfunktion gekommen.
**Schlussfolgerung:** Eine begrenzte Radiotherapie ermöglicht die sichere simultane Verabreichung von 1000 mg/m² GEM.

**Schlüsselwörter:** Pankreaskarzinom · Gemcitabin · Begrenzte Bestrahlung · 3D-CRT

[1] Department of Radiation Oncology, Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka, Japan,
[2] Department of Radiology, National Hospital Organization Osaka National Hospital, Osaka, Japan,
[3] Department of Internal Medicine, Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka, Japan,
[4] Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka, Japan.

Received: August 24, 2006; accepted: April 3, 2007

## Introduction

The annual mortality from pancreatic cancer continues to nearly equal the incidence, and few significant advances have been made in nonoperative treatment of these patients [12]. Combined-modality treatment of pancreatic cancer has largely been based on data from serial Gastrointestinal Tumor Study Group trials of 5-fluorouracil (5-FU) [12, 25]. The benefits of combined-modality treatment compared with radiotherapy alone were demonstrated and hereafter, 5-FU and combined irradiation have been a standard therapy for patients with unresectable disease [22, 25, 29].

Gemcitabine (GEM) is a novel deoxycitidine analog with a broad spectrum of cytotoxic activity [1]. Through several phase III trials, GEM has been indicated as a first-line treatment for patients with advanced pancreatic cancer including metastatic tumor, because it improves survival [28] and has clinical advantages (pain reduction, improved performance status, weight gain) over 5-FU treatment [6, 19].

GEM is also a potent radiosensitizer with mechanisms that have not been fully identified [4, 18, 20, 30]. Several clinical trials using GEM instead of 5-FU in concurrent radiotherapy have been performed [2, 5–11, 13, 27, 31]. However, the optimal schedule has not yet been clearly established. As distant metastasis is mostly a component of failure, earlier and extensive application of systemic agents may be the best strategy for controlling micrometastases. In accordance with this notion, the use of a standard dose (1 g/m$^2$) of GEM was considered. However, at the same time, as not only tumor cells, but also normal tissue are GEM-sensitive, standard dose administration may also enhance normal tissue damage. To overcome this limitation, we reduced the radiation field with the aid of three-dimensional planning, which is now becoming a standard treatment. This means that prophylactic wide-field radiotherapy for regional nodes, which induces serious adverse reactions, may not always be necessary. Instead, adequate targeting of the gross tumor volume (GTV), with a minimal margin of normal tissue, may be more important in radiotherapy. Based on this concept, we conducted a phase I study and found 50 Gy/25 fractions/5 weeks of radiotherapy were tolerable with concurrent administration of weekly 1,000 mg/m$^2$ GEM [14]. Following that study, we here report our successive results of concurrent chemoradiotherapy using full-dose GEM by limited-field three-dimensional radiotherapy planning for patients, either preoperative [23] or unresectable, with advanced pancreatic cancer.

Table 1. Patient characteristics. F: female; M: male.

Tabelle 1. Patientencharakteristika. F: weiblich; M: männlich.

| Characteristics | Strata | Values |
|---|---|---|
| Sex (n) | M/F | 13/9 |
| Age (years) | Range (median) | 53–75 (66) |
| Performance status (n) | 0/1 | 16/6 |
| Tumor location (n) | Head/body/tail | 16/4/2 |
| T- and N-category (n) | T2 N1/T3 N0/T3 N1/T4 N0 | 1/10/2/9 |
| Clinical stage[a] (n) | IIa/IIb/III | 10/3/9 |

a2002 UICC TNM

## Patients and Methods

Between July 2002 and August 2003, 22 patients with histologically or cytologically confirmed pancreatic adenocarcinoma received concurrent chemoradiotherapy using 1,000 mg/m$^2$ GEM once a week (three times during 4 weeks). This study included 13 men and nine women, whose age ranged from 53 to 75 years (median 66 years; Table 1). All patients showed good performance status (World Health Organization [WHO]) 0 or 1 and were followed up for at least 23 months or until their death (3–28 months; median 14 months). Twelve patients received this treatment for preoperative chemoradiotherapy (eight stage IIA, two stage IIB, two stage III), while ten patients had been diagnosed as unresectable (two stage IIA, one stage IIB, seven stage III; 2002 UICC TNM classification, 6th version). Stage was determined by helical CT and ultrasonography, and chest X-ray. CT criteria for the nonresectability of the tumor were inclusion of tumor encasement of the celiac trunk and/or superior mesenteric artery and obstruction or bilateral invasion of the portal vein. Paraaortic lymph node swelling is also a contraindication to surgery.

Radiation fields were determined as follows: for patients with unresectable pancreatic cancer, the planning target volume (PTV) was comprised of the addition of a 1- to 1.5-cm margin to GTV (primary tumor and apparent lymph node shortest diameter > 1 cm). Radiation fields were determined by adding a 3- to 5-mm margin to PTV (Figure 1). In general, four fields (anterior, posterior, left and right lateral fields) were used for patients with unresectable disease.

For preoperative radiation therapy, the PTV was determined based on GTV and the celiac axis, superior mesenteric artery and paraaortic area (Figure 2). The posterior margin was placed 1–1.5 cm behind the anterior margin of vertebral bodies, right and left lateral margins were 0–1.0 cm outside the vertebral body at the celiac level, and 1–1.5 cm at the superior mesenteric artery level with at least a 1-cm margin from the left side of the abdominal aorta. The anterior border included the primary lesion and a 0.5- to 1-cm margin anterior to the superior mesenteric artery and celiac axis. Superior and inferior borders were created at the levels of the celiac axis and the third portion of the duodenum. In most cases, five portals were used at angles of 0°, 60°, 150°, 210°, and 300°. The angle of at least one portal was modified to avoid the spinal cord, so that irradiation of the spinal cord had to be limited to 40 Gy. These fields were in the shape of a pentagon (Figure 2). Data obtained with treatment-planning CT (Hitachi W1000, Hitachi Medical Co., Tokyo, Japan) were transferred onto Cadplan (Varian Medical System Co., Palo Alto, CA, USA). Volume of PTV was measured by Cadplan. Radiation therapy was delivered through two to